Quantitative Chirality/Enantioselectivity Relations in Large Random Supramolecular Structures**

Omer Katzenelson and David Avnir*[a]

Abstract: We study the relationship between shape and enantioselectivity, employing quantitative geometric chirality measurements. The model we use comprises of the boundary surfaces of two-dimensional (2D) chiral, large, random selectors (diffusion limited aggregates), interacting with homologous series of small 2D-chiral S-shaped probes (the selectands). We show how the enantioselectivity of the selectors depends on the chirality of the selectands and report the following findings: I) The enantioselectivity of a chiral selector can switch preference from the "right" to the "left" enantiomer within a homologous series of selectands. II) At this switch point the chiral selector is functionally achiral. III) Within a homologous series of chiral selectands, there is a "resonance of recognition", namely, the classical key–lock concept is replaced by a picture of various degrees of recognition. IV) The degree of enantioselectivity and the switch in handedness preference are the outcome of a complex interplay between the details of the specific geometry of the selector and the selectand, and the global shape parameter of chirality measure. V) It is shown that isochiral selectands, namely selectands of the same chirality value, may be recognized differently by a chiral selector. VI) It is proposed that a more realistic way to treat the issue of minimal points needed for chiral interaction is resolution based. VII) It is shown how to attach handedness to purely random objects.

Keywords: chiral resolution · enantioselectivity · quantitative chirality · supramolecular chemistry

Introduction

We study the relationship between chirality and enantioselectivity. The novel aspect of this study is its quantitative level: We measure the degree of structural chirality of certain selectors and selectands, and show how varying the chirality content affects chiral recognition. We recall that molecular recognition is labeled "chiral" only if the selector (the surface of a chiral chromatographic material, the surface of a chiral catalyst, a chiral active site of an enzyme, etc.), reveals enantioselectivity towards the two enantiomers of the selectand (the substrate of the selector, usually a chiral or a prochiral molecule). This label is needed because chiral selectors may recognize molecular features of chiral selectands, which have nothing to do with their chirality. In this case the selector is incapable of distinguishing between the enantiomers upon interaction. This may happen, for instance, when the number of interaction points involved in the recognition of a certain moiety of the selectand is smaller than the minimal number of points needed for recognizing that the selectand is devoid of an improper element of symmetry; or when the points of the former are located differently than the latter. It is therefore known to practitioners of chiral chemistry, that it is not trivial and not automatically expected that chiral selectors and selectands will exhibit enantioselectivity upon interaction. Understanding the features necessary for this elusive recognition is a quest still going on, and to which we wish to add this report. The main observation that has guided us in this task is that even if enantioselectivity does exist, it can be anywhere on a *continuous* scale from barely detectable to fully developed (namely good recognition of one enantiomer and near zero recognition of the other). The location of the degree of enantioselectivity within this continuous range is dictated by a complex interplay between chemical, functional, and structural parameters, which act in synergism. In this study we focus on the structural parameter from a novel angle, guided by the following rational: If enantioselectivity is a continuous property, let us explore the effects of chirality by the same token, namely as a continuous, measurable quantity. Quantitative structural chirality is briefly described in the section on measurement of chirality.

There are two principle approaches in studying complex interactions between a number of parameters. One is the

[a] Prof. D. Avnir, O. Katzenelson
Institute of Chemistry and the Lise Meitner Minerva Center for Computational Quantum Chemistry
The Hebrew University of Jerusalem
Jerusalem 91904 (Israel)
Fax: (+972)-2-652-0099
E-mail: david@chem.ch.huji.ac.il

[**] Part III of references [1].

  0947-6539/00/0608-1346 $ 17.50+.50/0

study of the whole; the other, which we adopt here, is to isolate the effects of the specific parameters. Since such isolation may be quite difficult in the experiment, modeling has served such purposes successfully. While modeling cannot fully mimic a real situation, it does provide insight on possible trends brought upon by changes of the desired isolated parameter. Indeed, computational analysis of chiral recognition has a well established fruitful history, which traditionally followed two lines: One line has focused on the energy parameter[2–5] and another on geometry and structure.[6–9] Quite often, the latter provided guidelines for the former, and a celebrated example is Fischer's 1894 proposition that a drug interacts with its receptor just as a key fits into a lock[10] and Pauling's 1948 proposition[11] that the geometry of the active site is the complement of the transition state of the bound molecule. Following the order of these developments, this study purely applies geometrical considerations. The extension to energy considerations will be the topic of future reports. Some examples for the successful application of structural considerations in recognition studies are the docking methods developed by Kuntz et al.,[12] Connoly et al.[7] and Katchalski-Katzir et al.[6]

A short reminder on the distinction between chirality and enantioselectivity should be given at this point. While chirality reflects shape and topological characteristics of the structure by itself, be it the selector or the selectand, enantioselectivity relates to functional chirality namely, to the effective structures of the selector and selectands, as "seen" by each other. Thus, enantioselectivity is case specific. It should also be noted that, defining the handedness of a probe, for example with the CIP set of rules, does not assure that recognition of a homologous line of that probe with the same selector will preserve its handedness preference. This is due both to the inherent tentativeness of handedness definitions[13] and to the fact that recognition is sensitive to the specific geometric details of the relevant interacting fragment; this is a main issue in this report, and we return to it below.

The way to measure geometrical chirality is to use geometrical functions and structural parameters, some of which are employed below. On the other hand, functional chirality is determined quantitatively from the extent of actual enantioselectivity, that is from the diastereomeric (geometric) fit.[6, 12] As stated above, the relation between chirality and enantioselectivity is not trivial, even on the purely structural level. It is dictated by specific geometric features such as bond angles, dihedral angles, bond lengths, molecular size and volume, etc., all of which determine the effective geometrical complimentary at the region of contact.

The two novel aspects of this study are the following: First, we use a measure of the degree of chirality, a recently developed global shape parameter based on the quantitative measurement of symmetry,[14–18] in order to determine on a quantitative level how chirality does affect enantioselectivity. It is not at all a priori self-understood that a global shape measure is capable of relating to recognition. However, as we have shown recently for a number of enzymes, the degree of chirality of inhibitors, correlates nicely with their inhibition efficacy.[19] The consequences of the observation of this novel type of correlation are far reaching, and have been discussed in reference [19]. Yet one issue still remains open and has been part of the motivation for the study described here, that is: Given a chirality value and given the global, thermodynamic-like nature of this measure, there is an infinite number of structures this value may relate to. Therefore, in order to gain useful insight from this parameter, one would be better off limiting to homologous families of structures. It is important then to understand the family-specific behavior, which is what we do here.

The second novel aspect is our interest in the enantioselectivity properties of large, random selectors (Figure 1a).[1] Due to the cascading fractal properties of these structures we expect that enantioselectivity will be dependent on both the degree of chirality of selectands and on their size. The interplay between this and other specific geometric parameters and the global parameters is investigated.

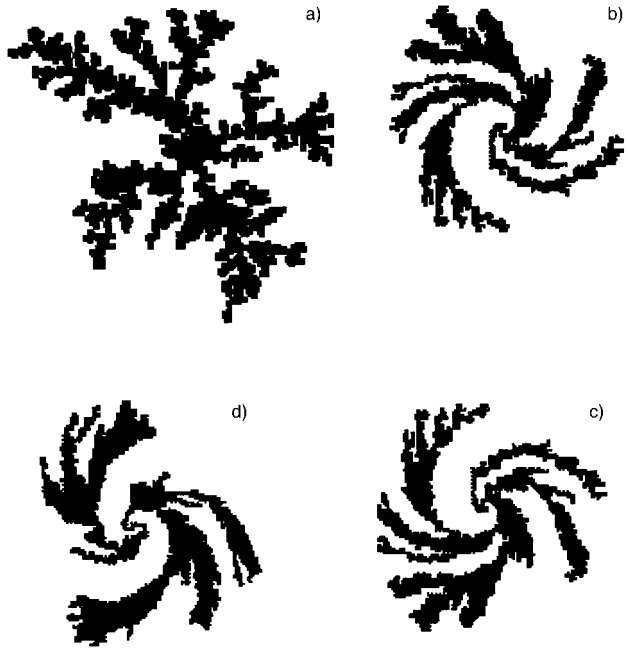

Figure 1. Large random selectors—diffusion limited aggregates. a) An incidentally chiral selector: No chiral bias was added to its construction algorithm. b) An inherently chiral selector: Biased diffusion along spiral lines was added to its construction. c) The virtual enantiomer of b), obtained by its mirror reflection. d) A natural enantiomer of b), obtained by repeating the process that led to b), but in the opposite enantiomeric way. The chirality values of the four structures are 0.99, 4.00, 4.00, and 4.79. Following Bursill,[50] we label b) as "R" (Right, the arms of the structure curl in the direction of the fingers of the right hand, counterclockwise, when the thumb points up), and c) and d) as "L" (left).

Brief summary of the chirality properties of random objects

It is important for the understanding of the enantioselectivity properties of the structures shown in Figure 1, to summarize briefly their main chirality properties.[1] These structures are fractal diffusion limited aggregates[20, 21] (DLAs), and are a prototype to other supramolecular structures which may be chiral such as liquid crystals, polymers, sub-monolayer adsorbate structures chiral chromatographic materials and patterns of bacterial colonies.[22] A central feature of all of these supramolecular structures is that their chirality need not be

the outcome of the chirality of the building blocks.[23] Thus, while the building block of the DLAs is an achiral pixel, the structure manifests chirality on larger scales. Interestingly, we found in our previous report[1a] that the degree of chirality of the DLAs changes barely with size, that is not only is the mass of these fractal-like structures invariant with scale, but so is their chirality.

Questions we shall ask then are what are the consequences of having a structure that is of ill-defined geometry, but yet chiral? How does chiral recognition manifest itself in such cases, if at all? What is the role of the specific handedness of the probe in recognizing the chirality of the selector? Does disorder interfere with enantioselectivity? In order to answer these questions we recall first that chirality analysis of the DLAs lead to the following observations/conclusions:[1]

1) Supramolecular random objects are always chiral even if there was no chiral bias in their construction.
2) A distinction is made between two types of chiral objects: Incidentally chiral objects, namely objects the chirality of which is a pure outcome of their randomness (Figure 1a), and inherently chiral objects, namely objects with a handedness-biased construction (Figure 1b). Measurement of the chirality value was found essential for the analysis of the transition between incidental chirality to inherent chirality.[1]
3) It is practically impossible to obtain the exact enantiomer of such structures (by either repetition of the construction process for an incidentally chiral object, or by repeating it in an opposite handedness mode for an inherent one). The only way to observe the exact enantiomer is to make a mirror-image picture; we termed this a virtual enantiomer (Figure 1c). A pair of natural enantiomers is then obtained by repeating the same (random) procedure of construction, but with opposite handedness; similar objects in their general shape are obtained, although they are not exact mirror images in their details (Figure 1d, which is the natural enantiomer of Figure 1b). We see that natural enantiomers have the interesting property of not being limited to a specific pair: An infinite number of enantiomers is possible.[24]
4) By measuring the degree of chirality on a quantitative scale—using the continuous chirality measure (CCM) methodology[15]—we arrived at another key result: The degree of chirality does not depend on the resolution of observation: De-focus the DLAs pictures and notice that the chirality prevails. However, resolution changes can be affected not only by focusing/defocusing but also by using probe molecules of varying size—this size dependent functional chirality is a main issue of this report. Mezey et al. have introduced alternative resolution-based chirality and similarity measures tailored for large systems.[25]

We also make use in this report of two additional concepts developed in earlier reports.[1, 26] The first is the concept of isochirality, namely, of structures possessing equal chirality values; note that this is made possible only by the use of quantitative measurement of chirality. Thus, two identical structures or a pair of enantiomers are trivially isochiral, however, completely unrelated structures may also be isochiral. The second concept, which we use here as well is that of non-handedness of chiral objects. Since the specific handedness of an enantiomer is only a matter of agreed-upon convention, there is, in principle, at least one chiral structure for which the handedness labeling must collapse (e.g., along a chiral enantiomerization pathway). No handedness assignment can be then given to that structure under the agreed convention. Non-handedness can also be functional, as in the case in which enantiomers do not identify the chirality of circularity polarized light at a certain wavelength; or when the enantioselectivity of a chiral pair is absent. It is this type of non-handedness that we treat below.

Background for the model

General description of the model: Details of the following general description are given in the sections below. Yet it is helpful to keep in mind at this stage the overall approach: We analyze the recognition of small, simple geometry chiral model probe molecules, the selectands, by the large chiral random DLAs just described, the selectors. The recognition level is determined by counting the number of contact points above a threshold value (defined below), between the selector and the selectand, upon legitimate (defined below) complex formation, for all of the complexes. Enantioselectivity is then determined by comparison of the recognition levels of the enantiomeric pairs of the selectands. Parameters tested were the size, shape, and degree of chirality of the selectand, and the degree of chirality of the selector and its history of construction (incidental vs. inherent chirality). Without losing generality and for sake of simplicity, modeling was carried out in two dimensions. Nevertheless, the results of this research are straighforwardly applicable to three dimensions.

Measurement of chirality: The rational, the practical solutions and the applications of chirality measurement by the continuous chirality measure (CCM) methodology, as a special case of the continuous symmetry measure (CSM) approach, were described repeatedly in our previous papers;[1] for a review, see ref. [27]; for other approaches, see ref. [28–47]). We shall therefore limit ourselves here and just mention that the measure evaluates the minimal distance that the vertices of a structure have to be translated to in order to acquire a desired symmetry, which in our case is the nearest achiral point group, usually the $\{E,\sigma\}$ mirror-symmetry group:

$$S_{\mathrm{CCM}} = \frac{100}{n\,D}\sum_{i=1}^{n} \| P_i - \bar{P}_i \|^2 \qquad (1)$$

The boundaries of the symmetry measure are zero (perfect symmetry, perfect achirality) to 100, although maximal chirality values approach lower limits. The interested reader may wish to consult references[14–16, 27] for full details of the property of this measure and for examples of its successful applications.

The selectors: We return now to the selectors: Four families of 2D-chiral DLAs served as selectors, including three groups of inherently chiral DLAs differing in their degree of chirality, and a group of incidentally chiral DLAs.[48, 49] As explained in

  0947-6539/00/0608-1348 $ 17.50+.50/0 *Chem. Eur. J.* **2000**, *6*, No. 8

our previous report,[1] inherent chirality is introduced into the purely random DLA by imposing a spiral shaped bias on the random-walkers that build the cluster. The construction parameters of the DLAs are N, the number of points building the aggregate; the spiral angular and radial curvature parameters Δa and Δr; and p, the probability of the random walker (see ref. [1] for details) to follow the spiral field. The parameters for the construction of the inherently chiral DLAs were $N = 3 \times 10^3$, $\Delta a = 90°$, $\Delta r = 0.2$, $p = 1$, and for the incidental parameter $N = 3 \times 10^3$ and $p = 0$. Due to the elements of randomness, even if a single set of parameters is chosen, a distribution of chirality S_{CCM} values (of the boundaries of the DLAs[49]) is obtained. For the inherent chirality study we have extracted from this distribution three sub-populations of 172, 53, 61 DLAs with S_{CCM} values in the ranges of 2.0 to 3.0, 3.0 to 4.0, and 4.0 to 5.0, respectively, with average S_{CCM} values of 2.702 ± 0.226, 3.052 ± 0.028, and 4.203 ± 0.172, respectively. The average boundary line lengths for these populations were $\langle N_L \rangle = 1731 \pm 136$, 1783 ± 150, 1780 ± 137 pixels, respectively. For a representative structure see Figure 1b. We arbitrarily chose to carry most of the simulations with selectors of "right" handedness (Figure 1b; this convention is taken from Bursill[50] and is explained in the caption of this figure; R- "right"; L- "left"). We confirmed that taking L-DLAs indeed produces identical results. For comparison, 183 DLAs of incidental chirality were also taken, with an average S_{CCM} value of 1.324 ± 0.209 and $\langle N_L \rangle = 3243 \pm 150$. (An example is shown in Figure 1a; an example of how the nearest achiral structure looks like—an outcome of applying the CCM methodology—is given in Figure 5 of ref. [1].)

The selectands: The homologous 2D-chiral probe series we chose comprises of the C_2 symmetric right-angle "S" shapes shown in Figure 2. The C_2 symmetry was introduced in order to equalize the opposite sides of the selectand towards interaction with the selector. We label the handedness of the selectands r- "right" or l- "left" (to be distinguished from R, L of the selectors), following the same rule applied for the selectors, as explained in the caption of Figure 2. This general shape provides in fact two possible homologous series. In one homologous series the ratio of lengths of one of the outer arms to the center arm (the "ratio") gradually changes from zero to infinity, while keeping the total length (the "size") constant. Another homologous series is obtained by gradual change of the size, while keeping a selected ratio constant. Since S_{CCM} is size normalized, it is constant for this homologous series, but varies within the ratio-changing series, as shown in Figure 2. Let us explain this variation: At very low and very high ratios, the shape approaches that of a straight line, and S_{CCM} drops therefore close to zero. Following a nearly bell-shaped curve (on a semi-logarithmic scale), it passes then through the most chiral shape, characterized by a ratio of outer-arm/center-arm of 0.7, with $S_{CCM} = 3.783$. Left to the maximum, the selectands are with ratios smaller than 0.7, and right to it, with ratios larger than 0.7. (The maximum S_{CCM} value does not coincide with the ratio of one; intuitively, a selectand of ratio 1 can be visualized as a superposition of two achiral equal length of the L-shaped

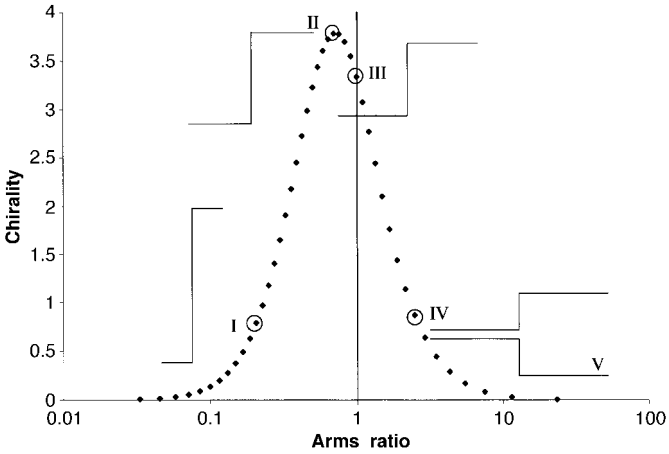

Figure 2. The selectands chirality as a function of the ratio of the arms at fixed total length (94 pixels). Examples of various ratios of the arms are shown: Selectands I and IV are nearly isochiral, having similar chirality contents. II is the most chiral one, and III has a ratio of the arms of 1. The handedness assignment of the selectands follows the same rule of Figure 1: Locate the right-hand thumb up at the center of the selectand; if the arms curl as the fingers, the selectand is labeled "r"; thus, selectands I – IV are "l" and V is the "r" enantiomer of IV. Under this definition, the full graph refers to an "l" homochiral homologous series.

arms). Figure 2 also helps to distinguish between the chirality parameter and the geometric parameter. In this set of selectands, for each chirality value there is a pair of isochiral selectands, with different ratios of the arms. Within the interval of ratios of the arms 0.5–1.0, the two isochiral structures are both with ratios < 1, and outside the interval one selectand in this pair with a ratio > 1 (Figure 2). It will be interesting to see (below) how the selector distinguishes, if at all, between these two isochiral structures. Finally, the enantioselectivity of the DLAs towards the series of selectands depends not only on their structural parameters, but also on the number of contact points between the selector and the selectand within their complex (the "complexation score"). Having all of these parameters in mind, it is already evident as we shall see below, that homologous trends do not necessarily imply simple monotonous enantioselectivity change behavior.

Returning to the size parameter, we note that its variation is of relevance to the fractal-like features of the DLAs: Their structure is a cascade of self-similar features of various sizes, and it has been shown in many previous studies that surface (boundary) accessibility of such structures to molecular interactions is highly size dependent.[51] To study the effects of size we took a homologous set of selectands with fixed ratio of 0.5, varying in size from 14 to 142 pixels ($\langle S_{CCM} \rangle = 3.291 \pm 0.121$). The second homologous set of selectands we took reflects our central theme, namely a set of selectands varying in the degree of chirality but fixed in size (94 pixels) with S_{CCM} values in the range 0.005 to 3.783 and dropping down again to 0.003, following the bell shape of Figure 2. In many realistic cases of a homologous chiral series, both he size and the degree of chirality change and the separation of the two effects, as done here is practically impossible.

The adsorption model and the determination of enantioselectivity

The selector-selectand complex and the complexation score: The probe is placed on the contour of the selector as shown in Figure 3. No penetration of the selectand into the selector is allowed. We call the selectand–selector pair, a complex, and define the complexation score as the number of contact points between the selectand and the selector. (The complexation score is related to the enthalpy of complexation, when all interaction forces are equal.) Enantiomers may have either a similar score at the complexation site, or a different one (Figure 3). In order to be counted, the score has to overcome a minimal threshold value (the "reaction barrier"). This was set up to be smaller than the size of the smallest selectand used, namely six pixels, since we found that smaller thresholds blur enantioselectivity; we return to the threshold issue and to the question of the minimal number of contact points needed for recognition later on in the Discussion. Interestingly, we found that the number of legitimate complexes obtained is insensitive to the selected mutual orientation, and that therefore orientation need not be optimized. This is due to the fact that the selector has an extremely convoluted geometry, which therefore, tends to average the orientation effect. Thus, in our simulation we took only the extremes, namely zero and ninety degree orientations (Figure 3). All contour points of the selector were tested as to their capability to form an allowed impenetrable complex, the complexation score was determined for each complex (Figure 3), and the total number of legitimate complexes above a selected threshold, N_r and N_l recorded. This was done on R-selectors with both r- and l-selectands, and we confirmed that the L-selectors gave the same output. (Placement of the selectands along the contour line of the selectors was done from the third dimension.)

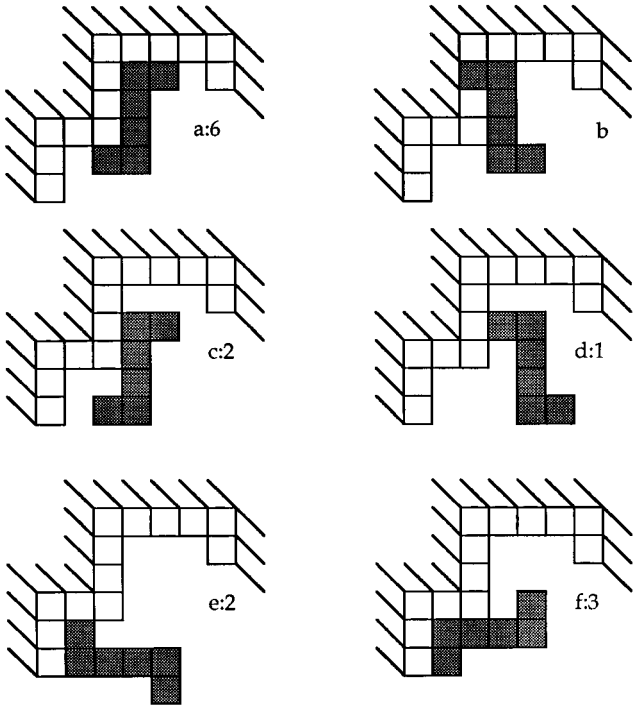

Figure 3. Placements of selectands (black) on the selector boundary line (white boxes). a) A legitimate placement of an l-selectand with a complexation score of 6. b) Illegitimate placement of the r-enantiomer at that location. c) Movement of the l-selectand by one step; the score drops to 2. d) The r-enantiomer can now be placed legitimately at that location, but its score is only 1. e) A 90° rotated placement of the l-selectand with regard to a) plus a translation step, the score is 2. f) Here, however, the rotated r-enantiomer has a higher score of 3.

In order to get an estimation on the complexation ability of the selector towards a specific selectand, we define the average score as

$$\langle Score \rangle = \frac{\sum_{Score>\text{threshold}} \text{Score}}{\sum_{\text{Score}>\text{threshold}} Complex\ formation} \quad (2)$$

that is, it is the sum of all complexation scores for that selectand, normalized to the number of legitimate complex formations above a threshold.

Determination of enantioselectivity: The quantitative enantioselectivity E is based on the comparison between R-r and R-l recognition level. This can be done in several related ways. We chose to determine it—as commonly done in chromatography—by using the ratio between N_r and N_l, the total number of legitimate complexes of the r- and l-selectands, as defined above:

$$E_n = \frac{N_r}{N_l} \quad (3)$$

Since randomness is involved, we average E_n over the number g of all selectors in the examined subgroup:

$$\langle E_n \rangle = \frac{\sum_{i=1}^{g} E_n^i}{g} \quad (4)$$

No enantioselectivity is reflected by value of 1. By convention, values greater than 1 mean "r" preference, while values smaller than 1 mean "l" preference. Alternatively, one could express enantioselectivity in various related definitions, for instance:

$$E_r = \frac{\langle Score(R-r) \rangle}{\langle Score(R-l) \rangle} \quad (5)$$

However, we found that this and other alternative definitions provide as expected similar results, so we chose the simplest, namely Equation (4). Finally, note that enantioselectivity measures functional chirality: The selectands may be geometrically chiral, yet the selector need not necessarily detect this chirality; we return to this point below.

Results and Discussion

The effect of the handedness of the selectand on enantioselectivity: Figure 4 shows how the enantioselectivity of two sets of R-selectors depends on the degree of chirality of the probes series of Figure 2. Intuition would perhaps suggest that higher probe chirality means better enantioselectivity; and that an R-selector will be recognized better than an r-selectand. The main result of this study is that this naive picture is far from being true: Above a certain level of chirality, enantioselec-

0947-6539/00/0608-1350 $ 17.50+.50/0 *Chem. Eur. J.* **2000**, *6*, No. 8

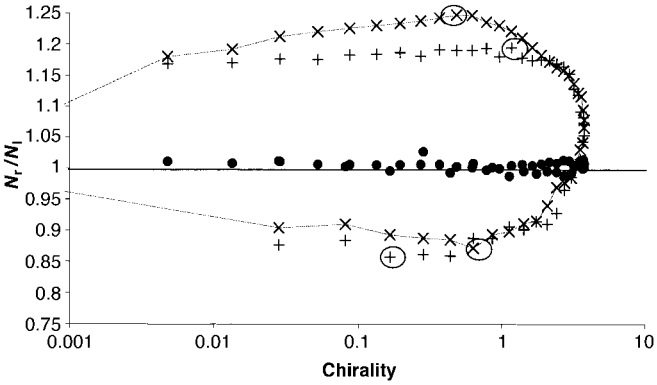

Figure 4. Enantioselectivity of R-selectors vs. selectand chirality. The selectors used were a group of 172 DLAs (×) with chirality values in the range of 2.702 ± 0.226 and a group of 61 DLAs (+) with chirality within the range of 4.203 ± 0.172. Each point represents the average of interactions of the specific selectand with all the selectors in the subgroup. The control group (●) comprises of 183 incidental DLAs with chirality of 1.324 ± 0.209, providing an uncertainty value of 0.008. Above the horizontal line the selectors prefer "r" selectands, while below it the preference is of "l". Encircled are the best recognized selectands: chirality content of 1.173 and 0.167 for r- (top) and l-enantioselectivity (bottom) for the (×) selectors, and 0.489 and 0.637, respectively, for (+).

tivity drops sharply and vanishes, where chirality is close to its maximal value; and within the pair of isochiral selectands, the R-selector prefers one to be r, but the other one to be l (see the two $\langle E_n \rangle$ values for each of the chirality levels in Figure 4). In particular, the limits of the selectors to enantioselect from within this chiral set of selectands, is evident: $\langle E_n \rangle$, by definition, is 1 for zero chirality (the left end of the figure) and it drops to 1 again for selectands with chirality values around 3.5 due to the functional nonhanded chirality of the selectors within this range (a result which will be explained shortly). Interestingly, this implies therefore, as seen in Figure 4, that for selector–selectands sets there must be a "resonance of recognition", namely, that there must be a selectand for which recognition was most effective, so that to the left and right, the enantioselectivity drops (Figure 4). Thus, enantioselectivity, like the chirality measure itself, is not a term of "either–or", but rather of "how much" on a continuous, gradual level. In other words, the classical key–lock concept of recognition, should contain gradations. A blank test of the enantioselectivity–chirality relations with respect to probe size showed the same type of relations (not shown). Finally, the fact that chirality plays a role in this observation is evident from the blank test carried out with incidentally chiral selectors: 183 such DLAs with chirality content of 1.324 ± 0.209 rendered $\langle E_n \rangle$ values around 1. The standard deviation of this blank test was found to be 0.008 and it was used as the uncertainty for the inherent chiral selectors. (It is smaller than 1 % of the $\langle E_n \rangle$ values and therefore cannot be seen in the Figure.) In order to understand the complex behavior of enantioselectivity as a function of chirality, we now go back to the analysis of the ratio of the arms effect on chirality (Figure 2), and analyze its effects on enantioselectivity.

Effects of the ratio of the arms of the selectand on enantioselectivity: These are shown in Figure 5; it is seen that for ratios <1 (see Figure 2), the sets of R-selectors show r-selectand preference, while for ratios >1, the l-selectand is preferred. (The background enantioselectivity for the incidental DLAs is 1.004 ± 0.008.) The resonance of recognition seen in Figure 4, is even more pronounced in Figure 5. The nonhandedness of Figure 4 is seen here around the ratio of 1: Despite of being chiral selectands, the selectors do not detect it. Since enantioselectivity also does not exist for the extreme ratios where the probe is an achiral line segment, enantioselectivity as a function of ratio, must pass through two extremes, one for the ratio <1 part, and one for the ratio >1; which, as seen in Figure 5, is the case. The best ratios for

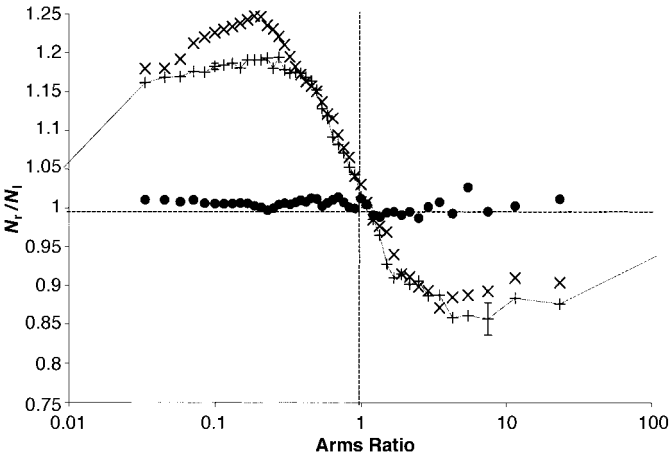

Figure 5. Enantioselectivity as a function of selectands ratio of the arm (for fixed size of 94 pixels; the selectands and selectors of Figures 2 and 4 are used here as well). The best enantioselectivities are for arm ratios of 0.274 (of r-handedness for the X selectors), 7.50 (l, X), 0.185 (r, +), and 3.50 (l, +). See text for discussion. The incidentally chiral DLAs are shown as well (●), providing an uncertainty level of 0.004.

enantioselectivity are shown in this Figure as well. These maximal values are obtained for both very short and very large arm ratios, which represent elongated shapes of good penetrability. In other words, the selector's cavities shapes dictate better preference towards selectands with a rather small or rather large ratio of the arms. Indeed the number of complex formation increases with the arm ratios (not shown). To understand the behavior of Figure 5 and its relation to Figure 4, one should note that the recognition act does not involve the whole of the selectand, but only the regions that penetrate and form a legitimate complex. Thus, in most instances, the penetrating complexing portion of the selectand is mainly one of the L-shaped ends of the selectand; the frequency of complexation that includes the whole probe's points is rare. It becomes clear now, why r-selectands are preferred for ratios <1, while l is preferred beyond this value, see Figure 6: For ratios <1, the handedness of the L-ends of the selectands, which form the complex, is the same as the handedness of the whole selectand; however, for ratios >1 the handedness of the complexed L-ends is opposite to the handedness of the whole. Thus, if only the handedness of the penetrating, interacting portion is taken into account, then preference of r-ends is preserved throughout the whole series. The lack of enantioselectivity around ratio of 1, reflects not

only by the bulkiness, but also by the achirality of equal L-shaped arms. Figure 6 shows also why selectands with ratios <1 have more distinct enantioselectivity values: Their penetrating tip is chiral while the penetrating tips of selectands with ratios >1 are achiral.

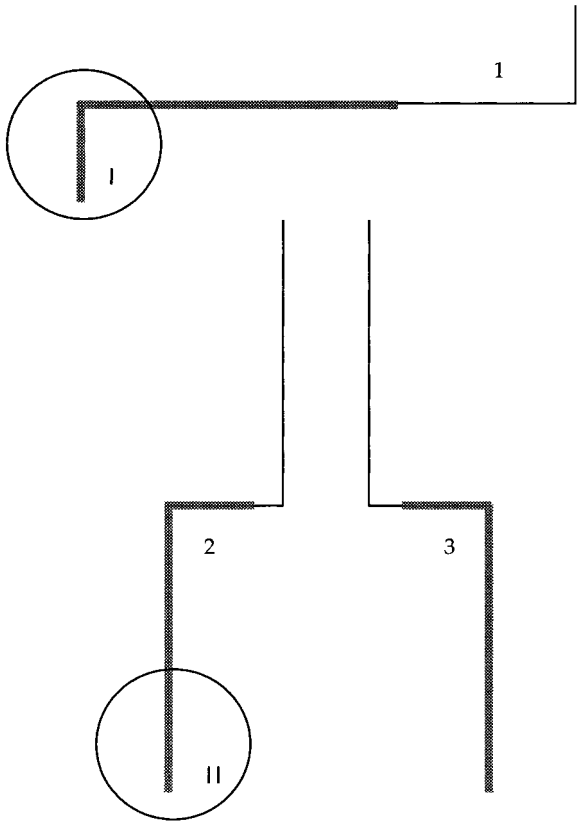

Figure 6. a) Explaining the switch in the preference of R-selectors from r-selectands to l-selectands, as the ratio of arms' changes: The handedness of (I) is r, and let us label the local handedness of the thick portion of it—the portion which participates in the complexation—arbitrarily also as r. As the ratio of arms' changes from <1 to >1, as in (2), the handedness of the whole is still r, but the handedness of the interacting darkened portion is opposite to that in (1), namely l. To preserve the preference of the selectors towards r-selectands as in 1), the handedness of the interacting portion for ratios >1 must also be r, which means, see (3), that the handedness of the whole is l. The higher recognition of the small arm ratios selectands is due to the fact that the penetrating tip I of selectand 1 is chiral while that of 2, II, is achiral.

Size effects of the selectand on enantioselectivity: While the building blocks of the DLAs are achiral pixels, the structure manifests chirality on larger scales. It is therefore expected that the enantioselectivity of DLAs, namely their functional chirality will be scale dependent, and will change with the size of the probe. This is indeed shown in Figure 7 for fixed ratio of the arms of 0.5 (and average chirality level of 3.291 ± 0.121) (at this point, see only the threshold of 6 in this Figure; the effect of lowering the threshold is discussed below) and for size ranges from 14 pixels up to 142 pixels, where the long arm of the selectand is approximately equal to the selector's radius. It is seen that there is a maximum around 90. The passage through a maximum is dictated by the fact that selectands, which are either too small or too large, will not

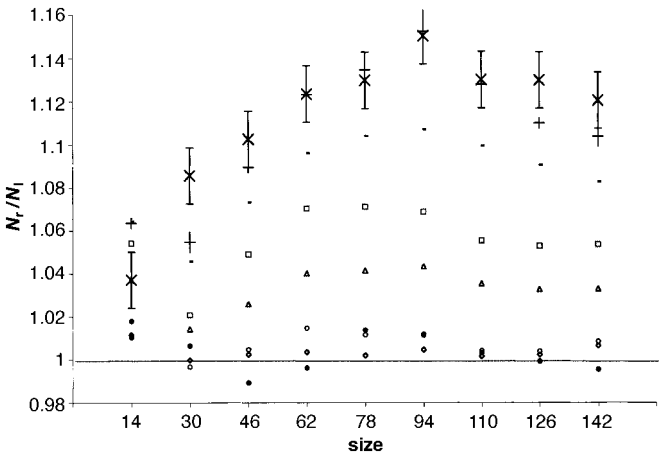

Figure 7. Selectands size effect (r-handed, fixed chirality value of 3.291 ± 0.121, ratio of the arms of 0.5) on the average enantioselectivities of two groups of inherently chiral selectors (X, +), as in Figure 4, at a complexation threshold value of 6. Also shown is the effect of lowering the threshold values from 6 to 1 (+, −, □, △, ○, ◇, respectively). The control group (●) comprises of 183 incidental DLAs with chirality of 1.324 ± 0.209, providing an uncertainty level of ± 0.013.

detect the chirality of the selectors: For the small size range, the selectands must reach the size where the achiral pixels do begin to show supramolecular structure, and it is seen that this happens around 15 pixels. For the upper range, a probe which is too coarse will again miss the structural details of the selector. Again, as a control group we used the incidental DLAs. Their $\langle E_n \rangle$ was 1.004. The uncertainty for the inherently chiral selectors was taken as half of the control group standard deviation 0.026.

The effects of the threshold value for complexation: A major issue in chromatographic literature has been the minimal number of contact points needed for chiral recognition.[52] Celebrated examples are the studies of Dalglish,[53] which Pirkle restated as[54] "chiral recognition requires a minimum of three simultaneous interactions between the chiral stationary phase (the selector) and at least one of the enantiomers, with at least one of these interactions being stereochemically dependent". By analogy, in our 2D case, the minimal contact points needed for enantioselectivity by a specific site on the selector, cannot be less than two.[54] However, we propose that trying to answer the question of the minimal number of contact points needed for chiral recognition in terms of a universal "magic number", may be an oversimplification in the case of complex geometry materials, such as in most of the chromatographic materials. The aspect to be considered in this context is the behavior of the selector as a population of sites. Figure 7 shows, in addition to size effects, how enantioselectivity of the full boundary of the selectors, with its many geometrical features, depends on the threshold level, namely on the number of minimal contact points with the selectand. It is seen that if there is no threshold, namely all complexes are counted (threshold of one point of contact), no enantioselectivity is apparent. As the threshold increases, enantioselectivity becomes more pronounced, and for the higher threshold values an extreme in recognition appears. It is important to realize that in a given population of selectands,

  0947-6539/00/0608-1352 $ 17.50+.50/0 *Chem. Eur. J.* **2000**, *6*, No. 8

many will not have their chirality recognized at all, at any threshold level. Furthermore, as the threshold increases, the absolute number of legitimate complexes drops sharply. In Figure 7, for example, the number of average complex formations for each selector decreases from 4852 through 4117, 2721, 1963 and 1304 down to 903 as the threshold increases from 1 to 6 for the r/R complexes, and the enantioselectivity "is on the shoulders" of a rather small population of all selectands: An average of 11.0 ± 2.7 % of the total complex formation ($N_r - N_l/N_r + N_l$; not shown). Since actual chromatography includes so many different features of the surface geometry of the chromatographic material, and so many orientations of contact with the selectands, we believe that the gradual picture of Figure 7 provides a broader and fuller answer to the question of the minimal number of contact points. Furthermore, according to our approach, the answer to the question of "what is the number of minimal points?" is not absolute, but a matter of the analytical tool resolution. Thus, whereas there is a barely detectable enantioselectivity for a threshold of two, for practical purposes the answer of minimal number of points may be, for example, four, for which enantioselectivity is better pronounced. A gradual, resolution-dependent answer seems to us of better practical value.

Orientation: As explained in last section, results of the simulation should have only small overall sensitivity to selectand orientation. Indeed, taking, for instance, a selectand of size 94 pixels, ratio of the arm = 0.5, $S_{CCM} = 3.23$ and a set of 60 selectors of chirality values in the range 4.203 ± 0.172, the preferences were for the r-selectand with enantioselectivity values of 1.23 ± 0.43 and 1.24 ± 0.38 for 0° and 90° orientations, respectively.

The chirality of the selectors: The preference of the inherently chiral selectors towards one of the enantiomers was consistent for selectors with chirality values of at least 2. For instance, for a selectand of size 94, ratio of arms of 0.5 and $S_{CCM} = 3.23$, an average enantioselectivity of 1.19 ± 0.11 was obtained for the group of 172 selectors with chirality values between 2 and 3 (2.702 ± 0.226), an average enantioselectivity of 1.18 ± 0.09 was obtained for the group of 53 selectors with chirality values between 3 and 4 (3.052 ± 0.028), and an average enantioselectivity of 1.25 ± 0.21 was obtained for the group of 61 selectors with chirality values between 4 and 5 (4.203 ± 0.172).

The case of the incidentally chiral selectors is different and interesting. Here, the individual handedness of each selector is dictated by the specific random process of its built-up and is manifested by handedness preference of a specific selectand. A population of purely random DLAs is expected to be roughly equally divided in its effective handedness. Thus, using the same selectand (with ratio of arms of 0.5) with 49 random DLAs, 25 selectors showed values smaller than one and therefore can effectively be labeled L-selectors, and 24 given values greater than one, leading to an R-label. Examples for left-handed randomness and right-handed randomness, are shown in Figure 8. It is a general practical way for the attachment of effective handedness to random structures.

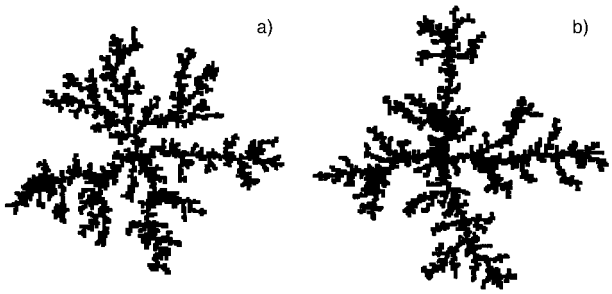

Figure 8. The handedness of pure randomness. Incidental chiral selectors can show opposite enantioselectivity towards the same selectand (size of 94 with ratio of arms of 0.5 in this case): a) An "R" DLA with $S_{CCM} = 0.576$ showing r-selectand preference of $E_n = 1.040$, and b) An "L" DLA with $S_{CCM} = 0.741$ showing l-selectand enantioselectivity with $E_n = 0.868$.

Above, Equation (2), we defined the average complexation score towards a specific selectand. Using this score we find that in all cases (Figures 4, 5, and 7) inherently chiral selectors have a higher complexation scores, namely that they interact better, than incidentally chiral selectors. Thus, for a threshold of 6, the average scores of inherent chirality interactions were $6.00 + 2.11$ and $6.00 + 1.84$ for the fixed size and for the fixed chirality selectands, respectively, while the average scores of incidental interaction were $6.00 + 1.52$ and $6.00 + 1.49$ for the fixed size and for the fixed chirality selectands, respectively.

Conclusion

Motivated by the success of the use of quantitative measures for symmetry in general[27] and chirality as a specific case,[19] we investigated how this measure correlates with enantioselectivity, and how it relates to the specific geometry details of the selectors and selectands. The main results of this study are summarized in the Abstract. They show the importance of a global shape descriptor, and identify the bounds within which it should be practiced. Following the theme of many of our previous publications in this field, we show in fact that the classical, sharp key–lock picture of Fischer[10] should serve as a starting point only; reality is much more complex, and a better understanding of enantioselectivity is achievable by gradual, gray level analyses that quantify not only exact geometry, but overall shape as well. In addition, we showed that for complex interactions (as described here), the question of resolution is of great importance (Figure 7) in providing an answer to the question of the minimal contact points needed for chiral recognition. Finally, the issues of enantioselectivity we highlighted in this study and the conclusions we have drawn, go beyond the specific set of selectors–selectands we have selected, and apply to recognition in general, and to recognition by complex systems in particular.

[1] a) O. Katzenelson, H. Zabrodsky, D. Avnir, *Chem. Eur. J.* **1996**, *2*, 174; b) D. Avnir, O. Katzenelson, H. Zabrodsky, *Chem. Eur. J.* **1996**, *2*, 744.
[2] Y. P. Pang, A. P. Kozikokowski, *J. Comput.-Aided Mol. Des.* **1994**, *8*, 669.
[3] L. Yeo, K. D. M. Harris, *Tetrahedron: Asymmetry* **1996**, *7*, 1891.

[4] G. M. Morris, D. S. Goodsell, R. Huey, A. J. Olson, *J. Comput.-Aided. Mol. Des.* **1996**, *10*, 293.
[5] K. B. Lipkowitz, R. Coner, M. A. Peterson, *J. Am. Chem. Soc.* **1997**, *119*, 11269.
[6] E. Katchalski-Katzir, I. Shariv, M. Eisenstein, A. A. Friesem, C. Aflalom, I. A. Vakser, *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 2195.
[7] M. L. Connolly, *Biopolymers* **1986**, *25*, 1229.
[8] R. L. DesJarlais, R. P. Sheridan, G. L. Seibel, J. S. Dixon, I. D. Kuntz, R. Venkataraghavan, *J. Med. Chem.* **1988**, *31*, 722.
[9] B. K. Shoichet, I. D. Kuntz, *Protein Eng.* **1993**, *6*, 723.
[10] E. Fischer, *Chem. Ber.* **1894**, *27*, 2985.
[11] L. Pauling, *Nature* **1948**, *161*, 707.
[12] I. D. Kuntz, J. M. Blaney, S. J. Oatley, R. Langridge, T. E. Ferrin, *J. Mol. Biol.* **1982**, *161*, 269.
[13] Y. Pinto, D. Avnir, unpublished results.
[14] H. Zabrodsky, S. Peleg, D. Avnir, *J. Am. Chem. Soc.* **1992**, *114*, 7843.
[15] H. Zabrodsky, D. Avnir, *J. Am. Chem. Soc.* **1995**, *117*, 462.
[16] H. Zabrodsky, S. Peleg, D. Avnir, *J. Am. Chem. Soc.* **1993**, *115*, 8278.
[17] K. B. Lipkowitz, D. Gao, O. Katzenelson, *J. Am. Chem. Soc.* **1999**, *121*, 5559.
[18] S. Grimme, *Chem. Phys. Lett.* **1998**, *297*, 15.
[19] S. Keinan, D. Avnir, *J. Am. Chem. Soc.* **1998**, *120*, 6152.
[20] P. Meakin in *The Fractal Approach to Heterogeneous Chemistry* (Ed.: D. Avnir), Wiley, Chichester, **1989**, p. 131.
[21] P. Meakin, *Heterogen. Chem. Rev.* **1994**, *1*, 99.
[22] E. Ben-Jacob, O. Shochet, I. Tenenbaum, I. Cohen, A. Czirok, T. Vicsek, *Fractals* **1994**, *2*, 15.
[23] G. Heppke, D. Moro, *Science* **1998**, *279*, 1872.
[24] In ref. [1] we added a statistical aspect to Kelvin's definition (L. Kelvin, *Baltimore Lectures* **1884**, 436), in order to accommodate the chirality properties of random objects such as the DLAs. Some further clarifications are as follows: The ultimate test of whether an object, random or otherwise, is chiral, is Kelvin's test of non-superimposability with the mirror image. In the bulk of chemistry, that mirror image molecule is obtainable by realization of the synthetic procedure in an opposite enantiomeric route. Unlike chiral molecules, which are synthesized such that the final structure is known exactly, in random processes, the exact outcome is unknown a priori. Therefore, the realization towards an enantiomer of a random structure becomes problematic. One has then to distinguish between two types of realizations: One, which leads to the exact enantiomer, as in the case of synthesis of chiral molecules, and one which repeats the process towards an object with a different exact structure though with a similar shape. A distinction was therefore made between a virtual enantiomer and a natural enantiomer, for these two realizations. We use the term "virtual" for the first case, because repetition of the random synthetic process can never lead to the exact enantiomer. For each specific family of incidental random objects there is a minimal level of chirality (the "noise" level of chirality). This does not hold for the virtual enantiomer: It is obtained by strict rules of exact copying as in molecular synthesis, using, for instance, actual photocopying or a preset rule of exact duplication of each random step with its mirror image step, either simultaneously or consecutively. In this context we recall that the chirality measure we employ here, is based on the comparison of an object with its exact (virtual, in our case) enantiomer.
[25] P. G. Mezey in *New Developments in Molecular Chirality* (Ed.: P. G. Mezey), Kluwer, Netherland, **1991**, p. 257 and p. 241.
[26] Y. Pinto, Y. Salomon, D. Avnir, *J. Math. Chem.* **1998**, *23*, 13; Y. Pinto, H. Z. Hel-Ol, D. Avnir, *J. Chem. Soc. Faraday Trans.* **1996**, *92*, 2523.
[27] D. Avnir, O. Katzenelson, S. Keinan, M. Pinsky, Y. Pinto, Y. Salmon, H. Z. Hel-Or, in *The measurement of Symmetry and Chirality: Conceptual Aspects* (Ed.: D. H. Rouvray), Wiley, New York, **1997**, p. 283.
[28] N. Weinberg, K. Mislow, *J. Math. Chem.* **1995**, *17*, 35.
[29] N. L. Allan, D. L. Cooper, *J. Chem. Inf. Comput. Sci.* **1992**, *32*, 578.
[30] P. J. Artymiuk, P. A. Bath, H. M. Grindlyey, C. A. Pepperrell, A. R. Poirrette, D. W. R. Thorner, D. J. Wild, P. Willett, F. Allen, R. Yaylor, *J. Chem. Inf. Comput. Sci.* **1992**, *32*, 617.
[31] P.-A. Guye, *C.R. Hebd. Seances Acad. Sci.* **1890**, *110*, 714.
[32] G. Derflinger, in *Chirality: From Weak Bosons to the α-Helix* (Ed.: R. Jansoscheck), Springer, Berlin, **1991**, Ch. 3.
[33] P. G. Mezey, in *A Global Approach to Molecular Chirality* (Ed: P. G. Mezey), Kluwer, Dordrecht, The Netherlands, **1991**, p. 257.
[34] V. E. Kuz'min, I. B. Stel'mach, M. B. Bekker, D. V. Pozigun, *J. Phys. Org. Chem.* **1992**, *5*, 295.
[35] M. Raji, A. Cosse-Barbi, *C.R. Acad. Sci. Paris* **1997**, *II*, 51.
[36] E. Ruch, *Acc. Chem. Res.* **1972**, *5*, 49.
[37] A. Cosse-Barbi, M. Raji, *Struct. Chem.* **1997**, *8*, 409.
[38] S. S. Tratch, N. S. Zefirov, *J. Chem. Inf. Comput. Sci.* **1996**, *36*, 448.
[39] R. Chauvin, *J. Math. Chem.* **1996**, *19*, 147.
[40] R. Chauvin, *J. Phys. Chem.* **1992**, *96*, 4706.
[41] M. Petitjean, *J. Math. Chem.* **1997**, *22*, 185.
[42] M. A. Osipov, B. T. Pickup, M. Fehervari, D. A. Dunmur, *Molec. Phys.* **1998**, *94*, 283.
[43] A. V. Luzanov, V. V. Ivanov, R. M. Minyaev, *J. Struct. Chem.* **1998**, *39*, 261.
[44] G. Gilat, in *The concept of structural chirality* (Ed.: D. H. Rouvray), Wiley, New York, **1997**, p. 325.
[45] G. Gilat, Y. Gordon, *J. Math. Chem.* **1994**, *16*, 37.
[46] G. Gilat, *J. Math. Chem.* **1994**, *15*, 197.
[47] G. Gilat, *J. Phys. A*, *Math. Gen.* **1989**, *22*, L545.
[48] E. Ben-Jacob, I. Cohen, O. Shochet, A. Tenenbaum, A. Czirok, T. Vicsek, *Phys. Rev. Lett.* **1995**, *75*, 2899.
[49] T. Nagatani, F. Sagues, *J. Phys. Soc. Jap.* **1990**, *95*, 3447.
[50] L. A. Bursill, J. L. Rouse, A. Needham, in *Spiral Symmetry* (Eds.: I. Hargittai, C. A. Pickover), World Scientific, Singapore, **1992**, p. 295.
[51] P. Pfeifer, M. Obret, in *The Fractal Approach to Heterogeneous Chemistry* (Ed.: D. Avnir), Wiley, Chichester, **1989**, p. 31.
[52] D. R. Taylor, K. Maher, *J. Chromatogr. Sci* **1992**, *30*, 67.
[53] C. E. Dalglish, *J. Chem. Soc.* **1952**, *47*, 3940.
[54] W. H. Pirkle, T. C. Pochapsky, *Chem. Rev.* **1989**, *89*, 347.

Received: May 28, 1999 [F1815]

  0947-6539/00/0608-1354 $ 17.50+.50/0