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AMIDE BOND SURROGATES: PSEUDOPEPTIDES AND MACROCYCLES

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ABSTRACT: Peptide backbone modifications, involving either α-carbon changes or amide bond replacements, furnish new hybrid structures (pseudopeptides) with often profoundly altered physical and biological properties. Using both direct (ψ-condensation) and indirect (preformed pseudodipeptide) solid phase approaches, new cyclic enkephalin analogs have been prepared with a thiomethylene amide bond surrogate in the interior portion of the molecule. A drastic loss in potency observed with one of the analogs, when contrasted with the generally favorable utility of such replacements in small cyclic hosts, suggests a functional role for the 3-4 amide bond. The incorporation of three ψ[CH_2S] replacements into an 18-membered ring was effected in order to demonstrate the feasibility of synthesizing a new class of pseudopeptide (heterodetic) thioether macrocycles.

INTRODUCTION

Recent structure-function studies in peptide chemistry have increasingly focused on peptide backbone modifications, as opposed to side chain replacements, as alternative synthetic approaches to drug design. Amide bond replacements (surrogates) of various types have been devised and selectively incorporated into host molecules to yield new structures that contain both amide and non-amide linking elements. We have referred to such structures as pseudopeptides (1).

Part of the impetus for this effort has been to replace the weak links in the chain, namely the amide bonds themselves, that, due to enzymic activity, render most peptides orally inactive and result in typical in vivo half lives of only 1-10 minutes (2). It has also proven important to probe the possible functional role of the peptide backbone, and to test the consequences that new flexible linkages such as -CH_2CH_2, CH_2S, or CH_2NH might have on conformation and potency (1). Conversely, it has also been of interest to establish the ability of more rigid units such as the alkylidene moiety (CH=CH) or the "retro-amide" (NHCO) to mimic the parent peptide unit (3,4).

Synthetic routes leading to dipeptide analogs (pseudodipeptides) in many cases allow one to incorporate these δ-amino acids into existing solution or solid phase procedures. Alternatively, the Merrifield solid phase methodology (5) has occasionally been adapted to permit direct formation of the surrogate structures in a totally resin-bound approach.

By incorporating one or more of the amide bond replacements within a peptide chain, new linear hybrid species are created that can be cyclized via amide formation to yield a potentially large variety of macrocycles. In this publication, we wish to report some of our recent work dealing with the synthesis of both biologically active pseudopeptide hormone analogs, as well as a few examples of linear pseudopeptides that illustrate the feasibility of cyclizing these molecules to produce these new macrocycles.

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Abbreviations used:
Fmoc: 9-fluorenylmethyloxycarbonyl; Boc: tert-butyloxycarbonyl; Pen: β,β'-dimethylcysteine; Meb: 4-methylbenzyl; Bzl: benzyl; But: tert-butyl; Z: benzyloxycarbonyl; Dcb: dichlorobenzyl; HOBt: 1-hydroxybenzotriazole; DPPA: diphenylphosphoryl azide; DMAP: 4-dimethylaminopyridine; DMF: N,N'-dimethylformamide; TFA: trifluoroacetic acid; DCC: dicyclohexylcarbodiimide; FAB-MS: fast atom bombardment-mass spectrometry; RP-HPLC: reversed phase-high performance liquid chromatography. Other abbreviations correspond to standard nomenclature used for naturally occurring amino acids. All amino acids except glycine are of the L-configuration unless otherwise specified.

The parent hosts for the biologically active analogs described here are two cyclic enkephalin derivatives. One, Tyr-cyclo(D-Lys-Gly-Phe-Leu), Figure 1, is a potent, backbone to side chain cyclized derivative, first described by DiMaio and Schiller (6), which has greater affinity for the morphine-like "mu" receptor than for the enkephalin "delta" receptor. The second host is the Hruby-Mosberg (7) side-chain-to-side-chain cyclized enkephalin derivative, Tyr-cyclo(D-Pen-Gly-Phe-D-Pen)-OH (DPDPE), which features the sterically hindered, rigid amino acid penicillamine (β,β-dimethylcysteine) at positions 2 and 5 and an intramolecular disulfide ring closure. This compound has virtually no affinity for the mu receptor, but interacts strongly with the delta receptors present in the mouse vas deferens tissue.

Also described in this paper is the first example of a cyclic model compound containing three amide bond surrogates. This molecule, and the strategies used for its synthesis, are believed to represent a general route leading to new classes of macrocycles, with configurationally defined side chains.

Among the amide bond replacements referred to in this study are the reduced amide ($\psi[CH_2NH]$), the thiomethylene ether ($\psi[CH_2S]$), the corresponding sulfoxides ($\psi[CH_2$-(R) or (S)-SO]), as well as the retroamide (ψ[NHCO], thioamide (ψ[CSNH]) and ethylidene derivatives (trans-ψ[CH=CH]). The "psi-bracket" nomenclature, first described in 1981 (8), and accepted (in slightly modified form) by the IUPAC commission on nomenclature (9) will be used through this work to refer to the pseudopeptide structures.

RESULTS AND DISCUSSION

Most of our recent work has dealt with the $\psi[CH_2S]$, ψ[CSNH], and $\psi[CH_2NH]$ amide surrogates. A great many linear (10,11) and cyclic (12,13,14,15) peptide analogs containing the thiomethylene ether linkage have confirmed that this flexible replacement is most efficacious in constrained, cyclic hosts. A recent conformational analysis of a model cyclic pseudopentapeptide has shown the ability of the $\psi[CH_2S]$ to coexist with intramolecularly hydrogen bonds in β and γ turns in nearby loci (16).

A similar conformational analysis of the ψ[CSNH] moiety has also been undertaken, and its similarity and differences with the amide group have been correlated (17). We (18) and others (19) have prepared leucine enkephalin and other peptides with thioamide replacements and have seen both increases, decreases, and selectivity changes with respect to biological potency. Similar studies are now underway in our laboratory with $\psi[CH_2NH]$ pseudopeptides.

Among the linear $\psi[CH_2S]$ enkephalin series, only the 4-5 replacement (Phe$\psi[CH_2S]$Leu) retained substantial biological potency (11). Although the 3-4 $\psi[CH_2S]$ surrogate was not active, the enhanced biostability of a 3-4 Gly$\psi[CH_2S]$Phe analog was exploited in the synthesis of an effective leucine enkephalin antagonist (N,N-Bis-allyl)-Tyr-Gly-Gly$\psi[CH_2S]$Phe-Leu-OH (20).) When the 4-5 thiomethylene unit was combined with cyclization, the result (Figure 1) was a

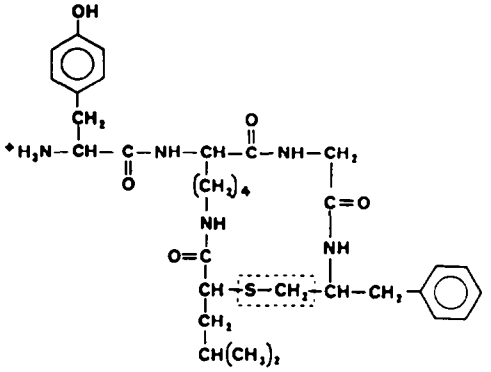


FIGURE 1. Structure of DiMaio-Schiller cyclic enkephalin analog with $\psi[CH_2S]$ replacement between Phe^4 and Leu^5 (Reference 12).

compound that was exceptionally potent (but thus not selective) in both mu and delta receptor assays and which, in vitro, displayed high opioid activity (13). Since the 3-4 linear replacement did not result in an active compound, but cyclization had dramatically increased the potency of a 4-5 analog, we were interested in the synthesis of a cyclic pseudopeptide containing the $\psi[CH_2S]$ surrogate at the Gly^3-Phe^4 position. Also, since the Tyr-cyclo(D-Lys-Gly-Phe-Leu) (DiMaio-Schiller) parent was mu selective, we also wondered about the effect of incorporating the $\psi[CH_2S]$ group in the 3-4 position of the highly delta selective parent, Tyr-cyclo(D-Pen-Gly-Phe-D-Pen)-OH.

Finally, in both linear and cyclic compounds, the change in configuration of the C-terminal amino acid residue from the natural L to the unnatural D configuration usually leads to a moderate increase in delta selectivity. Would such a correlation be retained among the pseudopeptide analogs?

In order to synthesize our pseudopeptide analogs more easily, we decided to explore the possibility of resin-bound formation of the thiomethylene ether linkage. Recently, Coy and coworkers have described a novel method for preparing $\psi[CH_2NH]$ analogs by solid phase methods--it involves the Schiff base condensation of a Boc-amino aldehyde with the resin bound terminal amine, followed by in situ reduction with $NaBH_3CN$ (21). We have recently confirmed the utility of this method in the preparation of $\psi[CH_2NH]$-enkephalin analogs (22). If $\psi[CH_2S]$ analogs could be prepared on the resin, this might also facilitate the preparation of a great many more such analogs.

Accordingly, for the synthesis of Tyr-cyclo(D-Lys-Glyψ[CH_2S]Phe-Leu), two synthetic strategies were adopted. One (the indirect solid phase method), involved the usual condensation of preformed Boc-Glyψ[CH_2S]Phe-OH onto the growing resin bound peptide chain (in this case simply D-Leu-OCH_2-C_6H_4-polystyrene or L-Leu-OCH_2-C_6H_4-polystyrene). The alternative approach involved condensation of (R)-$BrCH(CH_2C_6H_5)CO_2H$ (derived from (R)-phenylalanine) (23) onto the resin, followed by S_N2 displacement with Boc-$NHCH_2CH_2SH$ in DMF to yield Boc-Glyψ[CH_2S]L-Phe-Leu-resin. A solution phase version of Boc-Serψ[CH_2S]Phe-OH synthesis using an analogous procedure has been previously reported (24). The thiol addition was repeated twice to ensure maximum coupling yield.

After this step, each synthesis was continued as usual, with subsequent couplings with Boc-D-Lys(Z)-OH and Fmoc-Tyr(But)-OH. The two linear sequences were cleaved from the resin by anhydrous hydrogen fluoride (25) and both linear sequences, still containing the acid stable fluorenylmethyloxycarbonyl function (26) at the amino terminus, were found to be identical, although the yield of the pseudodipeptide coupling approach was slightly higher. The peptides were separately intramolecularly cyclized using a previously reported procedure (27). In the case of the linear compound, Fmoc-Tyr-D-Lys-Glyψ[CH_2S]Phe-Leu-OH (8), prepared by the ψ-condensation method (see experimental) two products were obtained, one (the minor component) we believed, corresponded to the D-Leu analog, presumably formed during cyclization. This was subsequently confirmed by a synthesis of the identical compound, but this time using Boc-D-Leu-OH bound to the resin.

It should be noted that when DMAP was used during the cyclization of the D-Leu analog, two products were formed. When DMAP was not used, then only one major product (corresponding to the minor component of the L-Leu case) was isolated. We are currently examining the sequence dependence of epimerization that can occur with catalytic amounts of DMAP used during these cyclizations.

Once the cyclization was complete (the qualitative ninhydrin test (28) was negative), the products were passed through ion exchange resin, and 55% piperidine was used to remove the base-labile terminal protecting group.

The synthesis of Tyr-cyclo(D-Pen-Glyψ[CH_2S]Phe-D-Pen)-OH (Figure 2) was performed in a similar manner except that Boc-Glyψ[CH_2S]Phe-OH was coupled as the pseudodipeptide. Following HF treatment (to cleave the pseudopeptide chain from the resin and to remove the S-p-methoxybenzyl

protecting groups from the penicillamines), the disulfide bridge was formed via high dilution oxidation with potassium ferricyanide (29). All peptides were purified by gel filtration on Sephadex G-15 or LH-20, using 30 or 50% acetic acid as eluant, and by reversed phase high performance liquid chromatography with 0.05% TFA/acetonitrile gradient systems as described in Methods. Final structures were subjected to FAB mass spectrometry, thin layer chromatography, and quantitative amino acid analysis, as well as spectroscopic analysis (^1H and ^{13}C nmr, as appropriate) for additional characterization.

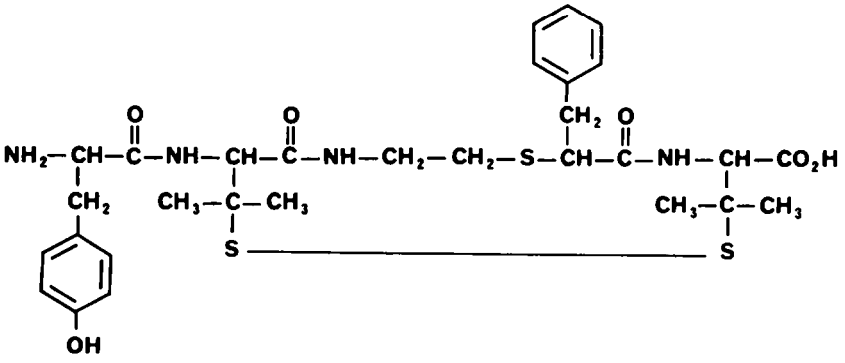


FIGURE 2. Structure of Hruby-Mosberg δ-selective enkephalin analog parent modified with a ψ[CH_2S] replacement between Gly^3 and Phe^4.

Full details concerning the biological activities will be reported elsewhere. However, in our preliminary findings (Table I) it is apparent that in both series, replacement of the 3-4 amide bond has been accompanied by a large drop in potency. The decrease is more striking when the activity of 3, Tyr-cyclo(D-Lys-Glyψ[CH_2S]Phe-Leu) (GPI IC_{50} = 124 nM), is contrasted with the high potency of the Pheψ[CH_2S]Leu analog (GPI IC_{50} = 3.19 nM), which was even more active than its cyclic parent, especially in the MVD assay (13). In each assay, the Glyψ[CH_2S]Phe^{3-4} analog is about 50x less active than its 4-5 pseudopeptide isomer, but 20x more mu selective than leucine enkephalin itself.

TABLE 1. Biological activities of enkephalin-related peptides and pseudopeptides.

Amide Replacement Position	Compound	Guinea Pig Ileum IC_{50}* [nM]	Guinea Pig Ileum Relative Potency	Mouse Vas Deferens IC_{50}* [nM]	Mouse Vas Deferens Relative Potency	Reference
3-4	Tyr-D-Lys-Glyψ[CH_2S]Phe-Leu (3)	124	2	1070	0.01	This work
3-4	Tyr-D-Lys-Glyψ[CH_2S]Phe-D-Leu (6)	9250	0.03	1160	0.01	This work
4-5	Tyr-D-Lys-Gly-Pheψ[CH_2S]Leu	3.19	77	2.32	4.9	13
--	Tyr-D-Lys-Gly-Phe-Leu	4.8	51	141	0.08	6
3-4	Tyr-D-Pen-Glyψ[CH_2S]Phe-D-Pen-OH (8)	>10,000	<0.02	>30,000	<0.004	This work
--	Tyr-D-Pen-Gly-Phe-D-Pen-OH	6930	0.04	2.19	5.2	7
--	H-Tyr-Gly-Gly-Phe-Leu-OH leucine enkephalin	246	1	11.4	1	6

* mean of three determinations (S.E.M. values not shown).

With regard to the D-Leu diastereomer (6), the GPI potency was even lower, indicating a shift toward greater MVD (delta receptor) affinity. This is in agreement with the usual behavior of D-Leu^5 analogs, as seen, for example in the compound DADLE (D-Ala^2,D-Leu^5-enkephalin), the prototypical delta-selective agonist (30). Such a change can no longer be attributed only to increased flexibility of $\psi[CH_2S]$ units, and the magnitude of the decrease seems to argue in favor of a functional role for the peptide bond at this position.

The even more dramatic decrease in potency for the $\psi[CH_2S]$ analog of DPDPE (compound 11) is more difficult to explain. Another factor that must be considered is the influence of geometry upon hormone receptor binding (31). As further indication of a possible major conformational change in the DPDPE pseudopeptide, one might expect to see evidence of this from proton or carbon-13 nmr, but thus far the spectral data (Figure 3) have been useful mainly for structural characterization. Interestingly, the pseudopeptide analog does show a similar proton nmr

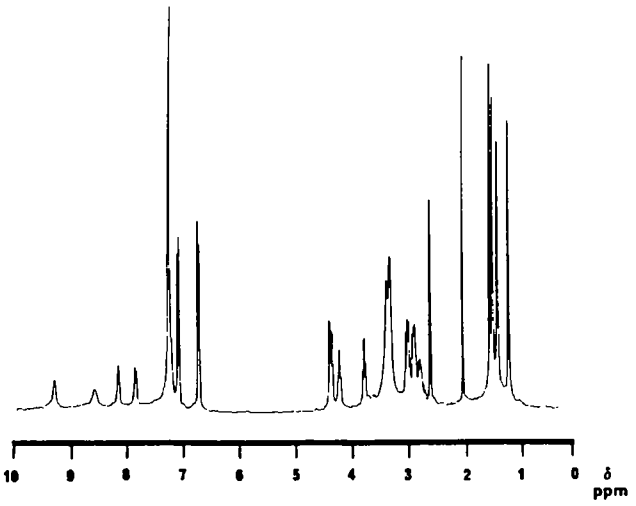


FIGURE 3. Proton NMR spectrum of Tyr-D-Pen-Gly$\psi[CH_2S]$Phe-D-Pen-OH in DMSO d_6. The four penicillamine methyl groups are clearly resolved at δ1.2-1.6.

spectral pattern to that of the parent analog (in D_2O (32)), including the characteristic upfield chemical shift for one of the four penicillamine methyl groups, attributable to the tyrosine ring current effect (33). Further details including the results of a 2-D spectral analysis will be reported elsewhere.

Two other possible explanations for the potency differences between the peptides and pseudopeptides could include 1) the greatly enhanced lipophilicity of $\psi[CH_2S]$ analogs, as documented by an internal $\psi[CH_2S]$ replacement in the linear series (11): this could perhaps be ruled out by the preparation of the corresponding more polar $\psi[CH_2SO]$ analogs; and 2) the essentiality of the carbonyl oxygen: this could be tested by the synthesis of the cyclic Gly$\psi[CH_2NH]$Phe replacement. Both of these preparations are currently in progress in our laboratory.

It should also be noted that other workers have previously postulated an essential role for the Gly-Phe bond. A retro amide replacement in the 3-4 position of a cyclic enkephalin analog, Tyr-cyclo(D-A$_2$bu-Glyψ[NHCO]Phe-Leu) (A$_2$bu = 2,4-diaminobutyric acid), was far less active than its amide parent, even though 4-5 and 5-2 $\psi[NHCO]$ analogs possessed high potency (34). This argues against the flexibility hypothesis. N-Methylphenylalanine-4 analogs are also quite potent (35), and this precludes essentiality of the NH as a hydrogen bond donor. That the carbonyl group of the 3-4 amide plays a key role, perhaps as a critical hydrogen bond acceptor moiety, as first postulated by Hudson and coworkers (36) seems to fit the above scenario. But

the equipotent GPI activity reported for a Glyψ[CSNH]Phe enkephalin analog (relative potency = 1.12) (19), is not consistent with this hypothesis to the extent that sulfur is generally considered to be a relatively poor hydrogen bond acceptor (38).

Also of interest is the question of receptor selectivity. As seen in Table I, the lack of potency for the DPDPE-based 3-4 replacement toward either mu or delta receptors makes selectivity arguments moot. But backbone changes have been shown to influence receptor selectivity in an often unpredictable manner. For example, in the series of linear enkephalin ψ[CSNH] pseudopeptides synthesized, a particularly striking selectivity was observed. The simple replacement of an amide oxygen by sulfur in one member of the series, H-Tyr-Glyψ[CSNH]Gly-Phe-Leu-OH, rendered the compound much more active towards the δ receptor (37). In contrast, the 4-5 thioamide replacement was approximately equipotent to its amide counterpart in both assays. Belleau and coworkers have reported that the 3-4 thioamide replacement results in diminished activity at the delta receptor (19). It is apparent that the role of the amide bond in biologically active peptides will require considerably more experimental input and analysis before it can be relatively well understood.

Pseudopeptide Macrocycles

Macrocycles have been synthesized by various methods involving acyclic ring closures or ring expansion strategies (39). Use of these systems as ligands for cations has been most extensively studied (40), but there is growing interest in their ability to bind neutral guest species (41). Cyclic peptides are ideal candidates for preparing varying ring size systems (42), and cyclic pseudopeptides add a new dimension to this endeavor. Not only are the advantages of configurationally defined side chains usually preserved, but in addition the ability to incorporate new coordinating elements into the ring backbone adds a new dimension to these studies. A further advantage is the potential of applying established and efficient methods of peptide synthesis, including solid phase methodology, for their preparation.

As a paradigm of such systems, we have selected the 18-membered thioether structure, cyclo(Glyψ[CH_2S]Phe)$_3$, as an initial synthetic target. The relative symmetry and simplicity of this molecule, combined with an appropriate balance of hydrophobic and chromophoric residues, were seen as attractive features. Furthermore, as discussed earlier, the Glyψ[CH_2S]Phe unit has been prepared both directly and by solid phase methods, and thus the macrocycle could be prepared by two separate synthetic routes, and their relative advantages and disadvantages could be directly compared.

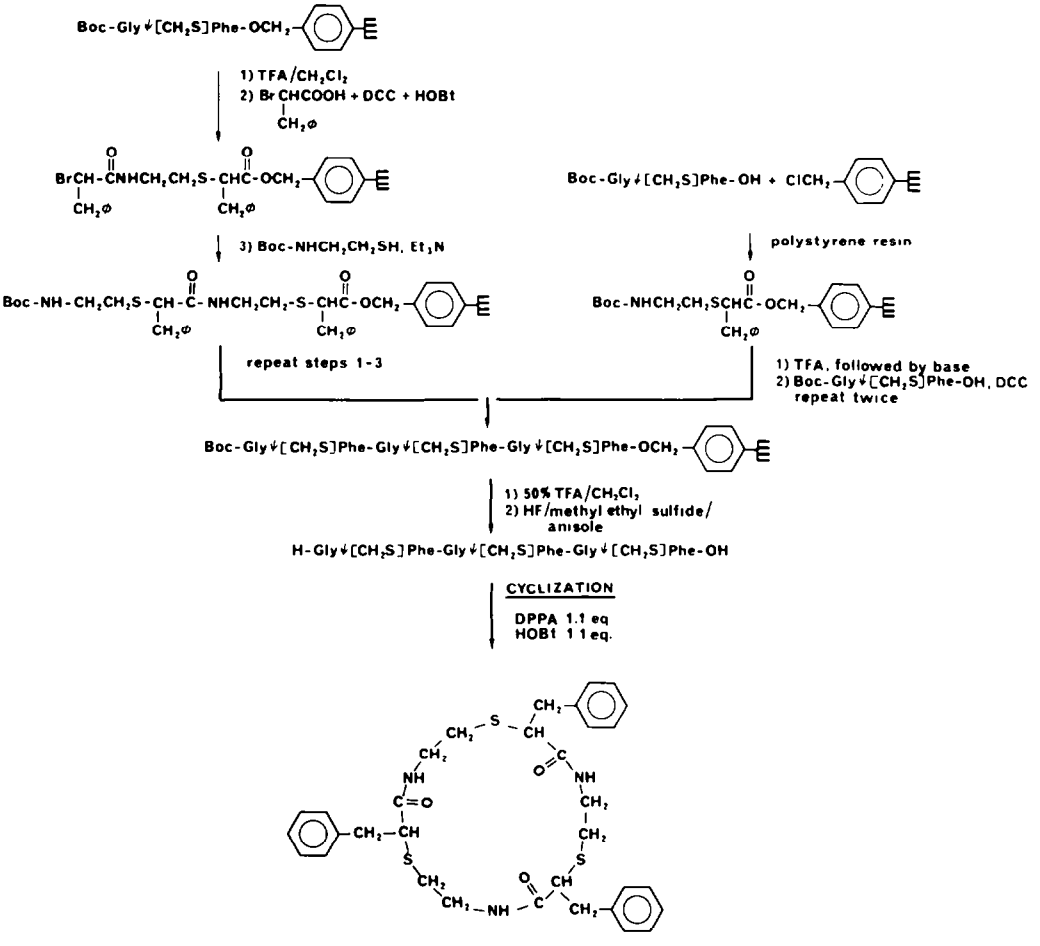
Scheme 1 illustrates our synthetic approach. In each case, the protected pseudodipeptide was attached to the Merrifield resin by the Gisin procedure (43). Although we had previously shown that the pseudodipeptide Boc-Pheψ[CH_2S]Leu-OH was vulnerable to epimerization during resin attachment (11), we were interested to see if a simpler pseudodipeptide would behave similarly. This α-carbon scrambling has only been a problem for the initial pseudodipeptide-resin coupling. No evidence of epimerization has ever been observed during subsequent solid phase couplings of pseudodipeptides (when not at the C-terminal residue), nor have we observed epimerization upon using other attachment methods (i.e., Marglin procedure (44)), that do not involve cesium salt formation.

After completion of the Gisin attachment and subsequent resin washes, the incorporation level of the pseudodipeptide onto the resin was approximated from weight gain, and also determined by amino acid analysis. The average value was determined to be 0.69 mmol/g of resin. Following Boc-deprotection with trifluoroacetic acid, the next two units were added by traditional DCC-mediated coupling procedures, using the Merrifield method.

HF cleavage of the linear pseudohexapeptide from the polystyrene resin gave two products, H-Glyψ[CH_2S]Phe-Glyψ[CH_2S]Phe-Glyψ[CH_2S]Phe-OH, and a second stereoisomer, presumably, H-Glyψ[CH_2S]Phe-Glyψ[CH_2S]Phe-Glyψ[CH_2S]D-Phe-OH. These were sometimes separable on tlc using system A but not on reversed phase HPLC using the TFA/acetonitrile gradient. We may therefore tentatively conclude that Boc-protected ψ[CH_2S] pseudodipeptides are prone to racemization or

epimerization at the α-carbon position, during cesium salt mediated resin attachment. This probably occurs following the attachment of the pseudodipeptide to the resin, and may be due to cesium-sulfur coordination, with a concomitant increase in vicinal proton acidity. We have previously shown (with Boc-Pheψ[CH_2S]Leu-OH) that this epimerization occurs prior to HF cleavage (12), and more recently we have demonstrated that recovered unreacted pseudodipeptide shows no evidence of epimerization (Mapelli and Spatola, unpublished observation).

In a parallel synthesis (Scheme 1), an alternative procedure (ψ-condensation) was followed in which two of the three pseudodipeptide units were prepared directly on the resin. After first coupling Boc-Glyψ[CH_2S]Phe-OH to the chloromethylated resin as before, the Boc group was removed with TFA. Next, the bromo acid, (R)-BrCH($CH_2C_6H_5$)CO_2H, was coupled with DCC to the pseudodipeptide, and a second pseudodipeptide unit was formed by subsequent reaction with N-protected cysteamine (Boc-NHCH$_2$CH$_2$SH). This procedure was repeated to form the linear



SCHEME 1. Two solid phase-based procedures for the synthesis of macrocycle cyclo(Glyψ[CH_2S]Phe)$_3$.

pseudohexapeptide. After cleavage with anhydrous HF, a product was isolated that was considerably more heterogeneous than that obtained in the first synthesis. However, most of the minor products, presumably representing truncated sequences, were readily removable by a single gel filtration. Nevertheless, by HPLC, a peak corresponding to the expected linear sequence was present and this represented the major product. The equality of the two compounds was confirmed by tlc and further by analytical RP-HPLC analysis including coelution with the linear sequence from the direct (preformed pseudodipeptide) method.

Cyclization of H-(Glyψ[CH_2S]Phe)$_3$-OH to cyclo(Glyψ[CH_2S]Phe)$_3$ was carried out in DMF using a mixture of DPPA and 1-hydroxybenzotriazole. The course of the cyclization could be conveniently monitored by disappearance of a ninhydrin positive spot (R_f = 0.77 in system A) and the appearance of a ninhydrin negative but UV-positive spot at higher R_f (0.90 in system A). (Curiously, we have routinely obtained excellent ninhydrin colors [and reproducible amino acid analysis data] with H-Glyψ[CH_2S]Xxx-OH pseudodipeptides but not with more sterically bulky Xxxψ[CH_2S]Yyy-OH analogs.) Upon reaction workup, we confirmed that two major products were formed, in a 40:60 mixture, with the minor compound apparently corresponding to cyclo(Glyψ[CH_2S]Phe-Glyψ[CH_2S]Phe-Glyψ[CH_2S]D-Phe). The major compound was the expected stereoisomer, cyclo(Glyψ[CH_2S]L-Phe)$_3$.

A third synthesis (not shown) was carried out in which Boc-Glyψ[CH_2S]Phe-OH was coupled to a chloromethylated resin using the Marglin procedure (Et_3N in DMF). In this case, racemization of the α carbon of the modified phenylalanine residue is not expected. After two further Boc-Glyψ[CH_2S]Phe elongations and HF cleavage, the same linear product as before was obtained. In this case only one product, corresponding to the major isomer H(Glyψ[CH_2S]Phe)$_3$-OH, was isolated.

The products of these syntheses were characterized by amino acid analysis, by chromatographic and spectrometric methods, and by fast atom bombardment mass spectrometry. Both major and minor peaks had the expected molecular weights, and no evidence of major sulfoxide (ψ[CH_2SO]) contamination was detected. The proton nmr data is supportive of the primary structure, but only marginally supportive of the predicted C_3 symmetry for the cyclo(Glyψ[CH_2S]Phe)$_3$ system.

These results demonstrate that 18-membered macrocycles, and presumably both larger and smaller variants, may be conveniently synthesized by solid phase procedures. In the present example, the products contain three ψ[CH_2S] surrogates, but a recent synthesis of a somatostatin analog with two ψ[CH_2NH] replacements (21) demonstrates the feasibility of still other types of cyclic pseudopeptides. While the detailed physical and biological properties of these compounds have yet to be established, it does appear that these synthetic approaches should enlarge the scope of easily attainable macrocycles, and spur the investigation of such species as unique and selective host compounds, for drug modification and/or transport, and eventually as templates for enzyme mimetics. With regard to the latter goal, we believe that the combination of backbone modifications together with a wide choice of sterically defined amino acid side chain residues with appropriate organic functional groups, can be used to prepare new designed host molecules.

MATERIALS AND METHODS

Pseudopeptides described in this work were prepared by solid-phase peptide methods using an automated synthesizer. Protected amino acids or pseudopeptides (Boc-Leu-OH, Boc-D-Leu-OH, Boc-D-Pen(Meb)-OH, and Boc-Glyψ[CH_2S]Phe-OH) were esterified to chloromethylated resin (Bio-Rad Bio-Beads SX-1) using the Gisin procedure (43). Protected amino acids were purchased from Bachem (Fmoc-Tyr(But)-OH and Boc-D-Lys(Z)-OH) or Peninsula (Boc-Tyr(Dcb)-OH, Boc-Leu-OH, Boc-D-Leu-OH). D-Penicillamine was purchased from Vega and converted to Boc-Pen(Meb)-OH using p-methylbenzyl bromide/sodium followed by treatment with $(Boc)_2O$ (Sigma). Boc-Glyψ[CH_2S]Phe-OH was prepared as previously described for Glyψ[CH_2S]Xxx derivatives (24,45). Boc-cystamine was prepared from cystamine (Sigma). Triethylamine was distilled from ninhydrin; dichloromethane was distilled from potassium carbonate; dimethylformamide was distilled under reduced pressure. Other solvents and reagents were of analytical grade. The following solid phase synthesis protocol was used: 1) CH_2Cl_2, 3 x 2 min; 2) TFA/anisole/mercaptoethanol/CH_2Cl_2, 40:2:10:38, v/v, 5, 25 min; 3) CH_2Cl_2, 4 x 2 min; 4) TEA/CH_2Cl_2, 1:10, v/v, 2 x 5 min; 5) CH_2Cl_2, 3 x 2 min; 6) DMF, 2 x 2 min; 7) coupling with Boc-amino acid (2.5 eq), HOBt (2.5 eq), DCC (2.5 eq) in DMF, 120 min; 8) DMF, 3 x 2 min; 9) second coupling: Boc-amino acid (2.5 eq), HOBt (2.5 eq), DCC (2.5 eq) in DMF, 12 h; 10) DMF, 3 x 2 min; 11) CH_2Cl_2, 3 x 2 min; 12) EtOH, 4 x 2 min; all couplings were monitored by the Kaiser test (28).

Thin layer chromatography was carried out on silica gel plates (Merck, Kieselgel F-60, 5 x 20 cm), and the products were detected by UV, ninhydrin or iodine vapor. The following systems were used; A) 1-butanol-acetic acid-water (4:1:5, v/v, upper phase); B) $CHCl_3$:MeOH:AcOH (85:15:5). Analytical reversed phase HPLC was performed using a C-18 Vydac column (4.6 x 250 mm) with linear gradients (flow rate 1 ml/min) in solvent systems: I: 30-50% (20 min) v/v 0.05% TFA in acetonitrile/0.05% trifluoroacetic acid (aqueous), II:40-60% (20 min) v/v 0.05% TFA in acetonitrile/0.05% trifluoroacetic acid (aqueous), III: 10-30% v/v acetonitrile/0.25 M H_3PO_4/TEA (aqueous), pH = 3.5, using a Hitachi automated liquid chromatograph (L-5000). Preparative RP-HPLC was accomplished on a C-18 Dynamax column (21.4 x 250 mm) with one of the following linear gradients (40 min; flow rate 4 ml/min); IV: 40-60%; V: 30-45%; VI: 10-60%; VII: 30-85% using in each case v/v 0.05% TFA in acetonitrile/0.05% TFA (aqueous) using a Varian 5000 liquid chromatography system monitored at 254 or 280 nM. For quantitative amino acid analyses, peptide (ca. 0.5 mg) were hydrolyzed with constant boiling hydrochloric acid (0.4 ml) containing phenol (0.02 ml) and norleucine (as internal standard) in evacuated and sealed ampules for 24 h at 110°C. The analyses were performed on a Dionex-300 analyser. Optical rotations were determined using a Perkin Elmer Model 241-MC polarimeter with 1.0 dm pathlength cell at 20°C.

The following general procedure for peptide-resin synthesis was used for each of the enkephalin analogs whose preparation is described below.

1. Synthesis of Cyclic Enkephalins Using Preformed Pseudodipeptides

Fmoc-Tyr(But)-D-Lys(Z)-Glyψ[CH_2S]Phe-Leu-polymer (1)

Boc-Leu-OH was attached to the chloromethylated resin using the Gisin procedure, substitution levels were calculated by weight gain as 0.87 mmol/g (1.05 mmol/g established by quantitative amino acid analysis). Using 1.50 g (1.5 mmol) of Boc-Leu-resin, three cycles of deprotection, neutralization and coupling were performed with Boc-Glyψ[CH_2S]Phe-OH, Boc-D-Lys(Z)-OH, and Fmoc-Tyr(But)-OH using the procedure above. Upon completion of the solid phase synthesis, peptide resin was washed with: EtOH (3 x 30 ml), diethyl ether (3 x 30 ml) and dried. This gave 2.52 g (82%) of 1. Amino acid analysis: Tyr 0.82; Lys 0.84; Glyψ[CH_2S]Phe 1.06; Leu 1.00.

Fmoc-Tyr-D-Lys-Glyψ[CH_2S]Phe-Leu-OH (2)

Peptide resin 1 (1.30 g) was treated with anhydrous HF in the presence of 5% anisole (v/v) and 5% methyl ethyl sulfide (v/v) for 30 min at 0°C. After removal of HF and scavengers under reduced pressure the solid residue was washed with Et_2O (3 x 20 ml) and then extracted with 50% AcOH (4 x 50 ml). An oily product, obtained after lyophilization, was passed through the Sephadex LH-20 column using 50% AcOH. The major peak was pooled and lyophilized, yielding 215 mg (32%). Part of this product (20 mg) was purified using preparative RP-HPLC (system IV) to give 10 mg of homogeneous material, 2. Amino acid analysis: Tyr 0.88; Lys 1.01; Glyψ[CH_2S]Phe 0.96; Leu 1.00. FAB-MS: MH^+ = 852, found 852. R_fA = 0.79, R_fB = 0.21. RP-HPLC, k'_{II} = 3.10.

H-Tyr-cyclo[D-Lys-Glyψ[CH_2S]Phe-Leu] (3)

To the solution of Fmoc-Tyr-D-Lys-Glyψ[CH_2S]Phe-Leu-OH 2 (130 mg, 0.15 mmol) in DMF (150 ml), adjusted to pH 7.0 in TEA at -30°C, was added dropwise DPPA (0.036 ml, 0.17 mmol) in 10 ml of DMF, followed by addition of HOBt (60 mg, 0.17 mmol) and DMAP (2 mg). The reaction mixture was stirred for 48 h at -15°C. A mixed bed ion exchange resin slurry (10 ml in water) was added and stirring was continued for 2 h at RT. Filtration and evaporation to dryness yielded an oil. The oil was treated with 55% piperidine in DMF (20 ml) at RT. After 30 min, the mixture was evaporated under reduced pressure. The oily residue was purified by elution through a Sephadex LH-20 column with 50% AcOH. The major peak was pooled as lyophilized, yielding 71 mg. Part of the product was purified by preparative RP-HPLC (system V). The homogeneous material was collected and lyophilized, yielding 33 mg (36%) of 3. Amino acid analysis: Tyr 0.89; Lys 0.95; Glyψ[CH_2S]Phe 1.02; Leu 1.00. $[\alpha]_D^{20}$ = +60.8 (c = 0.25, 1M AcOH). FAB-MS: MH^+ = 612, found 612.2. R_fA = 0.73, R_fB = 0.23. RP-HPLC: k'_I = 7.82, k'_{III} = 10.57.

Fmoc-Tyr(But)-D-Lys(Z)-Glyψ[CH_2S]Phe-D-Leu-polymer (4)

Boc-D-Leu-OH was attached to the chloromethylated resin using the Gisin procedure to give a substitution level (calculated by weight gain) of 0.89 mmol/g (1.22 mmol/g was established by amino acid analysis). Synthesis was initiated with 1.03 g (1.25 mmol) of Boc-D-Leu-resin as described above. Upon completion of the solid phase synthesis, peptide resin was washed with: EtOH (3 x 20 ml), diethyl ether (3 x 20 ml) and dried. Yield 1.66 g (62%) of 4.

Fmoc-Tyr-D-Lys-Glyψ[CH_2S]Phe-D-Leu-OH (5)

Peptide resin 4 (0.66 g) was treated with anhydrous HF in the presence of 5% anisole (v/v) and 5% methyl ethyl sulfide (v/v) for 30 min at 0°C. After workup, the oily product was passed through a Sephadex LH-20 column using 50% AcOH. The major peak was pooled and lyophilized. Yield 103 mg (25%) of 5. Amino acid analysis: Tyr 1.03; Lys 0.96; Glyψ[CH_2S]Phe 0.96; Leu 1.00. FAB-MS: MH^+ = 852, found 852. R_fA = 0.73, R_fB = 0.15. RP-HPLC k'_{II} = 2.83.

H-Tyr-cyclo[D-Lys-Glyψ[CH_2S]Phe-D-Leu] (6)

Fmoc-Tyr-D-Lys-Glyψ[CH_2S]Phe-D-Leu-OH 5 (100 mg, 0.12 mmol) was cyclized with DPPA (0.03 ml, 0.14 mmol) and worked up as described for 3. The oily product was purified by elution through a Sephadex LH-20 column with 50% AcOH. The major peak was pooled and lyophilized, yielding 45 mg of the white solid. The product was further purified by preparative RP-HPLC (system V). The homogeneous material was collected and lyophilized to give 25 mg (34%) of 6. Amino acid analysis: Tyr 1.03; Lys 0.96; Glyψ[CH_2S]Phe 0.96; Leu 1.00. $[\alpha]_D^{20}$ = +123.4 (c = 0.25, 1M AcOH). FAB-MS: MH^+ = 612, found 612. R_fA = 0.74, R_fB = 0.19. RP-HPLC: k'_I = 5.12, k'_{III} = 10.01.

2. Synthesis of Cyclic Enkephalins Using Solid Phase (ψ-Condensation) Method

Fmoc-Tyr(But)-D-Lys(Z)-Glyψ[CH_2S]Phe-Leu-polymer (7) (ψ-condensation solid phase method)

The synthesis was started with 0.50 g (0.50 mmol) of Boc-Leu-resin. (R)-BrCH($CH_2C_6H_5$)CO_2H was coupled to the resin using 2.5 eq. acid and a standard SPPS cycle, with 2.5 eq. bromo acid and 2.5 eq. DCC in DMF/CH_2Cl_2. A double coupling (see general SPPS procedure) was used (steps 7-12). Freshly prepared Boc-NH-CH_2-CH_2-SH (by reduction of Boc-cystamine, 2 mmol) was dissolved in 10 ml of DMF and was shaken for 48 h in the presence of triethylamine (0.21 ml, 1.5 mmol). After washing the resin with DMF (3 x 2 min), an analogous coupling with Boc-NH-CH_2-CH_2-SH (2 mmol) was repeated. The progress of CH_2S bond formation was controlled by quantitative amino acid analysis. Upon completion of the next two solid phase cycles (Boc-D-Lys(Z)-OH and Fmoc-Tyr(But)-OH), peptide resin was washed with: EtOH (3 x 20 ml), diethyl ether (3 x 20 ml), and dried, yielding 0.82 g (79%) of 7. Amino acid analysis: Tyr 0.84; Lys 1.00; Glyψ[CH_2S]Phe 0.98; Leu 1.01.

Fmoc-Tyr-D-Lys-Glyψ[CH_2S]Phe-Leu-OH (8) (solid phase method)

Peptide resin 7 (0.70 g) was treated with anhydrous HF using the procedure described for 2. The oily product was passed through a Sephadex LH-20 column using 50% AcOH. The major peak was pooled and lyophilized, yielding 215 mg (60%) white powder. Part of this product (20 mg) was purified using preparative RP-HPLC (system II). A white homogeneous lyophilized powder (10 mg) was obtained (8). This product was compared to the product from the pseudodipeptide method (see synthesis of 2) and found to be identical by tlc and analytical HPLC. Amino acid analysis: Tyr 0.99; Lys 1.04; Glyψ[CH_2S]Phe 1.01; Leu 1.00. FAB=MS: MH^+ = 852, found 852. R_fA = 0.79; R_fB = 0.21. RP-HPLC: k'_{II} = 3.10.

H-Tyr-cyclo(D-Lys-Glyψ[CH_2S]Phe-Leu) (9)

The preparation of cyclic pseudopeptide 9 by cyclization of the linear Fmoc-pseudopeptide 8 was carried out analogously to the procedure used for 3, but now starting with 85 mg (0.097 mmol) of 8. Following cyclization, the Fmoc group was removed with 55% piperidine in DMF as before. After final purification on a Sephadex LH-20 column with 50% AcOH, the major peak was pooled and lyophilized, yielding 36 mg of white powder. Part of the product (30 mg) was purified by preparative RP-HPLC (system V). The homogeneous material was collected and lyophilized. Yield

19 mg (32%) of 9. Amino acid analysis: Tyr 0.89; Lys 0.92; Glyψ[CH_2S]Phe 1.05; Leu 1.00. FAB-MS: MH^+ = 612, found 612.2. R_fA = 0.73, R_fB = 0.23. RP-HPLC: k'_I = 7.82, k'_{III} = 10.57.

3. Synthesis of Penicillamine-Containing Cyclic Enkephalin Analogs Using Preformed Pseudodipeptides

H-Tyr-D-Pen(Meb)-Glyψ[CH_2S]Phe-D-Pen(Meb)-polymer (10)

Boc-D-Pen(Meb)-OH was attached to the chloromethylated resin using the Gisin procedure to give a substitution level (calculated by weight gain) of 0.75 mmol/g. Synthesis was started with 2.00 g (1.5 mmol) of Boc-D-Pen(Meb)-resin. Upon completion of solid phase synthesis, the peptide resin was deprotected, neutralized, and washed with CH_2Cl_2 (3 x 20 ml), EtOH (3 x 20 ml), and diethyl ether (3 x 20 ml), and dried, yielding 2.74 g (97%) of 10. Amino acid analysis: Tyr 1.00; Glyψ[CH_2S]Phe 0.77.

H-Tyr-cyclo(D-Pen-Glyψ[CH_2S]Phe-D-Pen)-OH (11)

The peptide resin 7 (1.42 g) was treated with anhydrous HF in the presence of 5% anisole (v/v) and 5% methyl ethyl sulfide (v/v) for 45 min at 0°C. After removal of HF and scavengers under reduced pressure, the solid residue was washed with Et_2O/mercaptoethanol (3 x 20 ml, 100:1, v/v) and then extracted with 50% AcOH (4 x 50 ml, purged with nitrogen) and lyophilized. The residue was dissolved in 30 ml nitrogen-flushed AcOH and then diluted with water up to 1200 ml. 2 N aqueous ammonia was added gradually to give a final pH of 7.5. The solution was treated (under nitrogen) with 0.01 M $K_3Fe(CN)_6$ until a permanent yellow color was generated, and then stirred for an additional 2 h. Next, the pH was adjusted with glacial acetic acid to 5, anion exchange resin (75 ml, AG 3 x 4, acetate form) was added (to remove excess ferri- and ferrocyanide ions) and the mixture filtered and lyophilized. The crude product was desalted on a Sephadex G-15 column (2.5 x 100 cm) with 30% AcOH. The major peak was pooled, lyophilized to give 130 mg of off-white powder. Part of it (100 mg) was purified by preparative RP-HPLC (system VI). The major peak, after collection and lyophilization, gave 20 mg of 11. Amino acid analysis; Tyr 1.00; Glyψ[CH_2S]Phe 0.69. FAB-MS: MH^+ 649, found 649. Tlc: R_fA = 0.78; R_fB = 0.22. HPLC: k'_{III} = 7.78.

4. Synthesis of Pseudopeptide Macrocycles

H-(Glyψ[CH_2S]Phe)$_3$-polymer (12)

Boc-Glyψ[CH_2S]Phe-polymer (1.44 g, 1.0 mmol) was subjected to two cycles of SPPS using Boc-Glyψ[CH_2S]Phe-OH (Scheme 1). Upon completion of the synthesis, the peptide resin was treated with TFA to remove the N-terminal Boc group, neutralized, and washed with EtOH (3 x 20 ml), diethyl ether (3 x 20 ml), and dried. Yield 1.59 g (48%) of 12.

H-(Glyψ[CH_2S]Phe)$_3$-OH (13)

Peptide resin (12, 1.50 g) was treated with anhydrous HF in the presence of 5% anisole (v/v) and 5% methyl ethyl sulfide (v/v) for 45 min at 0°C. After workup the oily product was passed through a Sephadex LH-20 column using 50% AcOH. The major peak was pooled and lyophilized. Yield 350 mg (61%) of 13. FAB-MS: MH^+ = 640, found 640. R_fA = 0.77, R_fB = 0.89. RP-HPLC: k'_I = 4.58.

Cyclo(Glyψ[CH_2S]Phe)$_3$ (14)

The linear pseudohexapeptide (13, 250 mg, 0.36 mmol) was dissolved in 150 ml DMF and cyclized using DPPA (0.09 ml, 0.41 mmol) in the presence of HOBt (55 mg, 0.36 mmol) for 24 h at -15°C. Water (10 ml) was added and all solvents were evaporated under reduced pressure. Part of the oily residue was purified using preparative RP-HPLC (system VII). Two major fractions were collected (4.5 mg and 2.5 mg). The fraction eluting first (13.2 min) on analytical RP-HPLC (system II), proved to be ninhydrin negative and UV positive; its structural parameters were confirmed by nmr and FAB-MS. FAB-MS: MH^+ = 622, found 622. TLC: R_fA = 0.90, Analytical RP-HPLC: k'_{II} 3.48. ^1H NMR: ($CDCl_3$): δ 7.50 (m, 5H, arom), δ 6.82 (bs, 1H, NH), δ 3.58 (m, 1H, α-CH), δ 3.42 (m, 2H, NH-CH_2 or CH_2S), δ 2.68 (m, 2H, NH-CH_2 or CH_2S), δ 3.26 (m, 1H, β-CH_2), δ 2.96 (m, 1H, β-CH_2). The minor product was concluded to be the diastereomer.

H-(Glyψ[CH_2S]Phe)$_3$-polymer (15) (ψ-condensation method)

The synthesis of 15 was carried out as described for 12 using Boc-Glyψ[CH_2S]Phe-polymer (1.44 g, 1.0 mmol) as the starting resin. In this case, peptide elongation was carried out by the preparation of the pseudodipeptide on the resin (two cycles using the double coupling procedure as previously described for the synthesis of 12). In this case couplings were not monitored during the synthesis. After completion of SPPS an additional sequence of deprotection and neutralization was effected, after which the peptide resin was dried giving 1.59 g (48%) of 15.

H-(Glyψ[CH_2S]Phe)$_3$-OH (16)

Peptide resin (15, 1.50 g) was treated with anhydrous HF according to the procedure described for compound 13. Crude compound (0.48 g) was passed through the Sephadex LH-20 column in 20% AcOH. Three major peaks were eluted from the column. The first of these peaks contained material which was ninhydrin positive and had the expected UV absorbance; appropriate fractions were pooled and lyophilized, yielding only 140 mg (23% of theoretical) of a white hygroscopic powder. This product (16) was compared to the linear compound 13 (prepared by the preformed pseudodipeptide method) by tlc and RP-HPLC and found to be identical. TLC: R_fA = 0.77, R_fB = 0.89. RP-HPLC: k'_I = 4.58.

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REFERENCES

1. Spatola, A. F. "Chemistry and Biochemistry of Amino Acids, Peptides and Proteins"; Weinstein, B., Ed.; Marcel Dekker: New York, 1983; Vol. 7, pp. 267-357.
2. Bennett, H. P. J.; McMartin, C. Pharmalogical Reviews, **1979**, 30, 247.
3. Hann, M. M.; Sammes, P. G.; Kennewell, P. D.; Taylor, J. B. J. Chem. Soc. Chem. Commun., **1980**, 234.
4. (a) Momany, F. A.; Aubuchon, J. R. Biopolymers, **1978**, 17, 2609. (b) Freidinger, R. M.; Veber, D. F. J. Am. Chem. Soc., **1979**, 101, 6129.
5. Erickson, B. W.; Merrifield, R. B.; "The Proteins"; Neurath, H.; Hill, R. L.; Eds.; Academic Press: New York, 1976; Vol. 2, pp. 255-527.
6. DiMaio, J.; Schiller, P. W. Proc. Natl. Acad. Sci., USA, **1980**, 77, 7162.
7. Mosberg, H. I.; Hurst, R.; Hruby, V. J.; Gee, K.; Yamamura, H. I.; Galligan, J. F.; Burks, T. F. Proc. Natl. Acad. Sci., USA, **1983**, 80, 5871.
8. Spatola, A. F.; Bettag, A. L.; Agarwal, N. S.; Saneii, H.; Vale, W. W.; Bowers, C. Y. "LH-RH Peptides as Female and Male Contraceptives"; Zatuchni, G. I.; Shelton, J. D.; Sciarra, J. J., Eds.; Harper and Row: New York, 1981; pp. 24-36.
9. IUPAC-IUB Nomenclature Recommendations, Eur. J. Biochem., **1984**, 138, 9.
10. Spatola, A. F.; Agarwal, N. S.; Bettag, A. L.; Yankeelov, Jr., J. A.; Bowers, C. Y.; Vale, W. W. Biochem. Biophys. Res. Commun., **1980**, 97, 1014.
11. Spatola, A. F.; Saneii, H.; Edwards, J. V.; Bettag, A. L.; Anwer, M. K.; Rowell, P.; Browne, B.; Lahti, R.; von Voigtlander, P. Life Sci., **1986**, 38, 1243.
12. Spatola, A. F.; Edwards, J. V. Biopolymers, **1986**, 25, S229.
13. Edwards, J. V.; Spatola, A. F.; Lemieux, C.; Schiller, P. W. Biochem. Biophys. Res. Commun., **1986**, 136, 730.
14. Spatola, A. F.; Mapelli, C.; Humphrey, J.; Edwards, J. V.; Burks, T. F.; Schook, J. E. "Peptides, 1986"; Theodoropoulos, D., Ed.; W. de Gruyter: Berlin, 1986, pp. 381-384.
15. Gero, T. W.; Spatola, A. F.; Torres-Aleman, I.; Schally, A. V. Biochem. Biophys. Res. Commun., **1984**, 120, 840.

16. Spatola, A. F.; Anwer, M. K.; Rockwell, A. L.; Gierasch, L. M. J. Am. Chem. Soc., **1986**, 108, 825.
17. Sherman, D. B.; Porter, R. A.; Spatola, A. F. "Proceedings of the Tenth American Peptide Symposium"; Marshall, G., Ed.; St. Louis, MO, in press.
18. Clausen, K.; Thorsen, M.; Lawesson, S. O.; Spatola, A. F.; J. Chem. Soc. Perkin I, **1984**, 785.
19. LaJoie, G.; Lepine, F.; Lemaire, S.; Jolicoeur, F.; Aube, C.; Turcotte, A.; Belleau, B. Int. J. Peptide Protein Res., **1984**, 24, 316.
20. Shaw, J. S.; Miller, L.; Turnbull, M. J.; Gormley, J. J.; Morley, J. S. Life Sci., **1982**, 1259.
21. Coy, D. H.; Sasaki, Y.; Murphy, W. A.; and Heiman, M. "Peptides, 1986"; Theodoropoulos, D., Ed.; W. de Gruyter: Berlin, **1986**, pp. 143-146.
22. Spatola, A. F.; Darlak, K.; Mapelli, C.; Formaggio, F.; Burks, T. F.; Shook, J. E. Tenth American Peptide Symposium; Abstract #P111; Washington University in St. Louis, May 23-28, 1987.
23. Pfister, K., Howe, E. E.; Robinsin, C. A.; Shabica, C.; Pietrusza, E. W.; Tishler, M.; J. Am. Chem. Soc., **1949**, 71, 1096.
24. Spatola, A. F.; Bettag, A. L. J. Org. Chem., **1981**, 46, 2392.
25. Sakakibara, S. "Chemistry and Biochemistry of Amino Acids, Peptides and Proteins"; Weinstein, B., Ed.; Marcel Dekker: New York, 1971; Vol. 1, pp. 51-75.
26. Carpino, L. A.; Han, G. Y. J. Am. Chem. Soc., **1970**, 92, 5748.
27. Brady, S. F.; Varga, S. L.; Freidinger, R. M.; Schwenk, D. A.; Mendlowski, M.; Holly, F. W.; Veber, D. F. J. Org. Chem., **1979**, 44, 3101.
28. Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P.I. Anal. Biochem., **1970**, 34, 595.
29. Du Vigneaud, V.; Winestock, G.; Murti, V. V. S.; Hope, D. B.; Kimbrough, Jr., R. D.; J. Biol. Chem., **1960**, 235, 64.
30. Gorin, F. A.; Balasubramanian, T. M.; Cicero, T. J.; Schwietzer, J.; Marshall, G. R. J. Med. Chem., **1980**, 23, 1113.
31. Schiller, P. W. "The Peptides"; Udenfriend, S.; Meienhofer, J., Eds.; Volume VI, Academic Press: Orlando, **1984**, 219-268.
32. Mosberg, H. I. Int. J. Peptide Protein Res., **1987**, 29, 282.
33. Mosberg, H. I.; Schiller, P. W. Int. J. Peptide Protein Res., **1984**, 462.
34. Richman, S. J.; Goodman, M.; Nguyen, T. M.-D.; Schiller, P. W. Int. J. Peptide Protein Res., **1985** 25, 648.
35. Dutta, A. S.; Gormley, J. J.; Hayward, C. F.; Morley, J. S.; Shaw, J. S.; Stacey, G. J.; Turbull, M. T. Life Sci., **1977**, 21, 559.
36. Hudson, D.; Sharpe, R.; Tien, P.; Hallett, A.; Szelke, M. Molecular Endocrinology"; MacIntyre, I.; Szelke, M.; Eds.; Elsevier/North Holland Biomedical Press: Amsterdam, 1979; pp. 57-70.
37. Clausen, K.; Spatola, A. F.; Lemieux, C.; Schiller, P. W.; Lawesson, S.-O. Biochem. Biophys. Res. Commun., **1984**, 120, 305.
38. Walter, W.; Voss, J. "The Chemistry of Amides"; Zabicky, J., Ed.; Interscience: London, 1970; pp. 383-475.
39. Story, P. R.; Busch, P. Advances in Organic Chemistry, **1972**, 8, 67.
40. Lehn, J. M. Pure Appl. Chem., **1979**, 51, 987.
41. Aarts, V. M. L. J., van Staveren, C. J.; Grootenhuis, P. D. J.; van Eerden, J.; Kruise, L.; Harkema, S.; Reinhoudt, D. N. J. Am. Chem. Soc., **1986**, 108, 5035.
42. Ovchinnikov, Y. A.; Ivanov, V. T. "The Proteins", Neurath, H., and Hill, R. L., Eds., Academic Press: Orlando, 1982; Vol. 5, pp. 307-641.
43. Gisin, B. F. Helv. Chim. Acta, **1973**, 56, 1476.
44. Marglin, A. Tetrahedron Lett., **1971**, 3145.
45. Yankeelov, Jr., J. A.; Fok, K.-F.; Carothers, D. J. J. Org. Chem., **1978**, 43, 1623.