Chirality

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Systems Chemistry: Kinetic and Computational Analysis of a Nearly Exponential Organic Replicator**

Maik Kindermann, Insa Stahl, Malte Reimold,
*Wolf Matthias Pankau, and Günter von Kiedrowski**

In 1997, Wang and Sutherland reported a case of self-replication in a Diels–Alder reaction.[1] This system is remarkable for at least two reasons: it constituted the first example of nearly exponential growth and it had the potential to exhibit two kinds of self-replication, homochiral autocatalysis and heterochiral cross-catalysis, because a chiral diene was employed. Unfortunately, the authors could not complete a stereochemical analysis of their system. We report herein mechanistic and stereochemical studies of variants of the Wang–Sutherland replicator. We show that only one of four potential diastereomers is formed within our detection limits and that this diastereomer is involved in both autocatalytic and cross-catalytic channels. A computational study suggests that the system can be classified as an example of a nearby

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[*] Dr. M. Kindermann,[+] I. Stahl,[+] M. Reimold, Dr. W. M. Pankau,
Prof. Dr. G. von Kiedrowski
Lehrstuhl für Organische Chemie I
Ruhr-Universität Bochum
Universitätsstrasse 150, 44780 Bochum (Germany)
Fax: (+49) 234-32-14355
E-mail: kiedro@rub.de

[+] These authors contributed equally to this work.

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strong-exponential replicator according to our minimal replicator theory.[2]

Self-replication of chiral information has recently received considerable interest from its relevance to the question of the breaking of chiral symmetry on the early earth[3]. An impressive implementation of Frank's model[4] now exists in the reaction of Soai et al., whose kinetic analysis has recently revealed important insight.[5] Further examples of self-replication with prochiral precursors were successfully designed for cycloaddition reactions.[6] Self-replication experiments with peptides as chiral building blocks constituted the first example of homochiral autocatalysis in the sense that templating requires the same helicity, as in the case of template precursors.[7] The general predictability of homochiral autocatalysis versus heterochiral cross-catalysis is, however, far less pronounced for molecules without a predefined secondary structure (Figure 1).

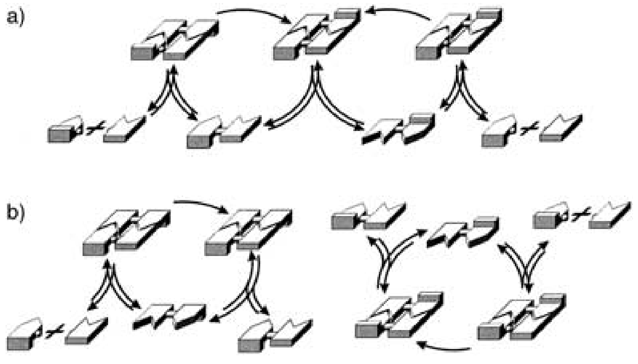

Figure 1. Principle of a) heterochiral cross-catalysis and b) homochiral autocatalysis.

We therefore became interested in the re-evaluation of the Wang–Sutherland system with respect to its stereochemical features.[1] Our approach is guided by our curiosity as to whether a combination of kinetic, computational, and theoretical methods could lead to a better understanding of structurally simple but dynamically complex systems. The components and principle reactions of the Wang–Sutherland system are shown in Figure 2a.

We thought that the replacement of the heterocyclic recognition units by amidopyridines and carboxylic acids, independently used by the research groups of Hamilton[8] and Philp,[6] could also expand the solubility range in the Wang–Sutherland system. Our components and reaction products as

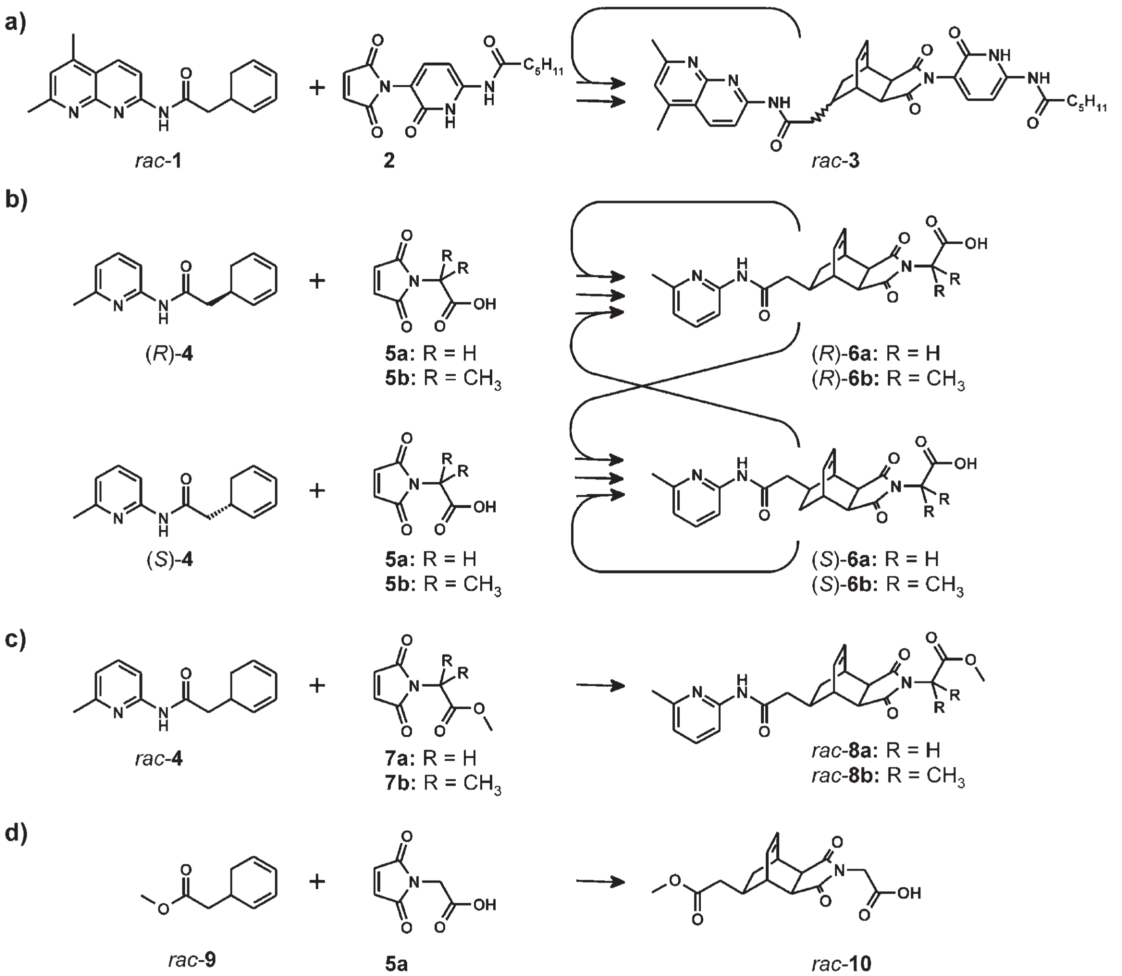

Figure 2. Reaction schemes; each arrow indicates a distinguishable reaction channel. a) Self-replicating Diels–Alder reaction as described by Wang and Sutherland.[1] b) Our self-replicating system taking into account both enantiomeric forms. c),d) Control reactions with compounds without recognition properties.

well as model components without recognition properties are depicted in Figure 2b–d.

We synthesized diene **4** in racemic form and as separate enantiomers from the corresponding 2,4-cyclohexadienylacetic acids[9] (Supporting Information). The reactions were monitored by ^{1}H NMR spectroscopy and evaluated by using our SimFit program.[10] A stack plot of the ^{1}H NMR spectrum of the reaction of *rac*-**4** with **5a** to give *rac*-**6a** is depicted in Figure 3, which shows the olefinic protons of *rac*-**6a**.

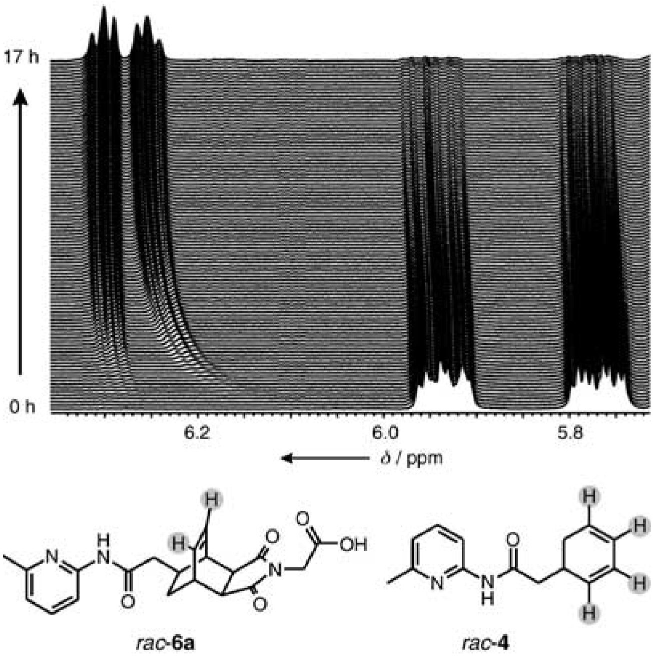

Figure 3. Stack plot of ^{1}H NMR spectra of the reaction of *rac*-**4** with **5a** to give *rac*-**6a** ($CDCl_3$, 600 MHz, 293 K).

Three phenomena are directly observable: an induction period, an autocatalytic increase of integrals, and a concomitant change of chemical shifts for the development of the product signal. The latter caused by the transition of free templates at low concentrations to template complexes and duplexes at higher product concentrations. Minimal modeling according to $A + B + pC \rightarrow (1+p)C$ for the autocatalytic channel and $A + B \rightarrow C$ for the non-autocatalytic channel, both for the building blocks **5a** and **5b** as B, leads to the profiles of theoretical concentration over time shown in Figure 4 and yields the kinetic parameters given in Table 1. From this data it is evident that our variants are reasonable approximations of the Wang–Sutherland system, of which the autocatalytic reaction order p over similar conversion was 0.8.[1]

Wang and Sutherland assumed an *endo* product but were not sure whether one or two diastereomers that differ in the configuration of the stereocenter carrying the recognition site at the [2.2.2]bicyclo-octene scaffold are formed. We were able to crystallize a single racemic diastereomer of the reaction product, *rac*-**6b**, the X-ray crystallographic structure is shown in Figure 5.[11]

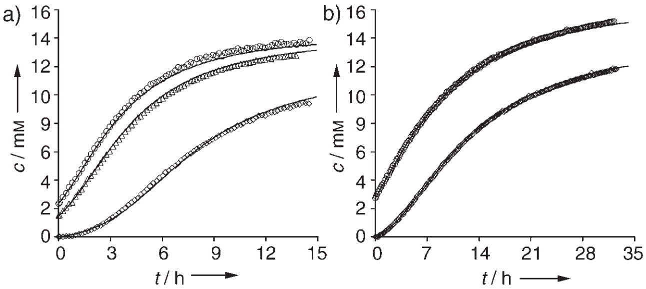

Figure 4. Profiles of concentration over time for the reaction of a) *rac*-**4** and **5a** ($CDCl_3$, 293 K, 15 mM) with template *rac*-**6a** initially added at 0 (□), 10 (△), and 15% (○); b) *rac*-**4** and **5b** ($CDCl_3$, 313 K, 15 mM) with template *rac*-**6b** initially added at 0 (□) and 17% (○). Continuous lines represent theoretical curves.

Table 1: Results of the analysis of the reactions of *rac*-**4** and **5a**/**5b** based on the value of p.

R	p	k_a [$M^{-(p+1)}s^{-1}$]	k_b [$M^{-1}s^{-1}$]	RMS [%]	T [K]
H	0.89	$(4.48 \pm 0.01) \times 10^{-1}$	$(2.10 \pm 0.02) \times 10^{-4}$	2.21[a]	293
CH_3	0.9	$(6.32 \pm 0.13) \times 10^{-2}$	$(4.43 \pm 0.23) \times 10^{-4}$	1.09[b]	313

[a] Based on experiments with added template initially at 0, 10, and 15%. [b] Based on experiments with added template initially at 0 and 17%.

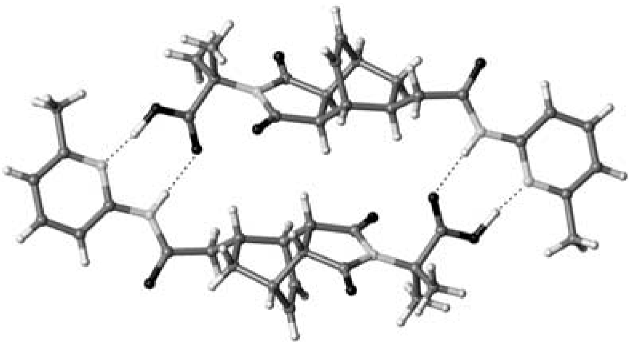

Figure 5. X-ray crystallographic structure of *rac*-**6b**.

There is no indication for another diastereomer above the detection limits of NMR spectroscopy. Transition-state modeling of simplified model reactions at the B3LYP/6-31G* level of calculation confirmed that the diastereomer with NN orientation is expected (Supporting Information).[12] An interesting detail from these studies is the presence of weak bifurcating hydrogen bonds, which stabilize the transition state of the model reaction between 5′-methyl-1,3-cyclohexadiene and *N*-carboxymethylmaleimide, but these are absent in the product. Weak bifurcating hydrogen bonds were also found to stabilize the transition state of the noncatalyzed template-formation reaction in the NNN conformation.[12] As expected, model reactions with the building blocks **7a** and **9** that are without recognition properties revealed no autocatalysis, but allowed us to determine the second-order rate constants: (*rac*-**4** + **7a** → *rac*-**8a**, $T = 293$ K, $k = 4.49 \times 10^{-5} M^{-1} s^{-1}$; **9** + **5a** → **10**, $T = 293$ K, $k = 5.83 \times 10^{-5} M^{-1} s^{-1}$).

We studied the individual reactions involving diene (*R*)-**4** and (*S*)-**4** in the presence of the template (*R*)-**6a** at 10%.

From Figure 6 it is clear that the effect of the template is similar in both cases, which indicates the presence of autocatalytic and cross-catalytic reaction channels. This finding could be confirmed by kinetic modeling and fitting both data sets simultaneously to the model shown in Figure 7. The ratio of the association constants of the template duplexes ($K_{2\,\mathrm{hetero}}/K_{2\,\mathrm{homo}} \approx 2$) suggests that homochiral and heterochiral duplexes are nearly equally populated.

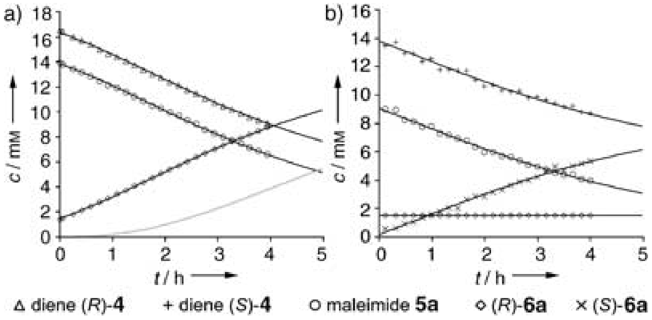

Figure 6. Profiles of concentration over time for the reaction of maleimide **5a** with: a) diene (*R*)-**4** to give (*R*)-**6a** (the gray line demonstrates product formation without any template added initially); b) diene (*S*)-**4** to give (*S*)-**6a**, both with 10% of template (*R*)-**6a** added initially ($CDCl_3$, 293 K, 15 mM). The first four hours of the reaction time were analyzed.

Background reactions

$k_{\mathrm{fix}} = 5.83\ \mathrm{x}\ 10^{-5}\ \mathrm{M}^{-1}\mathrm{s}^{-1}$

AR + B → CR
AS + B → CS
2 AR_B → AR_B_CR
2 AS_B → AS_B_CS
AR_B + AS_B → AR_B_CS
AR_B + AS_B → AS_B_CR
AR_B + B → B_CR
AS_B + B → B_CS
AR_B + AR → AR_CR
AR_B + AS → AR_CS
AS_B + AR → AS_CR
AS_B + AS → AS_CS

Template-directed reactions

auto-catalysis

$k_{\mathrm{auto}} = 3.77\ \mathrm{x}\ 10^{-3}\ \mathrm{s}^{-1}$

AR_B_CR → CR_CR
AS_B_CS → CS_CS

cross-catalysis

$k_{\mathrm{cross}} = 4.87\ \mathrm{x}\ 10^{-3}\ \mathrm{s}^{-1}$

AR_B_CS → CR_CS
AS_B_CR → CR_CS

Single amidopyridine-carboxylate equilibria

$K_1 = 1.38\ \mathrm{x}\ 10^{2}\ \mathrm{M}^{-1}$

AR + B ⇄ AR_B
AS + B ⇄ AS_B
AR + CR ⇄ AR_CR
AR + CS ⇄ AR_CS
AS + CR ⇄ AS_CR
AS + CS ⇄ AS_CS
B + CR ⇄ B_CR
B + CS ⇄ B_CS
AR_CR + B ⇄ AR_B_CR
AR_CS + B ⇄ AR_B_CS
AS_CR + B ⇄ AS_B_CR
AS_CS + B ⇄ AS_B_CS
B_CR + AR ⇄ AR_B_CR
B_CS + AR ⇄ AR_B_CS
B_CR + AS ⇄ AS_B_CR
B_CS + AS ⇄ AS_B_CS

Template-duplex equilibria

homochiral

$K_{2\,\mathrm{homo}} = 4.80\ \mathrm{x}\ 10^{4}\ \mathrm{M}^{-1}$

CR + CR ⇄ CR_CR
CS + CS ⇄ CS_CS

heterochiral

$K_{2\,\mathrm{hetero}} = 8.05\ \mathrm{x}\ 10^{4}\ \mathrm{M}^{-1}$

CR + CS ⇄ CR_CS

Figure 7. Full model that considers the complexes participating in the reaction ($CDCl_3$, 293 K, 15 mM), which results from a distinction between the diene enantiomers (A = diene **4**, B = maleimide **5a**, C = template **6a**). RMS = 1.44%.

The limited structural and dynamic complexity of our replication system led us to the question of whether computational chemistry could help to explain the kinetic data on the base of an energy profile. We took the following approach: a conformational search of the noncomplexed template at the MMFF94 level followed by further refinement at the B3LYP/6-31G* level led to two conformational families, NNN and NNX,[12] which differed in the orientation of the carboxy group relative to the C–C double bond.

Formation of template duplexes requires the units of recognition to be in a specific orientation, coaligned in the same direction. Considering that each of these template conformations can exist in both enantiomeric forms, we systematically constructed the six possible duplexes by manual docking and energy refinement at MMFF94, PM3, and B3LYP/6-31G* levels, successively. As expected, duplexes arising from inner-family combinations were found to be centrosymmetric in the heterochiral case and to have rotational symmetry in the homochiral case. The lowest-energy duplex has the conformation that was found in the crystal structure of **6b**. Transition states were derived from a transition-state search of the model reaction between *N*-methylmaleimide and 5-methyl-1,3-cyclohexadiene; all atoms, except those of the methyl groups, were frozen and the skeleton of frozen atoms were inserted into the respective position of the corresponding duplex. Geometry optimization of the nonfrozen atoms was carried out at the B3LYP/6-61G* level. The termolecular complexes were derived from the transition states by relaxation after unfreezing. Figure 8 shows the result of our computational study. For the case of the nontemplated reactions, we were able to find the true transition states. These are greater than 6 kcal mol^{-1} below the approximated counterparts in Figure 8. We observed that the energy difference between the respective conformations is similar to their counterparts in the approximated transition states with frozen atoms. Therefore, we trust the approximated transition states.

The largest energy differences between autocatalytic and cross-catalytic pathways were found at the level of transition states and template duplexes. Interestingly, transition-state stabilization by weak bifurcating hydrogen bonds (approximately −1.5 kcal mol^{-1}) leads to the lowest-energy conformations only in the autocatalytic cases. For the cross-catalytic cases the effect is counterbalanced by repulsive and/or dihedral distortion, which leads to a less optimal geometry at the recognition sites. The cross-catalytic pathway shows the lowest enthalpy of activation. There is always a clear enthalpic preference of termolecular complexes over the respective template duplexes. Further work on the temper-

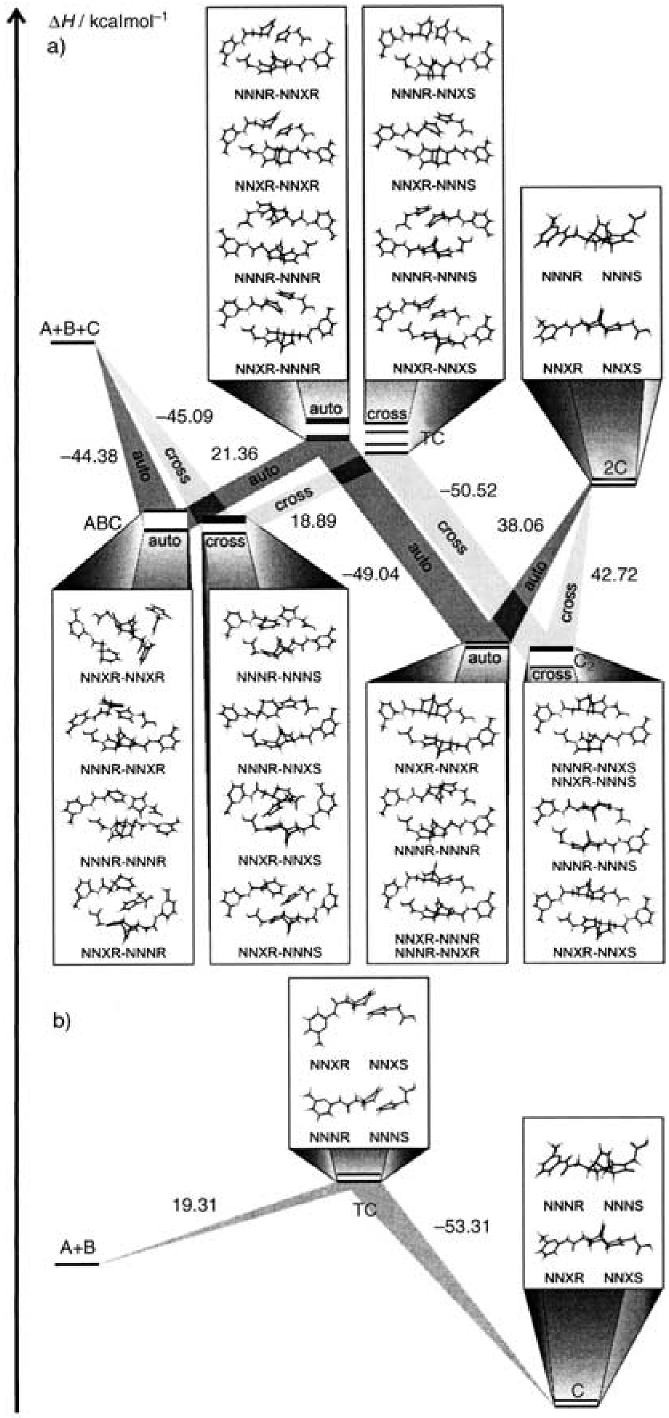

Figure 8. Energy profiles correlated to the corresponding structures (B3LYP/6-31G*) of the reaction of **4** and **5a** through the a) autocatalytic and b) nontemplate-directed pathways.

ature dependence of rates and equilibria in a slightly modified self-replicating system has revealed evidence that the above enthalpic preference is not overcompensated by the respective entropy differences.[13]

In summary, our re-evaluation of the Wang–Sutherland replicator with simplified variants reveals that the exponential dynamics arise from conformational constraints. Whereas the termolecular complexes offer enough freedom for an optimal docking of the reactants with the template, template duplexes are conformationally restricted in their finding of an optimal orientation of the recognition units. A similar rationale was reported to explain the nearly exponential growth of a peptide replicator.[14] Our replicator theory predicts that for systems with a negligible background channel, the question of parabolic versus exponential growth is solely answerable by the stabilities of the ground-state and not the transition-state complexes involved.[2] Further cases are needed to prove that exponential growth can be literally designed by taking into account the conformational control of ground states. If this recipe can be generalized, it could open a door to a field that may be termed "systems chemistry", namely, the design of prespecified dynamic behavior.

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[1] B. Wang, I. O. Sutherland, *Chem. Commun.* **1997**, 1495–1496.

[2] G. von Kiedrowski, *Bioorg. Chem. Front.* **1993**, *3*, 113–146.

[3] I. Weissbuch, L. Leiserowitz, M. Lahar, *Top. Curr. Chem.* **2005**, in press.

[4] F. C. Frank, *Biochim. Biophys. Acta* **1953**, *11*, 459–463.

[5] a) K. Soai, T. Shibata, H. Morioka, K. Choji, *Nature* **1995**, *378*, 767–768; b) D. G. Blackmond, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 5732–5736.

[6] a) V. C. Allen, D. Philp, N. Spencer, *Org. Lett.* **2001**, *3*, 777–780; b) R. J. Pearson, E. Kassianidis, A. M. Z. Slavin, D. Philp, Org. *Biomol. Chem.* **2004**, *2*, 3434–3441.

[7] A. Saghatelian, Y. Yokobayashi, K. Soltani, M. R. Ghadiri, *Nature* **2001**, *409*, 797–801.

[8] F. Garcia-Tellado, S. Goswami, S. K. Chang, S. J. Geib, A. D. Hamilton, *J. Am. Chem. Soc.* **1990**, *112*, 7393–7394.

[9] Synthesis of *rac*-2,4-cyclohexadienylacetic acid: a) C. Jonasson, M. Roenn, J.-E. Baeckvall, *J. Org. Chem.* **2000**, *65*, 2122–2126; (*R*)-2,4-cyclohexadienylacetic acid: b) M. Asaoka, K. Kobayashi, H. Takei, *Bull. Chem. Soc. Jpn.* **1994**, *67*, 1141–1146; (*S*)-2,4-cyclohexadienylacetic acid: c) J. E. Baeckvall, R. Gatti, H. E. Schink, *Synthesis* **1993**, 343–348, 642.

[10] For other SimFit applications, see: a) D. Sievers, G. von Kiedrowski, *Nature* **1994**, *369*, 221–224; b) D. Sievers, G. von Kiedrowski, *Chem. Eur. J.* **1998**, *4*, 629–641; c) H. Schöneborn, J. Bülle, G. von Kiedrowski, *ChemBioChem*, **2001**, *2*, 922–927; d) K. Severin, D. H. Lee, A. J. Kennan, M. R. Ghadiri, *Nature* **1997**, *389*, 706–709; e) S. Yao, I. Gosh, R. Zutshi, J. Chmielewski, *Angew. Chem.* **1998**, *110*, 489–492; *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 478–481; f) see Pearson et al.[6b]

[11] Crystallographic data for *rac*-**6b**: crystal size $= 0.2 \times 0.2 \times 0.3\,\text{mm}^3$; crystal system = monoclinic; space group $= P2_1/n$; unit cell dimensions: $a = 5.8973(17)$, $b = 26.318(8)$, $c = 18.881(5)$ Å, $\beta = 91.693(6)°$; $V = 2929.1(15)\,\text{Å}^3$; $\rho_{\text{calcd}} = 0.931\,\text{g cm}^{-3}$; $2\theta_{\max} = 45°$; $\lambda\text{Mo}_{K\alpha} = 0.71073$ Å; $T = 213(2)$ K; reflections collected = 10714, unique reflections = 3803, for 2983 reflections observed ($I > 2\sigma(I)$); The structure was solved by direct methods and refined by full-matrix least squares by using SHELXTL-97; 271 parameters; the non-H atoms were refined anisotropically, H atoms were included but not refined; final *R* indices: $R_1 = 0.0670$, $wR_2 = 0.1893$; *R* indices (all data): $R_1 = 0.0785$, $wR_2 = 0.1998$; goodness-of-fit on $|F^2| = 1.079$; max-

imum positive and negative peaks in ΔF map were $\rho_{\max} = 0.348\ e\,\text{Å}^{-3}$ and $\rho_{\min} = -0.292\ e\,\text{Å}^{-3}$. CCDC 269658 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

[12] We use a four-letter notation to describe the configuration and conformation of template molecules. The first letter is either N or X to indicate an *endo* or *exo* Diels–Alder product, respectively. The latter distinction is based on the relative orientation of the C=C double bond to the maleimide ring. We employed the C=C double bond also as a reference to describe the relative orientation of the 5-pyridinylaminocarbonylmethyl substituent (second letter) and of the carboxymethyl substituent (third letter). *R*/*S* (fourth letter) is determined by the absolute configuration of the precursor diene.

[13] I. Stahl, *Analysis and classification of minimal self-replicating systems based on Diels–Alder ligation chemistry*, PhD thesis, Ruhr-Universität Bochum, **2005**: http://www-brs.ub.ruhr-uni-bochum.de/netahtml/HSS/Diss/StahlInsa/.

[14] R. Issac, J. Chmielewski, *J. Am. Chem. Soc.* **2002**, *124*, 6808–6809.