corresponding Gerischer distribution curve only up to 10^{-3} of its maximum value, i.e., to a value where the customary linear representation of Gerischer's distribution curve still does not rise above the zero line. Considering the activation energy ($\lambda/4$) for the exchange current[1,9] at a metal electrode, this implies also that time-resolved measurements in the dark with 10-ns time resolution can only resolve adiabatic exchange currents with large reorganization energies, $\lambda > 0.75$ eV. The present knowledge, about the line shape corresponding to the energy dependence of the rate constant equivalent to Gerischer's distribution curve, is not sufficient for any molecule or redox ion to extrapolate from a measured value that is a factor of 10^{-3} smaller all the way up to the maximum value. The experiment described here gives a clear indication of the relevant time scale (<10 ps) at the maximum of Gerischer's distribution curve that is widely used as pictorial representation of the energy dependence of electron-transfer rate constants at electrodes.

Acknowledgment. We thank two members of our group for their invaluable help in this work, J. Lehnert for excellent technical assistance and R. Kietzmann for carrying out the computer fits. We are grateful to the Fonds der Chemischen Industrie for financial support.

Registry No. *n*-SnS_2, 1315-01-1; cresyl violet, 18472-84-4.

A Spectroscopic Study of the Interaction of D- and L-*N*-(3,5-Dinitrobenzoyl)valine Methyl Ester with *n*-Butylamide of (*S*)-2-[(Phenylcarbamoyl)oxy]propionic Acid: Direct Evidence for a Chromatographic Chiral Recognition Rationale

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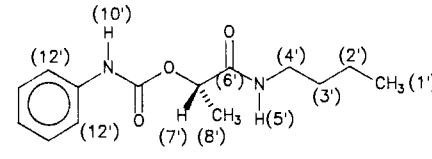
Abstract: The *n*-butylamide of (*S*)-2-[(phenylcarbamoyl)oxy]propionic acid forms diastereoisomeric adducts with the two antipodes of *N*-(3,5-dinitrobenzoyl)valine methyl ester. Their structures in solution have been studied by ^1H, ^{13}C, and ^{15}N NMR spectroscopy, and by their comparison it has been possible to gain some insights into the chiral recognition mechanism by which the enantiomers of *N*-(3,5-dinitrobenzoyl)amino acid methyl esters are separated by use of a chiral stationary phase obtained by immobilizing (*S*)-2-[(phenylcarbamoyl)oxy]propionic acid onto γ-aminopropylsilica gel.

Recently we reported[1] the preparation of a new chiral stationary phase (CSP) obtained by immobilizing (*S*)-2-[(phenylcarbamoyl)oxy]propionic acid (carbamalactic acid) on γ-aminopropylsilanized silica and its use for the chromatographic separation of *N*-(3,5-dinitrobenzoyl)amino acid methyl esters. As it has been pointed out by other authors[2] and ourselves,[3] it is possible to gain insights into the chiral recognition mechanism by studying the interactions between the chiral residue linked to the chromatographic support (assumed as a good, soluble model of the CSP) and the antipodes of a substrate which is resolved on the CSP itself.

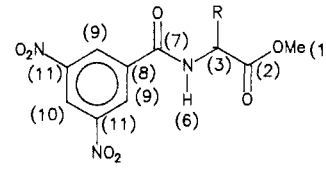
We report here the results of a study of interactions occurring, in solution, between the *n*-butylamide of (*S*)-2-[(phenylcarbamoyl)oxy]propionic acid (CBL) and L- or D-*N*-(3,5-dinitrobenzoyl)valine methyl ester (L-Val or D-Val, respectively) (Chart I) using NMR spectroscopy. Although a number of methods for detecting diastereoisomeric interactions have been developed, NMR spectroscopy constitutes one of the more sensitive probes used to reveal[2] the occurrence of such interactions and, more importantly, to gain detailed information on their nature. The determination of the structure of the diastereoisomeric adducts, CBL/L-Val and CBL/D-Val, provides information on their relative stability, and then, assuming that these adducts are similar to the selector–selectand and complexes, elution orders can be rationalized and a chromatographic chiral recognition mechanism can be proposed.

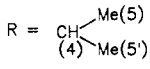
Chart I



n-butylamide of (S)-2-(phenylcarbomoyloxy)propionic acid

(CBL)





N-(3,5-dinitrobenzoyl)valine

methylester

(VAL)

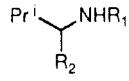
Results and Discussion

Our recent investigations on the CSP derived from carbamalactic acid, undertaken to explore the dependence of chromatographic separation on the structure of the amino acid derivatives, demonstrated that the presence of 3,5-dinitrobenzoyl and methyl ester groups on the substrate is essential for the chiral recognition.[1]

(1) Salvadori, P.; Pini, D.; Rosini, C.; Uccello-Barretta, G.; Bertucci, C. *J. Chromatogr.* **1988**, *450*, 163.
(2) Pirkle, W. H.; Pochapsky, T. C. *J. Am. Chem. Soc.* **1987**, *109*, 5975 and ref 17 therein.
(3) Salvadori, P.; Rosini, C.; Pini, D.; Bertucci, C.; Altemura, P.; Uccello-Barretta, G.; Raffaelli, A. *Tetrahedron* **1987**, *43*, 4969.

0002-7863/90/1512-2707$02.50/0

Table I. Dependence of Capacity Factor (K_1') and Separation Factor (α) on Structure[a] for Compounds Having General Formula



R_1[b]	R_2	K_1'	α
DNB[c]	COOMe	4.75	1.31
DNB[c]	CN	9.50	1.05
Bz[c]	COOMe	3.75	1.00
PFB	COOMe	1.00	1.00
p-NO_2B	COOMe	5.50	1.00

[a] In all the cases the eluent was hexane/2-propanol (95/5). [b] DNB, 3,5-dinitrobenzoyl; Bz, benzoyl; PFB, pentafluorobenzoyl; p-NO_2B, p-nitrobenzoyl. [c] Data from ref 1.

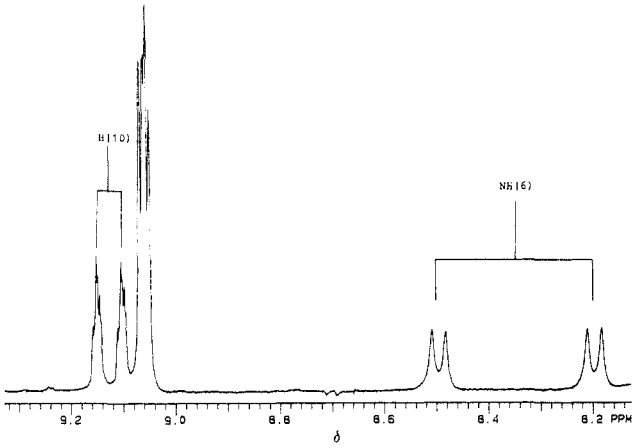


Figure 1. ^{1}H NMR spectrum of the equimolar mixture D- and L-Val/CBL, (300 MHz, 25 °C, $CDCl_3$, δ in ppm referred to TMS as internal standard).

As shown in Table I for a series of compounds related to valine, replacement of the 3,5-dinitrobenzoyl group with benzoyl, p-nitrobenzoyl, or pentafluorobenzoyl groups, as well as the use of a cyano group, in place of a carbomethoxy group, strongly reduces the separability factor.

These facts not only indicated the relevance of 3,5-dinitrobenzoyl and methyl ester moieties in the mechanism of chiral discrimination upon such CSP but also suggested the use of the two antipodes of an amino acid derivatized by means of these groups (L-Val and D-Val) for investigating by NMR the occurrence and nature of the interactions with the soluble model of the CSP (i.e. CBL).

The occurrence of the interactions between CBL and L-Val or D-Val was clearly detected by inspection of proton, carbon, and nitrogen spectra of equimolar mixtures CBL/D- and L-Val. As shown in Figure 1, the proton spectrum shows two sets of resonances for each proton or set of equivalent protons of the amino acid derivative. This indicates that, as a consequence of the interaction with CBL, the proton nuclei of Val, originally enantiotopic, become diastereotopic and give rise to the observed spectral inequivalence. The ^{1}H NMR analysis of equimolar mixtures CBL/L-Val and CBL/D-Val (at the same temperature and overall concentration) allowed the assignment of the resonances due to D and L antipodes, respectively (Table II). Analogously, the ^{15}N NMR resonance of the NH(6) group of Val, which is found at 106 ppm in the free substrate, duplicates at 108 and 109 ppm (Figure 2) in the CBL/D,L-Val mixture. Nitrogen spectra of the mixtures CBL/D-Val and CBL/L-Val (at the same concentration, molar ratio, and temperature) demonstrated that the absorption at 108 ppm is due to the D antipode of Val, while the resonance at 109 ppm is due to L-Val. Duplication of all carbon resonances of Val is also produced by interaction with CBL, as shown in Table III.

Information about the nature of the interactions occurring in the two adducts was gained from analysis of the changes of the spectral parameters of L- and D-Val upon interaction with CBL.

Table II. ^{1}H NMR Chemical Shifts (δ, ppm Referred to TMS) of Val in the Free, L-Val/CBL, and D-Val/CBL Mixtures ($CDCl_3$, 25 °C, 1:1 Molar Ratio, 0.03 M)

	free	L-Val/CBL	D-Val/CBL
COOMe	3.79	3.81	3.82
CH(3)	4.81 [J(3,4) 5.1; J(3,6) 8.6][a]	4.74 [J(3,4) 8.6; J(3,6) 8.6][a]	4.80 [J(3,4) 5.1; J(3,6) 8.6][a]
CH(4)	2.33	2.32	2.31
Me(5)	1.00	1.02	0.98
Me(5')	1.00	1.03	1.00
CH(9)	9.24	9.05	9.06
CH(10)	9.40	9.10	9.15
NH(6)	7.96	8.50	8.20

[a] Coupling constants in hertz.

Table III. ^{13}C NMR Chemical Shifts (δ, ppm Referred to TMS) of Val in the Free, L-Val/CBL, and D-Val/CBL Mixtures ($CDCl_3$, 25 °C, 1:1 Molar Ratio, 0.03 M for Each Component)

	free	L-Val/CBL	D-Val/CBL
OMe(1)	52.38	52.16	52.18
C=O(2)	172.71	172.68	172.31
CH(3)	57.99	58.99	58.58
CH(4)	31.03	30.49	30.72
Me(5)	17.69	18.45	18.16
Me(5')	18.71	18.85	18.81
NH—CO(7)	160.46	163.16	163.03
C(8)	136.66	136.75	136.95
C(9)	127.04	127.42	127.33
C(10)	148.19	148.00	148.11
C(11)	120.90	120.67	120.76

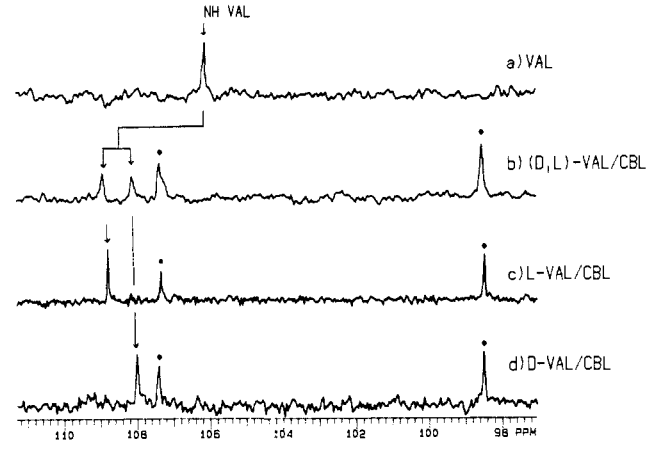


Figure 2. ^{15}N NMR spectrum (30.4 MHz, 25 °C, $CDCl_3$, $HCONH_2$ external standard) of (a) free Val and equimolar mixtures of (b) D- and L-Val/CBL, (c) L-Val/CBL, and (d) D-Val/CBL. Asterisks indicate resonances due to NH groups of CBL.

As shown in Table II, in the two diastereoisomeric pairs, the protons of the 3,5-dinitrobenzoyl group of Val are shifted upfield with respect to the free substrate, while the ^{1}H and ^{15}N resonances of the amide group NH(6) show a downfield shift. Reasonably the upfield shift of 3,5-dinitrobenzoyl group is due to the proximity of shielding regions of CBL (such as that created by the ring current of its phenyl group), while attractive intermolecular interactions with CBL, via formation of hydrogen bonds, bring about the downfield shift of the proton and nitrogen resonances of the NH(6) group.

The isopropyl group is probably far from any CBL group, remaining relatively unaffected by interaction with CBL.

It is noteworthy that all the more significant effects (i.e., shielding of 3,5-dinitrobenzoyl nuclei and deshielding of NH nuclei) are more evident (Table II) in the adduct formed with L-Val than that formed from D-Val. This may be an indication that, even if very similar centers of interaction with CBL are involved for each of the two antipodes, the intermolecular interaction with CBL is stronger for L-Val than for D-Val. It is also

Table IV. Effect of Temperature on the Chemical Shifts of Amide Protons of Free Val, Free CBL, L-Val/CBL, and D-Val/CBL Adducts ($CDCl_3$, 1:1 Molar Ratio, 0.03 M)

	$\delta(-60\ °C) - \delta(+25\ °C)$[a]			
	Val	CBL	L-Val/CBL	D-Val/CBL
NH(6)	+0.63		+1.81	+0.84
NH(10′)		+1.42	+2.18	+1.76
NH(5′)		+0.56	+1.50	+0.76

[a] Ppm referred to TMS.

Table V. Relevant Intra- and Intermolecular $^1H\{^1H\}$ NOE Data in L-Val/CBL and D-Val/CBL Adducts (1:1 Molar Ratio, $CDCl_3$, 25 °C)

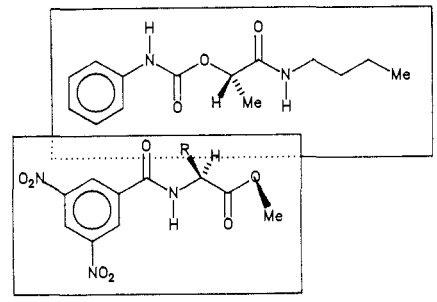
	observed					
	L-Val/CBL			D-Val/CBL		
saturated	intra	inter	NOE %	intra	inter	NOE %
NH(6)	CH(9)		17	CH(9)		16
	CH(3)		8	CH(3)		4
	CH(4)		5		CH(12′)	2
		CH(7′)	3		CH_3(8′)	2
CH(9)	NH(6)		28	NH(6)		18
		CH(12′)	5		CH(12′)	3
CH(3)	CH(4)		10	CH(4)		20
	NH(6)		10	NH(6)		7
		CH(7′)	3		CH_2(4′)	2
					CH_3(8′)	5
OCH_3	CH(4)		6			
		CH_2(2′)	4			
		CH_3(1′)	4			
NH(10′)	CH(12′)		13			
	CH(7′)		4			
		CH(9)	3			
		CH(3)	2			
NH(5′)	CH_2(4′)		10			
	CH(7′)		9			
	CH_3(8′)		5			
		CH(3)	2			
CH(7′)	CH_3(8′)		21			
	NH(5′)		4			
		CH(3)	3			
CH(12′)	CH(13′)		17			
	NH(10′)		7			
		CH(9)	3			

worth to note that the proton CH(3), bonded to the chiral center of Val, shows a different coupling pattern in the two adducts. In the pair CBL/D-Val, as in free Val, its resonance is a doublet of doublets due to the coupling with NH(6) (J ca. 8 Hz) and CH(4) (J ca. 5 Hz) protons. In the adduct formed with L-Val, this resonance appears as a triplet with J ca. 8 Hz. Thus the CH(3)–NH(6) coupling constant is the same in each adduct, while the CH(3)–CH(4) coupling constant is different. This increased coupling presumably stems from the L-Val/CBL interaction causing a change in the conformational behavior of L-Val. It appears that interaction entails the population of L-Val conformer having CH(3) and CH(4) transoid to each other, thus leading to the higher J value.

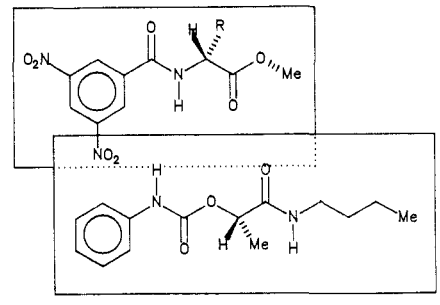
The analysis of the chemical shift dependence of amide protons on temperature affords some additional indication about the nature of the CBL–Val interactions. As shown in Table IV, in free Val and in CBL/D-Val or CBL/L-Val adducts, these protons undergo a remarkable downfield shift as the temperature is lowered. This confirms the involvement of the NH(6) proton of Val and NH(10′) or NH(5′) proton of CBL in attractive intermolecular interactions, the extent of which are increased by lowering the temperature. However, more importantly, the temperature gradient is greater for CBL/L-Val than that observed in the other adduct and in free Val.

In order to establish the overall structure of the two adducts, careful measurements of inter- and intramolecular $^1H\{^1H\}$ NOE enhancements in the CBL/L-Val and CBL/D-Val mixtures, in $CDCl_3$ at room temperature, were carried out. These data are reported in Table V.

Chart II



R = Pr^i



As far as the CBL/L-Val adduct is concerned, the NOE data can be summarized and discussed as follows (see Chart I for proton numbering). Saturation of the aromatic protons H(9) of L-Val causes an intermolecular NOE effect on the aromatic protons H(12′) of CBL. The reverse is also true. By saturating the proton NH(10′), CH(7′), or NH(5′) of CBL, intermolecular NOE effects of the same order of magnitude (≃2%) are produced on the proton CH(3) of L-Val, while no enhancement of the isopropyl resonances is observed. It is noteworthy that the saturation of NH(6) produces relevant intramolecular NOE (17%) on the aromatic protons CH(9) (indicating that the former is in proximity to proton CH(9) and, hence, nearly coplanar with respect to the aromatic ring) and a significant intermolecular effect on CH(7′) of CBL. The saturation of NH(6) also enhances the resonances of CH(3) and CH(4); hence these two protons are close to NH(6). In addition, the irradiation of the OMe protons produces intramolecular enhancements of Pr^i resonances.

From all these observations some important conclusions were drawn (Chart II): (i) The 3,5-dinitrobenzoyl group of L-Val and the phenyl group of CBL are close to each other, as indicated by the reciprocal intermolecular NOEs. In addition, they are stacked upon one another, as indicated by the previously discussed shielding of the aromatic protons (Table II). (ii) The alkyl chains of L-Val and CBL are cisoid, as revealed by the NOEs observed in the alkyl protons of CBL on saturation of alkyl resonances of L-Val; in addition, the CH(3) bond of L-Val is directed toward the CH(7′) bond of the CBL moiety; hence these two bonds are placed internally to the adduct. Accordingly, no intermolecular NOEs are observed on the isopropyl group of L-Val or CH_3(8′) group of CBL, indicating that these groups are external to the adduct, in a region of space which is relatively unhindered. (iii) Finally, the OMe linkage points toward the Pr^i and then toward the outside of the diastereoisomeric complex.

As far as the CBL/D-Val adduct is concerned, the analysis of the relevant NOE effects is reported again in Table V and Chart II reports its picture. The most important conclusions which can be drawn are as follows: (i) As found in the other pair, the irradiation of the aromatic protons of D-Val produces NOE effects on the aromatic protons of CBL, thus indicating stacking of the two rings. In other words, the situation of the aromatic fragments of the two moieties seems to be quite similar in the two adducts. (ii) The most striking difference between them is that a significant NOE effect can be measured at CH_3(8′) of CBL (and not at CH(7′) as observed in the previous adduct!!) upon saturation of CH(3) of D-Val. From these observations it can be concluded

that, in this adduct, the CBL residue is approached by the D-Val molecule by means of the opposite side with respect to the previous diastereoisomeric situation.

In summary, the above results allowed us to establish the occurrence and the nature of the diastereoisomeric interactions leading to the formation of the two adducts CBL/L-Val and CBL/D-Val, whose conformation is accurately defined.

The two diastereoisomeric adducts assume very similar conformation in solution (Chart II). The 3,5-dinitrobenzoyl group of D- or L-Val and the aromatic ring of CBL are stacked, and their side chains are cisoid. In both pairs the CH(3) group of Val is directed toward the CBL moiety, while the isopropyl group is external with respect to the adduct. The sole difference is that in the CBL/L-Val adduct the CH(3) group of L-Val is directed toward the CH(7′) group of CBL, whereas in CBL/D-Val it is directed toward the $CH_3(8')$ group of CBL.

From these observations, it can be concluded that the formation of the two diastereoisomeric complexes arises from attractive $\pi-\pi$ interaction between the aromatic nuclei of Val and CBL and formation of intermolecular hydrogen bonds as well as dipole–dipole interactions. Probably, as a consequence of the stacking between aromatic nuclei, the —C(=O)—NH(6)— group of Val and —NH(10′)—C(=O)— of CBL are involved in dipole–dipole attractive interaction,[4] while the NH(5′) bond of CBL gives rise to an intermolecular hydrogen bond with $—C(=O)—O—CH_3(1)$ group of Val. Only the steric interactions between CH(3) of L-Val and CH(7′) of CBL or CH(3) of D-Val and $CH_3(8')$ of CBL generate significant differences in the stability of the two diastereoisomeric adducts. Taking into account that a CH/CH_3 repulsion (occurring in the complex with D-Val) can be reasonably considered more destabilizing than a CH/CH repulsion (occurring in the complex with L-Val), we can state that the former adduct should be less stable than the latter. This stability difference fits well the chromatographic results, indeed upon the CSP derived from CBL the most retained antipode is L-Val, which, in solution, interacts more strongly with the soluble model of the chiral residue of the chromatographic support. The chromatographic experiments have been carried out both in hexane/2-propanol and hexane/chloroform mixtures, obtaining the same elution order and similar separation factors (α 1.3). Therefore the NMR studies in chloroform can be reasonably used to interpret the chromatographic separations.

(4) Pirkle, W. H. *Tetrahedron Lett.* **1983**, *24*, 5707.

Conclusions

Since it has been recently pointed out[5] that the knowledge of only one (the most stable) adduct cannot provide a complete explanation for chiral recognition, the most important result of this investigation is that, for the first time, a complete experimental characterization, by NMR spectroscopy, has been carried out on *both* the diastereoisomeric adducts formed by the soluble CSP analogue and the analyte enantiomers. In this case a chiral recognition rationale has been formulated by which the origin of the chromatographic separation of the enantiomers of *N*-(3,5-dinitrobenzoyl)amino acid methyl esters by means of a CSP derived from (*S*)-2-[(phenylcarbamoyl)oxy]propionic acid can be fully understood.

These results can be useful in the design of new, more effective CSPs derived from other α-hydroxycarboxyl acids having natural or synthetic origin. Furthermore, they provide experimental data on the two diastereoisomeric complexes, and this can constitute an interesting challenge to researchers involved[5,6] in the theoretical elaboration of chiral recognition rationales.

Experimental Section

The compounds named as CBL, L-Val, and D-Val have been prepared following a procedure already described.[1]

The NMR measurements were performed in $CDCl_3$ as solvent on a Varian VXR spectrometer operating at 300 MHz for ^1H, 75 MHz for ^{13}C, and 30.4 MHz for ^{15}N. Temperature was controlled with an accuracy ±0.1 °C. ^{15}N NMR spectra were obtained by using inverse-gated decoupling experiments, to suppress the unfavorable $^{15}N\{^1H\}$ nuclear Overhauser enhancement. ^{15}N NMR chemical shifts are referred to $HCONH_2$. The $^1H\{^1H\}$ NOE experiments were performed on carefully degassed samples in the difference mode. The decoupler was placed at the required frequency to saturate the proton in question. The decoupler power used was the minimum required to saturate the spin of interest. A waiting time varying from 10 to 30 s was used to allow the system to reach the equilibrium. Each NOE experiment was repeated at least four times, and results reproducible to ±1 have been obtained.

Registry No. CBL, 124443-73-8; L-Val, 69632-46-8; D-Val, 69632-45-7.

(5) (a) Lipkowitz, K. B.; Demeter, D. A.; Landwer, J. M.; Parish, C. A.; Darden, T. *J. Comput. Chem.* **1988**, *9*, 63. (b) Topiol, S.; Sabio, M.; Moroz, J.; Caldwell, W. B. *J. Am. Chem. Soc.* **1988**, *110*, 8367.

(6) Lipkowitz, K. B.; Demeter, D. A.; Zegarra, R.; Larter, R.; Darden, T. *J. Am. Chem. Soc.* **1988**, *110*, 3446.