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# Recognition of enantiomers by Chirasil-Val and oligopeptide analogues as studied by gas-phase calorimetry and $^1H$ NMR spectroscopy in solution

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## ABSTRACT

**A series of oligopeptides, L-Val$_n$-NH$^t$Bu (n = 1–4), were linked to poly($\beta$-methyl)siloxy-$\alpha$-methylpropanoic acid copolymer; the resulting chirally modified polydimethylsiloxanes were used for the resolution of enantiomers by gas chromatography. Chiral recognition proved most effective for $n$ = 1 (known as Chirasil-Val), as judged from the resolution factors ($\alpha$) and the thermodynamic parameters $\Delta\Delta H, \Delta\Delta S$ and $\chi = \Delta\Delta H/\Delta H'$, where $AH' = \Delta H_{Chir} - \Delta H_{SE30}$. Likewise, $^1H$ NMR spectroscopy in carbon tetrachloride solution revealed a maximum chemical shift non-equivalence of the amide N-H signal of racemic N-TFA-amino acid methyl esters on addition of the chiral polymer, for $n$ = 1. From circular dichroism spectroscopy of L-Val$_n$-NH$^t$Bu (n = 2, 3, 4 and 6) and polyoxyethylene-bound pivaloyl-L-Val$_n$-Gly-NH-POE$_{3000}$ ($n$ = 2–8), it is concluded that the peptide moiety of the stationary phase mostly adopts the unfavourable random coil conformation, whereas the P-sheet structure was only partially found and only for n ≥ 6.**

## INTRODUCTION

The separation of enantiomers by gas chromatography on a chiral stationary phase is one of the challenges of contemporary analytical chemistry. In particular, three types of chiral phases have been introduced to this end: amide phases [1–5], metal complexes [6,7] and modified cyclodextrins [8–10]; among these, essentially the first two bear the potential of peak reversal on an enantiomeric pair of the stationary phase [6], a useful aid to the unambiguous determination of high enantiomeric purities [11–15].

Thermodynamic analysis of retention data [16–21], in addition to the measurement of the chemical shift non-equivalence of enantiomers in $^1H$ NMR spectroscopy on addition of the chiral stationary phase [22,23], is one of the most common methods for the investigation of the chiral recognition mechanism. In stationary phases derived from oligopeptides, circular dichroism (CD) spectroscopy may shed some further light on the conformation of the chiral selector.

Although, in the early days of chiral peptide phases, several attempts were made to exploit the potential of dipeptides [16] for the resolution of the enantiomers of amino acid derivatives, a systematic investigation of a particular series of oligopeptides seems necessary to clarify the significance of this approach. Here, we describe the preparation of four chiral stationary phases, *i.e.*, **N-poly($\beta$-methyl)siloxy-$\alpha$-methylpropanoyl-L-(valine)$_n$-*tert.*-butylamide** copolymer ($n$ = 1-4, denotated phases I-IV, respectively), of the Chirasil-Val type [2,3], and their interaction with enantiomers of N-trifluoroacetyl amino acid esters.

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## EXPERIMENTAL

### *Materials*

The chemicals were purchased from Merck, Fluka, Aldrich, Janssen Chimica, Bachem, Nova Biochem, Serva, Hüls and Baker. Beads of polystyrene grafted with polyoxyethylene (PS–$POE_{3000}$ TentaGel) were obtained from Rapp Polymere (Tubingen, Germany).

### *Peptide syntheses*

The low-molecular mass oligopeptides were synthesized in the classical way [24], starting from L-valine methyl ester (L-Val-OMe) and *tert.*-butyloxycarbonyl-L-valine (BOC-L-Val-OH), with dicyclohexylcarbodiimide (DCCI) and hydroxybenzotriazole (HOBt) as the coupling reagent. All compounds gave the $^1H$ NMR spectra expected.

HOBt [25], L-Val-OMe [26], BOC-L-Val-OH [27], BOC-$Val_2$-OH [24], and *tert.*-butyloxycarbonyl-L-valine *tert.*-butylamide (BOC-Val-$NH^tBu$) [28] were prepared according to the literature.

### *BOC-$(Val)_2$-$NH^tBu$*

A solution of 0.082 mol of HOBt in 70 ml of tetrahydrofuran and 42.5 ml of a 2 *M* solution of 0.085 mol of DCCI in dichloromethane were added to a solution of 0.082 mol of BOC-L-Val-OH in 75 ml of dichloromethane. The mixture was cooled at 4°C for 30 min. The precipitate of dicyclohexylurea was removed by filtration and the solution was added to a solution of 0.082 mol of H-Val-$NH^tBu$ in 140 ml of dichloromethane. A volume of 18.1 ml (0.164 mol) of N-methylmorpholine was added dropwise within 30 min while cooling in an ice-bath. The mixture was stirred for 1 h at 0°C, then stirred overnight at ambient temperature. The solvent was removed *in vacuo*, the residue was dissolved in ethyl acetate, washed with aqueous solutions of potassium carbonate, citric acid and sodium chloride and dried over anhydrous sodium sulphate. The peptide was recrystallized from toluene, yield 60%, m.p. 186°C.

### *BOC-$(Val)_2$-OMe*

BOC-$(Val)_2$-OMe was synthesized in an analogous manner from 0.151 mol of BOC-L-Val-OH and 0.151 mol of H-Val-OMe, yield 50%, m.p. 164°C.

### *BOC-$(Val)_3$-$NH^tBu$*

A solution of 0.016 mol of HOBt in 10 ml of tetrahydrofuran and 9 ml of a 2 *M* solution of 0.018 mol of DCCI in dichloromethane were added to a solution of 0.016 mol of BOC-L-Val-OH in 15 ml of dichloromethane. The mixture was cooled at 4°C for 30 min. The precipitate of dicyclohexylurea was removed by filtration and the solution was added to a solution of 0.016 mol of H-$(Val)_2$-$NH^tBu$ in 10 ml of dimethylformamide. A volume of 3.6 ml (0.032 mol) of N-methylmorpholine was added dropwise within 30 min while cooling in an ice-bath. The mixture was stirred for 1 h at 0°C, then stirred overnight at ambient temperature. The solvent was removed *in vacuo*, ethyl acetate was added and the precipitate was isolated by centrifugation. The peptide was recrystallized from toluene, yield 74%.

### *BOC-$(Val)_4$-$NH^tBu$*

A solution of 0.051 mol of HOBt in 50 ml of dimethylformamide and 27.5 ml of a 2 *M* solution of 0.055 mol of DCCI in dichloromethane were added to a solution of 0.051 mol of BOC-L-$(Val)_2$-OH in 50 ml of dichloromethane. The mixture was cooled at 4°C for 30 min. The precipitate of dicyclohexylurea was removed by filtration and the solution was added to a solution of 0.051 mol of H-$(Val)_2$-$NH^tBu$ in 80 ml of dichloromethane. A volume of 11.2 ml (0.102 mol) of N-methylmorpholine was added dropwise within 30 min while cooling in an ice-bath. The mixture was stirred for 1 h at 0°C, then stirred overnight at ambient temperature. The solvent was removed *in vacuo*. The product was recrystallized from a mixture of methanol and dimethylformamide, yield 73%.

### *BOC-$(Val)_6$-$NH^tBu$*

A solution of 0.020 mol of HOBt in 60 ml of tetrahydrofuran and 11.5 ml of a 2 *M* solution of 0.023 mol of DCCI in dichloromethane were added to a solution of 0.020 mol of BOC-

L-$(Val)_2$-OH in 100 ml of dimethylformamide. The mixture was cooled at 4°C for 30 min. The precipitate of dicyclohexylurea was removed by filtration and the solution was added to a solution of 0.020 mol of H-$(Val)_4$-$NH^tBu$ in 150 ml of dimethylformamide. A volume of 4.4 ml (0.040 mol) of N-methylmorpholine was added dropwise within 30 min while cooling in an ice-bath. The mixture was stirred for 1 h at 0°C, then stirred overnight at ambient temperature. The solvent was removed *in vucuo. The* product was washed with methanol, yield 75%.

### *Cleavage of the BOC protecting group*

*The* BOC group was removed by treatment with 1.2 *M* hydrochloric acid in acetic acid for 2 h at 20°C. The solvent was evaporated *in vucuo.* The residue was dissolved in water, washed with ethyl acetate and the water was removed *in vucuo.* The final product was dried in a desiccator over potassium hydroxide.

The following compounds were prepared according to this procedure.

*H-Val-NH'Bu · HCl.* This was prepared from 0.11 mol of BOC-Val-NH'Bu and 250 ml of 1.2 *M* hydrochloric acid in acetic acid, yield 89%, m.p. 201°C. Elemental analysis: found, C 51.50, H 10.25, N 12.72, Cl 16.57%; calculated for $C_9H_{21}N_2OCl$, C 51.79, H 10.14, N 13.42, Cl 16.98%.

*H-$(Val)_2$-$NH^tBu$ · HCl.* This was prepared from 0.062 mol of BOC-$(Val)_2$-$NH^tBu$ and 150 ml of 1.2 *M* hydrochloric acid in acetic acid, yield 93%, m.p. 161°C. Elemental analysis: found, C 53.14, H 10.11, N 12.61, Cl 11.07%; calculated for $C_{14}H_{30}N_3O_2Cl$, C 54.62, H 9.82, N 13.65, Cl 11.52%.

*H-$(Val)_3$-$NH^tBu$ . HCl.* This was prepared from 0.0116 mol of BOC-$(Val)_2$-$NH^tBu$ and 40 ml of 1.2 *M* hydrochloric acid in acetic acid, yield 76%, m.p. >280°C.

*H-$(Val)_4$-$NH^tBu$ · HCl.* This was prepared from 0.037 mol of BOC-$(Val)_2$-$NH^tBu$ and 150 ml of 1.2 *M* hydrochloric acid in acetic acid, yield 98%, m.p. >280°C. Elemental analysis: found, C 56.89, H 10.41, N 14.03, Cl 7.20%; calculated for $C_{24}H_{48}N_5O_4Cl$, C 56.95, H 9.56, N 13.84, Cl 7.00%. Hydrolysis (6 *M* HCl, for 24 h at 110°C) and derivatization showed 1.09% D enantiomer by GC [2].

### *Polyoxyethylene peptides (POE peptides)*

*The* POE peptides were synthesized by the solid-phase method via the 9-fluorenylmethoxycarbonyl (Fmoc) strategy, with diisopropylcarbodiimide (DIC) and HOBt as the coupling reagent. Oligopeptides larger than tetrapeptides were prepared by means of a MilliGen 9050 PepSynthesizer automated peptide synthesizer equipped with an NEC APC IV computer. TentaGel was used as a solid-phase resin. The protecting group was removed with piperidine. The peptides were cleaved from the resin with trifluoroacetic acid (TFA) containing 5% of thioanisole.

### *Derivatization of the amino acids prior to GC analysis*

A 1-mg amount of the amino acid was placed in a l-ml Reacti-Vial (Macherey-Nagel). After addition of 100 μl of a solution of hydrochloric acid in n-propanol [prepared by reaction of acetyl chloride and n-propanol (1:5, v/v) for 30 min at 0°C], the vial was closed tightly and the reaction was performed for 30 min at 110°C. The solvent was removed in a stream of nitrogen and 100 μl of trifluoroacetic anhydride (TFA anhydride) were added. The mixture was kept for 10 min at 110°C. After cooling to ambient temperature, the solvent was removed in a stream of nitrogen and the residue was dissolved in 100 μl of dichloromethane.

### *Stationary phases*

Following the general methodology [28], 2 g of poly-(β-methylsiloxy-α-methylpropanoic acid) copolymer was dissolved in 20 ml of dimethylformamide and 20 ml of dichloromethane and a twofold excess of carbonyldiimidazole (CDI) was added. After stirring for 1 h, a twofold excess of the peptide was added and stirring was continued for a further 3 days at ambient temperature. The mixture was diluted with 150 ml of dichloromethane, washed with an aqueous solution of acetic acid (10%) and concentrated *in vacuo*. The residue was dissolved in 150 ml of pentane and insoluble components were removed by filtration. The solution was washed with aqueous solutions of potassium carbonate, acetic acid and sodium chloride, dried over anhydrous sodium sulphate and concentrated *in vucuo.*

### *Gas chromatography*

Glass capillary columns were treated with colloidal $SiO_2$ before coating with a 0.3% solution of the stationary phase in pentane–dichloromethane (9:1). Measurements were performed on a Carlo. Erba Fractovap Model 2101 gas chromatograph equipped with a flame ionization detector and a Hewlett-Packard Model 3390 A electronic integrator. The carrier gas was hydrogen.

Circular dichroism (CD) spectra were recorded on a Jasco Model 5720 A spectropolarimeter, $\lambda$ = 250-180 nm, solvent trifluoroethanol, $d$ = 0.02 cm.

Proton nuclear magnetic resonance ('H NMR) spectra were recorded on a Bruker AC 250 instrument at 250 MHz, with $C^2HCl_3$ as solvent and $C_6{}^2H_6$ as deuterium lock. A 10.4-$\mu$mol amount of the phase (molarity referring to the peptide side-chain) was dissolved in 900 $\mu$l of tetrachloromethane in an NMR funnel, giving an 11.6 m$M$ solution. For the spectrum of the mixture, a solution of 2.51 mg (10.4 $\mu$mol) of N-TFA-leucine methyl ester in 500 $\mu$l of tetrachloromethane was added, giving a solution 7.4 m$M$ in both components.

## RESULTS AND DISCUSSION

The peptides were obtained as described under Experimental (e.g., see the synthetic scheme in Fig. 1 for phases II and III). BOC-$(Val)_4$-$NH^tBu$ was prepared from BOC-$(Val)_2$-OH and H-$(Val)_2$-$NH^tBu$ in an analogous manner. In all solvents, the solubility decreases with increasing number of valine units. BOC-$(Val)_3$-$NH^tBu$ appeared insoluble in dichloromethane yet soluble in methanol. After cleavage of the BOC group, the peptide could be dissolved in dichloromethane. The tetrapeptide, with and without a protecting group, was found to be almost insoluble in all common solvents.

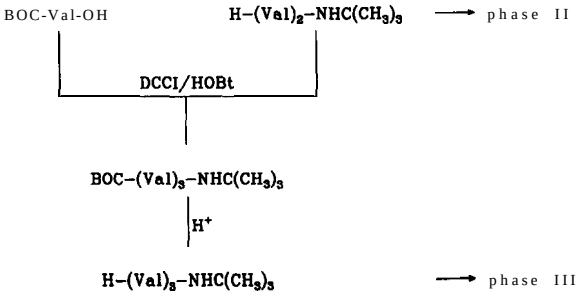


**Fig. 1. Synthesis scheme for phases II and III.**

The synthesis of poly($\beta$-methylsiloxy-$\alpha$-methylpropionic acid) copolymer and the coupling to the peptide moiety was carried out in the usual way [28]. The coupling reactions with the di-, tri and tetrapeptides were partly incomplete in comparison with H-Val-NH'Bu, owing to steric hindrance. Although the reaction was carried out several times under various conditions, the coupling yield did not exceed 41%.

The hexapeptide synthesis was hampered by the very low solubility of the peptide, and the higher homologues, in our hands, did not give any satisfactory results. In order to overcome the problem of coupling the peptide moiety to the polysiloxane, we tried to synthesize the peptide step by step, starting from poly($\beta$-methyl)siloxy-a-methylpropanoic acid Val-'Bu ester copolymer. However, this approach failed owing to the limited stability of the polysiloxane backbone. Moreover, the contemporary methods for peptide synthesis usually do not start from the amino end, but from the carboxylic end.

In another approach, polyoxyethyleneoxide (POE) was introduced to avoid the problem of insolubility. The POE-linked peptides were synthesized via the solid-phase method. Although the POE peptides are not useful for enantiomer resolution by GC, the solubility effect of the POE chain is beneficial for studies of the peptide conformation by CD spectroscopy.

The resolution of an enantiomeric pair $R$ and $S$ of a volatile solute, e.g., an amino acid derivative (see Fig. 2), on a chiral stationary phase (see Fig. 3) is based on the fact that the partition coefficients $K_{(R)}$ and $K_{(S)}$ of the enantiomers of the solute between liquid and gas phases is different. In practice, the GC investigations were limited to phases containing up to four valine units (see Table I). As shown in Fig. 4, the resolution factors ($\alpha$) for phase II are particularly small at 90°C. At lower temperature (70°C), the a-value is slightly increased. The

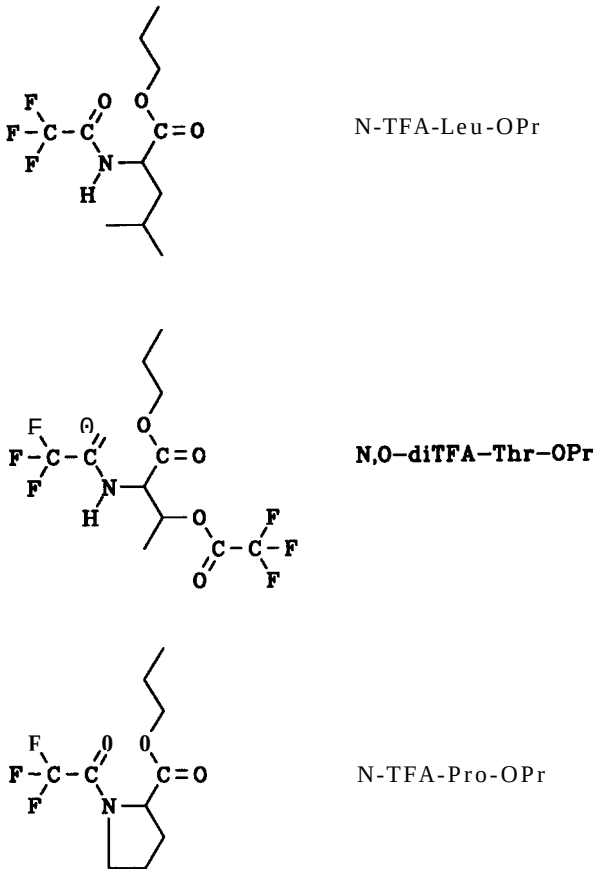


Fig. 2. **Solutes.**

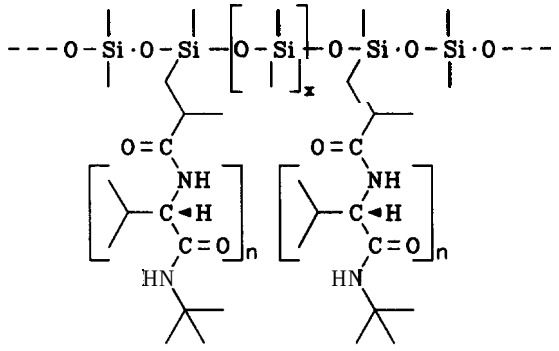

**Fig. 3. Stationary phases I-IV (*n* = 1-4, respectively).**

phases II and III show similar a-values, whereas phase IV exhibits a significantly enhanced capability for enantiomeric separation; here the resolution factors are close to those of phase I,

**TABLE I**

**NUMBER OF THEORETICAL PLATES PER METRE ($n_{eff}$/m) AND NET CAPACITY FACTORS (k'), DETERMINED WITH PENTADECANE AT 100°C**

**Duran glass capillary columns, coated with N-poly(β-methyl)siloxy - a - methylpropanoyl- L - (valine), - *tert.* - butyl - amide copolymer (*n* = 1-4, phases I-IV, respectively; phase Ia, 25% coupling), typically 20 m x 0.3 mm I.D. (phase I, 18 m; phase Ia, 27 m); flame ionization detection; carrier gas, 0.35 bar $H_2$.**

| Stationary phase | $n_{eff}$/m | k' |
|---|---|---|
| Phase I | 1843 | 6.46 |
| Phase Ia | 3618 | 9.29 |
| Phase II | 3175 | 11.21 |
| Phase III | 2089 | 5.82 |
| Phase IV | 1829 | 7.51 |

better known as Chirasil-Val. The results are summarized in Table II. In order to shed some more light on the resolution mechanism, gas-phase calorimetry was performed, as follows.

As discussed previously [19,21], the difference in the free enthalpy of interaction of the enantiomers with respect to the chiral solvent can be derived [21] from retention data (eqns. 1 and 2):

$$-\Delta\Delta G^0 = -\Delta\Delta H^0 + T\Delta\Delta S^0 = RT \ln\left[\frac{K_{(R)}}{K_{(S)}}\right] = RT \ln \alpha \quad (1)$$

where $-\Delta\Delta G^0$ is the enantiomeric difference in free enthalpy for transition of 1 mol of solute from the gas to the liquid phase, $R$ is the universal gas constant and $T$ is the temperature (K);

$$a = \frac{t'_{R(R)}}{t'_{R(S)}} \quad [\text{for } t'_{R(R)} > t'_{R(S)}] \quad (2)$$

where a is the resolution factor, $t'_{R(R)}$ is the net retention time of the $R$ enantiomer and $t'_{R(S)}$ is the net retention time of the $S$ enantiomer.

The determination of the resolution factor ($\alpha$) for at least three different temperatures ($T$) allows one to calculate the difference in the enthalpy ($\Delta\Delta H^0$) and entropy ($\Delta\Delta S^0$) of interaction of the enantiomers with the stationary phase, according to the equation

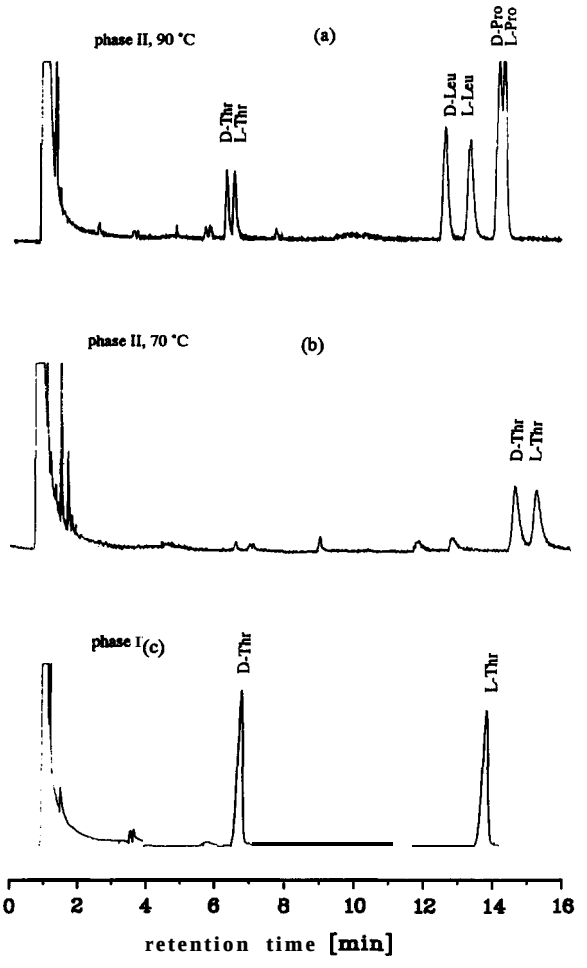


**Fig. 4. Gas chromatograms of N,O-TFA n-propyl ester of (a) D,L-Thr, D,L-Leu, D,L-Pro on phase II at WC, (b) D,L-Thr on phase II at 70°C and (c) D,L-Thr on phase IV at 70°C. Carrier gas, 0.35 bar $H_2$; flame ionization detection; Duran glass capillaries, as described in Table I.**

$$\ln \alpha = \frac{-\Delta\Delta H^0}{R} \cdot \frac{1}{T} + \frac{\Delta\Delta S^0}{R} \qquad (3)$$

A comparison of the enthalpies measured on the chiral phases with those measured on the unsubstituted dimethylpolysiloxane SE-30 as a standard yields the term -AH' that reflects the increase in interaction due to the chiral groups of the polymeric stationary phase:

$$\text{-AH'} = -\Delta H^0_{\text{Chir}} + \Delta H^0_{\text{SE-30}} \qquad (4)$$

where -AH' is the specific interaction, $-\Delta H^0_{\text{Chir}}$ is the mean enthalpy from -AH&, and -AH&, on a chiral phase and $-\Delta H^0_{\text{SE-30}}$ is the enthalpy on the SE-30 phase. This term, previously termed a "specific interaction", is somehow related to the chiral discrimination observed, and one may define a chiral recognition factor $\chi$ by normalization of the $-\Delta\Delta H$ values with respect to the specific interaction -AH' [21]:

$$\chi = \text{-AAHI-AH'} \qquad (5)$$

All thermodynamic parameters were computed using a laboratory-written FORTRAN program. As compiled in Table III, the resolution factors $\alpha$ on the dipeptide phase (phase II, $n = 2$) and the tripeptide phase ($n = 3$, phase III) are in the same range and significantly lower than on Chirasil-Val ($n = 1$). Phase IV ($n = 4$) yields $\alpha$-values almost as large as those measured on Chirasil-Val, although the coupling reaction for phase IV is rather incomplete, and therefore the loading with chiral groups is much lower than that of Chirasil-Val. One may expect from this finding that a perfectly coupled phase IV would be superior to Chirasil-Val, although at present there is no method available to reach this goal. A graphical comparison of the a-values for 100°C, with a view to greater clarity, is presented in Fig. 5.

In Fig. 6, the terms $-\Delta\Delta H$, -AH' and $\chi$ are presented graphically in a bar diagram. A comparison of the influence of the amino acid residue on the enthalpic properties reveals a common pattern that is valid for all four phases investigated. The magnitudes of $-\Delta\Delta H$ and -AH' are slightly higher for N-TFA-leucine n-propyl ester than for N,O-TFA-threonine n-propyl ester, whereas those for N-TFA-proline n-propyl ester are significantly decreased.

The impact of the number of valine units (n) in the chiral phase, likewise, shows a uniform picture. For $n = 2$ and 3, in general, the -AH' values are smaller than for $n = 1$ (Chirasil-Val), but fairly similar. In both instances, there is a strong decrease in $-\Delta\Delta H$ yet only a slight loss of -AH'; hence the $\chi$ values end up relatively small. Although the di- and tripeptide, like Chirasil-Val [4], undergo intramolecular interaction to form cyclic conformations, leaving the chiral groups saturated, and hence less prone to

TABLE II

NET CAPACITY FACTORS ($k'$) AND DEAD TIMES ($t_M$) FOR N,O-TFA-AMINO ACID PROPYL ESTERS ON PHASES I-IV

For chromatographic conditions, see Table I.

| Stationary phase | Parameter | 70°C | 80°C | 90°C | 100°C | 110°C |
|---|---|---|---|---|---|---|
| Phase I | $t_M$ (min) | | 0.67 | 0.67 | 0.68 | 0.69 |
| | $k'$ | | | | | |
| | D-Thr | | 9.36 | 5.42 | 3.19 | 1.97 |
| | L-Thr | | 11.58 | 6.51 | 3.72 | 2.25 |
| | D-Leu | | 20.66 | 11.51 | 6.51 | 3.91 |
| | L-Leu | | 29.30 | 15.63 | 8.49 | 4.93 |
| | D-Pro | | 16.21 | 9.94 | 6.15 | 3.99 |
| | L-Pro | | 16.78 | 10.27 | 6.34 | 4.10 |
| Phase Ia | $t_M$ (min) | 1.44 | 1.46 | 1.48 | 1.49 | |
| | $k'$ | | | | | |
| | D-Thr | 26.42 | 13.38 | 7.15 | 4.02 | |
| | L-Thr | 29.20 | 14.58 | 7.71 | 4.28 | |
| | D-Leu | 59.09 | 28.78 | 14.84 | 8.07 | |
| | L-Leu | 70.36 | 33.61 | 16.98 | 9.05 | |
| | D-Pro | 36.30 | 20.33 | 11.86 | 7.18 | |
| | L-Pro | 36.96 | 20.67 | 12.05 | 7.29 | |
| Phase II | $t_M$ (min) | 0.98 | 0.99 | 0.92 | 1.04 | |
| | $k'$ | | | | | |
| | D-Thr | 11.88 | 6.45 | 4.16 | 2.22 | |
| | L-Thr | 12.46 | 6.71 | 4.31 | 2.29 | |
| | D-Leu | 27.51 | 14.40 | 8.97 | 4.73 | |
| | L-Leu | 29.29 | 15.23 | 9.43 | 4.96 | |
| | D-Pro | 32.13 | 17.73 | 11.45 | 6.22 | |
| | L-Pro | 32.13 | 17.91 | 11.55 | 6.28 | |
| Phase III | $t_M$ (min) | 0.93 | 0.94 | 0.95 | 0.96 | |
| | $k'$ | | | | | |
| | D-Thr | 7.54 | 4.13 | 2.37 | 1.49 | |
| | L-Thr | 7.87 | 4.28 | 2.43 | 1.49 | |
| | D-Leu | 15.25 | 8.16 | 4.59 | 2.79 | |
| | L-Leu | 16.84 | 8.84 | 4.88 | 2.95 | |
| | D-Pro | 19.77 | 11.21 | 6.61 | 4.19 | |
| | L-Pro | 20.28 | 11.47 | 6.75 | 4.26 | |
| Phase IV | $t_M$ (min) | 0.91 | 0.93 | 0.94 | 0.96 | 0.98 |
| | $k'$ | | | | | |
| | D-Thr | 11.56 | 6.17 | 3.41 | 2.02 | 1.23 |
| | L-Thr | 14.31 | 7.35 | 3.94 | 2.27 | 1.36 |
| | D-Leu | 27.60 | 13.74 | 7.33 | 4.16 | 2.45 |
| | L-Leu | 41.36 | 19.41 | 9.79 | 5.29 | 2.98 |
| | D-Pro | 23.99 | 13.34 | 7.84 | 4.67 | 2.90 |
| | L-Pro | 24.70 | 13.75 | 8.06 | 4.78 | 2.96 |

interact with the solute, there is still a considerable solute-solvent interaction left; however, this is not very enantioselective. For a tentative explanation, we assume the peptide moiety to occur in a variety of conformations, giving rise to multiple selector-selectand complexes. Moreover, the free carboxylic functions still present in the phase, as we know from incompletely cou-

TABLE III

THERMODYNAMIC PARAMETERS OF INTERACTION OF THE ENANTIOMERS OF N,O-TFA-AMINO ACID PROPYL ESTERS OF THR, LEU, AND PRO ON CHIRASIL-VAL HOMOLOGUES WITH PHASES I-IV, AS CALCULATED FROM TABLE II

| Stationary phase | Parameter | Thr | Leu | Pro |
|---|---|---|---|---|
| Phase I ($n$ = 1; Chirasil-Val) | $-\Delta\Delta H$ (kJ/mol) | 3.19 ± 0.06[a] | 4.57 ± 0.09 | 0.20 ± 0.01 |
| | $\Delta\Delta S$ [J/(K · mol)] | -7.3 ± 0.2 | −10.0 ± 0.2 | -0.31 ± 0.01 |
| | $T_{iso}$ (°C) | 166 ± 2 | 182 ± 3 | 408 ± 10 |
| | $-\Delta H^0_{Chir}$ (kJ/mol) | 60.99 ± 0.36 | 65.68 ± 0.48 | 53.77 ± 0.31 |
| | -AH' (kJ/mol) | 5.0 ± 1.0 | 10.9 ± 0.9 | -2.6 ± 1.6 |
| | X | 0.63 ± 0.12 | 0.42 ± 0.04 | 0.08" 0.05 |
| | $\alpha$ | | | |
| | 25°C[b] | 1.512 ± 0.010 | 1.889 ± 0.016 | 1.049 ± 0.001 |
| | 100°C' | 1.168 ± 0.002 | 1.305 ± 0.003 | 1.031 ± 0.001 |
| | 180°C[b] | 0.974[d] ± 0.005 | 1.006 ± 0.007 | 1.019 ± 0.001 |
| Phase II ($n$ = 2) | $-\Delta\Delta H$ (kJ/mol) | 0.61 ± 0.10 | 0.59 ± 0.04 | 0.06 ± 0.02 |
| | $\Delta\Delta S$ [J/(K · mol)] | -1.4-to.3 | -1.2 ± 0.1 | -0.10 ± 0.05 |
| | $T_{iso}$ (°C) | 168 ± 22 | 217 ± 17 | 387 ± 203 |
| | $-\Delta H^0_{Chir}$ (kJ/mol) | 59.38 ± 0.60 | 62.42 ± 0.74 | 56.82 ± 0.09 |
| | -AH' (kJ/mol) | 3.4 ± 1.1 | 7.6 ± 1.1 | 0.5 ± 1.6 |
| | X | 0.18 ± 0.06 | 0.08 ± 0.01 | |
| | $\alpha$ | | | |
| | 25°C[b] | 1.083 ± 0.009 | 1.098 ± 0.004 | 1.014 ± 0.001 |
| | 100°C[c] | 1.031 ± 0.004 | 1.047 ± 0.002 | 1.009 ± 0.001 |
| | 180°C[b] | 0.995[d] ± 0.009 | 1.012 ± 0.004 | 1.005 ± 0.001 |
| Phase III (n = 3) | $-\Delta\Delta H$ (kJ/mol) | 1.10 ± 0.27 | 1.80 ± 0.13 | 0.27 ± 0.01 |
| | $\Delta\Delta S$ [J/(K . mol)] | -2.8 ± 0.8 | -4.4 ± 0.4 | -0.6 ± 0.1 |
| | $T_{iso}$ (°C) | 114 ± 17 | 134 ± 7 | 196 ± 9 |
| | $-\Delta H^0_{Chir}$ (kJ/mol) | 59.30 ± 0.88 | 62.02 ± 0.88 | 56.22 ± 0.68 |
| | -AH' (kJ/mol) | 3.4 ± 1.3 | 7.2 ± 1.2 | -0.12 ± 1.8 |
| | X | 0.33 ± 0.15 | 0.25 ± 0.05 | |
| | $\alpha$ | | | |
| | 25°C[b] | 1.107 ± 0.028 | 1.121 ± 0.015 | 1.040 ± 0.001 |
| | 100°C[c] | 1.013 ± 0.011 | 1.049 ± 0.006 | 1.018 ± 0.001 |
| | 180°C[b] | 0.952[d] ± 0.024 | 0.947[d] ± 0.012 | 1.003 ± 0.001 |
| Phase IV ($n$ = 4) | $-\Delta\Delta H$ (kJ/mol) | 3.53 ± 0.14 | 5.79 ± 0.09 | 0.11 ± 0.07 |
| | $\Delta\Delta S$[J/(K . mol)] | -8.5 ± 0.4 | -13.5 ± 0.3 | -0.1 ± 0.2 |
| | $T_{iso}$ (°C) | 141 ± 5 | 155 ± 2 | 1252" -469 |
| | $-\Delta H^0_{Chir}$ (kJ/mol) | 62.76 ± 0.61 | 68.87 ± 0.72 | 57.94 ± 0.18 |
| | -AH' (kJ/mol) | 6.8 ± 1.1 | 14.1 ± 1.1 | 1.6 ± 1.6 |
| | X | 0.52" 0.08 | 0.41 ± 0.03 | |
| | a | | | |
| | 25°C[b] | 1.490 ± 0.023 | 2.033 ± 0.018 | 1.036 ± 0.008 |
| | 100°C' | 1.120 ± 0.008 | 1.272 ± 0.004 | 1.027 ± 0.004 |
| | 180°C[b] | 0.916[d] ± 0.013 | 0.915[d] ± 0.008 | 1.021 ± 0.007 |

[a] Standard deviation, calculated according to ref. 29.
[b] Extrapolated data.
[c] Interpolated data.
[d] Beyond $T_{iso}$.

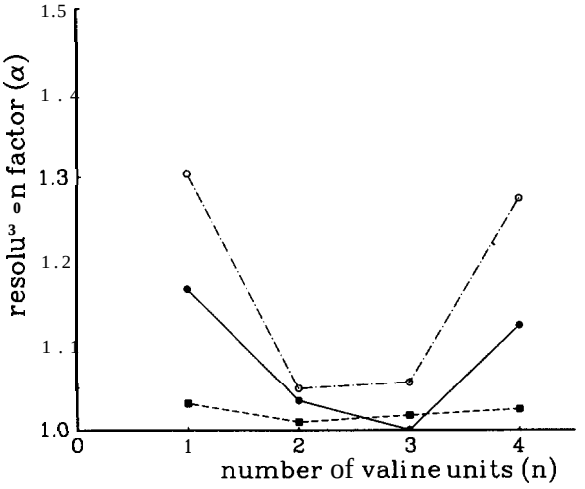


**Fig. 5. Resolution factors ($\alpha$) of the phases I-IV for N,O-TFA n-propyl esters of (●) Thr, (0) Leu and (■) Pro, as determined for 100°C.**

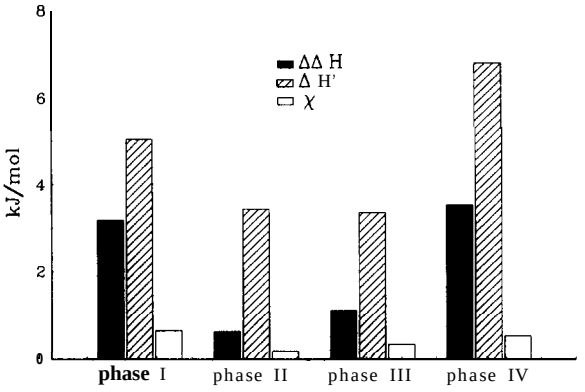


**Fig. 6. Thermodynamic parameters $-\Delta\Delta H$, -AH' and $\chi$ of the polysiloxane phases I-IV for Thr.**

pled batches of Chirasil-Val, exert a detrimental effect on the separation mechanism. Despite this shortcoming, however, for phase IV the -AH' values even exceed those determined for Chirasil-Val. In other words, the increase in the solute-solvent interaction as compared with the reference phase polydimethylsiloxane SE-30 must be significantly higher for $n = 4$ than for $n = 1$. The $-\Delta\Delta H$ values are also increased. Two possible reasons have to be considered. First, the tetrapeptide, owing to its sheer bulk, will be more efficient in masking the residual carboxylic functions. Second, it favours a more extended, partially ordered secondary structure. Both effects may contribute to an enhanced enantioselectivity towards the amino acid derivatives examined.

It has recently been shown [22] that the addition of Chirasil-Val to racemic N-TFA-amino acid methyl esters in carbon tetrachloride solution leads to a downfield shift (AS ) and a splitting ($\Delta\Delta\delta$) of the amide proton of the solute enantiomers in the $^1$H NMR spectrum. This is due to the fact that the originally external enantiotopic nuclei of a given enantiomeric pair become diastereotopic by formation of non-isolable diastereomeric solvates. As the chemical shift of the solute is a measure of the strength of the hydrogen bridges formed to the chiral polymer, whereas the chemical shift non-equivalence approximately reflects the difference in binding constants, such investigations should provide more information about the influence of the peptide chain on the chiral recognition mechanism. In particular, it would be interesting to see whether the pattern observed in the gas-phase calorimetric data was paralleled by similar trends in the NMR spectroscopic measurements.

The NMR spectra of the polysiloxane phases, dissolved in carbon tetrachloride, are displayed in Fig. 7. The initial concentration of each polymer was 11.6 m$M$, referring to the peptide side-chain. After addition of an equimolar amount of N-TFA-leucine methyl ester, giving a 7.4 m$M$ solution in each component, the spectra shown in Fig. 8 were recorded. Spectra of N-TFA-leucine methyl ester at both concentrations (7.4 and 11.6 m$M$) revealed that the self-association of this component is negligible, as judged from the constant chemical shift of the amide proton signal ($\delta$ = 6.41 ppm). On the other hand, there is a significant downfield shift ($\Delta\delta$), and a more or les pronounced difference in the shift ($\Delta\Delta\delta$) of the enantiomers, owing to the presence of the chiral stationary phase (see Table IV). The difference ($\Delta\Delta\delta$) obtained with phase II is much smaller than that with Chirasil-Val, whereas phase III does not cause any signal splitting, and the downfield shift is apparently small in both instances. For phase IV, a tentative assignment leads to A6 and $\Delta\Delta\delta$ values even higher than those observed on addition of Chirasil-Val. All these findings are in good agreement with the trend in the $-\Delta\Delta H$ values determined by gas chromatography, and may find a

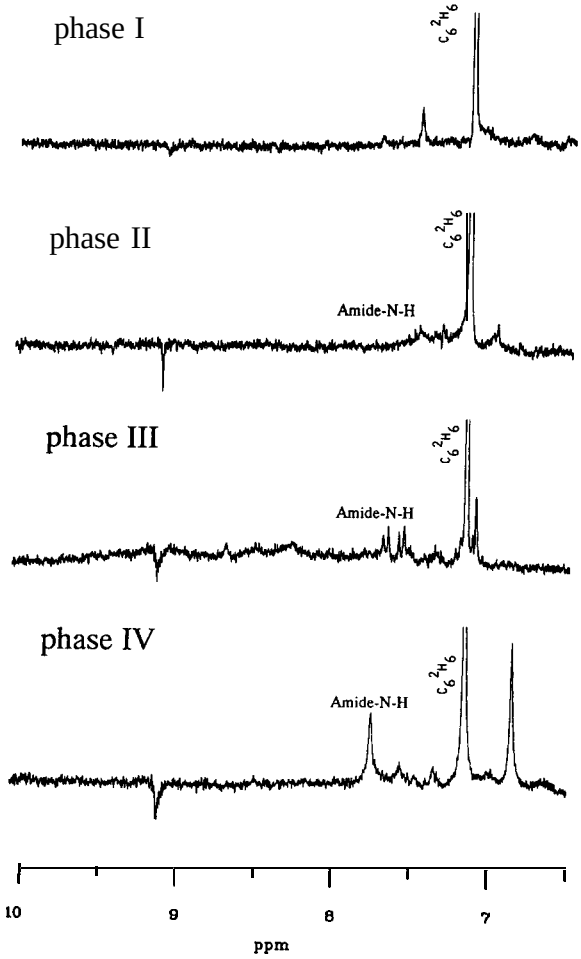


Fig. 7. $^1H$ NMR spectra of the polysiloxane phases I-IV, 11.6 m*M* solution, referring to the peptide side-chain, in carbon tetrachloride; 250 MHz; deuterium lock, $C_6^2H_6$.

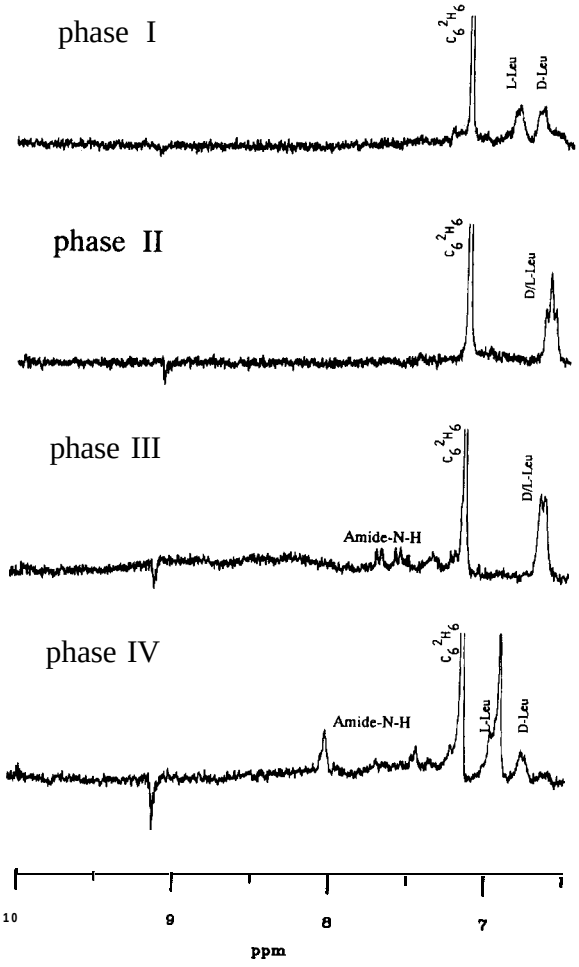


Fig. 8. $^1H$ NMR spectra of phases I-IV after addition of N-TFA-leucine methyl ester, molar ratio of both components 7.4 m*M*. 250 MHz; solvent, carbon tetrachloride; deuterium lock, $C_6^2H_6$.

TABLE IV

AVERAGE DOWNFIELD CHEMICAL SHIFT ($\Delta\delta$) AND DIFFERENCE IN THE CHEMICAL SHIFT ($\Delta\Delta\delta$) OF THE AMIDE PROTON OF ENANTIOMERS OF N-TFA-LEUCINE METHYL ESTER IN $^1H$ NMR SPECTROSCOPY ON ADDITION OF THE CHIRAL POLYSILOXANE, CONCENTRATION 7.4 m*M* IN EACH COMPONENT

Solvent: carbontetrachloride.

| Stationary phase | $\Delta\delta$ (L) (ppm) | $\Delta\delta$ (D) (ppm) | $\Delta\delta$ (ppm) | AM (ppm) | $\Delta\Delta\delta/\Delta\delta$ |
|---|---|---|---|---|---|
| Phase I | 0.42 | 0.28 | 0.35 | 0.14 | 0.40 |
| Phase II | 0.21 | 0.18 | 0.20 | 0.03 | 0.15 |
| Phase III | 0.24 | 0.24 | 0.24 | 0 | 0 |
| Phase IV | 0.53 | 0.33 | 0.43 | 0.20 | 0.47 |

similar explanation. Although it is not surprising that the enantioselectivity decreases from $n = 1$ to 3, further experimental evidence is required in order to understand the favourable properties of the tetrapeptide phase.

To this end, CD measurements were performed on two series of model peptides. The CD spectra of the peptides H-(Val),-'Bu (n = 2-4) are depicted in Fig. 9. While the spectrum of H-$(Val)_2$-'Bu does not show any characteristic bands, the di- and tripeptide both display a minimum at 200 nm. This points to a random coil structure.

In the spectra of the POE peptides (Fig. 10) bearing three to five valine units, the random coil structure is evident (minimum at 217 nm, maximum at 198 nm), while the peptides with six and seven valine units have approximately 60% and 80% &structure, respectively. The octavaline peptide (Fig. 11) has a pure p-structure (maximum at 192 nm, $\pi \rightarrow \pi^*$ transition, minimum at 208 nm, $n \rightarrow \pi^*$ transition, zero value at 199 nm), in good agreement with the literature [30,31]. In the CD spectra of pivaloyl-L-$Val_7$-Gly-NH-POE,,,, and pivaloyl-L-$Val_8$-Gly-NH–$POE_{3000}$ there is a double minimum at 206/216 nm and 208/217 nm, respectively. As these peptides cannot adopt both structures at the same time [30], we assume an equilibrium between the P-sheet and the $\alpha$-helix conformation.

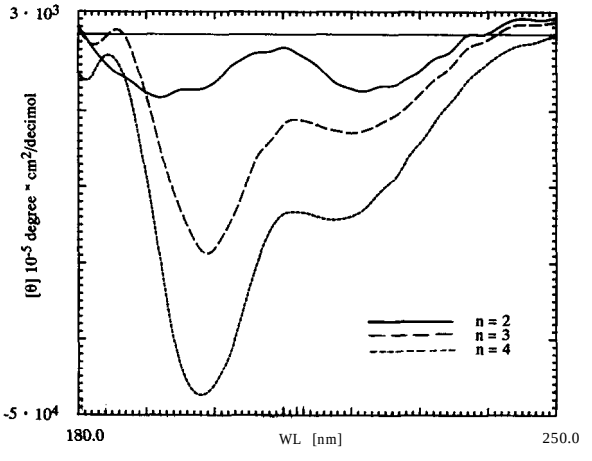


Fig. 9. CD spectra of H-$Val_n$-'Bu ($n$ = 2–4), $10^{-3}$ *M.* $\lambda$ = 250-180 nm; solvent, trifluoroethanol; ***d* = 0.02 cm.**

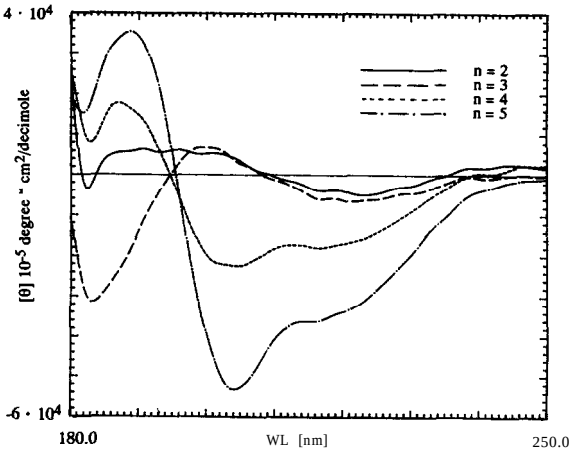


Fig. 10. CD spectra of pivaloyl-$Val_n$-Gly–$POE_{3000}$ ($n$ = 2–5), $10^{-3}$ M. $\lambda$ = 250-180 nm; solvent trifluoroethanol; ***d*** = 0.02 cm.

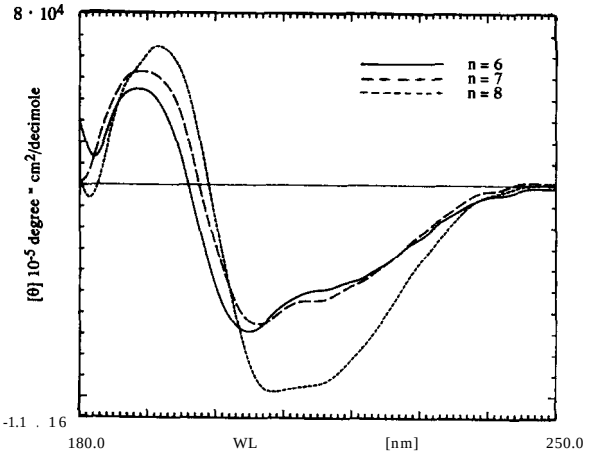


Fig. 11. CD spectra of pivaloyl-Val,-Gly-POE,,, ($n$ = 6–8), $10^{-3}$ *M.* $\lambda$ = 250-180 nm; solvent, trifluoroethanol; ***d* = 0.02** cm.

Analogous investigations [32] on stationary phases based on the sequence Gly-Val-Pro revealed very poor chiral recognition of the enantiomers of N-TFA-leucine n-propyl ester and of N-TFA-proline n-propyl ester, while peak resolution occurred for the racemic N-TFA-threonine n-propyl ester. The lack of $^1H$ NMR signal splitting of the amide proton of N-TFA-leucine methyl ester and CD investigations suggest that these peptides mainly form a random coil structure.

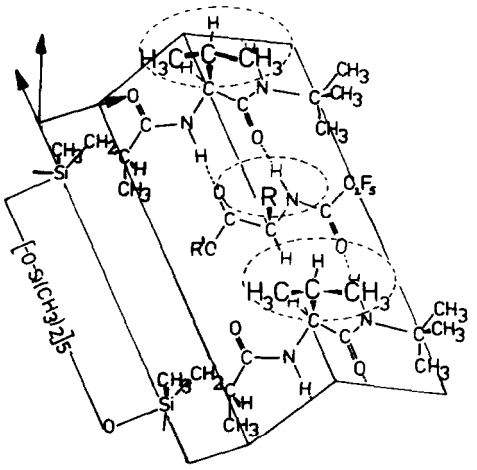

Fig. 12. Diastereomeric association complex between an amino acid derivative and Chirasil-Val [34].

## CONCLUSIONS

For Chirasil-Val [2,33], a P-plated sheet-like diastereomeric association complex between the stationary phase and an amino acid derivative (as depicted in Fig. 12) [34] served to describe tentatively the resolution mechanism. Later it was discovered that one strong attraction is already sufficient to bring about effective enantiomer discrimination [4]. From the present systematic investigation on oligopeptide solvents and previous findings on the thermodynamic behaviour of di- and tripeptide solutes on Chirasil-Val [35–38] it is clear that the contribution of a highly ordered suprastructure in the solvent-solute association complex is only significant for oligopeptides, and not for simple amino acid or dipeptide derivatives. In particular, the tetrapeptide phase appears to be a promising supplement to existing chiral stationary phases, provided the problems associated with its synthesis will find a proper solution.

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