Chromatographic Isolation and Enantioselective Separation of Atropisomeric Polybrominated Biphenyls (PBBs)

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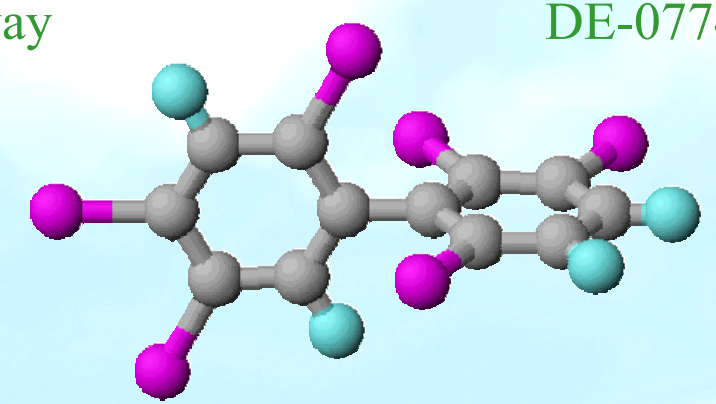

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Overview

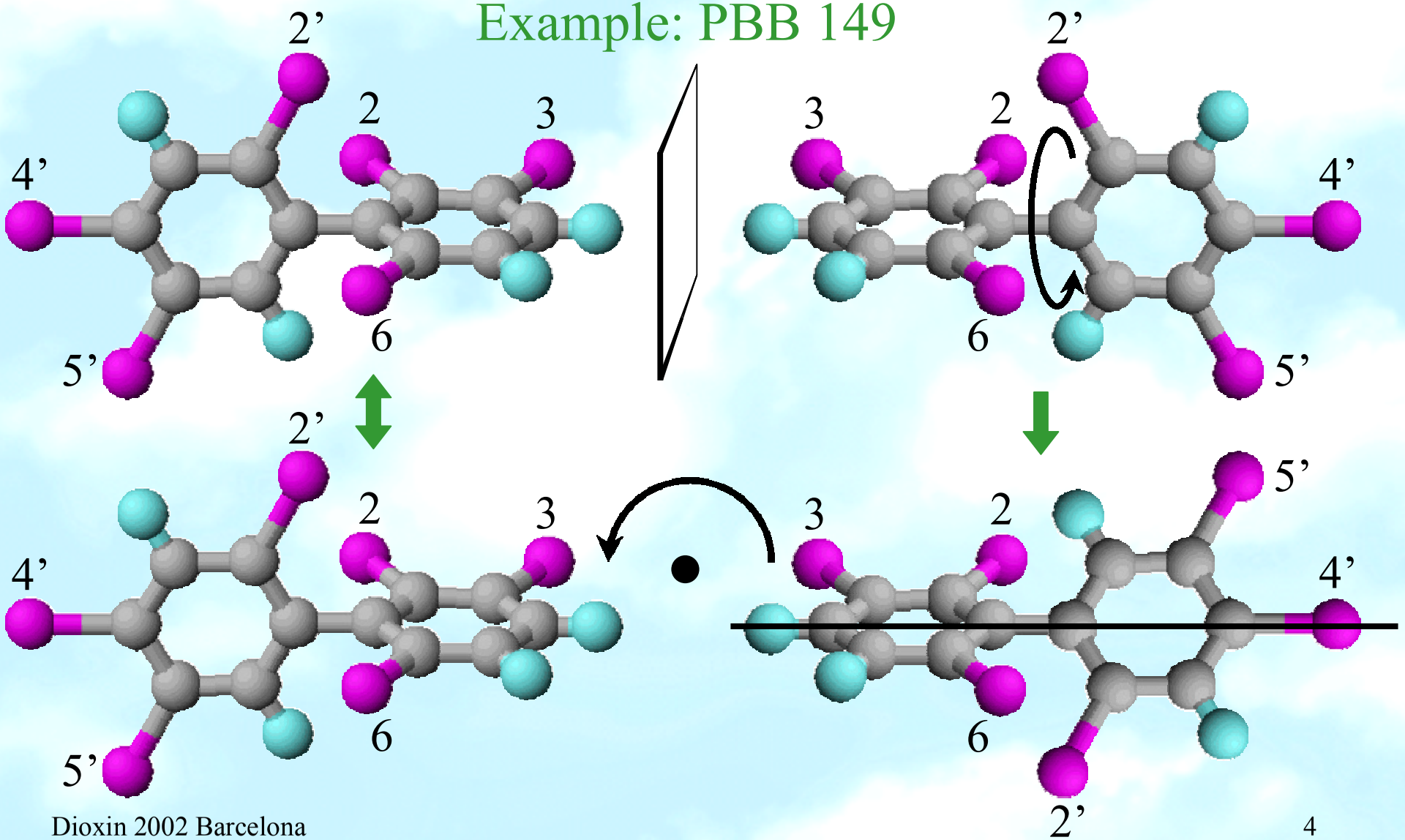
- Introduction
  - Polybrominated biphenyls (PBBs)
  - Atropisomerism
- Experimental
- Results and Discussion
  - Characterisation of Firemaster BP-6®
  - Isolation of PBB congeners
  - Enantioselective separation by HPLC and GC
- Conclusions and Outlook

Introduction
Polybrominated Biphenyls (PBBs)

- Structure
  - Analogous to PCB (Br instead of Cl); 209 congeners
- Use
  - Flame retarding additives (mainly technical mixtures) to polymers in the 1970s and 1980s
  - Employed in dyes, lacquers and in textile, electronic and motor industry
- Toxicity
  - Low acute toxicity, but high bioaccumulation rates
  - Tumour promoters
  - Neurotoxic effects

Introduction: Atropisomerism

Example: PBB 149



Introduction
Analytical Challenges

- Potential atropisomeric PBB congeners are not commercially available as single standards
- Enantiomer separation
  - Best suited chiral stationary phase (CSP)?
- HPLC lacks a sensitive detector for PBBs
- GC requires high elution temperatures (>200 °C)
  - Maximum operation temperatures for CSP
  - Enantiomerisation of PBBs which might form stable atropisomers at physiological temperatures?

Experimental

- Firemaster BP-6® (Fm BP-6®)
  - Technical hexabromobiphenyl (about 20 congeners)
  - PBB 153 dominates (approx. 60 %)

- LC-fractionating
  - Glass column (10 mm i.d.) with 4 g Envicarb/Celite (1:1)
  - Mobile phase: *n*-hexane

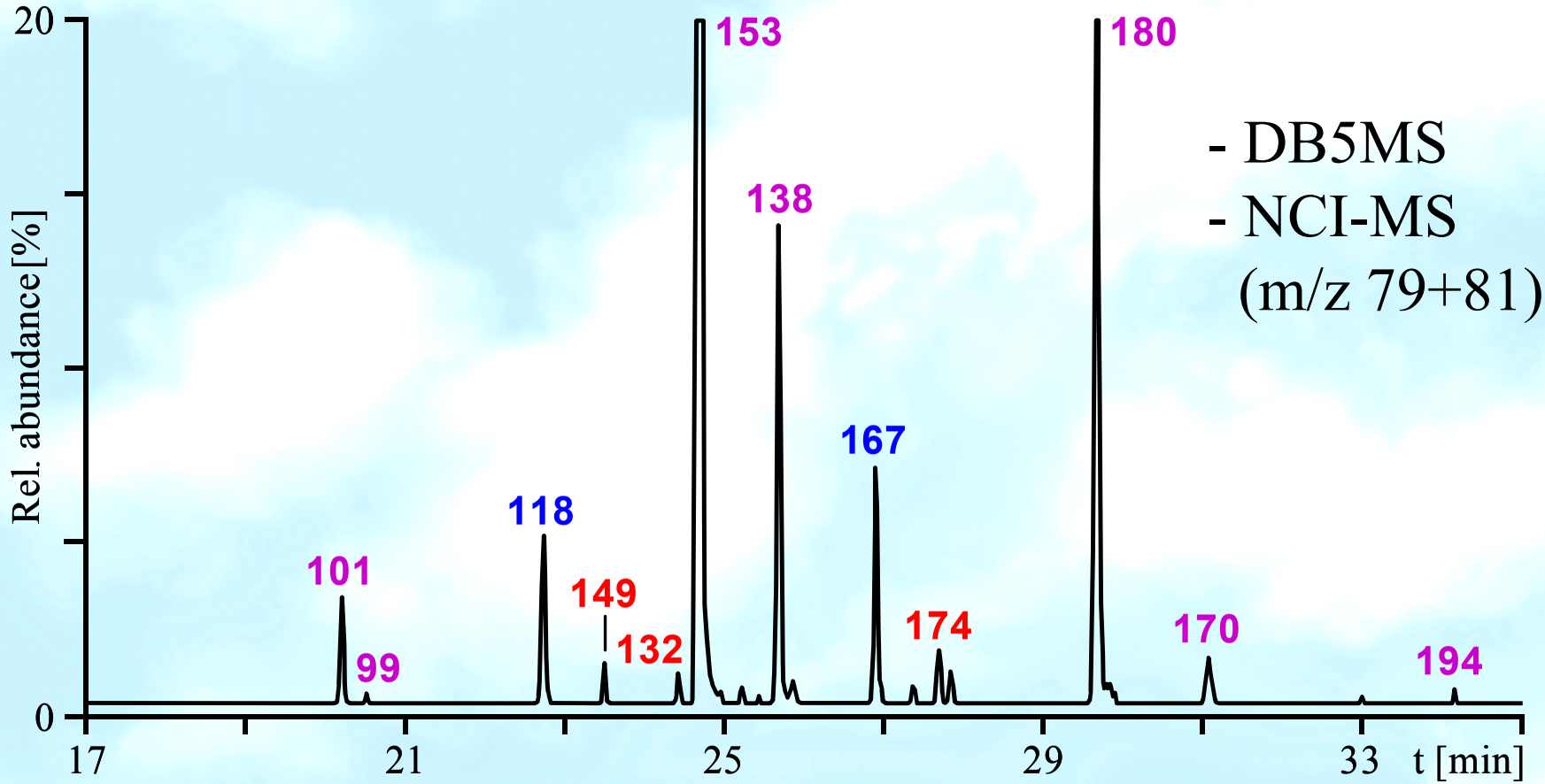
Experimental

- HPLC
  - For isolation of PBB congeners: Three C_{18} columns (250 × 4.6 mm each) in series
  - For separation of atropisomers: Best results with permethylated β-cyclodextrin (β-PMCD) on silica; also α-PMCD and β-PMCD tested
  - Mobile phase: acetonitrile-water (isocratic)
  - UV detection at 220 nm

Experimental

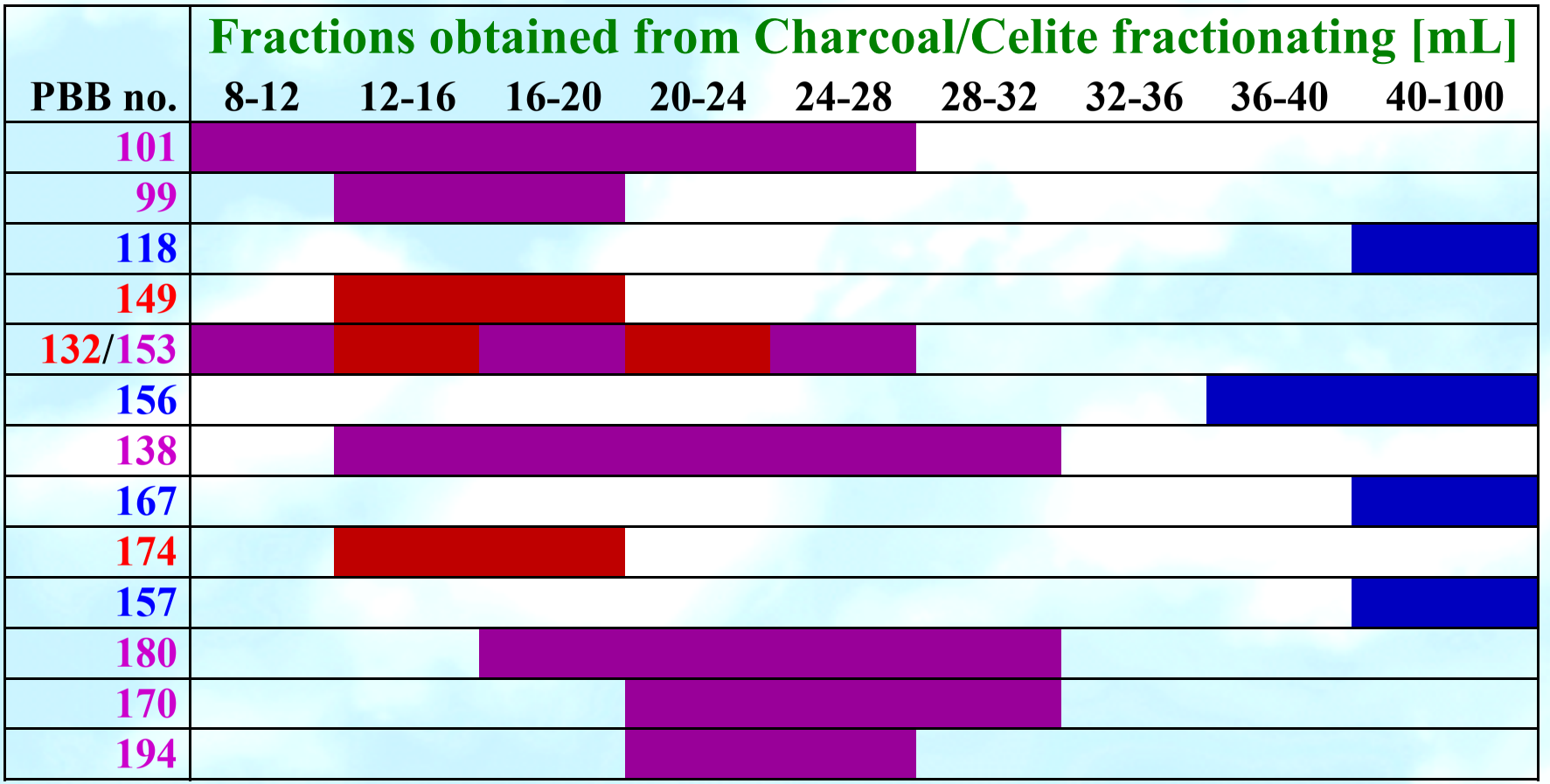
- GC
  - For characterisation of Fm BP-6®: DB5MS and CP-Sil 2 columns
  - For separation of atropisomers: Best results with 35 % randomly modified heptakis(6-*O*-*tert*.-butyldimethylsilyl-2,3-di-*O*-methyl)-β-cyclodextrin in OV 1701 (β-TBDM); also other cyclodextrin columns tested
  - Electron capture detection (ECD) or negative ion chemical ionisation mass spectrometric detection (NCI-MS)

Results and Discussion
Characterisation of Fm BP-6® by GC



Blue: Mono-*ortho* substituted PBB
Purple: Di-*ortho* substituted PBB
Red: Tri-*ortho* substituted PBB

Results and Discussion
Fractionating on Charcoal/Celite



Blue: Mono-*ortho* substituted PBB
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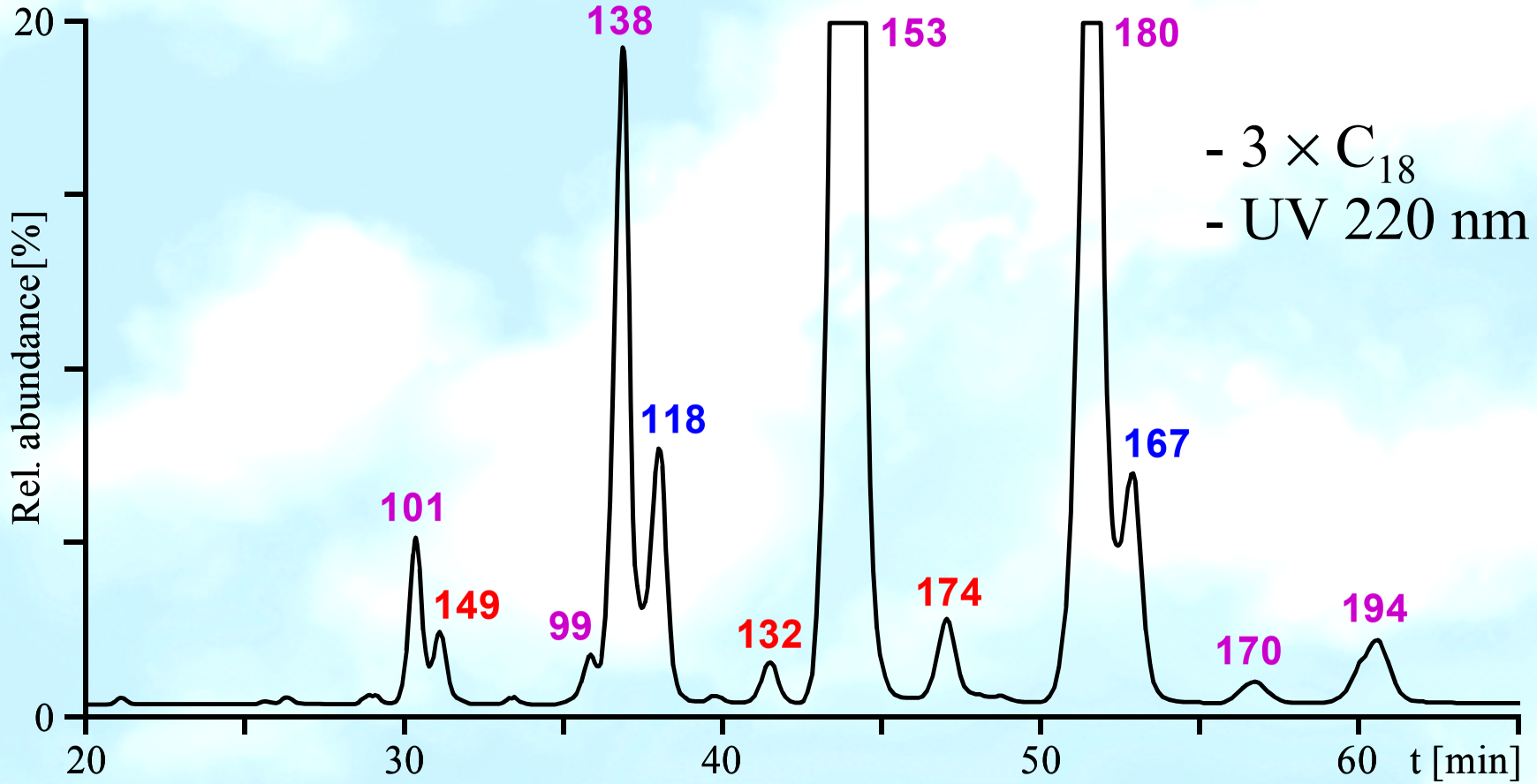
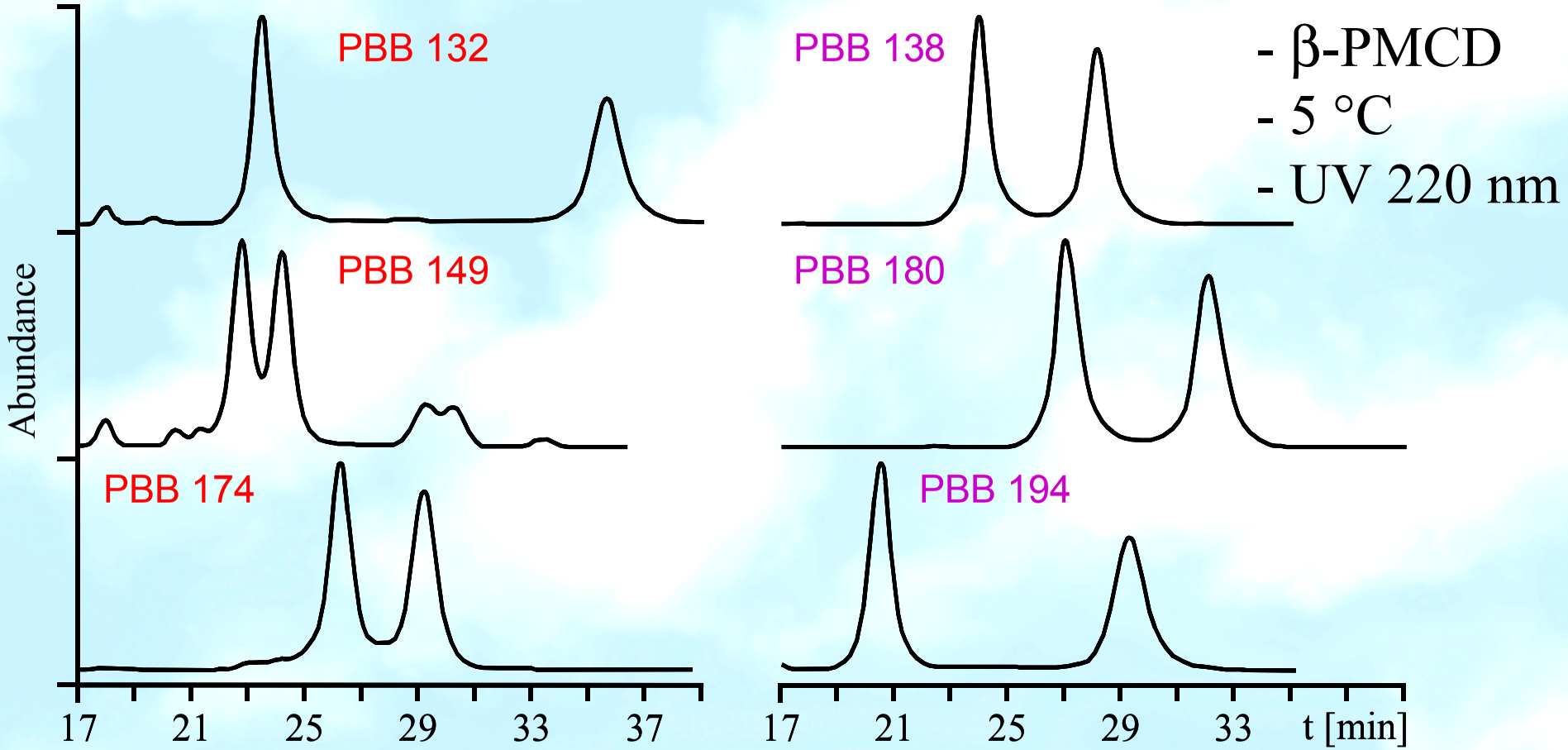
Results and Discussion
Isolation of PBB Congeners by HPLC



Blue: Mono-*ortho* substituted PBB
Purple: Di-*ortho* substituted PBB
Red: Tri-*ortho* substituted PBB

Results and Discussion: Enantioselective Separation of isolated PBBs by HPLC

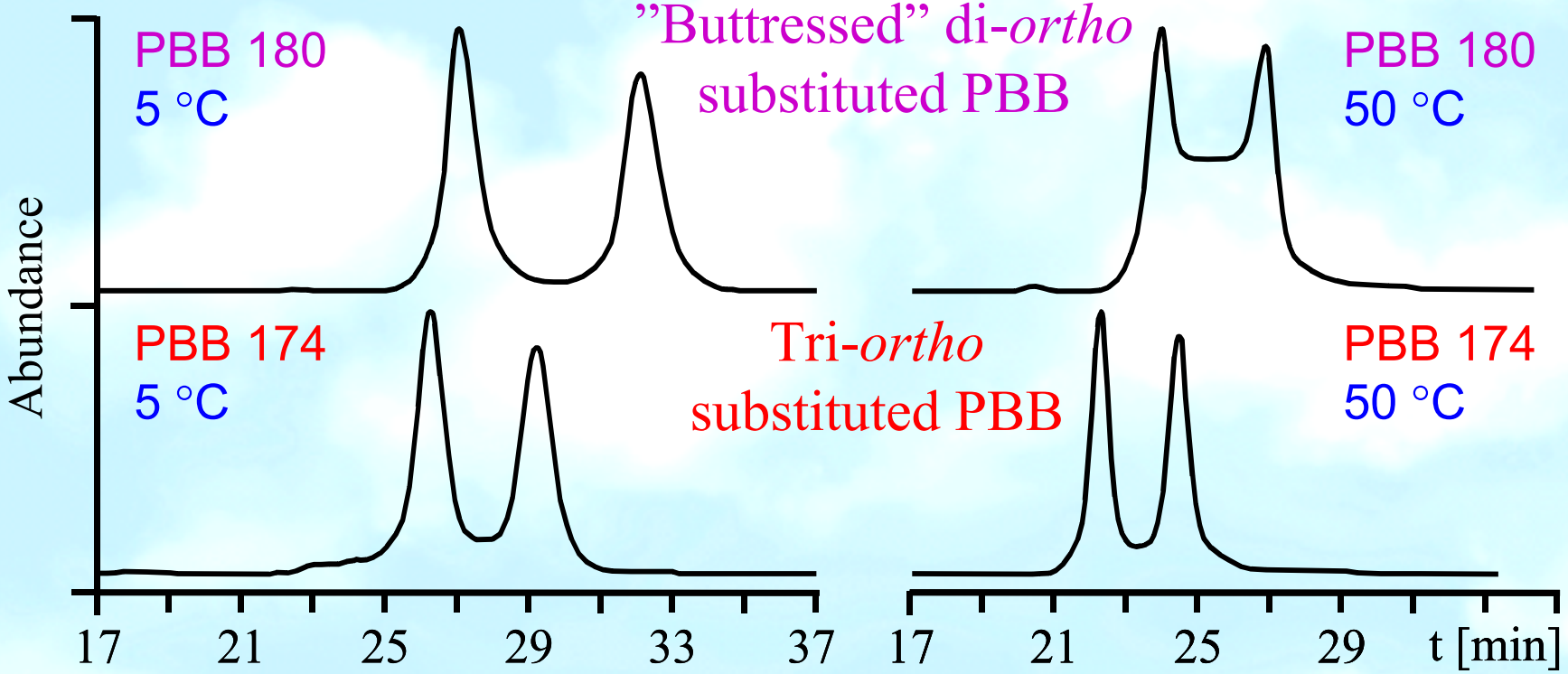


Tri-*ortho* substituted PBBs

"Buttressed" di-*ortho* subst. PBBs

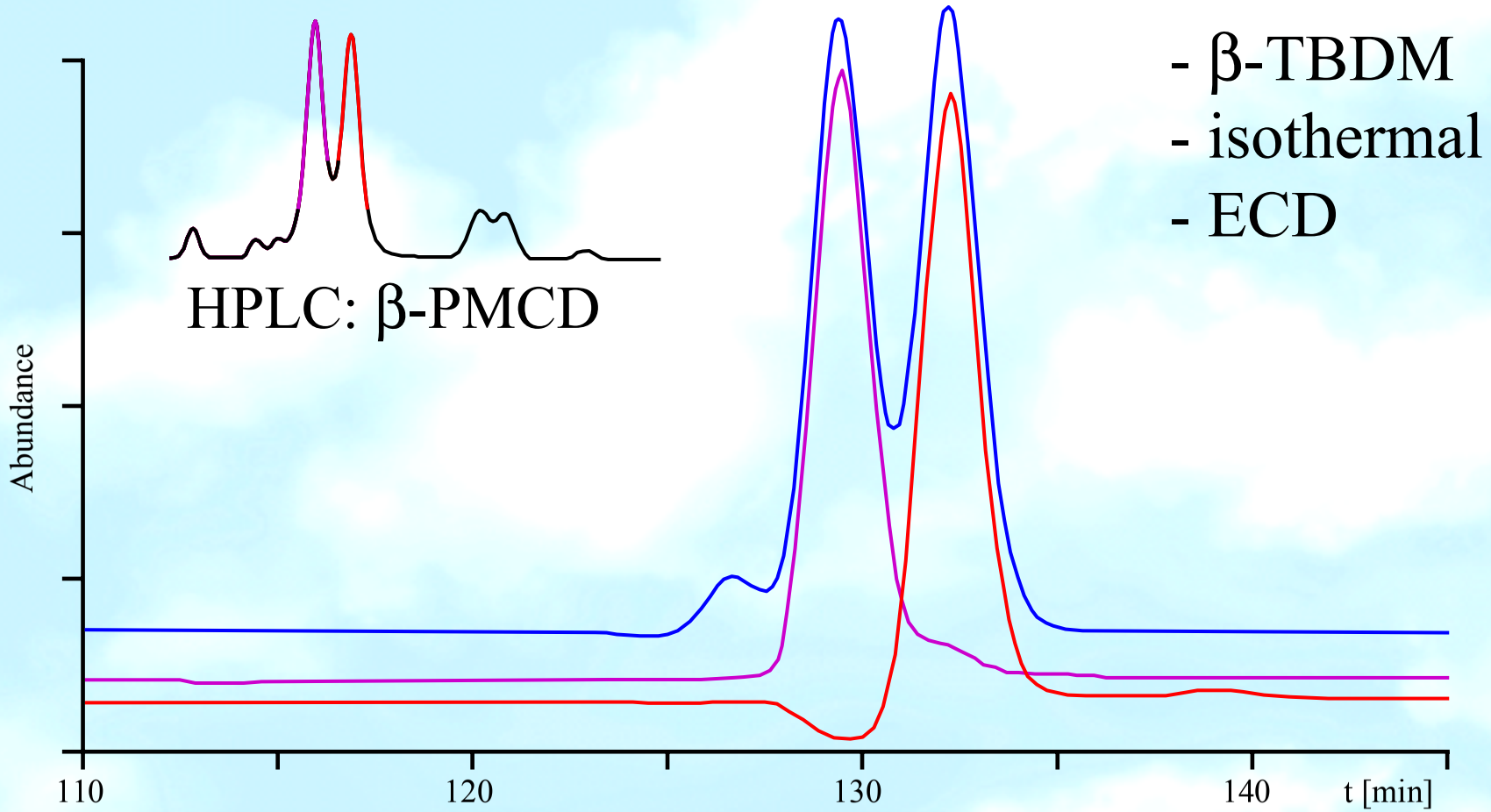
Results and Discussion
Temperature Dependence

- β-PMCD
- UV 220 nm



Results and Discussion
Enantioselective Separation by GC

PBB 149



Conclusions

- Twelve PBB congeners isolated, six of them were separated into atropisomers by HPLC
- Enantioselective separation of PBBs by GC is very challenging but succeeded for PBB 149
- Tri-*ortho* and buttressed di-*ortho* substituted PBBs proved to form stable atropisomers at physiological temperatures
- Enantioselective processes may therefore play a role for these compounds in the environment

Outlook

- Enantioselective HPLC can be used to obtain pure PBB atropisomers for toxicity testing. However, HPLC/UV is not sensitive enough for environmental trace analysis of PBBs.

- GC meets the selectivity and sensitivity requirements of environmental analysis. More work is necessary to improve chiral GC separations of PBBs to finally study enantioselective processes in the environment.

Acknowledgments

H. Bergmann, B. Luckas and co-workers (University of Jena, Germany) for providing HPLC equipment

M. Oehme (University of Basel, Switzerland) for providing chiral HPLC columns

The German Academic Exchange Service and the Research Council of Norway for financial support (project 313-PPP-N1-01)