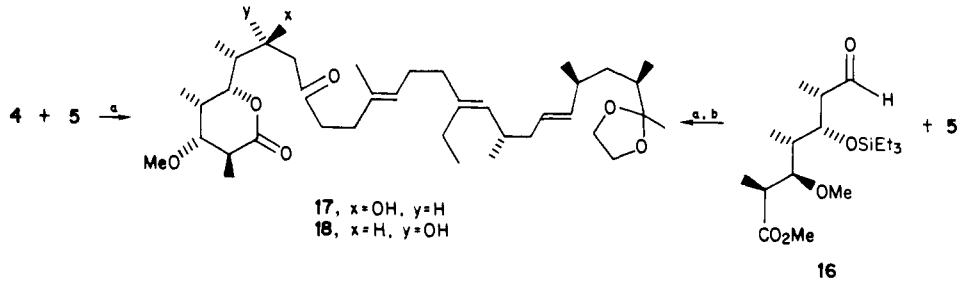
Scheme VI[a]



[a] Reagents: (a) LDA, THF, -78 °C; (b) n-$Bu_4N^+F^-$, THF.

thesis of **2b** which takes advantage of the biochemical methodology developed in these labs for the preparation of the key chiral intermediates.

Retrosynthetic analysis of the triene **2b** is shown in Scheme II. Our strategy entails the successive assembly of the prefabricated chirons (**6b**, **9**, **10**) which contain the stereochemical and structural features of **2**. On the basis of the literature precedent,[6] we envisaged that the final aldol condensation of **4** and **5** should predominantly produce triene **2b** with the desired Cram stereochemistry at C-6 and C-7. The five contiguous chiral centers of the fragment **4** could be elaborated from the chiron **6b**, readily available via enzymatic enantioselective hydrolysis.[7] The (*all-E*)-triene **5** could be formed using sulfone methodology to combine components **7** and **8**, which, in turn, may be prepared from the biochemically derived chirons **9**[8] and **10**,[9] respectively.

Condensation[10] of the boron enolate of (*S*)-phenyl thiopropionate with **6b** gave **11** (65%) as the major product with the desired 2,3-syn and 3,4-anti configuration. Transesterification and methylation of **11** afforded **12**, which, upon acid-catalyzed deprotection, lactonized spontaneously. Swern oxidation produced the lactone–aldehyde **4**, whose stereochemical assignment was confirmed by comparison to a sample of **4** derived from degradation of Monensin A (Scheme III).[11]

Addition of 1-buten-2-ylmagnesium bromide to **9** afforded the allylic alcohol, which upon ortho-ester Claisen rearrangement[12] produced the ester **13** (83% from **9**). Repetition of this addition–rearrangement sequence with the aldehyde, derived from **13** and 1-propen-2-ylmagnesium bromide, gave **14** (55% from **13**). To avoid overreduction, the ester grouping **14** was cleaved prior to reductive debenzylation. Reesterification and oxidation yielded the desired aldehydic fragment **7** (68% from **14**, 75% conversion) (Scheme IV).

The chiral half-ester–acid **10** was transformed into **15** following standard methodology[13] using mild acid catalysis to avoid epimerization of the α-methyl ketone. Reduction of **15** was followed by a direct conversion[14] of the resulting alcohol to the phenyl sulfide whose oxidation to **8** (64% from **15**) required buffered conditions to retain the ketal (Scheme V). The union of **7** and **8** was accomplished by the Kocienski–Lythgo–Julia procedure.[15] Thus, the anion of **8** (n-BuLi, THF, −78 °C) underwent smooth addition to **7**, and the product was trapped with benzoyl chloride. The resulting sulfone benzoate intermediate upon reductive elimination [Na(Hg), CH_3OH, EtOAc] gave the *E* olefin (35%). The ester was in turn transformed to the methyl ketone **5** via cuprate addition to the derived acid chloride [(a) NaOH; (b) $(COCl)_2$; (c) $(CH_3)_2CuLi$; 85% overall].

Aldol condensation[16] (LDA, THF, −78 °C) of **4** and **5** afforded a 9:1 mixture of diastereomeric aldols (80%; 81% conversion). The major diastereomer was assigned **17** on the basis of structural correlation with the major diastereomer from the aldol condensation of **5** and **16**[11] under identical conditions (2.6:1; 88%; 76% conversion) (Scheme VI). This assignment is in accord with theoretical predictions and earlier observations.[6b] The ketal and lactone of **17** were cleaved [(a) PPTS, 5:1 acetone–H_2O; (b) 4:1 THF–0.05 N NaOH, 90%] to complete the synthesis of the putative precursor **2b**.[17]

This convergent synthesis not only provides access to **2b** but also vividly demonstrates the value of enzymatic methods in complex natural product synthesis. Incorporation experiments using isotopically labeled **2b** to verify the triene–triepoxide biosynthetic model of Monensin A are now in progress.

Acknowledgment. We thank Professors W. C. Still and D. Collum for kindly providing experimental details and spectra of synthetic intermediates and the Eli Lilly Co. for a generous gift of Monensin A. This investigation was supported in part by Grant HL25772 of the National Institutes of Health.

(5) Van Tamelan, E. E. *Acc. Chem. Res.* **1968**, *1*, 111.
(6) (a) Fukuyama, T.; Akasaka, K.; Karanewsky, D. S.; Wang, C. L. J.; Schmid, G.; Kishi, Y. *J. Am. Chem. Soc.* **1979**, *101*, 262. (b) Collum, D. B.; McDonald, J. H., III; Still, W. C. *J. Am. Chem. Soc.* **1980**, *102*, 2120.
(7) Enantioselective hydrolysis of the acetoxy ester of (±)-**6a** with purified pig pancreatic lipase afforded (−)-6a (ee = 0.97) in high yields. Wu, C.; Wang, Y. F.; Donaubauer, J.; Sih, C. J., unpublished data.
(8) VanMiddlesworth, F.; Wang, Y. F.; Zhou, B. N.; DiTullio, D.; Sih, C. J. *Tetrahedron Lett.* **1985**, 961.
(9) Chen, C. S.; Fujimoto, Y.; Sih, C. J. *J. Am. Chem. Soc.* **1981**, *103*, 3580.
(10) Hirama, M.; Garvey, D.; Lu, L.; Masamune, S. *Tetrahedron Lett.* **1979**, 3937. Evans, D. A.; Nelson, J. V.; Vogel, E.; Taber, T. R. *J. Am. Chem. Soc.* **1981**, *103*, 3099.
(11) Collum, D. B.; McDonald, J. H., III; Still, W. C. *J. Am. Chem. Soc.* **1980**, *102*, 2117.
(12) Johnson, W. S.; Werthemann, L.; Bartlett, W. R.; Brocksom, T. J.; Li, T. T.; Faulkner, D. J.; Petersen, M. R. *J. Am. Chem. Soc.* **1970**, *92*, 741.
(13) Walba, D. M.; Wand, M. D. *Tetrahedron Lett.* **1982**, 4995.
(14) Nakagawa, I.; Hata, T. *Tetrahedron Lett.* **1975**, 1409.
(15) Kocienski, P. J.; Lythgo, B.; Waterhouse, I. *J. Chem. Soc., Perkin Trans. 1* **1980**, 1045.
(16) The lactone aldehyde **4** is extremely unstable and should be freshly prepared just prior to use.
(17) All compounds herein described gave satisfactory elemental or MS analyses and their NMR spectra were consistent with the assigned structures.

Enzymatic Synthesis of Unusual Sugars:[1] Galactose Oxidase Catalyzed Stereospecific Oxidation of Polyols

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Recently, there has been a large amount of interest in the synthesis of unnatural sugars.[2-4] Since the majority of natural sugars occur in only one enantiomeric form, unnatural sugars are

(1) Supported by the National Science Foundation Grant CHE-8318217 and the Robert A. Welch Foundation Grant A-1004.
(2) Szarek, W. A.; Hay, G. W.; Vyas, D. M.; Ison, E. R.; Hronowski, L. J. J. *Can. J. Chem.* **1984**, *62*, 671–674.
(3) Ko, S. Y.; Lee, A. W. M.; Masamune, S.; Reed, L. A., III; Sharpless, K. B.; Walker, F. J. *Science (Washington, D.C)* **1983**, *220*, 949–951.

0002-7863/85/1507-2997$01.50/0

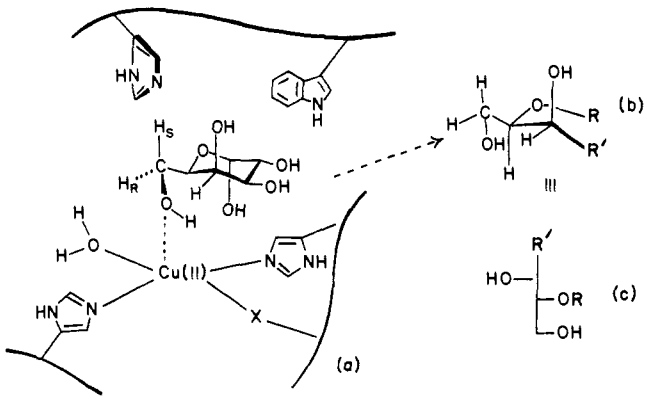


Figure 1. (a) Active site of D-galactose oxidase. (b,c) Configuration theoretically required for a polyol to be a substrate of D-galactose oxidase.

understandably expensive. However, despite their expense, the unnatural sugars are important because of their potential for use as safe, noncaloric sweeteners and as precursors for many natural products.[5,6]

Two general methods have recently been developed for the synthesis of unusual L-sugars: one is based on asymmetric epoxidation;[3] the other is an asymmetric Diels–Alder reaction.[7] The former procedure involves chain extention, asymmetric epoxidation, base-catalyzed epoxide ring opening, and oxidation. Typical yields for most L-hexoses are around 10% and ~3% for L-galactose and L-glucose.[3] The latter involves silylation, a Lewis acid catalyzed Diels–Alder reaction, and hydroxylation. Typical yields are in the range of 40–60%. However, the product is racemic.[7] Other methods involving more complicated procedures have also been reported.[4,5c,d]

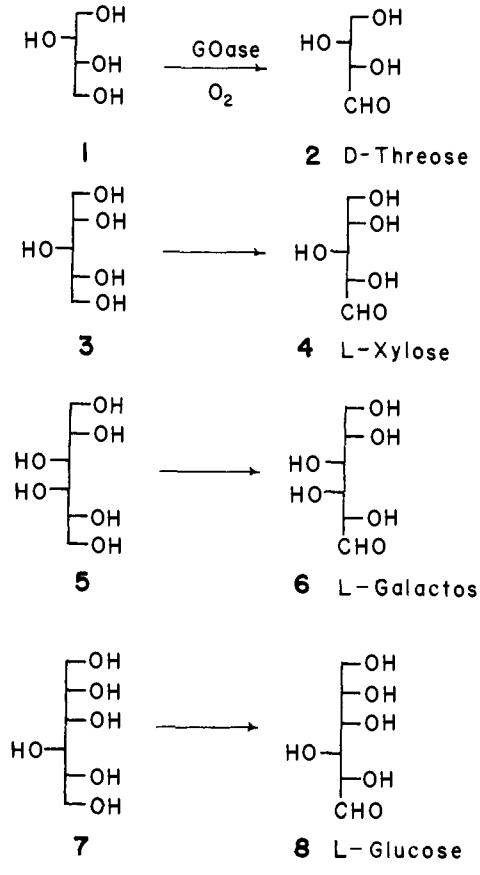
We here report an alternative route to unusual sugars, particularly L-sugars, from polyols using D-galactose oxidase (GOase, EC 1.1.3.9) as an oxidation catalyst. The Cu(II) enzyme GOase catalyzes the reaction

$$\text{D-galactose} + O_2 \rightarrow \text{D-galactohexodialdose} + H_2O_2^{8}$$

Based on the information obtained from the study of substrate specificity of GOase using D-galactose and its derivatives as substrates and chemical modification of the enzyme, the configuration of the active site containing D-galactose has been reported (Figure 1a).[9] The enzyme also catalyzes the oxidation of various alcohols. The primary alcohol groups of glycerol and DL-3-chloro-1,2-propanediol, for example, can be oxidized stereospecifically to L-aldehydes.[10] We have further investigated a number of other possible polyol substrates[11,12] from which we have developed a general structure of polyols (Figure 1b,c) required for the stereospecific oxidation.

Scheme I indicates four alditols containing the required configuration, which are of particular interest.[13] D-(+)-threitol (**1**) and xylitol (**3**) were stereospecifically oxidized to D-(+)-threose (**2**) and L-(−)-xylose (**4**), respectively. Galactitol (**5**) and L-(+)-glucitol, (**7**) were poor substrates for the enzyme, so catalytic amounts of ferricyanide (0.050 mM)[14] were therefore added to increase the reaction rate.

Scheme I



For the representative synthesis of L-xylose, catalase (EC 1.11.1.6) (3.6 mg, ~5700 units) and GOase (0.3 mg, ~6 units) were added to a 3.00-mL phosphate buffer solution (50 mM, pH 7.0) containing 50 mM xylitol. The solution was allowed to react at room temperature for 5 days. The reaction was stopped and the enzymes precipitated by heating the vial in boiling water for 30 min. The solution was centrifuged, and the supernatant was recovered and concentrated under vacuum to a solid residue. HPLC analysis[15] of the reaction products indicated that 50% of the xylitol was converted to L-xylose, and no other side products were detected.[16] The specific rotation measured was $[\alpha]^{25}_{D}$

(4) (a) Roush, W. R.; Brown, R. J. *J. Org. Chem.* **1982**, *47*, 1371–1372. (b) Lee, A. W. M.; Martin, V. S.; Masamune, S.; Sharpless, K. B.; Walker, F. J. *J. Am. Chem. Soc.* **1982**, *104*, 3515–3516. (c) Minami, N.; Ko, S. S.; Kishi, Y. *J. Am. Chem. Soc.* **1982**, *104*, 1109. (d) Danishefsky, S.; Kerwin, J. F., Jr. *J. Org. Chem.* **1982**, *47*, 1597.

(5) (a) Shallenberger, R. S.; Acree, T. E.; Lee, C. Y. *Nature (London)* **1969**, *221*, 555–556. (b) Rudney, H. *Science (Wasington, D.C)* **1940**, *92*, 112–113. (c) Levin, G. V. U.S. Patent 4 262 032, April 14, 1981. (d) Szarek, W. A.; Jones, J. K. N. U.S. Patent 4 207 413, June 10, 1980.

(6) Hadfield, A. F.; Sortorelli, A. C. *Carbohydr. Res.* **1979**, *72*, 235–242.

(7) (a) Danishefsky, S.; Bednarski, M. *Tetrahedron Lett.* **1984**, *25*, 721–724. (b) Danishefsky, S.; Webb, R. R., II. *J. Org. Chem.* **1984**, *49*, 1955–1958.

(8) Hamilton, G. A. In "Metal Ions in Biology"; Spiro, T. G., Ed.; Wiley: New York, 1981; Copper Proteins Vol. 3; pp 193–218.

(9) Ettinger, M. J.; Kosman, D. J. In "Metal Ions in Biology"; Spiro, T. G., Ed.; Wiley: New York, 1981; Copper Proteins Vol. 3; pp 219–261.

(10) Klibanov, A. M.; Alberti, B. N.; Marletta, M. A. *Biochem. Biophys. Res. Commun.* **1982**, *108*, 804–808.

(11) Bergmeyer, H. U.; Gawehn, K.; Grassl, M. In "Methods of Enzymatic Analysis", Bergmeyer, H. U., Ed.; Academic Press: New York, 1974; pp 425–522. All possible substrates were tested at 0.050 M concentration in the assay.

(12) The following compounds tested positive as substrates: glycerol, (*S*)-1,2-propanediol, 3-chloro-1,2-propanediol, 3-fluoro-1,2-propanediol, 3-bromo-1,2-propanediol, D-α-*O*-benzyl glycerol, 2-methyl-1,3-propanediol, 1-buten-3,4-diol, and D-glyceraldehyde. The following compounds tested negative as substrates: (*R*)-1,2-propanediol, 2-amino-1,3-propanediol, L-threitol, erythritol, D-glucose, (*S*)-1,2-butanediol, (*R*)-1,2-butanediol, D-fructose, L-xylose, L-galactose, D-arabinose, and D-ribose.

(13) Preparation of these polyols is straightforward. Compound **1** was prepared by $LiAlH_4$ reduction of diethyl D-tartrate.[20a] Compounds **3** and **5** are commercially available and inexpensive, or they can be prepared by reduction of readily available D-xylose and D-galactose, respectively. Compound **7** was prepared by reduction of D-gulonolactone,[20b] which is commercially available and relatively inexpensive.

(14) Hamilton, G. A.; DeJersey, J.; Adolf, P. K. In "Oxidases and Related Redox Systems"; King, T. E., Mason, H. S., Morrison, M., Eds.; University Park Press: Baltimore, 1973; pp 103–124.

(15) HPLC analysis was performed by using a Waters μ-Bondapak carbohydrate column (0.4 × 30 cm) with refractometer detection and aqueous acetonitrile (H_2O/CH_3CN, 85% v/v) as solvent. For a flow rate of 2 mL/min the retention times (min) were as follows: threitol, 4.5; D-threose, 5.7; xylitol, 6.6; L-xylose, 5.4; L-glucitol, 9.2; L-glucose, 10.2; galactitol, 10.2; L-galactose, 10.9.

-17.6°, which was in agreement with the value reported previously, $[\alpha]^{25}_D$ -18° (c 9.9, H_2O). The ^{13}C NMR of the reaction product is identical with that of L-xylose.[17] The alditol–aldose mixture can be easily separated by passing through a Dowex 50 (Ba^{2+} form) column using water as eluent.[18]

Similar procedures were applied to compounds **1**, **5**, and **7**. Although no side reactions were observed for each reaction, the reaction yield was 10–15% based on HPLC analysis and measurement of optical rotation. Like many other enzymatic reactions, product inhibition occurred in these reaction. To overcome this problem, we note that a column reactor can be used,[19] in which the reactant is passed through the column containing immobilized enzyme, and the product collected from the eluent is separated from the unreacted polyol,[18] which is then recirculated through the column for further reaction.

In summary, this work illustrates a novel use of an enzyme for stereospecific oxidation of polyfunctional compounds in aqueous medium. The process could be useful for preparation of various unusual L-sugars from readily available polyhydroxy compounds.

(16) It has been reported that reactions with D-galactose oxidase rarely go to completion: Schlegel, R. A.; Gerbeck, C. M.; Montgomery, R. *Carbohydr. Res.* **1968**, *7*, 193–199.

(17) The ^{13}C spectrum (in ppm from DSS) was essentially the same as that reported: (a) Serianni, A. S.; Nunez, H. A.; Barker, R. *Carbohydr. Res.* **1979**, *72*, 71–78. (b) Nunez, H. A.; Walker, T. E.; Fuentes, R.; O'Connor, J.; Serianni, A.; Barker, R. *J. Supramol. Struct.* **1977**, *6*, 535–550.

(18) Jones, J. K. N.; Wall, R. A.; Pittet, A. O. *Can. J. Chem.* **1960**, *38*, 2285–2294.

(19) Chibata, I. C. "Immobilized Enzymes"; Wiley: New York, 1978.

(20) (a) Klosterman, H.; Smith, F. *J. Am. Chem. Soc.* **1952**, *74*, 5336. (b) Frush, H. L.; Isbell, H. S. *J. Am. Chem. Soc.* **1956**, *78*, 2844.

Book Reviews

The Chemical Physics of Solid Surfaces and Heterogeneous Catalysis. Volume 3B. Chemisorption Systems. Edited by D. A. King (University of Liverpool) and D. P. Woodruff (University of Warwick). Elsevier Scientific Publishing Co.: Amsterdam and New York. 1984. xii + 320 pp. $96.25. ISBN 0-444-42178-5.

This volume is one of a series of authoritative, carefully prepared reviews of areas of modern surface science. Previously published volumes have dealt with Clean Solid Surfaces (Volume 1), Adsorption on Solid Surfaces (Volume 2), and Fundamental Studies of Heterogeneous Catalysis (Volume 4). This volume addresses several important adsorption systems which have been widely studied under well-controlled conditions. The chapters are the following: 1. Metals on Metals (E. Bauer); 2. Hydrides and Oxides of Nitrogen on Metal Surfaces (R. M. Lambert and M. E. Bridge); 3. Hydrocarbons on Metals (J. C. Bertolini and J. Massardier); 4. Adsorption on Oxides (G. Heiland and H. Luth); 5. Halogens on Solids (H. H. Farrell); and 6. Adsorption on Semiconductors (R. H. Williams and I. T. McGovern).

The reviews are well written and clearly illustrated. Although the presentations assume at least a cursory knowledge of the tools, methods, and results of modern surface science, an attempt has been made to obviate barriers between the separate disciplines which contribute to surface science. For example, band bending at semiconductor surfaces, a textbook-level topic for many, is reviewed briefly to aid the general reader in understanding adsorption on compound semiconductors. This reviewer applauds the care with which the subject matter has been developed from fundamentals through discussions of specific experimental results to (where possible) formulation of general rules. More than 700 references are given, mostly published prior to 1981. A small number of more recent publications have been included as notes added in proof.

Chemisorbed species are commonly intermediates in heterogeneous catalysis, so these reviews will obviously be valuable to workers in that field. However, chemisorption systems are just as relevant to many other areas, as diverse as friction, lubrication and adhesion, and photoelectrochemical energy conversion, and this volume can be recommended to a wide audience.

Robert C. Plumb, *Worcester Polytechnic Institute*

The Protein Folding Problem. Edited by D. B. Wetlaufer. Westview Press: Boulder, CO. 1984. xiv + 203 pp. $30.00. ISBN 0-86531-798-1.

Until recently, the title of this book would have led one to anticipate a discussion of the question, "can we theoretically predict protein structures from their sequences?" The focal question of the current monograph might be phrased, "what experimental efforts and interpretations of data are yielding insights and generalizations that enhance our understanding of protein folding?" This book is based on a symposium sponsored in early 1982 at the American Association for the Advancement of Science Annual National Meeting. It has eight articles discussing protein folding from a variety of perspectives. The specialized theoretical and computational discussions, which dominated this field a few years ago, are notable by their absence in this volume.

Jane Richardson gives a concise, and well illustrated, summary of our understanding of folding patterns in protein structural domains, with some suggestions as to how domain topologies may follow naturally from folding pathways. Donald Wetlaufer discusses current evidence for the suggestion that protein folding is a modular process and that structural domains are the folding modules. In a similar vein, Sherman Beychok considers the exon structure of genes coding for proteins, and referencing data on globin genes specifically discusses the question of whether exons code for functional domains in proteins. Irwin Kuntz summarizes data from such diverse sources as calorimetry, magnetic resonance, and proton exchange in a description of our current understanding of protein thermodynamics and dynamic motion in proteins.

A description of hierarchical structure and assembly of collagen is given by Karl Piez. There are also chapters on principles of biological growth by H. R. Crane and the helical hairpin hypothesis for folding and insertion of membrane proteins by Don Engelman and Tom Steitz.

Possibly the most novel approach to protein folding is the effort of John King and colleagues to apply genetics to the problem—more specifically, to try to decipher what they refer to as the genetic code for protein folding. In this volume, they discuss specifically their work on the tail spike protein of phage P22, for which they isolate a set of "temperature sensitive synthesis" mutants–mutants whose tail spike protein is functionally defective at the nonpermissive temperature only if synthesized at the nonpermissive temperature. It is argued that such mutants are expressing temperature-sensitive defects in protein folding; it will be interesting to see what insights this approach yields for the folding problem in the future.

Overall, this book gives a highly readable presentation of several different perspectives on the protein folding problem. The articles are written to be readily accessible to individuals who are not specialists in the field of protein structure. Taken as a whole, the material in the book provides the general audience with ample background material to become involved in the controversial question of how to address the problem of protein folding.

David B. McKay, *University of Colorado*

Advances in Liquid Crystals. Volume 6. Edited by Glenn H. Brown (Kent State University). Academic Press: New York. 1983. xiv + 268 pp. $80.00. ISBN 0-12-025006-3.

The general classification of liquid crystalline phases into smectic, nematic, and cholesteric is commonly known, but it deceptively suggests simpler phase behavior than is often observed. Consider these three facts. To date eight different smectic phases have been identified. The nematic phase in some systems has been observed to transform to a smectic phase on cooling, and then it appears again at lower temperatures (the "reentrant" phenomenon). It is not unusual for a pure mesogen to pass through two or three—even as many as five or six—stable fluid phases on heating between its crystalline melting temperature and appearance of the isotropic liquid.

The first and longest of the four reviews in the most recent volume of this informative series describes the fascinating phase behavior of these ordered fluids. This well-organized report was written by D. Demus and H. Sackman, who are among the foremost authorities of liquid crystalline polymorphism, in collaboration with S. Diele and S. Grande.