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# Advances in analysis and synthesis of *myo*-inositol-derivatives through resolution by crystallisation

Wolfgang Wewers,[a,*] Hartmut Gillandt[b,†] and Henner Schmidt Traub[a]

[a]*University of Dortmund, Department of Biochemical and Chemical Engineering, Chair of Plant Design, Emil Figge Str. 70, 44221 Dortmund, Germany*
[b]*Sirius Fine Chemicals (SiChem) GmbH, Fahrenheitstr. 1, 28359 Bremen, Germany*

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**Abstract**—A simple method for the preparation of both enantiomers of tetra-*O*-benzyl-*myo*-inositol is presented. This method is based on the resolution of stereoisomers by crystallisation. Starting with the known synthesis of four diastereomers using D-camphor dimethyl acetal as chiral auxiliary, one diastereomer is separated by crystallisation from methanol and is converted to D-1,4,5,6-tetra-*O*-benzyl-*myo*-inositol. A second synthetic route was carried out with the mother liquor of the crystallisation, which has so far been neglected in former sequences [*Am. Chem. Soc.* **1992**, 6361]. This approach leads to a non-racemic mixture of tetra-*O*-benzyl-*myo*-inositols, from which D-3,4,5,6-tetra-*O*-benzyl-*myo*-inositol can be separated by crystallisation from 1-propanol in its enantiopure form. The yield of this crystallisation step was determined by its eutectical point and the ratio of the diastereomers in the mother liquor of crystallisation step 1. For this purpose, an analytical HPLC separation for the fast elucidation of the diastereomeric ratio has been developed. With this analytical method, it is possible to optimise the ratio of the diastereomers by changing the reaction conditions.


## 1. Introduction

Inositol phosphates as molecules in biology have been known for 85 years.[2] A new area of research was started in 1983, after the discovery that *myo*-inositol 1,4,5-trisphosphate is a $Ca^{2+}$ releasing second messenger.[3]

Over the last 25 years, many other natural *myo*-inositol phosphates have been discovered. It has been realised that this group of phosphorylated *myo*-inositols plays an important role in various biological processes, such as cellular signal transduction, calcium mobilisation, chloride secretion, exocytosis, cytoskeletal regulation, insulin stimulation, intracellular trafficking of vesicles and anchoring of proteins to cell membranes.[4–8]

Herein, we report the preparation of key enantiopure molecules for the synthesis of two prominent $InsP_4$ enantiomers, D-*myo*-inositol 3,4,5,6-tetrakisphosphate Ins(3,4,5,6)$P_4$ and Ins(1,4,5,6)$P_4$ and the derivatives of both. Ins(3,4,5,6)$P_4$ behaves as an intracellular signal that inhibits the conductance of $Ca^{2+}$ activated $Cl^-$ channels in the plasma membrane. Modified membrane permeable derivatives of Ins(3,4,5,6)$P_4$ with antagonistic effects have been tested in the treatment of cystic fibrosis,[9] with Ins(1,4,5,6)$P_4$ levels in human colonic epithelial cells dramatically increasing in response to Salmonella invasion.[10]

Both enantiomers seem to play different roles in the cellular signal transduction. Herein, we report the use of the derivatives as pharmaceutically active substances and emphasise the necessity of a highly effective and simple way to synthesise the enantiopure key molecules.

## 2. Results and discussion

In former synthetic sequences, the preparation of enantiopure tetra-*O*-benzyl-*myo*-inositol derivatives was achieved by the separation of diastereomers. For this reason, D or L-camphor dimethyl acetal, used as a chiral auxiliary, has to be introduced into the *myo*-inositol structure. The synthetic strategy is shown in the left route of Figure 1 and was first described by Bruzik et al. in 1989 and improved upon in 1992.[11,1] Starting

* Corresponding author. Tel.: +49 (0231) 7556190; e-mail: wolfgang.wewers@bci.uni-dortmund.de
† Tel.: +49 (0421) 2208227; e-mail: hgillandt@sichem.de

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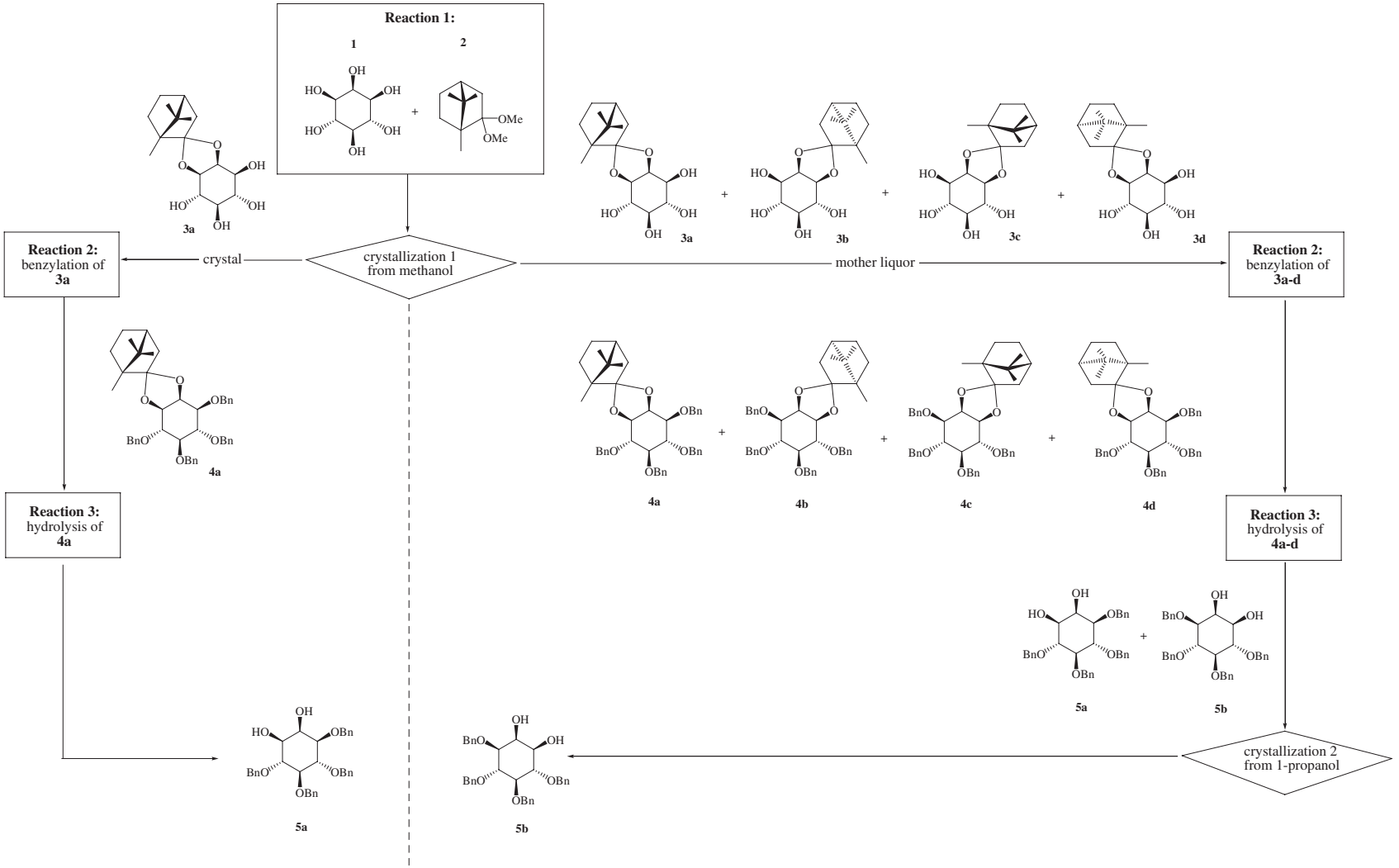


**Figure 1.** Synthesis of tetra-*O*-benzyl-*myo*-inositol.

from *myo*-inositol, a three step synthesis using D-camphor was performed. Reaction of *myo*-inositol **1** and D-camphor dimethyl acetal **2** gave a mixture of four diastereomeric tetraols **3a**–**d** in the ratio 47:23:13:27. Crystallisation of this mixture from methanol afforded pure diastereomer **3a**, which was then converted in a two step reaction to enantiopure D-1,4,5,6-tetra-*O*-benzyl-*myo*-inositol. The mother liquor of this first crystallisation step has until now been neglected.

A second possibility is the treatment of the four tetraols **3a**–**d** with benzyl chloride to produce the diastereomeric tetra-*O*-benzyl-derivatives **4a**–**d**. Derivatives **4a** and **4b** can be isolated by chromatography with silica gel, while **4c** and **4d** were obtained as a mixture.[11] Hydrolysis of the isolated diastereomers gave the enantiomerically pure tetra-*O*-benzyl-*myo*-inositols **5a** and **5b**. The non-resolved mixture of **4c** and **4d** was rejected.

These methods are exclusively used for the preparation of enantiomer **5a**, while **5b** was prepared by using more expensive L-camphor. For this reason, a method for the preparation of both enantiomers from D-camphor dimethyl acetal is presented in Figure 1.

As mentioned before, the left part shows the synthesis described by Bruzik et al.[11,1] which is used for preparation of **5a**, while the right part describes the new separation strategy to obtain enantiopure **5b**.

After crystallisation 1, the synthesis was continued with the mother liquor, which consisted of a mixture of tetraols **3a**–**d**. In the following two reaction steps (2 and 3) this mixture was converted to a non-racemic mixture of **5a** and **5b**. The ratio of the enantiomers is determined by the ratio of tetraols **3a**–**d** and results in a certain enantiomeric purity of **5b** before crystallisation. Eq. 1 shows the dependency of $Pur_{\mathbf{5b}}$ on the ratio of tetraols **3a**–**d**.

$$Pur_{\mathbf{5b}} = \frac{n_{\mathbf{5b}} \cdot 100\%}{n_{\mathbf{5a}} + n_{\mathbf{5b}}} = \frac{(n_{\mathbf{3b}} + n_{\mathbf{3c}}) \cdot 100\%}{n_{\mathbf{3a}} + n_{\mathbf{3b}} + n_{\mathbf{3c}} + n_{\mathbf{3d}}} \quad (1)$$

$Pur_{\mathbf{5b}}$ can be increased by decreasing the amount of **3a** and **3d** and increasing the amount of **3b** and **3c** in the feed. For this purpose, an analytical method must be found for fast analysis of the product ratio of reaction 1 and products of subsequent crystallisation step 1.

Attempts to separate **3a**–**d** by chromatography with common adsorbents (RP18, CN phases) failed and led to isolation of **3a** and a mixture of **3b**–**d**, although chromatographic separation with Hypercarb® and acetonitrile/water resulted in the resolution of all the tetraols shown in Figure 2.

Hypercarb columns are filled with porous particles of graphitised carbon and obtain very good selectivities for the separation of diastereomers.[12] This separation is used exclusively for analysis and not for preparative separation of the diastereomers because of the high price of Hypercarb columns, the limited available particle sizes and a limited stability, which was observed over long time experiments.[13]

Tetraols **3a** and **3d** elute as the first and second peaks, while the tetraols **3b** and **3c** eluted as the last peaks. Their elution order has not been elucidated yet. For further research, only the sum of tetraols **3b** and **3c** is needed because both molecules are converted to enantiomer **5b**.

The main advance of this work is crystallisation step 2 with 1-propanol where pure enantiomer **5b** is isolated as solid. For this reason, the enantiomeric purity $Pur_{eut}$

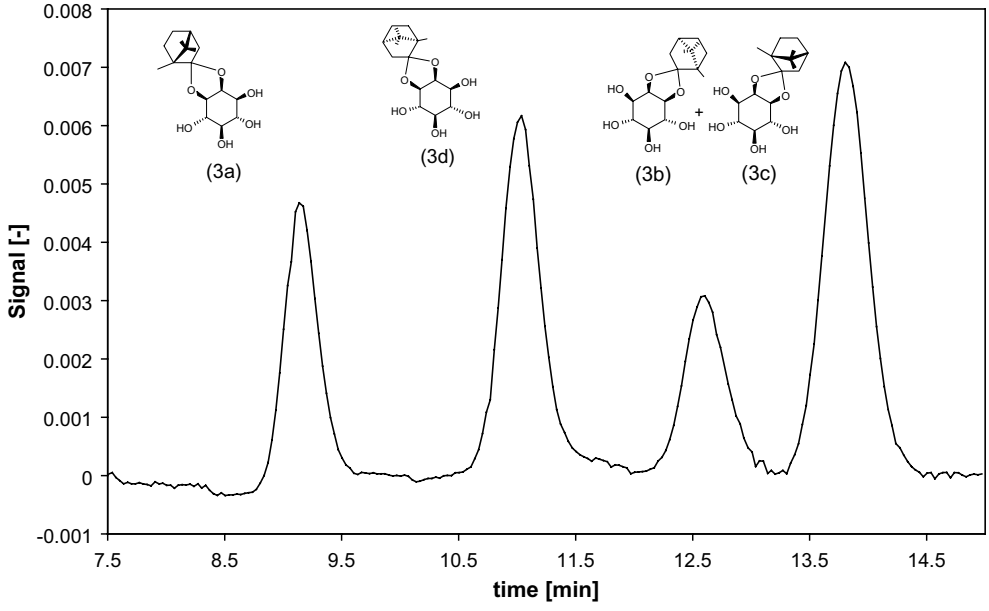


**Figure 2.** Separation of **3a**–**d** with Hypercarb® (acetonitrile:water, 20:80).

at eutectical composition of the ternary system is of special interest.

If $Pur_{\mathbf{5b}}$ is higher than $Pur_{eut}$, enantiopure **5b** can be isolated by crystallisation from 1-propanol until the purity of the mother liquor reaches $Pur_{eut}$.

$$\text{Yield} = \frac{m_{\text{crystalls},\mathbf{5b}}}{m_{\text{feed},\mathbf{5b}}} = \frac{(Pur_{\mathbf{5b}} - Pur_{eut})}{Pur_{\mathbf{5b}} \cdot (1 - Pur_{eut})} \quad (2)$$

The yield of this second crystallisation step can be calculated by Eq. 2.[14,15] It is generally increased if the difference $(Pur_{\mathbf{5b}} - Pur_{eut})$ is maximal. While $Pur_{\mathbf{5b}}$ is influenced by the ratio of the tetraols **3a**–**d**, $Pur_{eut}$ is determined by thermodynamics. For the ternary system, 1-propanol/**5a**/**5b** a value of 50% has been determined for $Pur_{eut}$. Thus, the ternary system is regarded as a conglomerate forming system,[16] where crystals of pure enantiomer can be withdrawn from the mother liquor until a racemic mixture is reached.

This process is demonstrated in Figure 3. A mixture of **5a**/**5b** in the ratio 70/30 was dissolved in pure 1-propanol at a temperature of 40 °C. The solution was stepwise cooled down to −10 °C. At ambient temperature, precipitation was observed. The solution was equilibrated at 25, 10 and −10 °C for at least 6 h and a sample of the supernatant analysed by HPLC. After the mother liquor had reached eutectical composition, the crystallisation stopped and the crystals were isolated by filtration. Figure 4 shows the analysis of the crystal and the mother liquor. The yield of the process reached 54%, which is very close to the theoretical value of 57%, which can be calculated by Eq. 2.

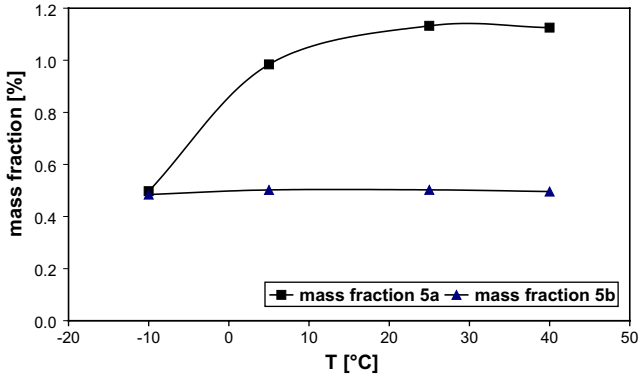


**Figure 3.** Cooling crystallisation of **5a**/**5b** with 1-propanol. Analysis of the mother liquor.

The crystallisation yield increases with $Pur_{\mathbf{5b}}$, which is maximised by the ratio (**3b**+**3c**)/(**3a**+**3d**). To increase the portion of **3b** and **3c**, reaction conditions were changed as shown in Figure 5.

Method a was previously described by Bruzik et al.[11,1] Due to the formation of diacetals, 3 equiv of D-camphor dimethyl acetal is needed. 77.5% of tetraols **3a** and **3d** is produced and thus this method can be used if enantiomer **5a** is needed. If the synthesis is continued, enantiomer **5a** can be isolated by crystallisation with a yield of 71% of the feed enantiomer **5a**.

In the experiment of Figure 3, enantiomer **5a** is isolated by crystallisation, but due to the symmetric behaviour of enantiomeric systems the isolation of **5b** is possible, if the mother liquor is enriched with **5b**. For the production of enantiomer **5b**, method b in Figure 5 should be used. Enantiomer **5b** results from the acetals **3b** and **3c**, which are present in a portion of 55%. The content of tetraol **3a** can be reduced by cooling crystallisation with methanol as described in Figure 1. Roughly 80% of **3a** can be removed from the mixture by crystallisation from methanol. The mother liquor of this step is enriched with tetraols **3b** and **3c** and would result in higher yields of **5b** in the second crystallisation step with 1-propanol (right part of Fig. 1). The synthesis sequence is continued with the enriched mother liquor (in reference to **3b** and **3c**) and at last $Pur_{\mathbf{5b}}$ reaches a value of ca. 68%, which results in a yield of 53% for the second crystallisation step.

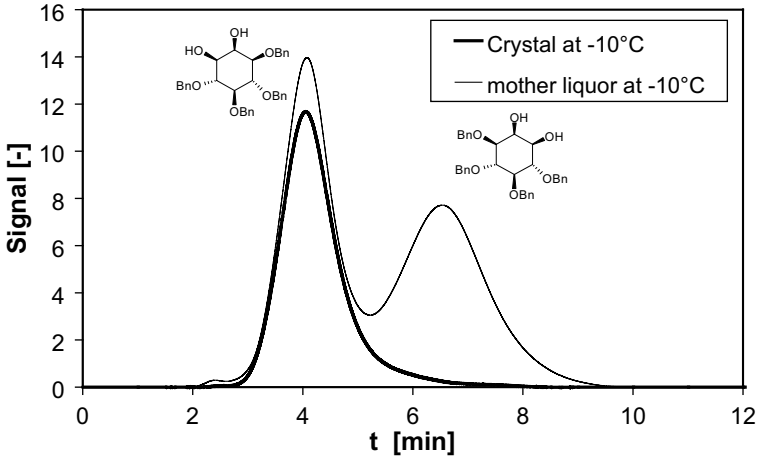


**Figure 4.** Analysis of mother liquor and crystal after the crystallisation process by HPLC (Chiralpak AD/1-propanol).[17]

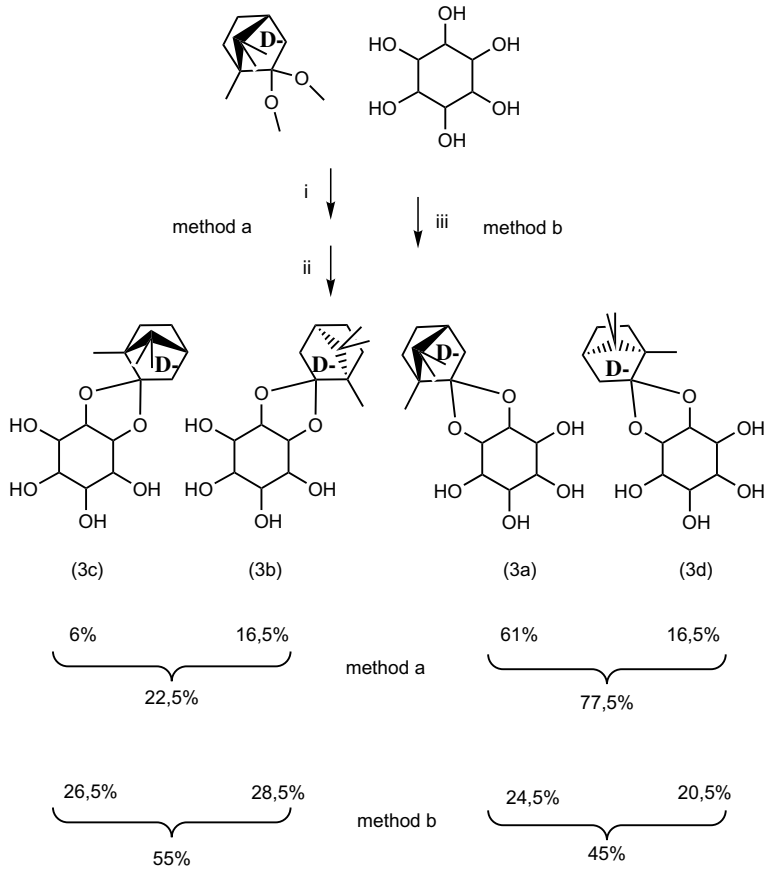


**Figure 5.** Reagents and conditions: (i) DMSO, 3 equiv D-camphor dimethyl acetal, cat. [*p*-TsOH], rt–75 °C, 2 h, HPLC control until almost no monoacetal was detected; $Et_3N$; (ii) $CHCl_3$/MeOH/$H_2O$ 90:10:2, cat. [*p*-TsOH], HPLC control until almost no diacetal was detected; $Et_3N$; (iii) *myo*-inositol dissolved in DMSO (110 °C); then 1.1 eq. D-camphor dimethyl acetal, cat. [*p*-TsOH], 80 °C, 60 min; then $Et_3N$.

## 3. Conclusions

In conclusion, a new option for the preparation of enantiopure tetra-*O*-benzyl-*myo*-inositol derivatives is presented. Both enantiomers were obtained with D-camphor dimethyl acetal as the chiral auxiliary and two effective crystallisation steps. No expensive L-camphor was needed. This resulted in a reduced number of synthetic steps, because the second route (with L-camphor dimethyl acetal) became redundant.

Resolution of the stereoisomers was obtained by two crystallisation steps. The first step was used to isolate tetraol **3a** in high yield from the diastereomeric mixture. The crystal of pure tetraol **3a** resulted in D-1,4,5,6-tetra-*O*-benzyl-*myo*-inositol. The mother liquor of crystallisation step 1 was enriched with the 1,2-D-camphor-acetals of *myo*-inositol. If the synthesis was continued with the mother liquor, a non-racemic mixture of tetra-*O*-benzyl-*myo*-inositols was obtained from which D-3,4,5,6 tetra-*O*-benzyl-*myo*-inositol was isolated by crystallisation from 1-propanol in the second crystallisation step. The eutectic point of this ternary system was agreed as a racemic mixture. This eutectical point could be observed for many other *myo*-inositol derivatives, which leads to new options for the enantiopure production of these molecules. Due to the advantageous eutectic point of 50% purity, pure enantiomers can be directly isolated from the enriched mixture. Enrichment can be reached by crystallisation and chromatographic separation of diastereomers after one of the first two steps or by choosing suitable reaction conditions during formation of diastereomers.

Next to the improved production process, advances in the analysis of *myo*-inositol derivatives are presented. For the first time, the separation of all four D-camphor-acetals of *myo*-inositol **3a**–**d** is possible by separation with Hypercarb® columns. Further analysis of tetra-*O*-benzyl-*myo*-inositols was obtained by HPLC separation with Chiralpak AD and 1-propanol.

## Acknowledgements

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## References

1. Bruzik, K. S.; Tsai, M. *J. Am. Chem. Soc.* **1992**, 6361–6374.
2. Posternak, S. *C.R. Acad. Sci.* **1919**, *169*, 138–140.

3. Streb, H.; Irvine, R. F.; Berridge, M. J.; Schulz, I. *Nature* **1983**, *306*, 67–69.
4. Markadieu, N.; Blero, D.; Boom, A.; Erneux, C.; Beauwens, R. *Am. J. Physiol.-Renal Physiol.* **2004**, *287*, F319–F328.
5. Irvine, R. F.; Schell, M. J. *Nature Rev. Mol. Cell Biol.* **2001**, *2*, 327–338.
6. Mills, S. J.; Riley, A. M.; Liu, C. S.; Mahon, M. F.; Potter, B. V. L. *Chem.-Eur. J.* **2003**, *9*, 6207–6214.
7. Traynor-Kaplan, A.; Schultz, C.; Meyerdierks, T.; Moody, M.; Schnaars, A.; Smith, J. Inositol derivatives for increasing chloride secretion and inhibiting inflammation, 2000.
8. Sureshan, K. M.; Yamasaki, T.; Hayashi, M.; Watanabe, Y. *Tetrahedron: Asymmetry* **2003**, *14*, 1771–1774.
9. Rudolf, M. T.; Dinkel, C.; Traynor-Kaplan, A. E.; Schultz, C. *Bioorg. Med. Chem.* **2003**, *11*, 3315–3329.
10. Eckmann, L.; Rudolf, M. T.; Ptasznik, A.; Schultz, C.; Jiang, T.; Wolfson, N.; Tsien, R.; Fierer, J.; Shears, S. B.; Kagnoff, M. F.; Traynor-Kaplan, A. E. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 14456–14460.
11. Bruzik, K.; Salamonczyk, G. M. *Carbohydr. Res.* **1989**, *195*, 67–73.
12. Ross, P.; Ax, O. *Laborpraxis Sep.* **2000**, 29–35.
13. Linnemann, J. Master thesis, Dortmund, 2003.
14. Strohlein, G.; Schulte, M.; Strube, J. *Sep. Sci. Technol.* **2003**, *38*, 3353–3383.
15. Mullin, J. W. *Crystallization*, 3rd ed.; Butterworth-Heinemann: London, 1993.
16. Schroer, J. W.; Wibowo, C.; Ng, K. M. *Aiche J.* **2001**, *47*, 369–387.
17. Wewers, W.; Gillandt, H.; Schmidt-Traub, H. SPICA, Aachen, 2004.