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Synthesis of N^{α}-(1-Phenyl-2-mercaptoethyl) Amino Acids, New Building Blocks for Ligation and Cyclization at Non-Cysteine Sites: Scope and Limitations in Peptide Synthesis

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Received March 31, 2004

A new and convenient method for the synthesis and incorporation of N^{α}-(1-phenyl-2-mercaptoethyl)-derivatized amino acids applicable to chemical ligation at non-cysteine sites is presented. N^{α}-Auxiliary derivatives of glycine and alanine were easily prepared using reductive amination approaches. Several strategies for the incorporation of these derivatives into peptide chains were investigated: coupling without protection, with acid-labile protection, with base-labile protection, and via a novel protection strategy using the thiazolidine derivative. All amino acid derivatives were successfully coupled to various peptide resins, and with the exception of those incorporating Boc-protected derivatives, all resins yielded the desired peptide fragments. However, the coupling of the two alanine derivative diastereomers generated some epimerization. Finally, N-terminal auxiliary glycine and alanine peptides were cyclized, and the corresponding native circular peptides were obtained upon successful removal of the auxiliary.

Introduction

The ability to ligate or cyclize peptide fragments at non-cysteine sites has become critical in the postgenomics era[1] and in the current efforts to develop enhanced drug candidates.[2] A rapid and efficient way to access proteins discovered by the modern paradigms of pharmaceutical research, notably proteome analyses, would represent a key component in the advance toward accelerated discovery of potential new therapeutic agents and drug targets.[3] Similarly, medical research would benefit from new methodology facilitating the production of cyclic peptides, which are becoming increasingly important as new antimicrobial agents,[4] in cancer research[5] and in various fields of medicinal chemistry.[6] The past decade has provided extensive demonstration of the utility of native chemical ligation (NCL)[7] for the rapid preparation of medium-sized proteins with a high level of homogeneity.[8]

This approach exploits a chemoselective reaction between two unprotected fragments, a C-terminal thioester and an N-terminal cysteine, in aqueous solution at neutral pH. In the first step of the reaction transthioesterification occurs at pH 6−8 by thiol exchange between the free thiol of the N-terminal cysteine and the thioester moiety on the other molecule. The newly generated thioester then undergoes an S to N acyl shift due to the proximity of the amino group to the thioester functionality, thus generating a native amide bond at the ligation site. By the same principle, NCL has been extended to the generation of cyclic peptides via an intramolecular reaction.[9]

A drawback with standard NCL is the intrinsic restriction imposed by the requirement for cysteine at the site of peptide ligation/cyclization. In proteins, as well as in many cyclic peptides, cysteine occurs with a relatively low frequency; many protein sequences do not have suitably disposed cysteines for the NCL strategy, and some lack cysteine residues entirely. Similarly, many cyclic peptides of high biological relevance do not contain cysteine residues. Although several methodologies for the

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10.1021/jo049471k CCC: $27.50 © 2004 American Chemical Society
Published on Web 11/13/2004

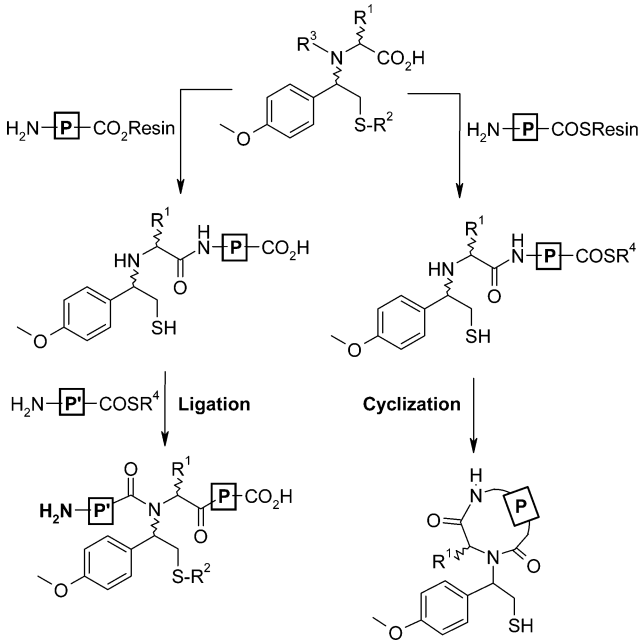
cyclization of peptides both on the solid phase and in solution have been described,[10] a technique enabling both NCL and cyclization of unprotected peptides in aqueous solution at an X−X site (where X = any amino acid) would greatly facilitate the chemical synthesis of new protein targets and would represent a valuable tool for both the discovery and the large-scale synthesis of cyclic peptides of potential therapeutic interest.

In this context, we have recently presented a novel and practical method that extends NCL to the joining of peptide segments at the X-Gly site,[11] by making use of a removable N^{α}-(1-phenyl-2-mercaptoethyl) auxiliary designed to closely mimic a native cysteine during ligation. ECL (extended chemical ligation) strategy is based on the same mechanistic pathway as NCL, generating an amide bond at the ligation/cyclization site. Preparation of the N^{α}-(1-phenyl-2-mercaptoethyl) peptide fragments for ECL is achieved by the synthesis of the appropriate benzylamine precursor and its subsequent reaction with the fully protected N-terminal bromoacetylated peptide. Final acid treatment only removes the auxiliary when on the amide bond, thus allowing unambiguous confirmation that the product is the desired material.[12]

This strategy presents a few limitations, however. Although it has been successfully applied to generate a native glycine residue at the ligation/cyclization site,[12,13] it would not be appropriate for the unambiguous preparation of N^{α}-(1-phenyl-2-mercaptoethyl) peptide fragments featuring a substituted residue at the N-terminus. Indeed, the reaction on the solid support of the benzylamine derivative of choice with a secondary 2-bromoacetyl residue precursor of the desired N-terminal amino acid (for example, the 2-bromopropionyl template is a precursor for alanine) could not reach completeness depending on the residue and the size of the peptide, and may generate side products via an elimination mechanism.[14] Furthermore, when on a thioester resin, an almost stoichiometric ratio between the benzylamine precursor and the bromine is required to minimize amidation of the thioester moiety.[13]

On the basis of these observations and on our previous results, we set out to design a general method for the synthesis of N^{α}-(1-phenyl-2-mercaptoethyl) amino acid derivatives (N^{α}(Aux)-AA-OH)[15] to enable the direct incorporation of the appropriate auxiliary-derivatized amino acid into either C^{α}-carboxyester or C^{α}-thioester peptide fragments. In this paper we present the chemical syntheses of these derivatives, comparing several N^{α} protection approaches, including use of acid- or base-labile protecting groups, direct coupling with unprotected derivatives, and thiazolidine formation as a novel protecting moiety for the 1,2-aminothiol of the auxiliary. We also present a preliminary evaluation of the potential of these novel building blocks with direct use in SPPS, and the syntheses of model circular peptides with glycine and alanine at cyclization sites.

SCHEME 1. N^{α}-(1-Phenyl-2-mercaptoethyl) Amino Acid Derivatives for ECL[a]



[a] P and P′ = peptides. R^1 = amino acid side chain, R^2 = thiol protecting group, R^3 = H or N protecting group, R^4 = alkyl chain.

Results and Discussion

To exploit the concept of preformed N^{α}-auxiliary-derivatized amino acids ready for coupling to a peptide resin by standard methods (Scheme 1), we prepared auxiliary-derivatized glycine and alanine. We chose alanine as the most suitable substituted candidate, because it is statistically one of the most abundant residues in natural proteins and it bears the smallest side chain among the substituted amino acids.

Apart from enabling the preparation of derivatives carrying both the amino acid and the auxiliary moiety necessary for ligation, our synthetic approach presents a further advantage. On the basis of a reductive amination key step, it involves the conjugation of an optically pure amino acid (alanine) with the auxiliary moiety, generating a new stereogenic center. This gives access to two resulting diastereomers, which can be separated, thus allowing a comparative study of their reactivity during the ligation/cyclization reactions.

Synthesis of N^{α}-Auxiliary-Derivatized Glycine and Alanine. The derivatized amino acids N^{α}(Aux)-Gly-OH and N^{α}(Aux)-Ala-OH were obtained by the concise, facile, and general strategy described in Scheme 2. The synthesis of the N^{α}(Aux)-Gly-OH derivative **4** can be accomplished either via its benzylamine precursor **3** or via the general procedure required for the side-chain-functionalized amino acids (Scheme 2). The benzylamine precursor **3** was synthesized from 1-(4-methoxyphenyl)-2-(4-methylbenzylsulfanyl)ethanone (**1**) through the oxime intermediate **2** using a preparation slightly modified from that previously reported.[11] Next, reductive amination with glyoxylic acid[16] gave racemic N^{α}(Aux)-Gly-OH **4** in

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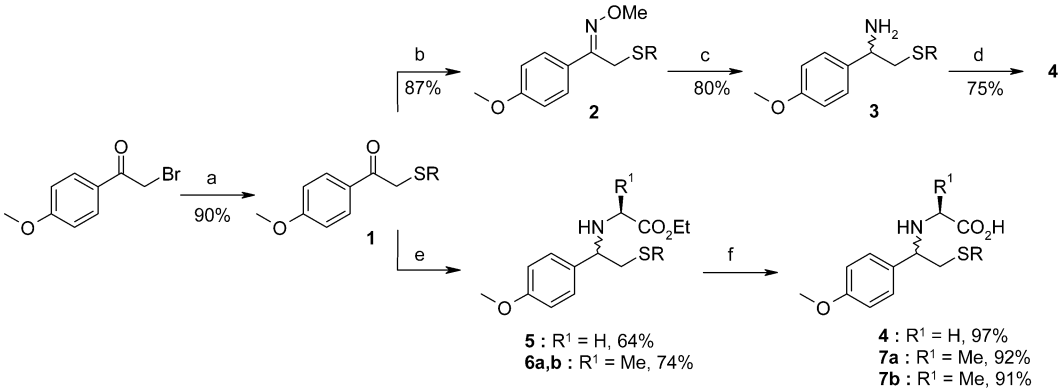
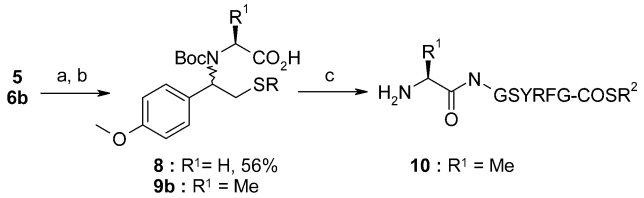
(15) When our work was in its final revision before submission, a related work exploiting a similar concept was published: Clive, D. L. J.; Hisaindee, S.; Coltart, D. M. *J. Org. Chem*. **2003**, *68*, 9247−9254.

SCHEME 2. Synthesis of Derivatized Amino Acids[a]



[a] Reagents and conditions: (a) *p*-$MeC_6H_4CH_2SH$, DIEA, DMF, rt, 4 h; (b) H_2NOMe, pyr, EtOH, reflux, 3 h; (c) BH_3·THF, THF, 3 h reflux; (d) glyoxylic acid, $NaBH_3CN$, MeOH, rt, 17 h; (e) $H_2NCH(R^1)CO_2Et$, BF_3·OEt, toluene, reflux, 6−8 h, then $NaBH_4$, MeOH, 0 °C, 2 h; (f) NaOH, dioxane/H_2O, rt, 17 h. R = *p*-$MeC_6H_4CH_2$−.

SCHEME 3 *N*-Boc Protection and Coupling[a]



[a] Reagents and conditions: (a) Boc_2O, NEt_3, EtOH/CH_2Cl_2, rt, 48 h; (b) NaOH, dioxane/H_2O, rt, 24 h; (c) H_2N-GSYRFG-COS-Resin, **9b**,TBTU, DIEA, DMF, rt, 1.5 h, TFA (2 × 1 min), then HF, *p*-cresol, 0 °C, 1 h. R = *p*-$MeC_6H_4CH_2$−, R^2 = $-CH_2CH_2CO$-Leu-OH.

75% yield from the benzylamine precursor. The general procedure for the preparation of the N^{α}(Aux)-Gly-OH **4** and N^{α}(Aux)-Ala-OH **7a,b** involves a reductive amination reaction between ketone **1** and the amino acid ethyl ester of choice. The diastereomers **6a** and **6b** from the reaction with L-alanine ethyl ester were separated by flash chromatography to give pure compounds **6a** (less polar) and **6b** (more polar). Subsequent saponification of **5**, **6a**, and **6b** afforded amino acids **4**, **7a**, and **7b** in, respectively, 97%, 92%, and 91% yield.

Synthesis and Side Reaction of Boc-N^{α}(Aux)-AA-OH. Our first choice for N^{α} protection of these amino acids was to use classical Boc protection (Scheme 3). The Boc-N^{α}(Aux)-Gly-OH derivative **8** was obtained by reaction of **5** with Boc-anhydride followed by the hydrolysis of the ester bond, producing the desired compound in 56% yield.

Our attempts to prepare the N^{α}-Boc-protected alanine derivative of **9b** gave relatively poor results. Indeed, under conditions identical with those used to synthesize Boc-N^{α}(Aux)-Gly-OH derivative **8**, **9b** was obtained only in 18% yield. The diminished reactivity of the alanyl template was certainly due to its increased steric hindrance. Nevertheless, having both the N^{α}-Boc-protected derivatives **8** and **9b**, we decided to start testing them by coupling to a peptide resin.

In a typical experiment, Boc-N^{α}(Aux)-Ala-OH **9b** was coupled to a model peptide resin of sequence GSYRFG-COS-Resin using the standard TBTU coupling method[17] (Scheme 3). After Boc removal using two 1 min 100% TFA cycles, standard HF treatment was performed to cleave the peptide from the resin. The resulting peptide showed a mass value corresponding to the N-terminal alanine peptide without the auxiliary group. Since it is known[11] that the auxiliary group can be cleaved only when on an amide, this result can be explained by the carbamate nature of the protected *N*-Boc group, suggesting that the TFA-induced auxiliary cleavage most probably takes place before or concomitantly with Boc removal. To find selective Boc cleavage conditions, Boc-N^{α}(Aux)-Gly-OH **8** was treated with different percentages of TFA (25%, 5%, and 1%) in dichloromethane, at room temperature. While in the first two cases both the auxiliary and the Boc group were completely cleaved in 30 min, leaving native glycine as the reaction product, we detected some unreacted material when the amount of TFA was reduced to 1%. Encouraged by the latter result, we investigated the possibility of using a super-acid-labile protecting group, which might allow its own selective cleavage in very mild conditions, leaving the auxiliary intact. To this end we tried to prepare the N^{α}-Bpoc-protected version of (Aux)-Gly-OH as reported.[18] Unfortunately, all our efforts to prepare such a derivative completely failed, even on the glycine template, and we decided to explore different strategies.

Synthesis of N^{α}-(1-Phenyl-2-mercaptoethyl) Peptide Segments. Due to the challenges of using N^{α}-acid-labile-protected auxiliary-derivatized amino acids, we decided to investigate coupling using both N^{α}-unprotected and N^{α}-base-labile-protected amino acids (Aux)-AA-OH.

Coupling Using N^{α}-Unprotected (Aux)-AA-OH. The coupling of unprotected derivatives **4**, **7a**, and **7b** (Scheme 4) involved a very judicious excess of the incoming derivative (1.1 equiv) and is based on the hypothesis that the activated N^{α}-unprotected (Aux)-AA-OH should be much more likely to couple to the unhindered primary amine on the solid support rather than to oligomerize by self-reacting with the secondary and much

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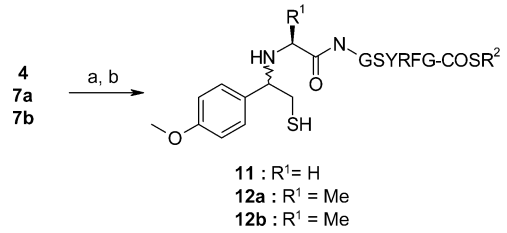
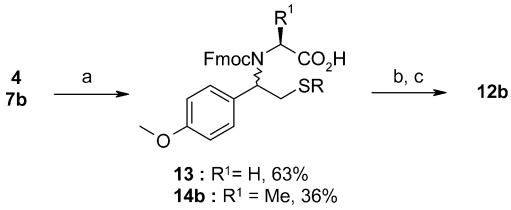
SCHEME 4. Coupling of N-Unprotected (Aux)-AA-OH[a]



[a] Reagents and conditions: (a) H_2N-GSYRFG-COS-Resin, TBTU, DIEA, DMF, rt, 1.5 h; (c) HF, *p*-cresol, 0 °C, 1 h. R^2 = $-CH_2CH_2CO$-Leu-OH.

SCHEME 5. *N*-Fmoc Protection and Coupling[a]



[a] Reagents and conditions: (a) FmocCl, DIEA, dioxane/H_2O, rt, 24 h; (b) H_2N-GSYRFG-COS-Resin, HOBt, DCC, DMF, rt, 1.5 h, then DBU (1% v/v), DMF, rt; (c) HF, *p*-cresol, 0 °C, 1 h. R = *p*-$MeC_6H_4CH_2-$.

more hindered amine of another incoming molecule. All the unprotected amino acids **4**, **7a**, and **7b** were coupled to a model peptide resin of sequence GSYRFG-COS-Resin in 1.1 equiv excess using standard TBTU activation for 1.5 h at room temperature. In each case, reaction completion was monitored by ninhydrin analysis. The crude peptide obtained after HF cleavage showed no evidence by HPLC analysis of uncoupled amino acid derivatives or double incorporation of the amino acid. The peptide **11** resulting from the coupling of racemic N^{α}(Aux)-Gly-OH **4** was obtained as a mixture of diastereomers. The coupling of each diastereomer N^{α}(Aux)-Ala-OH gave peptide diastereomers **12a** and **12b**, which had distinct retention times in HPLC.

Coupling Using N$^{\alpha}$-Base-Labile-Protected (Aux)-AA-OH. For the base-labile protection strategy (Scheme 5) to be compatible with both our strategies would lie in the possibility to remove the protecting group even on a C^{α}-thioester peptide. Recently, Clippingdale et al.[19] utilized DBU as an Fmoc deprotecting agent for the synthesis of C^{α}-thioester peptides via Fmoc chemistry chain assembly. Although such a methodology is applicable only to the preparation of small-sized peptides, its use in a single step presents practically no side reactions.

To prepare the Fmoc-protected amino acid derivatives, we utilized the highly reactive FmocCl. Thus, the desired derivatives Fmoc-N^{α}(Aux)-Gly-OH **13** and Fmoc-N^{α}(Aux)-Ala-OH **14b** were obtained in, respectively, 63% and 36% yield. Compound **14b** was then successfully coupled to a peptide resin C^{α}-thioester, and the Fmoc group was removed using 1% DBU in anhydrous DMF. No appreciable amount of resin was lost during the treatment. The desired compound **12b** was finally obtained after HF cleavage of the peptide.

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Thiazolidine Protection Scheme. To take advantage of the 1,2-aminothiol moiety of our auxiliary-derivatized amino acids **4**, **7a**, and **7b**, we set out to exploit a different protection scheme, which could possibly be easy, efficient, and general. Thiazolidine derivatives have been used to protect the β-aminothiol moiety of cysteine during syntheses of peptides.[20] Recently, the thiazolidine cyclic structure has also been successfully exploited as an N-terminal protection scheme for cysteine in the synthesis of a fully native folded protein of over 18 kDa.[21] In multifragment ligation strategies a key issue is the reactivity of the middle segment, which bears both N-terminal cysteine and C-terminal thioester reacting sites. In such a case, the N-terminal cysteine must be protected to avoid cyclization.[9] Accordingly, in a three or more fragment ligation, the 1,2-aminothiol of the N-terminal (Aux)-middle segment must be protected.[13] In the thiazolidine protection scheme the 1,2-aminothiol of the N-terminal cysteine is blocked via a heterocyclic ring, which provides a convenient temporary protection of this residue for peptide segments with a C-terminal thioester during ligation. In this paper we have extended the use of the thiazolidine protection to the 1,2-aminothiol of the N^{α}-(2-mercaptoethyl) amino acids. In fact, the thiazolidine formation with the secondary nitrogen of the N^{α}-auxiliary-derivatized amino acids generates a convenient temporary tertiary amine, suitable for its use directly in SPPS. The thiazolidine derivatives of glycine (**15**) and alanine (**16a** and **16b**) were conveniently prepared from their precursors **4**, **7a**, and **7b** in, respectively, 83%, 74%, and 76% yields, by a two-step procedure including removal of the sulfur protecting group and subsequent condensation with formaldehyde (Scheme 6). Unfortunately, NOE experiments on the two thiazolidine diastereomers **16a** and **16b** did not allow their stereochemistry determination.

To test the stability of our thiazolidine derivatives under coupling conditions, compound **15** was coupled in solution using standard TBTU activation to glycine methyl ester (H_2NCH_2COOMe), generating uniquely the desired product. Subsequently, the thiazolidine **15** was coupled to a model peptide on a Rink amide PEGA resin (Scheme 6). After cleavage of the peptide from the resin using standard Fmoc conditions (95% TFA, 2.5% TIS, 2.5% H_2O), analysis of the crude showed no traces of ring opening, thus demonstrating the stability of the thiazolidine derivative in strong acidic conditions, for example, the TFA cocktail required for the cleavages of peptides in Fmoc-like chemistries. Future work will address a more exhaustive study on the thiazolidine derivatives to test also their full compatibility with the Boc chemistry.

Finally, the free 1,2-aminothiol of the N-terminal auxiliary-derivatized glycinyl peptide was released by

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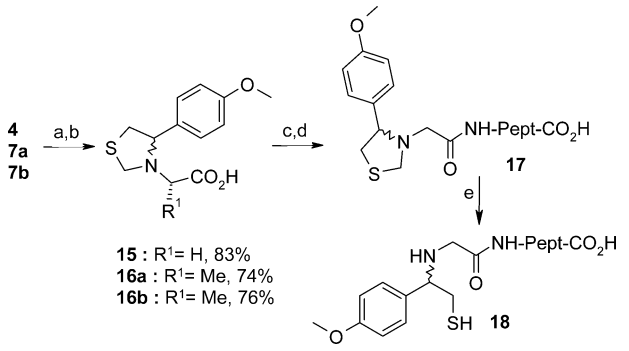
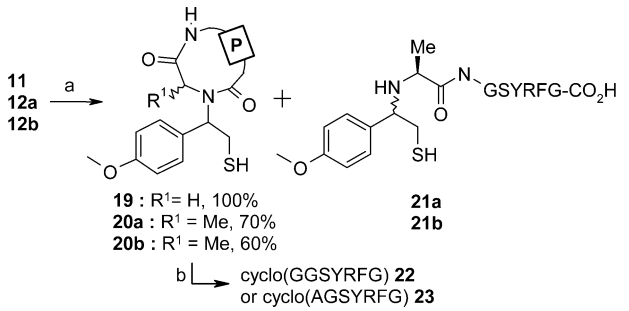
SCHEME 6. Synthesis of Thiazolidines, Coupling, and Resin Cleavage[a]



[a] Reagents and conditions: (a) HF, anisole, 0 °C, 1 h; (b) HCHO(aq), NaOH, EtOH/H_2O, rt, 1 h; (c) H_2N-GAQF$^{\beta}$A-CO_2-Resin, TBTU, DIEA, DMF, rt, 1.5 h; (d) TFA/TIS/H_2O (95/2.5/2.5), rt, 1 h; (e) $MeONH_2$·HCl, phosphate buffer, pH 3.5. R = *p*-$MeC_6H_4CH_2$−.

SCHEME 7. Cyclization of Peptide Thioesters[a]



[a] Reagents and conditions: (a) CH_3CN/phosphate−imidazole buffer, pH 7.4, rt, 0.5 h, or 37 °C, 0.5 or 17 h; (b) TFA/TIS (95/5), rt, 5 or 17 h.

treatment with *O*-methylhydroxylamine under acidic conditions (identical to those reported for N-terminal cysteine derivatives) directly in the postcleavage mixture.[20,21]

Cyclization Studies Using *N*$^{\alpha}$(Aux)-AA-OH Derivatives. The coupling of glycine and alanine derivatives **4**, **7a**, and **7b** to a model peptide resin of sequence GSYRFG-COS-Resin produced after HF cleavage an HPLC trace (as crude) on a C8 analytical column with a major single peak corresponding to the desired mass. The obtained peptides **11**, **12a**, and **12b** were then cyclized in a phosphate/imidazole buffer at pH 7.4, and the reactions were monitored by HPLC analysis. At each time point 2-mercaptoethanol (20% v/v) was added to the sample before injection to unambiguously distinguish the circular unrearranged thiolactone intermediate from the cyclized peptide with an amide bond.[13] While cyclization of peptide **11** generated only compound **19** as an expected diastereomeric mixture after 0.5 h at room temperature, cyclization of **12a** or **12b** afforded a mixture of cyclic compound **20a** or **20b** and linear product **21a** or **21b** resulting from the hydrolysis of the corresponding starting materials (Scheme 7, Table 1).

HPLC analysis of the crude peptides showed the presence of two distinct peaks (in different percentages, depending on the used starting material **12a** or **12b**) with the same expected mass for cyclic derivative **20a** or **20b**, suggesting an epimerization process (Table 1, Figure 1).

TABLE 1. Comparison of Cyclization Results for Peptide Diastereomers 12a and 12b

peptide thioester	cyclic peptide/ hydrolysis product ratio,[a] %	diastereomeric ratio,[a] %
12a	**20a/21a**, 70/30	**20a**, 85/15
12b	**20b/21b**, 60/40	**20b**, 70/30

[a] Ratios determined after reaction completion (17 h, 37 °C).

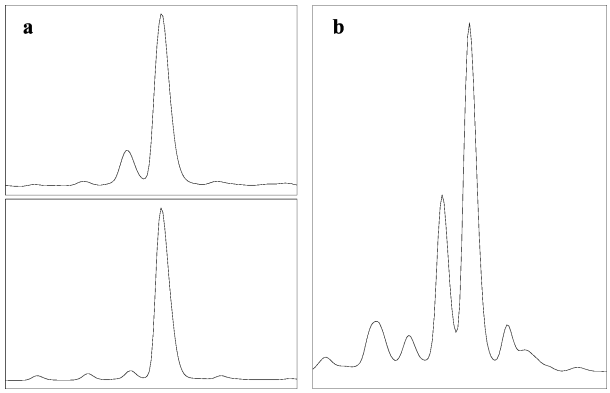


FIGURE 1. HPLC analysis of cyclization products: (a) **20a** before and after a second purification of starting material **12a**; (b) **20b** diastereomeric mixture.

TFA/TIS treatment to remove the auxiliary group was performed on desalted compounds **19**, **20a**, and **20b**. While the final product **22** from linear precursor **11** resulted quantitatively in a single peak in HPLC analysis, unfortunately crude **23** still showed the presence of two peaks with the same expected mass for the cyclized peptide without the auxiliary group. As reference standards we synthesized on the oxime resin[22] two cyclic peptide diastereomers of sequence AGSYRFG with alternatively D- and L-alanine. The comparison with these D- and L-alanine cyclic peptide reference standards allowed the determination of epimerization ratios generated for peptides **20a** and **20b**, respectively 15% and 30% (Figure 1).

These results reflect the different reactivity induced by the initial use of the two amino acid derivatives **7a** and **7b**, in both the coupling (epimerization process) and cyclization steps. Indeed, one diastereomer was less prompt to epimerization and favored peptide cyclization more than the other, thus validating our synthetic efforts to synthesize and separate amino acids **7a** and **7b**. According to their different NMR spectra and distinct HPLC retention times, amino acid derivatives **7a** and **7b** show no detectable racemization of their alanine moiety. Accordingly, the observed epimerization could not come from the starting amino acid **7a** or **7b**, but presumably occurred during either the coupling or the cyclization step. HPLC traces on a C8 analytical column of purified peptides **12a** and **12b** (as well as **11**) showed in each case a single symmetrical peak, and no trace of epimerized material was detected, regardless of the slope of the gradient. When however a C18 column was employed, both peptides **12a** and **12b** showed a non-symmetrical shape of the peak (shoulder). Finally, **12a** was repurified,

(22) Osapay, G.; Profit, A.; Taylor, J. W. *Tetrahedron Lett.* **1990**, *31*, 6121−6124.

and the purity of the fractions was checked on HPLC with a C18 column. A new sample from the pooled fraction was then cyclized, producing this time only one peak with the expected mass for the cyclized material (Figure 1), thus demonstrating that the racemization occurred during the coupling.

To reduce the extent of the racemization, we investigated the use of carbodiimide-mediated coupling (DCC) with alternatively HOBt and HOOBt (3-hydroxy-3,4-dihydro-4-oxo-1,2,3-benzotriazine) as activating agent. Unfortunately, preliminary results showed no significant reduction of epimerization. Reproducible results were obtained when the Fmoc-alanine derivative **14b** was employed. Future work will make a systematic study of different coupling reagents and conditions.

Conclusion

Our work greatly expands the applicability of the extended chemical ligation and peptide cyclization by making available the preformed auxiliary-derivatized alanine and glycine ready to be used in SPPS. These residues have been easily synthesized by a general three-step procedure that could be extended to other amino acids, irrespective of the nature of their side chains. Such derivatives in both their unprotected and protected forms have been successfully coupled to various peptide resins (C^{α}-thioester and -carboxyester). The alanine derivatives however have been found to generate some epimerization during the coupling. Finally, purified peptides with N-terminal auxiliary-derivatized glycine and alanine have been successfully cyclized, generating, after the cleavage of the auxiliary group, the desired cyclic peptides. Furthermore, the thiazolidine approach should permit the development of functional derivatives suitable for both single- and multiple-step extended chemical ligation. Additional studies on the coupling conditions as well as further exploitation of the thiazolidine protection scheme will be addressed in a future work.

Experimental Section

General Two-Step Procedure for Preparation of Compounds 5 and 6. A mixture of 1-(4-methoxyphenyl)-2-(4-methylbenzylsulfanyl)ethanone (**1**) (4 mmol), aminoester (1.35 equiv, 5.4 mmol), and boron trifluoride etherate (12 μL) was refluxed in toluene (13 mL) for 6−8 h under N_2 using a Dean−Stark apparatus. The reaction mixture was then cooled to room temperature and the solvent evaporated. The crude residue was dissolved in methanol (24 mL). To the resulting solution was added at 0 °C sodium borohydride (4 mmol), and the reaction mixture was stirred at 0 °C under N_2 for 1.5−2 h. Water was added and the aqueous layer extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated. Flash chromatography on silica gel (ether/pentane, 1/2 and then 1/1) afforded compound **5** or **6**.

Starting from ketone **1** (2.88 g, 10 mmol) and glycine ethyl ester (1.39 g, 13.5 mmol), *rac*-[1-(4-methoxyphenyl)-2-(4-methylbenzylsulfanyl)ethylamino]acetic acid ethyl ester (**5**) was isolated as an oil (2.38 g, 64%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.20 (d, 2H, J = 7.9 Hz), 7.18 (d, 2H, J = 8.5 Hz), 7.10 (d, 2H, J = 7.8 Hz), 6.84 (d, 2H, J = 8.6 Hz), 4.16 (q, 2H, J = 7.3 Hz), 3.78 (s, 3H), 3.74−3.68 (m, 3H), 3.28 (d, 1H, J = 17.4 Hz), 3.10 (d, 1H, J = 17.4 Hz), 2.66 (dd, 1H, J = 4.5, 13.6 Hz), 2.56 (dd, 1H, J = 9.3, 13.6 Hz), 2.31 (s, 3H), 1.24 (t, 3H, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.4, 159.1, 136.6, 135.0, 133.9, 129.1, 128.8, 128.4, 113.9, 60.6, 59.7, 55.2, 48.3, 39.3, 35.5, 21.0, 14.2. HRMS (CI): *m/z* calcd for $C_{21}H_{27}NO_3S$ (M + H) 374.1790, found 374.1796.

Starting from ketone **1** (2.5 g, 8.74 mmol) and L-alanine ethyl ester (1.38 g, 11.8 mmol), compound **6** was isolated as a 50/50 mixture of diastereomers (2.51 g, 74% after flash chromatography). The diastereomers **6a,b** were separated by flash chromatography on silica gel (ether/pentane, 1/2) to give pure 2-[1-(4-methoxyphenyl)-2-(4-methylbenzylsulfanyl)ethylamino]propionic acid ethyl esters **6a** (less polar) and **6b** (more polar). The following are data for diastereomer **6a**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.20 (d, 2H, J = 8.1 Hz), 7.17 (d, 2H, J = 8.8 Hz), 7.10 (d, 2H, J = 7.8 Hz), 6.83 (d, 2H, J = 8.6 Hz), 4.18 (q, 2H, J = 7.0 Hz), 3.79 (s, 3H), 3.70 (s, 2H), 3.67 (dd, 1H, J = 4.0, 9.6 Hz), 3.10 (q, 1H, J = 7.1 Hz), 2.61 (dd, 1H, J = 4.1, 13.7 Hz), 2.47 (dd, 1H, J = 9.8, 13.4 Hz), 2.31 (s, 3H), 1.28 (d, 3H, J = 7.0 Hz), 1.25 (t, 3H, J = 6.9 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 176.0, 159.0, 136.6, 134.8, 134.3, 129.1, 128.9, 128.3, 113.9, 60.5, 58.1, 55.2, 53.6, 39.3, 34.9, 21.1, 19.7, 14.3. HRMS (CI): *m/z* calcd for $C_{22}H_{30}NO_3S$ (M + H) 388.1947, found 388.1948. The following are data for diastereomer **6b**. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.20−7.15 (m, 4H), 7.09 (d, 2H, J = 7.9 Hz), 6.83 (d, 2H, J = 8.8 Hz), 4.02 (q, 2H, J = 7.1 Hz), 3.78 (s, 3H), 3.66 (pt, 1H, J = 6.8 Hz), 3.57 (s, 2H), 3.22 (q, 1H, J = 6.8 Hz), 2.71−2.65 (m, 2H), 2.31 (s, 3H), 1.22 (d, 3H, J = 6.8 Hz), 1.18 (t, 3H, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 175.1, 159.0, 136.6, 135.2, 134.0, 129.2, 128.8, 128.4, 113.8, 60.6, 59.4, 55.2, 54.2, 39.1, 36.4, 21.1, 18.2, 14.1. HRMS (CI): *m/z* calcd for $C_{22}H_{30}NO_3S$ (M + H) 388.1947, found 388.1944.

General Procedure for Preparation of Compounds 4 and 7. To a stirred solution of amino ester **5**, **6a**, or **6b** (1 mmol) in dioxane (2 mL) was added a 4 M NaOH solution (1.25 mL, 5 mmol). The resulting mixture was stirred for 17 h at room temperature. Water was added and the aqueous layer extracted with ethyl acetate. The aqueous layer was then acidified with a 1 M HCl solution and extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered, and concentrated, affording pure amino acid **4**, **7a**, or **7b**.

Starting from amino ester **5** (1.12 g, 3 mmol), *rac*-[1-(4-methoxyphenyl)-2-(4-methylbenzylsulfanyl)ethylamino]acetic acid (**4**) was isolated as a white powder (1 g, 97%). ^{1}H NMR (CD_3OD, 400 MHz): δ 7.30 (d, 2H, J = 8.6 Hz), 7.17 (d, 2H, J = 8.1 Hz), 7.12 (d, 2H, J = 7.8 Hz), 7.01 (d, 2H, J = 8.6 Hz), 4.30 (t, 1H, J = 7.6 Hz), 3.82 (s, 3H), 3.66 (s, 2H), 3.61 (d, 1H, J = 16.9 Hz), 3.55 (d, 1H, J = 16.9 Hz), 3.09 (dd, 1H, J = 7.1, 13.9 Hz), 2.94 (dd, 1H, J = 7.8, 13.9 Hz), 2.30 (s, 3H). ^{13}C NMR (CD_3OD, 100 MHz): δ 168.8, 162.5, 138.2, 135.8, 131.0, 130.3, 130.1, 126.1, 115.9, 62.4, 55.9, 46.5, 36.6, 34.5, 21.1. HRMS (CI): *m/z* calcd for $C_{19}H_{24}NO_3S$ (M + H) 346.1477, found 346.1480.

Starting from amino ester **6a** (387 mg, 1 mmol), 2-[1-(4-methoxyphenyl)-2-(4-methylbenzylsulfanyl)ethylamino]propionic acid **7a** was isolated as a white powder (331 mg, 92%). ^{1}H NMR (CD_3OD, 400 MHz): δ 7.24 (d, 2H, J = 8.6 Hz), 7.19 (d, 2H, J = 8.1 Hz), 7.13 (d, 2H, J = 8.0 Hz), 4.34 (t, 1H, J = 7.3 Hz), 3.82 (s, 3H), 3.71−3.63 (m, 2H), 3.50 (q, 1H, J = 7.1 Hz), 3.06 (dd, 1H, J = 7.3, 14.2 Hz), 2.93 (dd, 1H, J = 8.1, 14.2 Hz), 2.31 (s, 3H), 1.43 (d, 3H, J = 7.3 Hz). ^{13}C NMR (CD_3OD, 100 MHz): δ 172.0, 162.5, 138.2, 135.8, 131.1, 130.3, 130.1, 126.0, 115.9, 62.1, 55.9, 55.1, 36.5, 34.9, 21.1, 16.3. HRMS (CI): *m/z* calcd for $C_{20}H_{26}NO_3S$ (M + H) 360.1633, found 360.1627.

Starting from amino ester **6b** (387 mg, 1 mmol), 2-[1-(4-methoxyphenyl)-2-(4-methylbenzylsulfanyl)-ethylamino]-propionic acid **7b** was isolated as a white powder (327 mg, 91%). ^{1}H NMR (CD_3OD, 400 MHz): δ 7.29 (d, 2H, J = 8.9 Hz), 7.17−7.12 (m, 4H), 7.00 (d, 2H, J = 8.8 Hz), 4.09 (dd, 1H, J = 5.9, 9.1 Hz), 3.82 (s, 3H), 3.75 (q, 1H, J = 7.3 Hz), 3.63−3.56 (m, 2H), 3.15 (dd, 1H, J = 5.8, 13.9 Hz), 3.00 (dd, 1H, J = 9.3, 13.9 Hz), 2.32 (s, 3H), 1.47 (d, 3H, J = 7.3 Hz). ^{13}C NMR (CD_3OD, 100 MHz): δ 171.7, 162.4, 138.3, 136.3, 131.0, 130.4,

130.1, 126.6, 115.7, 61.9, 55.9, 55.4, 37.2, 34.58, 21.1, 20.8, 15.1. HRMS (CI): *m/z* calcd for $C_{20}H_{26}NO_3S$ (M + H) 360.1633, found 360.1630.

General Two-Step Procedure for Thiazolidine Synthesis. Standard HF cleavage of the thiol protecting *p*-methylbenzyl group of amino acid **4**, **7a**, or **7b** (1 mmol) in the presence of 500 μL of anisole as scavenger affords, after elimination of HF, a residue which is dissolved in a CH_3CN/H_2O mixture (50/50). After concentration, the resulting aqueous phase is extracted twice with ether to remove anisole. Concentration of the aqueous phase affords a white solid residue which is dissolved in a 2/1 water/ethanol mixture (4 mL). A 4 M NaOH solution (500 μL, 2 mmol) was then added, followed by HCHO (36% aqueous, 77 μL, 1 mmol). The reaction mixture was stirred at room temperature for 1 h and concentrated. The aqueous phase was then acidified with a 1 M HCl solution and extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$, filtered, concentrated, and purified by flash chromatography (ethyl acetate 100%), affording pure thiazolidine **15**, **16a**, or **16b**.

Starting from amino acid *rac*-**4**, (830 mg, 2.41 mmol), *rac*-[4-(4-methoxyphenyl)thiazolidin-3-yl]acetic acid (**15**) was isolated as a white powder (505 mg, 83%). ^{1}H NMR (CD_3OD, 400 MHz): δ 7.39 (d, 2H, J = 8.6 Hz), 6.92 (d, 2H, J = 8.6 Hz), 4.33−4.27 (m, 1H), 4.28 (d, 1H, J = 8.3 Hz), 4.05 (d, 1H, J = 8.3 Hz), 3.79 (s, 3H), 3.38 (d, 1H, J = 17.2 Hz), 3.26 (dd, 1H, J = 6.6, 10.6 Hz), 3.22 (d, 1H, J = 17.2 Hz), 3.13 (dd, 1H, J = 7.8, 10.6 Hz). ^{13}C NMR (CD_3OD, 100 MHz): δ 172.9, 161.4, 130.6, 130.4, 115.2, 72.2, 57.3, 55.8, 53.6, 35.3. HRMS (CI): *m/z* calcd for $C_{12}H_{16}NO_3S$ (M + H) 254.0851, found 254.0846.

Starting from amino acid **7a** (150 mg, 0.42 mmol), 2-[4-(4-methoxyphenyl)thiazolidin-3-yl]propionic acid **16a** was isolated as a white powder (83 mg, 74%). ^{1}H NMR (CD_3OD, 400 MHz): δ 7.30 (d, 2H, J = 8.6 Hz), 6.90 (d, 2H, J = 8.6 Hz), 4.35 (dd, 1H, J = 6.1, 8.1 Hz), 4.20−4.16 (m, 1H), 3.78 (s, 3H), 3.35−3.27 (m, 1H partly masked by CD_3OD), 3.16 (dd, 1H, J = 6.1, 10.4 Hz), 2.95 (dd, 1H, J = 8.6, 10.4 Hz), 1.34 (d, 3H, J = 7.3 Hz). ^{13}C NMR (CD_3OD, 100 MHz): δ 175.6, 161.1, 132.4, 130.2, 115.2, 69.3, 57.2, 55.7, 50.7, 38.2, 17.2. HRMS (CI): *m/z* calcd for $C_{13}H_{18}NO_3S$ (M + H) 268.1008, found 268.1008

Starting from amino acid **7b** (150 mg, 0.42 mmol), 2-[4-(4-methoxyphenyl)thiazolidin-3-yl]propionic acid **16b** was isolated as a white powder (85 mg, 76%). ^{1}H NMR (CD_3OD, 400 MHz): δ 7.38 (d, 2H, J = 8.6 Hz), 6.89 (d, 2H, J = 8.8 Hz), 4.35 (t, 1H, J = 6.1 Hz), 4.12 (d, 1H, J = 8.8 Hz), 4.06 (d, 1H, J = 8.8 Hz), 3.77 (s, 3H), 3.42 (q, 1H, J = 7.1 Hz), 3.22 (dd, 1H, J = 6.3, 10.6 Hz), 3.07 (dd, 1H, J = 5.8, 10.6 Hz), 1.27 (d, 3H, J = 7.1 Hz). ^{13}C NMR (CD_3OD, 100 MHz): δ 176.7, 161.0, 132.9, 129.9, 115.0, 70.2, 59.1, 55.7, 51.8, 36.2, 13.3.

General Procedure for the Coupling of Derivatized Amino Acids to Peptide C^{α}-Thioester or -Carboxyester Resins and Subsequent HF Cleavage. Amino acid 4, 7a, 7b, 9b, or 14b (0.11 mmol) was coupled for 1.5 h to peptide C^{α}-thioester (0.10 mmol) in DMF in the presence of TBTU (0.11 mmol) and DIEA (0.20 mmol). Completion of coupling was monitored by a ninhydrin test. In the case of protected amino acid 14b, the coupling reaction was performed in the presence of HOBt (0.22 mmol) and DCC (0.17 mmol) and was followed by Fmoc removal by treatment with 1% v/v DBU solution in DMF. The peptides were then deprotected and cleaved from the resin by treatment with anhydrous HF for 1 h at 0 °C with 500 μL of *p*-cresol as scavenger. After cleavage, the peptides were precipitated with ether, dissolved in an acetonitrile/water (50/50) mixture, and lyophilized. All peptides 10, 11, 12a, and 12b obtained from coupling with 9b, 4, 7a, 7b, and 14b were purified by reversed-phase HPLC, affording white powders characterized by MALDI mass spectrometry. Amino acid 15 (0.11 mmol) was coupled for 1.5 h to peptide carboxyester resin (0.1 mmol). Peptide 17 was then cleaved from the resin by treatment with a TFA/TIS/H_2O (95/2.5/2.5) mixture, precipitated with ether, dissolved in an acetonitrile/water (50/50) mixture, and lyophilized. Crude peptide 17 (2 mg, 2.71×10^{-6} mol) was dissolved in a 0.2 M Na_2HPO_4, 0.2 M $MeONH_2 \cdot HCl$ buffer, pH 3.5 (544 μL), and the reaction was stirred at room temperature. After 17 h, HPLC analysis of the mixture revealed complete conversion to peptide 18. MALDI TOF MS for peptide 10: *m/z* calcd for $C_{43}H_{63}N_{11}O_{12}S$ 957.44, found 958.50. MALDI TOF MS for peptide 11: *m/z* calcd for $C_{51}H_{71}N_{11}O_{13}S_2$ 1109.47, found 1111.14. MALDI TOF MS for peptide 12a: *m/z* calcd for $C_{52}H_{73}N_{11}O_{13}S_2$ 1123.48, found 1125.13. MALDI TOF MS for peptide 12b: *m/z* calcd for $C_{52}H_{73}N_{11}O_{13}S_2$ 1123.48, found 1125.19. Q-TOF MS for peptide 17: *m/z* calcd for $C_{34}H_{45}N_7O_9S$ 727.83, found 728.33. Q-TOF MS for peptide 18: *m/z* calcd for $C_{33}H_{45}N_7O_9S$ 715.82, found 716.35.

Acknowledgment. We thank the Swiss National Science Foundation for financial support. We also thank Irene Rossito-Borlat and Brigitte Dufour for technical assistance and the NMR team of the Organic Chemistry Department at the University of Geneva for the NMR spectra. We thank Dr. Matteo Villain and Dr. Hubert Gaertner for useful discussions. We finally thank Dr. John Hill of Kent Mass Spectroscopy for his advice.

Supporting Information Available: Experimental procedures for compounds **1**, **2**, **3**, **4**, **8**, **13**, **14b**, **19**, **20a**, **20b**, **21a**, **21b**, **22**, and **23**, NMR spectra of compounds **1**, **2**, **3**, **4**, **5**, **6a**, **6b**, **7a**, **7b**, **8**, **13**, **14b**, **15**, **16a**, and **16b**, mass spectra of peptides **10**, **11**, **12a**, **12b**, **17**, **18**, **19**, **20a**, **20b**, **21a**, **21b**, and **23b**, HPLC chromatograms of pure products **4**, **7a**, **7b**, **15**, **16a**, and **16b**, and crude peptide **17**, and cyclization reaction of peptide **12a** and crude peptide **23** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JO049471K