ELSEVIER

Journal of Chromatography A, 672 (1994) 59–65

JOURNAL OF CHROMATOGRAPHY A

Comparison between silica-bonded chiral stationary phases derived from 3,5-disubstituted N-benzoyl-(*S*)-phenylalanine and (*S*)-cyclohexylalanine in the resolution of racemic compounds by liquid chromatography

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(Received January 26th, 1994)

Abstract

A study was made of the role of the phenylalanine phenyl ring in the enantioselectivity of several chiral stationary phases (CSPs) whose chiral selectors consist of several N-(3,5-disubstituted)benzoyl derivatives of this amino acid covalently bonded to silica gel. Racemic compounds with π-acceptor, π-donor or both characters were resolved on two series of CSPs derived from N-(3,5-dimethyl)benzoyl, N-(3,5-dimethoxy)benzoyl and N-(3,5-dinitro)benzoyl-(*S*)-phenylalanine and (*S*)-cyclohexylalanine. In all instances the best enantioselectivities were obtained with CSPs derived from (*S*)-cyclohexylalanine. The results show that the phenyl ring in the phenylalanine moiety does not have an electronic role in the recognition of the racemic compound by the chiral selector on the CSP, a non-classical $\pi-\pi$ interaction between the 3,5-dinitrobenzoyl group in the racemic compound and in the CSP acts in the resolution of N-(3,5-dinitro)benzoyl derivatives of amino acids on CSPs with the same group and the change in the arrangement of solutes in the diastereomeric solute–stationary phase complexes can take place without an inversion of the elution order of enantiomers.

1. Introduction

The 3,5-dinitrobenzamides and particularly the N-(3,5-dinitro)benzoyl derivatives of racemic amino acids are generally well resolved on chiral stationary phases (CSPs) whose chiral selectors are N-(3,5-dinitro)benzoyl derivatives of amino acids [1–4]. That is, racemic compounds with a π-acceptor character are resolved on CSPs with the same π-acceptor character. This cannot be explained by the three-point interaction chiral recognition model in which one of the interactions is of π-acceptor–π-donor character [5–7]. The amino acids in the chiral selectors of these CSPs often have aromatic groups (phenylglycine, phenylalanine and tyrosine). The presence of a 3,5-dinitrobenzoyl group and an additional phenyl ring on the chiral selector of the CSP could give rise, *a priori*, to several modes of electronic interaction between the solute and the chiral entity in the CSP. The most important one in this kind of CSP is a classical π-acceptor–π-

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0021-9673/94/$07.00
SSDI 0021-9673(94)00178-C

donor interaction between the 3,5-dinitrobenzoyl group in the CSP and the aryl group in the racemic compound. However, a $\pi-\pi$ interaction between the phenyl ring of the CSP and the analytes [8], or a non-classical π-acceptor–π-acceptor interaction between the 3,5-dinitrobenzoyl groups in the racemic compounds and those in the CSPs, or even a classical π-donor–π-acceptor interaction between the 3,5-dinitrobenzoyl group in the racemic compound and the phenyl ring in the CSP could be considered.

On the other hand, it has been shown [9] that there is no inversion of the elution order of the enantiomers of the methyl esters of N-(3,5-dinitro)benzoyl derivatives of racemic amino acids when these are resolved on CSPs whose chiral selectors are N-(3,5-dimethyl)- or N-(3,5-dimethoxy)benzoylphenylalanine and 3,5-dimethylanilidophenylalanine. If the $\pi-\pi$ interaction were the driving force in the chiral recognition in this kind of CSP and analytes [10], the inversion of the fixation direction of the phenylalanine between the π-donor group and the spacer, which binds the chiral selector to the silica gel, would produce an inversion in the elution order of enantiomers. However, this order would be maintained if the π-acceptor–π-donor interaction were established between the 3,5-dinitrobenzoyl group in the racemic compound and the phenyl ring in the phenylalanine moiety in the CSPs based on N-(3,5-dimethyl)- or N-(3,5-dimethoxy)benzoylphenylalanine. In this instance, the dipole stacking would be orientated in the same direction as in the case in which the chiral selector is 3,5-dimethylanilidophenylalanine and the maintenance of the elution order of enantiomers could be explained [11,12].

The aim of this study was to elucidate the role of the phenyl group of the phenylalanine in the enantioselectivity of the CSP in which the chiral selector is derived from this amino acid. The chromatographic behaviour of two series of CSPs whose chiral selectors are derived from either (*S*)-phenylalanine or (*S*)-cyclohexylalanine was studied by means of the resolution of racemic compounds with π-acceptor, π-donor or both kinds of aromatic groups (Fig. 1). The CSPs also

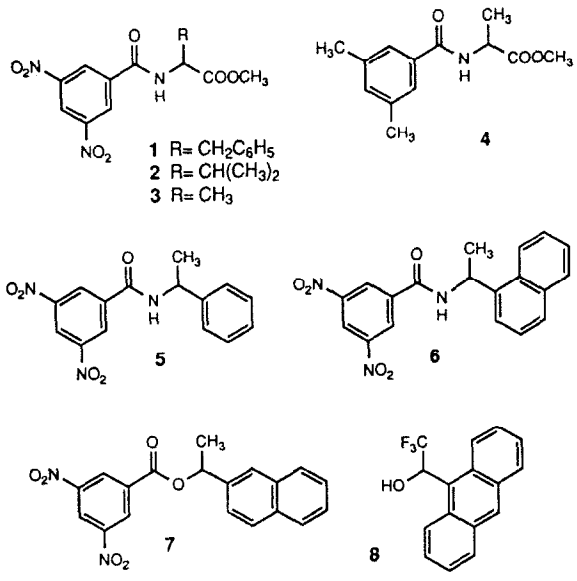

Fig. 1. Structures of racemic test compounds.

have either π-acceptor or π-donor character. An additional CSP whose chiral selector is N-(3,5-dinitrobenzoyl)alanine was prepared and tested to compare the steric effect of the methyl, the benzyl and the cyclohexylmethyl groups on the chiral centre of the CSPs (Fig. 2).

2. *Experimental*

NMR spectra were measured using a Varian Gemini-200 spectrometer. Tetramethylsilane (TMS) was used as the internal standard and the chemical shift, δ, is measured in ppm. Rotatory power was measured with a Perkin-Elmer (Überlingen, Germany) Model 241 polarimeter. Elemental analyses were performed at the Institut de Química Bioorgànica de Catalunya (Barcelona, Spain). The chromatographic experiments were carried out on an HPLC system consisting of a Waters Model 600E pump and a Waters Model 717 autosampler (Millipore, Milford, MA, USA) and equipped with a Waters Model 996 pohotodiode-array detector and a Perkin-Elmer Model 241LC polarimetric detector. The chiral stationary phases were packed into stainless-steel tubes (100 × 4.6 mm I.D.) by the slurry method by Interchim (Montluçon,

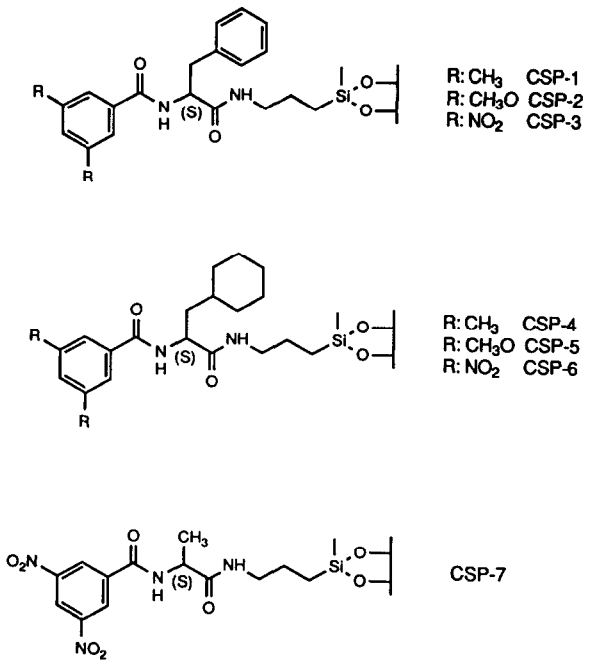

Fig. 2. Structures of chiral stationary phases.

France). The volume of sample injected was 1 μl, the flow-rate of the pump was 1 ml/min and the detection wavelength was 254 nm. The mobile phases consisted of various mixtures of *n*-heptane, chloroform and methanol. The void volume was determined using tri-*tert.*-butylbenzene [13].

2.1. Chemicals and reagents

Chiral selectors of CSPs 1–3 were prepared by the method described previously [9]. Compounds **1–3** (Fig. 1) were obtained by treating the methyl ester of each racemic amino acid with 3,5-dinitrobenzoyl chloride. Compound **4** (Fig. 1) was obtained by treating the methyl ester of racemic alanine with 3,5-dimethylbenzoyl chloride. Compounds **5–7** were obtained by treating the corresponding amines or alcohol with 3,5-dinitrobenzoyl chloride. Compounds **8** (Fig. 1) and 3-aminopropyltriethoxysilane were purchased from Aldrich.

3,5-Disubstituted N-benzoyl derivatives of (*S*)-cyclohexylalanine, chiral selectors of CSP 4–6, were prepared as follows. To a solution of 2.26 g (11 mmol) of (*S*)-cyclohexylalanine hydrochloride, obtained by catalytic reduction of (*S*)-phenylalanine [14], in 1 *M* NaOH, 11 mmol of the appropriate acyl chloride was added following the same method as for N-benzoyl derivatives of (*S*)-phenylalanine [9].

N-(3,5-Dimethylbenzoyl)-(S)-cyclohexylalanine (yield 51%)

^{1}H NMR (200 MHz): δ (C^2HCl_3) 0.7–2.0 (m, 13H, C_6H_{11} and CH_2), 2.35 (s, 6H, CH_3Ar), 4.84 (m, 1H, CH), 6.57 (d, 1H, NH), 7.15 (s, 1H, C^4H), 7.39 (s, 2H, C^2H and C^6H), 8.63 (ba, 1H, COOH). ^{13}C NMR (50.3 MHz): δ (C^2HCl_3) 20.9 (CH_3Ar), 25.7, 25.9 and 26.1 ($C^{3'}H_2$, $C^{4'}H_2$ and $C^{5'}H_2$), 32.3 and 33.2 ($C^{2'}H_2$ and $C^{6'}H_2$), 33.9 ($C^{1'}H$), 39.7 (CH_2), 50.5 (CH), 125.0 ($C^{2,6}H$), 133.5 (C^1), 133.6 (C^4H), 138.4 ($C^{3,5}$), 168.6 (CONH), 177.3 (COOH). $[\alpha]_D^{20} = -19.5°$ (c = 1.4, pyridine). M.p.: 97–98°C (diethyl ether–hexane). Analysis: calculated for $C_{18}H_{25}NO_3$, C 71.26, H 8.31, N 4.62; found, C 71.23, H 8.27, N 4.62%.

N-(3,5-Dimethoxybenzoyl)-(S)-cyclohexylalanine (yield 52%)

^{1}H NMR (200 MHz): δ ($C^2HCl_3/C^2H_3O^2H$) 0.7–2.0 (m, 13H, C_6H_{11} and CH_2), 3.84 (s, 6H, CH_3O), 4.84 (m, 1H, CH), 6.53 (d, 1H, NH), 6.61 (m, 1H, C^4H), 6.93 (d, 2H, $C^{2,6}H$). ^{13}C NMR (50.3 MHz): δ ($C^2HCl_3/C^2H_3O^2H$) 25.6, 25.8 and 26.0 ($C^{3'}H_2$, $C^{4'}H_2$ and $C^{5'}H_2$), 32.0 and 33.2 ($C^{2'}H_2$ and $C^{6'}H_2$), 33.9 ($C^{1'}H$), 39.3 (CH_2), 50.3 (CH), 55.2 (CH_3O), 103.7 (C^4H), 105.0 ($C^{2,6}H$), 135.8 (C^1), 160.8 ($C^{3,5}$), 167.7 (CONH), 175.6 (COOH). $[\alpha]_D^{20} = -7.0°$ (c = 1.6, pyridine). M.p.: 127–129°C (diethyl ether–hexane). Analysis: calculated for $C_{18}H_{25}NO_5$, C 64.46, H 7.51, N 4.18; found, C 64.47, H 7.48, N 4.20%.

N-(3,5-Dinitrobenzoyl)-(S)-cyclohexylalanine (yield 33%)

^{1}H NMR (200 MHz): δ ($C^2HCl_3/C^2H_3O^2H$) 0.8–2.2 (m, 13H, C_6H_{11} and CH_2), 4.80 (m, 1H, CH), 8.53 (d, 1H, NH), 9.11 (d, 2H, C^2H and

C^6H), 9.15 (d, 1H, C^4H). ^{13}C NMR (50.3 MHz): δ ($C^2HCl_3/C^2H_3O^2H$) 25.9, 26.1 and 26.2 ($C^{3'}H_2$, $C^{4'}H_2$ and $C^{5'}H_2$), 32.1 and 33.5 ($C^{2'}H_2$ and $C^{6'}H_2$), 34.2 ($C^{1'}H$), 39.1 (CH_2), 51.0 (CH), 121.0 (C^4H), 127.8 ($C^{2',6'}H$), 137.3 ($C^{1'}$), 148.5 ($C^{3',5'}$), 163.3 (CONH), 175.1 (COOH). $[\alpha]_D^{20} = -11.5°$ ($c = 1.4$, pyridine). M.p.: 173–174°C (diethyl ether). Analysis: calculated for $C_{16}H_{19}N_3O_7$, C 52.60, H 5.24, N 11.50; found, C 52.95, H 5.32, N 11.40%.

N-(3,5-Dinitrobenzoyl)-(S)-alanine

Following the same procedure, from 11.2 mmol of (*S*)-alanine and 3,5-dinitrobenzoyl chloride, the title compound was obtained in 85% yield. ^1H NMR (200 MHz): δ ($C^2HCl_3/C^2H_3O^2H$) 1.58 (d, 3H, CH_3), 4.73 (m, 1H, CH), 9.13 (d, 2H, C^2H and C^6H), 9.18 (d, 1H, C^4H). ^{13}C NMR (50.3 MHz): δ ($C^2HCl_3/C^2H_3O^2H$) 17.3 (CH_3), 48.9 (CH), 120.9 (C^4H), 127.7 ($C^{2,6}H$), 137.3 (C^1), 148.4 ($C^{3,5}$), 162.9 (CONH), 174.7 (COOH). $[\alpha]_D^{20} = +15.6°$ ($c = 1.4$, pyridine). M.p.: 156–157°C (diethyl ether). Analysis: calculated for $C_{10}H_9N_3O_7$, C 42.41, H 3.20, N 14.84; found, C 42.32, H 3.16, N 14.73%.

2.2. Chiral stationary phases

Following the described procedure [15], to a solution of 6 mmol of the appropriate chiral acidic compound in 18 ml of pyridine, 1.7 ml (7.2 mmol) of 3-aminopropyltriethoxysilane in 12 ml of pyridine was added. The mixture was stirred under reflux for 1.5 h. The solvent and the excess of 3-aminopropyltriethoxysilane were removed under reduced pressure (0.1 mmHg) and the residue was used in the following step without further purification. A 6-g mass of spherical silica (5 μm, 100 Å, Nucleosil 100-5; Macherey–Nagel) was slurried with toluene and the water was then removed azeotropically using a Dean–Stark trap. After complete removal of water, toluene was removed by distillation and a solution of 6 mmol of the appropriate chiral silane freshly prepared in 50 ml of pyridine was added. The mixture was stirred under reflux for 1.5 h and 3 ml of hexamethyldisilazane were added, and the mixture was left to react for a further 1 h at reflux temperature. The resulting bonded silica was collected by filtration and washed exhaustively in pyridine, ethanol, water, ethanol, acetone and diethyl ether, and dried *in vacuo* at room temperature.

The elemental analyses and the surface concentration of surface-bonded chiral entities (μmol/m^2) according to Unger *et al.* [16] of the stationary phases are given in Table 1.

3. Results and discussion

Chromatographic parameters for the separation of the test compounds are given in Table 2.

The racemic compounds **1**, **2** and **3**, with π-acceptor character, are resolved on all CSPs tested. However, the selectivity is higher when they are resolved on CSPs with π-donor character (CSPs 1, 2, 4 and 5). Conversely, **4**, with π-donor character, is better resolved on π-acceptor CSPs (CSPs 3, 6 and 7) than on π-donor CSPs (CSPs 1, 2, 4 and 5). On the other hand, when **4** is resolved on CSPs 6 and 7 the only $\pi-\pi$ interaction that could be established is of π-acceptor–π-donor type. Moreover, **8** is only resolved on CSPs with π-acceptor character. All these observations are as expected: a π-acceptor–π-donor interaction could favour the chiral recognition of solutes by the CSP considerably.

Compounds **2** and **3** are well resolved on CSPs 6 and 7 even if the only aromatic group present on these racemic compounds and on the CSPs is a 3,5-dinitrobenzoyl ring. In such a case, if a $\pi-\pi$ interaction takes part in the recognition of those solutes by the CSPs, it could only be established between both 3,5-dinitrobenzoyl rings. Some facts seem to indicate this possibility. In all instances the α values are of the same order. This seems to indicate that the difference in stability of solute–CSP adsorbates in all CSPs tested is similar, and therefore a similar recognition mechanism could be expected. Moreover, the elution order of the enantiomers of **2** and **3** on CSPs 6 and 7 is the same as on π-donor CSPs, where a reasonable

Table 1
Elemental analyses of chiral stationary phases

Stationary phase	Elemental analysis (%)			Ratio of carbon atoms per nitrogen atom		mmol of chiral moiety per g of stationary phase (from % N)	α_{exp} [b] (μmol/m^2)
	C	H	N	Analytical	Theoretical[a]		
CSP 1	8.42	1.44	1.10	8.93	10.5	0.39	1.16
CSP 2	8.01	1.33	0.95	9.84	10.5	0.34	1.11
CSP 3	10.36	1.36	2.11	5.73	4.75	0.38	1.26
CSP 4	8.55	1.64	1.03	9.68	10.5	0.38	1.20
CSP 5	10.05	1.59	1.10	10.66	10.5	0.39	1.31
CSP 6	9.47	1.71	2.02	5.47	4.75	0.36	1.21
CSP 7	8.34	1.66	1.96	4.96	3.25	0.35	1.13

[a] Methyl groups coming from the "end-capping" treatment were not taken into account.

[b] Surface concentration of surface-bonded chiral entities, $\alpha_{exp} = \frac{m}{M} \cdot \frac{10^6}{S_{BET}(1-m)}$, where m = mass of functional group (grams per gram of adsorbent), M = molar mass of the bonded functional group (g/mol) and S_{BET} = specific surface area of the starting support (m^2/g) [16].

π-donor–π-acceptor interaction could be established. That is, in both situations the arrangement of the diastereomeric adsorbates could be the same. The 3,5-dinitrobenzoyl group on **2** and **3** may establish a $\pi-\pi$ interaction with the 3,5-disubstituted benzoyl ring on the CSP (π-acceptor–π-donor interaction in the case of CSP 1, 2, 4 and 5 and π-acceptor–π-acceptor interaction in the case of CSPs 6 and 7). Moreover, **1** and **2** are not resolved and $\alpha = 1.02$ in the case of **3**, on a CSP whose chiral selector is N-acetylphenylalanine [3], where the 3,5-dinitrobenzoyl group present on CSP 3 has been replaced by an acetyl group.

From the comparative study of data obtained with CSPs derived from (*S*)-cyclohexylalanine (CSPs 4, 5 and 6), and those obtained with CSPs derived from (*S*)-phenylalanine (CSPs 1, 2 and 3), it can be deduced that the phenyl ring in the phenylalanine moiety does not establish a $\pi-\pi$ interaction with the aryl group in racemic compounds. In all instances, the selectivity values of cyclohexylalanine-derived CSPs are higher than those of phenylalanine-derived CSPs. This lack of $\pi-\pi$ interaction is confirmed by the comparison of the performances of CSPs 3, 6 and 7. The selectivity values of CSP 6 are higher than those of CSPs 3 and 7 and those of CSP 7 are the lowest. These differences seem to have a steric origin. In fact, there are results in the literature in which the presence of hindering groups near the chiral centre, either on the CSP or on the racemic compound, enhances the separation.

The racemic compounds **5–7**, which simultaneously bear a π-donor and a π-acceptor group, are not resolved on π-donor CSPs, in spite of the presence of a 3,5-dinitrobenzoyl group in their structures. This is unexpected if the similarity in the structures of **1–3** (better resolved on π-donor than on π-acceptor CSPs) and **5** and **6** (resolved only on π-acceptor CSPs) is taken into account. In this case the π-donor aryl group on racemic compounds **5–7** seems to interact with the 3,5-dinitrobenzoyl group on the CSPs, as is supposed to happen in compound **8**. It should be noted that, in this instance, the chiral recognition involves the formation of diastereomeric solute–CSP complexes with different arrangements of groups to that of compounds **1–3**. However, in all instances the first enantiomer eluted has the same configuration at the chiral centre (if the absolute configuration of **8** changes, it is because of the inversion in the priority order of substituents on the chiral centre). This is possible even if the diastereomeric solute–CSP complexes were those proposed. In fact, a rotation of 180° could change one arrangement into the other [12].

Table 2
Chromatographic parameters (capacity factor, k_1, selectivity factor, α, and resolution, R_s) for the separation of test compounds

Racemic compound	CSP-1			CSP-2			CSP-3			CSP-4			CSP-5			CSP-6			CSP-7			Mobile phase: $CHCl_3$[b]–heptane
	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	k'_1	α	R_s	
1	0.36	2.06	4.13	0.48	2.35	4.98	0.43	1.52	1.94	0.29	2.61	3.66	0.38	2.94	5.38	0.51	1.64	2.51	0.52	1.41	1.76	75:25
	R/−[a]			*R*/−			*R*/−			*R*/−			*R*/−			*R*/−			*R*/−			
2	0.29	1.94	3.13	0.40	2.01	3.32	0.42	1.46	1.79	0.25	2.25	3.03	0.33	2.35	3.49	0.52	1.49	2.13	0.48	1.37	1.56	75:25
	R/−			*R*/−			*R*/−			*R*/−			*R*/−			*R*/−			*R*/−			
3	0.83	1.74	4.44	1.05	1.96	5.44	0.62	1.56	2.66	0.74	2.08	4.38	0.87	2.63	5.88	0.90	1.61	3.21	0.82	1.36	1.78	75:25
	R/−			*R*/−			*R*/−			*R*/−			*R*/−			*R*/−			*R*/−			
4	6.53	1.07	–	7.51	1.07	–	7.02	1.51	2.30	5.09	1.09	–	4.26	1.12	–	7.63	1.52	2.73	6.71	1.44	3.25	10:90
	–			–			–			–			–			–			–			
5	2.41	1.00	–	2.88	1.00	–	1.73	1.13	1.01	1.92	1.00	–	2.50	1.00	–	2.41	1.28	2.12	2.95	1.06	–	50:50
							R/−			[c]						*R*/−			*R*/−			
6	1.94	1.00	–	2.40	1.00	–	2.53	1.13	1.11	1.51	1.00	–	1.97	1.00	–	3.10	1.44	3.02	3.52	1.13	1.30	50:50
							R/−									*R*/−			*R*/−			
7	1.29	1.00	–	1.94	1.00	–	5.84	1.10	1.03	0.86	1.00	–	1.32	1.00	–	3.51	1.18	1.85	3.83	1.05	–	10:90
							R/−									*R*/−			*R*/−			
8	0.96	1.00	–	1.21	1.00	–	1.39	1.58	3.98	1.13	1.00	–	1.18	1.00	–	1.86	1.60	4.17	2.17	1.25	2.27	75:25
							S/+									*S*/+			*S*/+			

[a] Absolute configuration/optical rotation sign of the first-eluted enantiomer in the solvent used as eluent.
[b] $CHCl_3$ to which 0.5% of MeOH was added.
[c] Using $CHCl_3$–heptane (25:75) as the mobile phase: k'_1 = 11.79 (*R*/−), α = 1.05.

4. Conclusions

In bonded-silica CSPs whose chiral selector is a phenylalanine derivative, the phenyl ring belonging to this moiety seems to have a steric role instead of acting as a π-interacting group in the chiral recognition mechanism.

According to the results obtained, racemic compounds belonging to different structural series, even if they are similar, could interact with CSPs following different patterns, *i.e.*, they could be recognized at different interacting groups.

Acknowledgements

Financial support from the Comisión Interministerial de Ciencia y Tecnologia and from the Generalitat de Catalunya (Project No. QFN91-4201) is acknowledged. T.G. thanks the Comissió Interdepartamental de Recerca i Innovació Technològica (Generalitat de Catalunya) for a doctoral fellowship.

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