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Chiral discrimination of complexes between benzyloxycarbonylglycyl-L-proline and 4-hydroxy-2-dipropylaminoindan in ion-pair chromatography

A molecular mechanics study

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ABSTRACT

The separation of the enantiomers of 4-hydroxy-2-dipropylaminoindan (USDA-46) was accomplished by ion-pair chromatography using benzyloxycarbonylglycyl-L-proline (L-ZGP) as a chiral selector in the mobile phase. Molecular mechanics calculations (MMX) were performed to model the formation of diastereomeric complexes of L-ZGP and the enantiomers of USDA-46. Complexes were generated by manual dockings of a variety of different conformations of each component and by an automated Monte Carlo search in MacroModel. The complexes were found to be stabilized by a reinforced ionic interaction, inter- and intramolecular hydrogen bonds and attractive aromatic interactions. Geometries and energies of complex conformations were calculated to determine dynamic measures of total and partial surface areas and dipole moments. The results were combined with data from a study on a series of phenolic aminotetralin derivatives. The previously established correlation between the separation coefficient α of the tetralin derivatives and the difference in dynamic unsaturated surface area of the L-ZGP complexes was corroborated by the results obtained with USDA-46.

1. INTRODUCTION

Many molecules involved in processes of importance for life are chiral. Further, enantiomers are likely to interact differently with biomolecules that are themselves chiral. Nevertheless, many biological processes have been and still are studied by use of racemates and a large number of drugs currently used in therapy are racemic mixtures [1]. An increasing awareness of, in particular, the potential advantages of stereochemically pure drugs has provided impetus for the development of efficient methods for asymmetric synthesis and for analytical and preparative separations of stereoisomers. Progress in these areas is promoted by an increased understanding of stereoselective processes and of the mechanisms underlying the phenomenon of chiral recognition and molecular recognition in general.

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Enantiomeric separations may be accomplished by conversion of a racemate into diastereomers that can be separated by regular chromatography. Other means of separations are available, such as the use of chiral stationary phases for gas and liquid chromatography. In these systems, the enantiomeric separation is due to differences in the stability of the diastereomeric complexes formed between the enantiomers and the chiral stationary phase. An alternative technique for liquid chromatography is based on the addition of a chiral additive to the mobile phase using an achiral stationary phase. Here the chiral additive complexes with the enantiomeric analytes but the mechanism for separation of the resulting diastereomeric complexes by this method is not known in detail. A conventional achiral solid phase is used and enantiomeric separations are possible when the diastereomers have different stabilities and/or distribution properties to the solid phase [2]. Pettersson and co-workers have shown the usefulness of the protected dipeptide benzyloxycarbonylglycyl-L-proline (L-ZGP) as a chiral additive to the mobile phase (dichloromethane) on the carbon phase Hypercarb [3–8]. L-ZGP has been used in separations of the enantiomers of β-blocking agents and related amines [3–6] and phenolic 2-dipropylaminotetralins [7,8]. An example of the excellent separations obtained with L-ZGP addition is shown in Fig. 1.

In a previous study, we modelled the formation of diastereomeric complexes between enantiomers of phenolic 2-dipropylaminotetralins and L-ZGP by molecular mechanics calculations [9]. We have now extended our theoretical studies to the structurally related 4-hydroxy-2-dipropylaminoindan (USDA-46) [10]. This indan derivative has been classified as a potent dopamine receptor agonist with the *R*-enantiomer being approximately 100 times more potent than the *S*-enantiomer [11]. The dynamic properties of the diastereomeric complexes of USDA-46 and L-ZGP were calculated and combined with previous data on complexes of L-ZGP and the phenolic 2-dipropylaminotetralins.

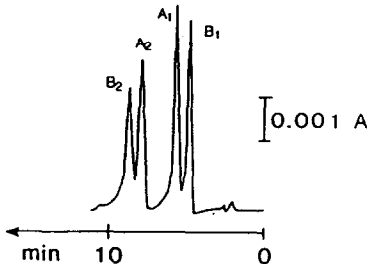


Fig. 1. Separation of racemic 8-OH-DPAT (A) and USDA-46 (B). Solid phase, Hypercarb; mobile phase, 10 m*M* L-ZGP in CH_2Cl_2 (80 ppm H_2O).

ION-PAIR CHROMATOGRAPHY

The liquid chromatographic system consisted of a Constametric III (LDC, Riviera Beach, FL, USA) pump, a Model 7125 injector (Rheodyne, Cotati, CA, USA) with a 20-μl loop and a Spectromonitor III UV detector (LDC) with a 12-μl cell. The Hypercarb column was purchased from Shandon (Astmoor, UK). The column and solvent reservoirs were thermostated at 25.0 ± 0.1°C with a HETO Type 2 Pt 243 TC waterbath (Birkerød, Denmark). The mobile phase flow-rate was 1.0 ml/min. The samples were dissolved in the mobile phase before being injected.

Dichloromethane (LiChrosolv) and molecular sieves (4A) were obtained from Merck (Darmstadt, Germany). Dichloromethane dried with molecular sieves had a water content of less than 30 ppm (w/w) (determined by Karl Fischer titration). The water contents of the eluents were adjusted by mixing dry and water-saturated (2100 ppm of water) dichloromethane.

N-Benzyloxycarbonylglycyl-L-proline (L-ZGP) was obtained from Nova Biochem (Switzerland).

MOLECULAR MECHANICS CALCULATIONS

Method

Energy-minimized geometries were obtained using the MMX force field as included in the PCMODEL program (version PI 3.1) [12][a] The calculations were performed without restrictions in the minimization process. The H-BND command was activated during the minimizations to

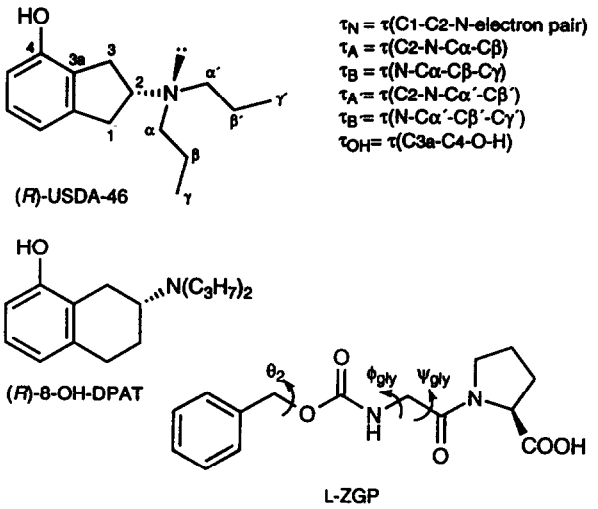
take into account potential hydrogen bonds, and the default value of the dielectric constant (1.5) was used. Hence the electrostatic effects might be overemphasized. However, as we are studying the differences between the binding of enantiomers, this effect is probably negligible.

Starting geometries of the diastereomeric complexes between the enantiomers of USDA-46 and L-ZGP were generated both by manual dockings and by an automatic conformational search (Monte Carlo). It was necessary to know the conformational preferences of both complex components as it is believed that the enantioselective binding of two chiral molecules is conformationally dependent. In the calculations of the complexes we used the charged ammonium ion of USDA-46 and the carboxylate anion of L-ZGP.

USDA-46

The conformational flexibility of USDA-46 has been studied by Karlén [14] using Allinger's MMP2 force field [15] as included in the MIMIC program [16]. Similar studies have been performed by Wikström *et al.* [17]. We made a comparative study of the conformational preferences of USDA-46 using the MMX force field. Geometries and relative energies of low-energy conformations were identical with those found by Karlén (Table I). Twelve conformations with steric energies below 2.5 kcal/mol (1 kcal = 4.184 kJ) were found. Karlén reported only six conformations since he did not take into account both phenolic rotamers. The non-aromatic ring of USDA-46 preferentially adopts an envelope conformation with a pseudo-equatorially oriented 2-dipropylamino group. The structural differences between conformations are due to rotation(s) of the OH and the propyl groups (τ_A, τ_B, $\tau_{A'}$, and $\tau_{B'}$; for definitions see formula and ref. 18). Eight of the twelve conformers are of similar energy ($\Delta E_s < 0.4$ kcal/mol).

[a] The PCMODEL program is available from Serena Software, Bloomington, IN 47402-3076, USA. The program has been reviewed by Freeman [13].



A small fraction (2%) of the conformations adopted envelope conformations with the dipropylamino group pseudo-axially dispositioned. Wikström *et al.* [17] reported that MM2 calculations on 4-hydroxy-2-aminoindan predict that conformations with an axially oriented amino group are preferred over those with an equatorially positioned substituent. The stabilization was believed to be a result of hydrogen bonding between the amino group and the aromatic ring. This stabilizing effect could not be reproduced using MMX calculations.

Initially, two confirmations (A and F) of each enantiomer of USDA-46 were used in the manual dockings with L-ZGP (Table I). Conformation A is characterized by $\tau_N \approx \tau_A \approx +60°$, $\tau_B \approx \tau_{A'} \approx \tau_{B'} \approx 180°$ and conformation F by $\tau_N \approx 180°$, $\tau_A \approx -60°$, $\tau_B \approx \tau_{A'} \approx \tau_{B'} \approx 180°$ [18]. The dipropylamino group is pseudo-axially positioned in conformation F. In the automatic dockings three conformations (A, D and F) of USDA-46 were used; conformation D is characterized by $\tau_N \approx +60°$, $\tau_A \approx \tau_B \approx 180°$, $\tau_{A'} \approx -60°$, $\tau_{B'} \approx 180°$.

L-ZGP

The preferred conformations on L-ZGP have been established using the MMX force field [19]. In our previous study we found that the conformational flexibility of L-ZGP in the complex was considerably restricted as only a limited

TABLE I

RELATIVE STERIC ENERGIES AND TORSION ANGLES FOR LOW-ENERGY (MMX) CONFORMATIONS[a,b] OF (*R*)-USDA-46

Conformation	ΔE_s (kcal/mol)	τ_N(°)	τ_A(°)	τ_B(°)	$\tau_{A'}$(°)	$\tau_{B'}$(°)
A	0.0[c]	58	65	179	179	−171
B	0.4	56	65	180	177	−57
C	0.4	63	−177	57	−65	−179
D	0.0[c]	62	−179	171	−65	−179
E	2.5	−57	−173	169	49	170
F	2.4	176	−51	−169	173	−170

[a] For definitions see formula and ref. 18.
[b] Only conformations with $\tau_{OH} = 0°$ with ΔE_s below 3.0 kcal/mol have been included.
[c] MMX steric energy = 13.7 kcal/mol.

number of conformations were found in low-energy complexes [9]. Two main groups of conformations were characterized by a *cis*-glycylproline amide bond arrangement, $\psi_{gly} \approx +60°$, and $\phi_{gly} \approx +60°$ or 180°. Both *cis*- and *trans*-carbamate groups were represented as well as benzyl group rotamers. These L-ZGP conformations allow the formation of an intramolecular hydrogen bond between the carbamate hydrogen and the carboxylate group. In our previous study, this hydrogen bond appeared to be of importance for the stability of complexes with the phenolic 2-dipropylaminotetralin derivatives [9], but it was not as important for the stability of free L-ZGP [19].

Manual dockings

The manual dockings were performed by alignment of the A or F conformations of both enantiomers of USDA-46 with the 48 conformations of L-ZGP described previously [19], followed by energy minimization. In the alignments we attempted to optimize important interactions for complex stability: ionic interactions between the ammonium group of USDA-46 and the carboxylate of L-ZGP, hydrogen bonding between the phenol and the carbamate functionalities and attractive aromatic interactions.

The complexes generated from conformation A of USDA-46 with relative steric energies below 3 kcal/mol were used as starting geometries for the other low-energy conformations (B, C and D; see Table I). Bond rotation in the propyl groups followed by energy minimization generated a more representative set of complex geometries compared with using only conformation A.

Automatic dockings

The automatic dockings of the enantiomers of USDA-46 with L-ZGP were performed using the BatchMin program included in the MacroModel package (version 3.0) [20]. The strategy used is described in Fig. 2. The MM2 force field included in MacroModel was used in energy minimizations as the MMX force field is not included in MacroModel. Initially we used both enantiomers of conformations A and D of USDA-46 and allowed bond rotation only in the phenol group. In contrast, all flexible bonds of L-ZGP were rotated in the conformational search. In addition, the two complex components (USDA-46 and L-ZGP) were allowed to rotate freely about an axis defined by N^+–H$\cdots$ $^-$O–C. These initial searches included 10 000 conformations of each diastereomeric complex. The most stable complex conformer found in each run was used as starting geometry in a new search of 10 000 conformations. To make the geometries generated by the automated procedure comparable to those generated by the manual method, they were reminimized with the MMX force field.

As indicated above, the most stable complex

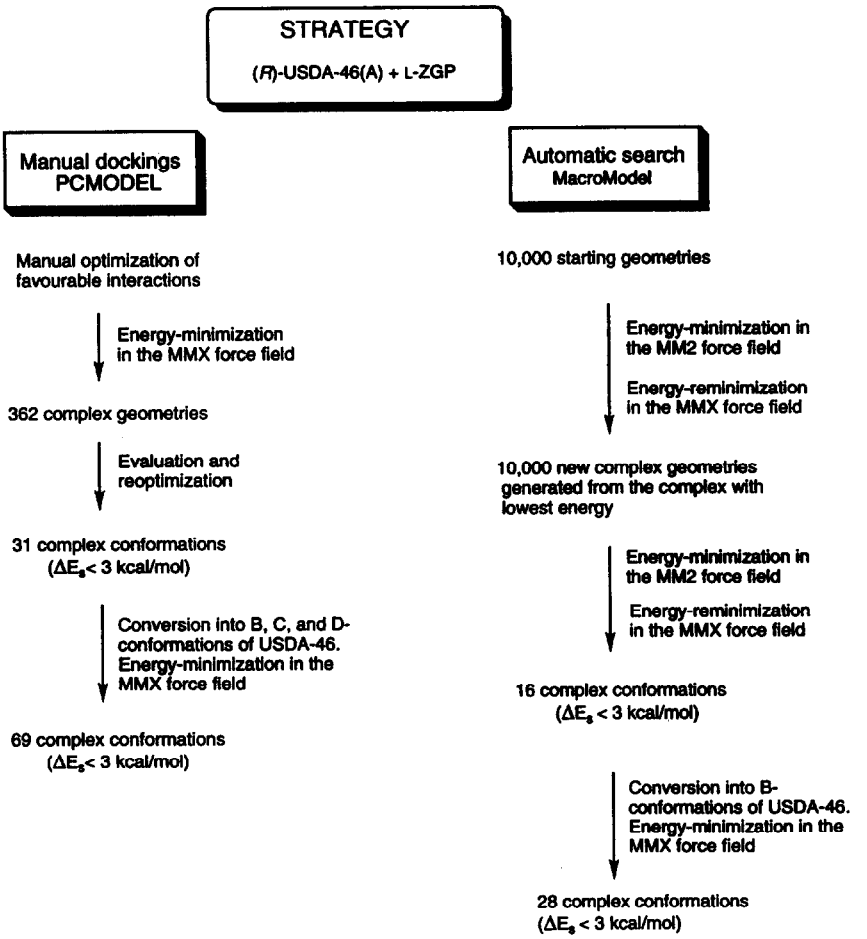


Fig. 2. General strategy used to generate complex conformations of (*R*)-USDA-46 and L-ZGP.

geometries found were used to generate complexes including conformations B [from USDA-46(A) complexes] and C [from USDA-46(D) complexes]. Conformation F, which has an axial dipropylamino group, was also used in the automatic dockings using the same strategy as described above.

RESULTS FROM MANUAL AND AUTOMATIC DOCKING EXPERIMENTS

Manual dockings

Initially, 362 complexes of (*R*)-USDA-46(A)–L-ZGP and 228 complexes of (*S*)-USDA-46(A)–L-ZGP were generated. Energy minimization gave 31 *R*-complexes and 24 *S*-complexes with relative steric energies below 3.0 kcal/mol. These low-energy complex geometries were used as starting points when generating complexes of USDA-46 in the B, C and D conformations. Energy minimizations gave a total of 69 *R*-complexes and 50 *S*-complexes within 3 kcal/mol of the minimum energy complex. (*S*)-USDA-46 gave the most stable complex [$\Delta E_s(S-R) = 0.6$ kcal/mol].

Conformation F of USDA-46 was also used in the manual dockings and 82 complexes of (*R*)-USDA-46(F)–L-ZGP and 58 (*S*)-USDA-46(F)–L-ZGP were generated. Energy minimization gave seven *R*-complexes and no *S*-complexes among those with relative steric energy below 3 kcal/mol. According to a Boltzmann distribution calculated at 25°C, only two complex conformations with the F conformation of USDA-46

contributed more than 1% to the conformational population.

Automatic search

A total of 20 000 geometries were generated for each enantiomer of USDA-46(A) and USDA-46(D) with L-ZGP. Refinements in MacroModel and reminimizations in MMX produced sixteen complexes of (*R*)-USDA46(A)–L-ZGP, fourteen of (*S*)-USDA46(A)–L-ZGP, twelve of (*R*)-USDA46(D)–L-ZGP and thirteen of (*S*)-USDA46(D)–L-ZGP with relative steric energies below 3.0 kcal/mol. In addition, 17 *R*- and 28 *S*-complexes of low energy were found with B and C conformations of USDA-46. This search gave an *S*-complex of lowest energy [$\Delta E_s(S-R) = 0.5$ kcal/mol] that was more stable than any of those found in the manual search. Interestingly, the most stable complex found contained a pseudo-axially positioned dipropylammonium group. This complex was obtained when (*S*)-USDA-46(D) was used as the starting conformation. The conformational change from an equatorial to an axial dipropylammonium group appears to be related to the formation of an intermolecular hydrogen bond between the phenolic group and the glycylproline amide bond. Starting from (*R*)- or (*S*)-USDA-46(D) we identified eight low-energy complexes with an axial dipropylammonium group.

Conformation F of USDA-46 was used to generate 20 000 complex geometries with axial dipropylamino groups. No low-energy complexes were found. In fact, the most stable complexes including USDA-46(F) had relative steric energies of about 4.5 kcal/mol.

Combination of results from the manual and automated searches

When the results of the manual and the automatic searches were combined, 30 low-energy complexes of (*R*)-USDA-46–L-ZGP and 29 of (*S*)-USDA-46–L-ZGP with $\Delta E_s < 3.0$ kcal/mol were found. A Boltzmann distribution calculated at 25°C showed that several of the complexes represented less than 1% of the total conformational population. When omitting these conformations only seventeen *R*- and seventeen *S*-complexes remained. However, they represent about 90% of both the *R*- and the *S*- conformations.

The low-energy complexes were stabilized by an electrostatic interaction, inter- and intramolecular hydrogen bonds and attractive aromatic interactions. The intramolecular hydrogen bond between the carbamate hydrogen and the carboxylate group in L-ZGP was present in all low-energy complexes. The glycylproline amide bond adopted a *cis* arrangement and the carbamate group preferred a *trans* orientation, $\psi_{gly} = +60°$, and $\phi_{gly} = +60°$ or 180°, *i.e.*, their conformations are identical with those found in the aminotetralin complexes [9]. The benzyl group adopted two favourable orientations, $\theta_2 = 85°(\pm 5°)$ or 180°. The phenol group of USDA-46 adopted predominantly conformations with $\tau_{OH} = 10–15°$.

In the low-energy complexes, the cyclopentene ring of USDA-46 was preferably positioned face to face with the pyrrolidine ring of L-ZGP, whereas the aromatic moieties were oriented edge to face. Hence the peptide backbone and the benzyl moiety were folded around USDA-46 in the complex. Fig. 3 shows the most stable diastereomeric complexes.

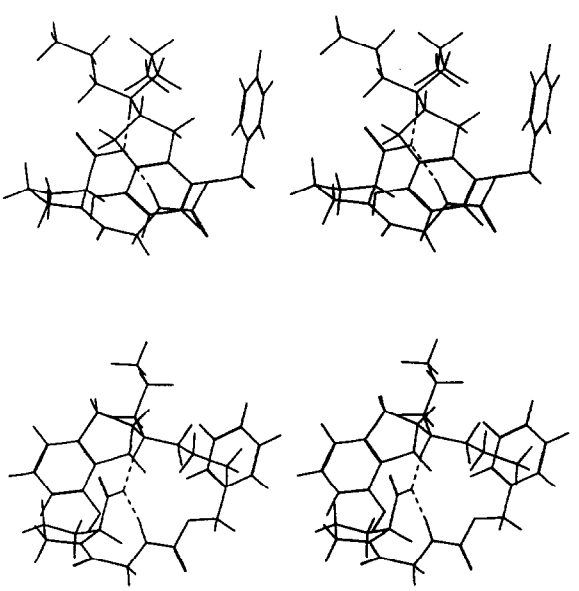


Fig. 3. Stereo views of the minimum-energy conformation of (top) (*R*)-USDA-46–L-ZGP complex and (bottom) (*S*)-USDA-46–L-ZGP complex.

DYNAMIC COMPLEX PROPERTIES

Dynamic surface areas [21] and dipole moments of the low-energy complexes were calculated. Since the complexes are formed from flexible molecules, the data were statistically averaged according to a Boltzmann distribution to give the dynamic properties of the diastereomeric complexes. Surface areas were calculated using a grid size of 0.1 Å. The total surface was divided into polar and non-polar surface areas and the non-polar surface was further divided into saturated and unsaturated non-polar surface areas. The data obtained in the present study were combined with data from the previous calculations of the 2-aminotetralin–L-ZGP complexes (Table II). Statistical analysis of potential correlations between the dynamic properties and the chromatographic data (α) indicated a correlation between the dynamic unsaturated surface area and the separation coefficient α ($r^2 = 0.80$) (Fig. 4). This correlation was found also in our previous study and, consequently, the present data corroborate the relationship between α and the unsaturated surface of the complexes.

CONCLUSIONS

As suggested by our previous calculations on complexes of 2-dipropylaminotetralin derivatives and L-ZGP, enantioselectivity in the ion-pair chromatography of phenolic dipropylaminotetralins using L-ZGP as a chiral selector appears to depend on differences in degree of adsorption of the diastereomeric complexes to the solid phase [9]. When combining the results from the aminotetralins with those of USDA-46 we obtain the same correlation coefficient. This implies

TABLE II

DYNAMIC PROPERTIES[a] OF ENERGY-MINIMIZED (MMX) DIASTEREOMERIC COMPLEXES OF USDA-46 AND SIX PHENOLIC ANIMOTETRALINS[b] AND L-ZGP

Results were obtained from a combination of data from manual dockings and an automated conformational search.

Complex of L-ZGP with	Total surface area (Å^2)	Partial surface area (Å^2)			Dipole moment (D)	ΔE ($E_S - E_R$)	α[c]
		Saturated surface	Unsaturated surface	Polar surface			
(2*R*)-USDA-46	550	383	70	97	12.2	−1.7	2.20
(2*S*)-USDA-46[d]	562	392	75	96	12.9		
(*R*)-8-OH DPAT[e]	587	413	79	96	11.7	−1.4	2.04
(*S*)-8-OH DPAT[d]	588	409	78	103	14.2		
(*R*)-LY-10[e]	594	418	73	101	12.8	−1.0	1.0
(*S*)-LY-10[d]	588	418	65	106	14.6		
(1*R*,2*R*)-ALK-4[e]	605	425	78	102	13.3	−0.2	1.16
(1*S*,2*S*)-ALK-4[d]	591	423	69	99	13.5		
(1*S*,2*R*)-ALK-3[d,e]	596	422	74	98	12.6	0.3	1.03
(1*R*,2*S*)-ALK-3	576	412	74	93	13.3		
(2*S*,3*S*)-CM-12[e]	594	417	78	98	12.9	−2.0	1.32
(2*R*,3*R*)-CM-12[d]	601	422	76	103	14.2		
(2*S*,3*R*)-CM-11[d,e]	591	426	72	95	10.5	0.4	3.46
(2*R*,3*S*)-CM-11	604	428	83	96	12.4		

[a] Calculated according to a Boltzmann distribution at 25°C.
[b] For structures see ref. 9.
[c] Solid phase, Hypercarb; mobile phase, 10 m*M* L-ZGP in CH_2Cl_2 (80 ppm H_2O).
[d] Denotes the most stable diastereomeric complex in each pair.
[e] 8-OHDPAT = 8-hydroxy-2-dipropylaminotetralin; LY-10 = 2-methyl 8-OHDPAT; ALK-4 = *trans*-1-methyl 8-OHDPAT; ALK-3 = *cis*-1-methyl 8OHDPAT; CM-12 = *trans*-3-methyl 8-OHDPAT; CM-11 = *cis*-3-methyl 8-OHDPAT.

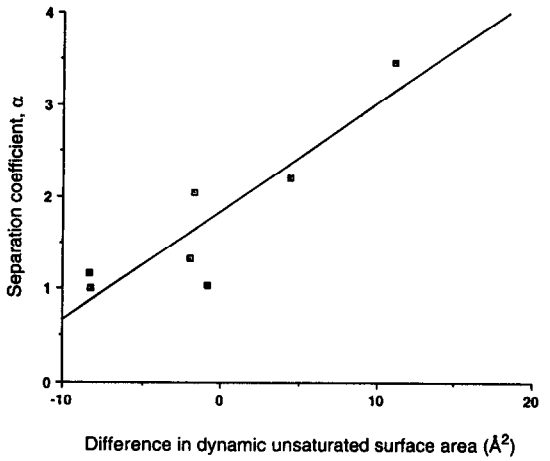


Fig. 4. Correlation diagram of the separation factors (α) and the difference in dynamic unsaturated surface area for the diastereomeric complexes obtained by a combination of results from manual and automatic searches of USDA-46 and the phenolic 2-dipropylaminotetralins [9] and L-ZGP (r^2 = 0.80).

that complexes formed from the aminoindan and L-ZGP are separated by a similar mechanism to that for the aminotetralin complexes. The differences in interatomic distances between the two types of derivatives is reflected by the formation of stable complexes containing an axial dipropylamino group with USDA-46. The corresponding conformation of the aminotetralin derivatives is not stabilized by an intermolecular hydrogen bond between the phenolic group and the glycylproline amide bond.

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REFERENCES

1 E.J. Ariens, in C. Brown (Editor), *Chirality in Drug Design and Synthesis*, Academic Press, London, 1990, p. 29.
2 C. Pettersson, in A.M. Krstulovic (Editor), *Chiral Separations by HPLC*, Ellis Horwood, Chichester, 1989, p. 124.
3 C. Pettersson and M. Josefsson, *Chromatographia*, 21 (1986) 321.
4 C. Petterson, A. Karlsson and C. Gioeli, *J. Chromatogr.*, 407 (1987) 217.
5 A. Karlsson, C. Pettersson and S. Björkman, *J. Chromatogr.*, 494 (1989) 157.
6 A. Karlsson and C. Pettersson, *J. Chromatogr.*, 543 (1991) 287.
7 A. Karlsson, C. Pettersson, S. Sundell, L.-E. Arvidsson and U. Hacksell, *Acta Chem. Scand., Ser. B*, 42 (1988) 231.
8 A. Karlsson, L. Björk, C. Pettersson, N.-E. Andén and U. Hacksell, *Chirality*, 2 (1990) 90.
9 A. Karlsson, K. Luthman, C. Pettersson and U. Hacksell, *Acta Chem. Scand.*, 47 (1993) 469.
10 U. Hacksell, L.-E. Arvidsson, U. Svensson, J.L.G. Nilsson, H. Wikström, P. Lindberg, D. Sanchez, S. Hjorth, A. Carlsson and L. Paalzow, *J. Med. Chem.*, 24 (1981) 429.
11 J.G. Cannon, R.G. Dushin, J.P. Long, M. Ilhan, N.D. Jones and J.K. Swartzendruber, *J. Med. Chem.*, 28 (1985) 515.
12 J.J. Gajewski, K.E. Gilbert and J. McKelvey, *Adv. Mol. Model.*, 2 (1990) 65.
13 P.K. Freeman, *J. Am. Chem. Soc.*, 111 (1989) 1942.
14 A. Karlén, *Doctoral Thesis*, Uppsala University, Uppsala, 1989.
15 N.L. Allinger, *J. Am. Chem. Soc.*, 99 (1977) 8127.
16 T. Liljefors, *J. Mol. Graphics*, 1 (1983) 111.
17 H. Wikström, J.-H. Lii and N.L. Allinger, *J. Med. Chem.*, 30 (1987) 1115.
18 A. Karlén, A.M. Johansson, L. Kenne, L.-E. Arvidsson and U. Hacksell, *J. Med. Chem.*, 29 (1986) 917.
19 K. Luthman and U. Hacksell, *Acta Chem. Scand.*, 47 (1993) 461.
20 F. Mohamadi, N.G.J. Richards, W.C. Guida, R. Liskamp, M. Lipton, C. Caufield, G. Chang, T. Hendrickson and W.C. Still, *J. Comput. Chem.*, 11 (1990) 440.
21 K.B. Lipkowitz, B. Baker and R. Larter, *J. Am. Chem. Soc.*, 111 (1989) 7750.