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OPTICAL ROTATORY PROPERTIES OF METHYLISOTHIOCYANATE-AMINO ACID ADDUCTS

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Abstract—Definite information concerning the optical configurations of amino acids in peptides has been obtained from an investigation of the circular dichroism of their adducts with methylisothiocyanate.

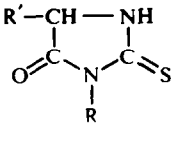
ALTHOUGH there is at present considerable doubt concerning the occurrence of D-amino acid residues in proteins, mainly due to the methods of hydrolysis and isolation which do not eliminate the danger of racemization,[1] the presence of D-amino acids in a variety of microorganisms in both the free state and in peptide linkage has been soundly demonstrated.[2]

Gas chromatography,[3,4] ion exchange chromatography,[1] mass spectrometry,[3] NMR spectroscopy,[5] TLC,[6] and stereospecific enzymatic reactions[7] have recently been used to distinguish enantiomeric amino acids directly or through suitable derivatives.

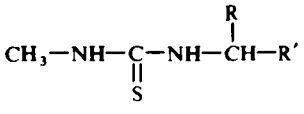
In this paper a simple and rapid method is reported, which allows correlation of absolute configurations of sequences of α-amino acids to be made through the examination of the circular dichroic spectra of their adducts with methylisothiocyanate. Moreover, if free sulfhydryl groups of cysteine residues are present, the optical rotatory properties of the resulting dithiocarbamates can be used to differentiate the enantiomeric forms of this amino acid.

RESULTS AND DISCUSSION

Reaction of methylisothiocyanate with amino groups of amino acids and peptides. A number of chromophorically substituted[8] derivatives of the optically transparent[9] amino group have been investigated by means of ORD and CD in order to determine the optical configurations of amino acid residues.[10] Nevertheless, many of them present some practical drawbacks, which limit even the restricted objective of the assignment of absolute configuration to N-terminal amino acid residues in peptides. The promising Djerassi's ORD and CD preliminary results[11] on phenylthiohydantoins (I; R = phenyl), prepared from phenylisothiocyanate and amino acids, have not been extended, mainly because compounds (I; R = Ph) are often obtained totally racemized.



I



II

Methylisothiocyanate is preferred since it has a somewhat higher reactivity towards amino groups, the time required for the reaction to be completed therefore being shortened;[12] it is considerably more soluble in water, which makes it possible to conduct the reaction in aqueous solution without resorting to the addition of organic solvents;[13] it does not contain a chromophore which could interfere in the CD determinations and, finally, it seems to give less racemized thiohydantoins (I; R = Me).[14]

For these reasons the optical rotatory properties of methylisothiocyanate amino acid- and peptide-adducts have been measured in aqueous solution at different pH in order to ascertain if the method could be applied in connection with the modified Edman degradation technique to determine the configuration of sequences of amino acid residues in natural compounds.

The reaction between methylisothiocyanate and α-NH_2 groups of amino acids and peptides in a weakly alkaline medium is known to give N-methylthiocarbamoyl derivatives (II; R′ = COO^-).[13] The —NH—C(=S)—NH—chromophore has been examined in detail by UV absorption spectroscopy and demonstrated to present a low intensity band (log $\simeq$ 2) at 280–290 mμ and a much higher intensity band (log $\simeq$ 4) at 238–248 mμ, which have been related to n $\rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions within the $\diagdown$C=S group, respectively.[15]

To the best of our knowledge, CD technique has never been applied to compounds containing thioureine moieties. Hence, in order to obtain information on the optical rotatory properties of the —NH—C(=S)—NH—chromophore CD spectra of N-methylthiocarbamoyl-L-alaninol (II; R = CH_3, R′ = CH_2OH) have been examined in a series of solvents of different polarity and different capability to form H-bonds. In the 250–380 mμ region a positive band is apparent; its location, close to that of the related UV absorption band, and its red-shift in going from trifluoroethanol (262·5 mμ) to water (263 mμ), ethanol (273·5 mμ), chloroform (277 mμ), acetonitrile (281 mμ), and ethyl ether (287·5 mμ) suggest that it is associated with an n $\rightarrow \pi^*$ transition within the chromophore. Moreover, in agreement with the theory,[10a] when a solvent containing an OH group is employed, H-bonds make the n $\rightarrow \pi^*$ transition more difficult by blocking the lone pair electrons involved.

The CD data of N-methylthiocarbamoyl (MTC)-amino acids at weakly alkaline pH are reported in Table 1 and four representative examples are shown in Fig 1. The results obtained can be summarized as follows: (a) N-methylthiocarbamoyl-amino acids of L-configuration have a positive Cotton effect and, consequently, those of the D-series a negative one; this observation has been tested on all the most common amino acids and was demonstrated to hold without exception; (b) the dichroic bands are centred at 262–263 mμ, with the exception of MTC-L-serine, which shows a broad band at 265–270 mμ; (c) the reported A_L–A_R values do not have absolute significance, since N-methylthiocarbamoyl-amino acids have not been isolated but measured directly in the reaction mixtures; (d) the amino side-chain group of lysine residues reacts with methylisothiocyanate (as demonstrated with N-α-carbobenzoxy-L-lysine by TLC), but the N-ε-methylthiocarbamoyl derivative do not complicate the stereochemical correlations, since it does not exhibit a dichroic band in the region of absorption of thioureines, the asymmetric C atom being too far removed from the inherently symmetric chromophore; (e) a second α-C atom of opposite configuration

TABLE 1. CD DATA ON AMINO ACID AND PEPTIDE DERIVATIVES

	Amino acid/ peptide	Methylthioureine[b] $\Delta A^c \cdot 10^3$	λ_{max}	Methylthiohydantoin[e] $\Delta A^f \cdot 10^3$	λ_{max}
(1)	L-Alanine	1·1	262	3·85	306
(2)	D-Alanine	−1·1	262	−3·85	306
(3)	L-Valine	1·0	263	3·9	303
(4)	L-Leucine	3·0	262	0·42	308[g]
(5)	D-Leucine	−3·0	262	−0·42	308[g]
(6)	L-Isoleucine	1·3	262	0·23	308[g]
(7)	L-Serine	0·15	265–270[d]	2·25	305[h]
(8)	L-Threonine	0·8	263	1·1	305[h]
(9)	L-Aspartic acid	1·6	263	2·4	309
(10)	L-Asparagine	1·0	263	2·5	304
(11)	L-Glutamic acid	1·5	263	2·8	306
(12)	L-Glutamine	0·9	263	8·45	302
(13)	L-Methionine	3·0	262	3·0	303
(14)	L-Lysine	1·6	262	4·45	305
(15)	L-Arginine	0·8	262	5·2	307
(16)	L-Proline	5·7	262	44·2	308
(17)	L-Histidine	—	—	2·05	315
(18)	L-Phenylalanine	—	—	0·38	309[g]
(19)	L-Tyrosine	—	—	11·0	305[h]
(20)	L-Tryptophan	—	—	12·6	302
(21)	Z-L-lysine[a]	—	—	—	—
(22)	L-Alanyl-D-Alanine	1·7	267	3·75	303

[a] N-α-Carbobenzoxy-L-lysine.

[b] Approximately 90 min at slightly alkaline pH; temp 23 ± 1°C; excess of methylisothiocyanate.

[c] Amino acid or peptide concentrations, 10^{-1} M; 1 mm. cell.

[d] Wavelength maximum not clear.

[e] Several hours at acidic pH.

[f] 1 cm. cell.

[g] The isolated compound has been measured in methanol solution; Δε values.

[h] shoulder.

is not able to alter the sign of the band; (f) in the presence of other groups absorbing in this spectral region the method can not be employed unequivocally. Moreover, an additional complication, which will be discussed in the next section, arises, if the starting material contains free —SH groups.

The rates of the reaction of methylisothiocyanate and amino acid residues have been also measured and found dependent on the degree of substitution of the amino moiety[16] (Fig 2), being rapid for the secondary amino group of proline (50% reacts in 70 seconds and 95% in 7 min) and much slower for the primary amino groups of a series of amino acids (for leucine, 50% reacts in about 9 min and 95% in approximately 40 min).

The drawback of the superposition of bands related to chromophores absorbing in the 250–300 mμ region shown by the N-methylthiocarbamoyl derivatives can be easily removed if the reaction mixtures, after about 90 min at slightly alkaline pH, are kept at acidic pH for a few hr. The resulting methylthiohydantoins (I; R = Me) are expected to show a CD Cotton effect above 300 mμ, as reported for their phenyl analogs.[11]

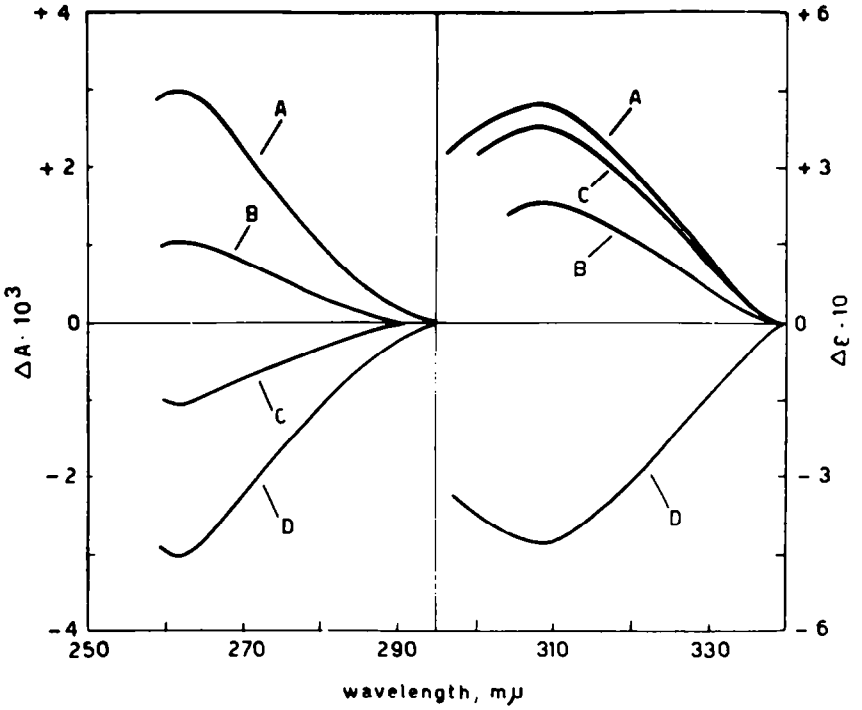


FIG 1. CD curves in the 250–295 mμ region: A, MTC-L-Leu; B, MTC-L-Ala; C, MTC-D-Ala; and D, MTC-D-Leu. CD curves in the 295–340 mμ region: A, MTH-L-Leu; B, MTH-L-Ileu; C, MTH-L-Phe; and D, MTH-D-Leu.

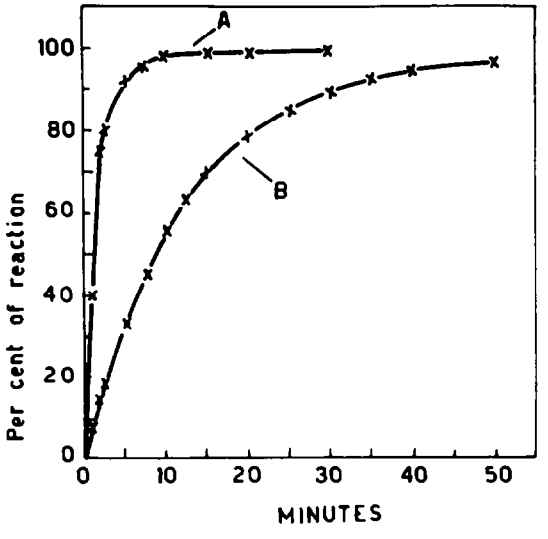


FIG 2. Reaction rates of thioureine formation from methylisothiocyanate and L-proline (A), and L-leucine (B). Slightly alkaline pH; amino acid concentrations, 10^{-1} M, and methylisothiocyanate concentration, $5 \cdot 10^{-1}$ M, in water-dioxane 9:1; wavelength: 275 mμ; temperature 23 ± 0·5°C; 0·5 mm cell.

TABLE 2. COTTON EFFECTS OF METHYLISOTHIOCYANATE/ALANYL-ALANINE ADDUCTS

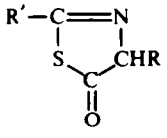
Dipeptide	Thioureine	Thiohydantoin	Thioureine	Thiohydantoin
L-Ala-D-Ala	267 mμ (+)[a]	319 mμ (+)[b]	262 mμ (−)[c]	320 mμ (−)[d]
D-Ala-L-Ala	267 mμ (−)[a']	319 mμ (−)[b']	262 mμ (+)[c']	320 mμ (+)[d']

[a] MTC-L-Ala-D-Ala, alkaline pH.
[b] MTH from L-Ala, chloroform.
[c] MTC-D-Ala, alkaline pH.
[d] MTH from D-Ala, chloroform.
[a'] MTC-D-Ala-L-Ala, alkaline pH.
[b'] MTH from D-Ala, chloroform.
[c'] MTC-L-Ala, alkaline pH.
[d'] MTH from L-Ala, chloroform.

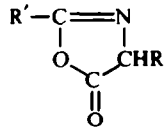
In fact (Table 1 and Fig 1), the 3-methyl-2-thiohydantoins (MTH) show a Cotton effect at 300–315 mμ in water or in methanol, which is positive for the L-series and negative for the D-series. The location of the band in a region of low absorption[13] and its shift from 308 to 315 mμ on changing the solvent from methanol to dioxan suggests that it is associated with an $n \rightarrow \pi^*$ transition within the chromophore.

The position and the dichroic intensities of these Cotton effects are merely indicative, since completion of the reactions and extent of racemization have not been assessed.

The method has been extended to a couple of diastereomeric dipeptides containing alternate L- and D-alanine residues in order to ascertain if it could be employed to assign configurations of sequences of amino acid residues in natural compounds. In both cases the methylthiohydantoins (I; R = Me), extracted with chloroform from the acidic aqueous solutions, exhibited CD Cotton effects centred at about 320 mμ of alternate signs (Table 2), corresponding to those expected on the basis of the data in Table 1. It must be emphasized that the Cotton effect under investigation does not exhibit inversion of sign on changing the solvent from water (or methanol) to chloroform (or dioxan), as reported for other thiocarbamyl-containing compounds.[17] Moreover, the sensitivity of the method is satisfactory, since it allows the use of about 1 mg/ml concentrations if the starting material has a molecular weight of 5000. This technique offers the additional possibility of investigating the problem of racemization during the Edman degradation, through the study of the optical rotatory properties of 4-thiazolinones (III; R′ = alkyl(aryl)—NH—)[18] and thiohydantoins (I); compounds (III) can be considered sulfur analogs of the azlactones (IV), which are known to be intermediates in the racemization in peptide synthesis.[19]

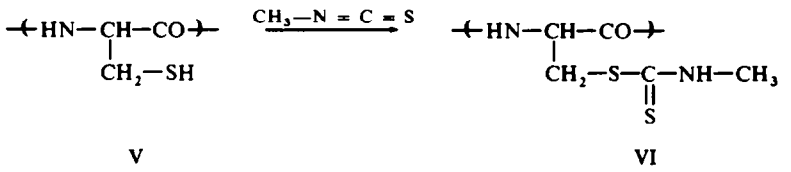


III



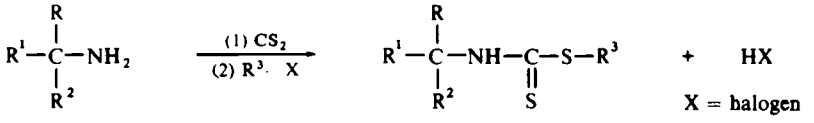
IV

Reactions of methylisothiocyanate with sulfhydryl groups in amino acid derivatives and in peptides. The reaction between methylisothiocyanate and the sulfhydryl group of cysteine residues (V) is known to give S-methylthiocarbamoyl derivatives (VI),[20] which contain a chromophorically substituted thiol function.



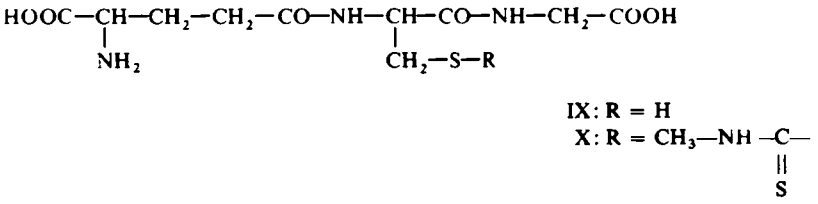
V VI

The dithiocarbamate chromophore has been extensively investigated by UV,[10a, 11, 21] ORD and CD methods.[10d, 11, 22–24] However, the compounds which have been examined were obtained by the reaction of amino groups in optically active compounds with carbon disulfide and alkyl halides (VII → VIII).



VII VIII

The —NH—C(=S)—S— moiety exhibits in water a weak UV absorption band at about 325 mμ, along with bands of greater complexity in the further UV at about 270 and 250 mμ with log ≃ 4. When the group is in an asymmetrical environment these transitions have optical activity, as demonstrated in Fig 3, which shows the CD spectra of glutathione (IX) and S-methylthiocarbamoyl-glutathione (X) in the 210–350 mμ region.



IX: R = H
X: R = CH_3—NH—C(=S)—

The three dichroic Cotton effects at 318, 267 and 246 mμ correspond to a shoulder at about 325 mμ (log ε = 1·7) and two maxima at 265 mμ (log ε = 3·89) and at 247 mμ (log ε = 3·88), respectively, in the UV absorption spectrum of the modified tripeptide. The pH of the solutions were kept at 4 to avoid glutathione oxidation,[25] and reaction of the α-NH_2 group with the isothiocyanate.[26]

Since the 325 mμ absorption, which most likely involves a promotion of a non-bonding electron of the S atom within the >C=S group to an anti-bonding orbital, exhibits low extinction, being associated with an electrically-forbidden and magnetically-allowed transition, and since it occurs in a spectral range which is transparent in a variety of natural compounds, we undertook to examine in details the CD of S-methylthiocarbamoyl-cysteine derivatives in the 310–390 mμ region.

Fig 4 shows the near UV dichroic curves of the adducts of methylisothiocyanate with L-cysteine and its methyl ester at pH 5·5, with glutathione (which contains an L-cysteine residue) at pH 4, and with N-acetyl-L-cysteine at pH 5·5. Two Cotton effects are apparent, the former negative and centred at 360–370 mμ and the latter, 10–50 times more intense, positive and centred at about 320 mμ, except in S-methylthiocarbamoyl-L-cysteine methyl ester where the long-wavelength CD band is totally

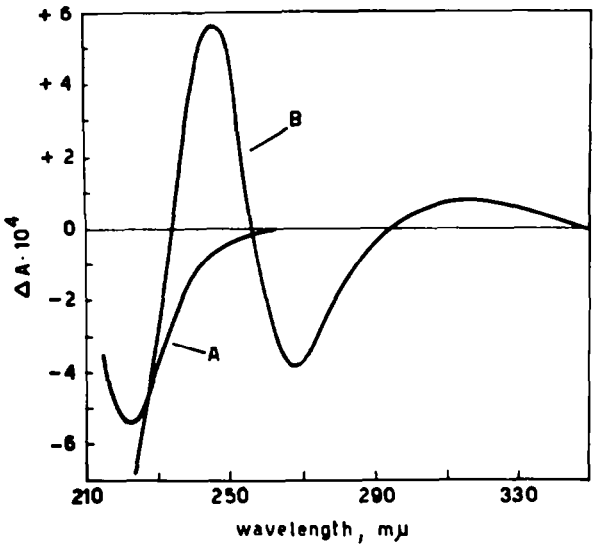


FIG 3. CD curves: A, glutathione, and B, S-methyl-thiocarbamoyl-glutathione at pH 4; tripeptide concentrations, 10^{-2} M; excess of methylisothiocyanate; 0·5 mm cell; temperature 23 ± 1°C.

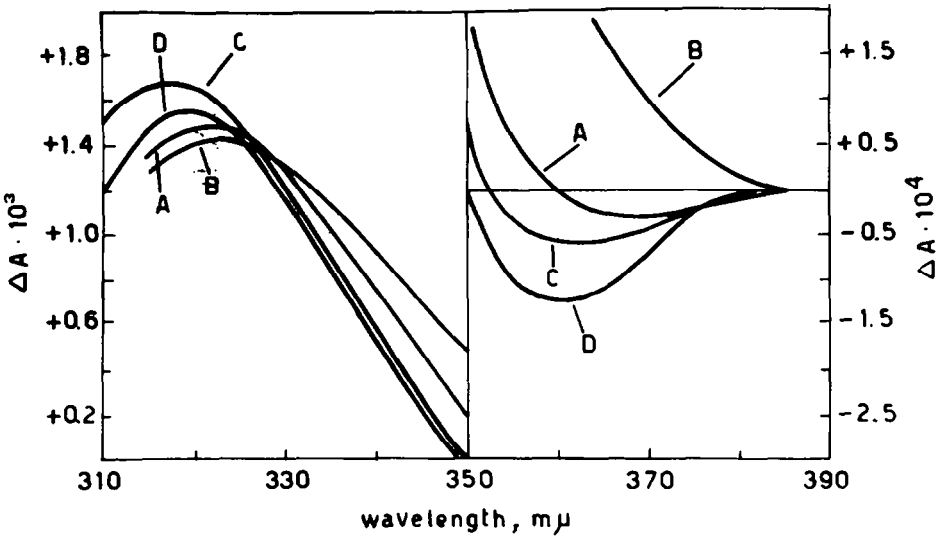


FIG 4. CD curves: A, S-methylthiocarbamoyl-L-cysteine at pH 5·5; B, S-methylthiocarbamoyl-L-cysteine methyl ester at pH 5·5; C, S-methylthiocarbamoyl-glutathione at pH 4; D, N-α-acetyl-S-methylthiocarbamoyl-L-cisteine at pH 5·5. Amino acid or peptide concentrations, 10^{-1}M; excess of methylisothiocyanate; 1 mm. cell; one hour (overnight for glutathione) at 23 ± 1°C.

absent. Moreover, if N-acetyl-S-methylthiocarbamoyl-L-cysteine is examined in dioxan solution only one Cotton effect, negative and centred at 337 mμ, is observed in this spectral region. These results extend to dithiocarbamates from sulfhydryl groups and isothiocyanates the dependence of the sign of the $n \rightarrow \pi^*$ Cotton effect

on the nature of the solvent, which has already been shown in other thiocarbonyl compounds.[17]

The double-humped CD curves reported in the literature[17, 27] have been tentatively explained as associated with $n \rightarrow \pi^*$ transitions of the thiocarbonyl chromophore of different solvated species and/or different rotamers. An equilibrium involving unsolvated and solvated forms might reasonably account for the appearance of two oppositely signed CD absorptions provided the two species have opposite Cotton effects. In addition, it has been suggested in an analogous case[28] that the short-wavelength CD band is that of the solvated species, and the long-wavelength band that of the unsolvated form, in agreement with the commonly accepted view that electrostatic and/or H-bonding effects arising from solvation by polar molecules almost invariably result in a blue shift of $n \rightarrow \pi^*$ transitions. A further control on the sign of the Cotton effect might be given by the spatial relationship between the thiocarbonyl chromophore and the various substituents. In the chromophoric derivative reported in this work the number of possible spatial arrangements is large, since the free rotation that exists around all single bonds allows for a number of conformational isomers. The dichroic spectra of such compounds would be a composite of the individual contributions of all the rotamers present. At this stage of our research, the distinction between one of these or other possibilities is not yet feasible.

The sign of the 320 mμ Cotton effect in water can be used to determine the configuration of the asymmetric C atom of cysteine residues, being positive for the L-amino acid (Fig 4) and obviously negative for its enantiomer. A recent fascinating aspect of stereospecificity-bioluminiscence relationships, represented by the work of McElroy *et al*,[29] illustrates the major role of cysteine configuration in the natural products field. In this and similar senses, D-cysteine may be present in other natural compounds and involved in special roles.

Cysteines have frequently been isolated from various animal sources, but the reports seldom specify their optical configurations. The above described method can rapidly supply this information. Moreover, the extension of the study of the optical rotatory properties of dithiocarbamate groups as thiol derivatives to the determination of the configuration of optically active mercaptans[30] and α-SH-acids,[31] and to conformational problems in the areas of thiocarbohydrates[32] and cysteine-containing polypeptides[33] appears particularly attractive.

EXPERIMENTAL

Distilled water, and spectrograde MeOH and dioxan (Merck) were used for the CD measurements. Amino acids and methylisothiocyanate were purchased from Fluka, AG, and peptides from Cyclo Chem. Corp.

UV measurements have been carried out on a Cary 15 recording spectrophotometer. CD spectra were obtained from a Roussel-Jouan Dichrographe-185 CD model. Data are given in terms of dichroic optical density, $A_L - A_R$, or circular dichroic absorption, $\varepsilon_L - \varepsilon_R$, according to the equation:

$$A_L - A_R = \frac{\Delta\varepsilon \cdot c \cdot d}{M}$$

where c is the concentration in grams per liter, d is the cell path in centimeters, and M is the mol wt.

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