CHARLES UNIVERSITY

FACULTY OF PHARMACY IN HRADEC KRÁLOVÉ

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY AND PHARMACEUTICAL ANALYSIS

DISSERTATION THESIS:

CHIRAL SEPARATIONS OF BORANE CLUSTER COMPOUNDS

(A collection of published papers accompanied by a commentary)

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Supervisor: doc. PharmDr. Radim Kučera Ph.D.

Hradec Králové, 2023

Statement

I declare that this thesis is my original work, which I have carried out independently under the guidance of my supervisor. All the literature and other sources I have drawn upon in the preparation of this thesis are listed in the reference list and duly cited. The thesis has not been used to obtain another or the same degree.

Mgr. Ondřej Horáček

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Abstract

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Title: Chiral separations of borane cluster compounds

The inorganic three-dimensional boron cluster compounds have recently gained the attention of scientists due to their interesting properties, e.g., exceptional thermal, chemical, and biological stability, unusual three center two electron bonds, and three-dimensional aromaticity. The subclass of boron clusters is carboranes, which possess at least one carbon atom in the cage, combining the properties of inorganic and organic compounds in one molecule. The carbon atom enables various substitution of the cluster by different organic substituents, resulting in a plethora of derivatives. Although thousands of compounds have been synthesized, the derivatives of anionic cobalt bis(dicarbollide) ($[Co(C_2B_9H_{11})_2]^-$) have recently been thoroughly investigated, particularly in medicinal chemistry, materials chemistry, electrochemistry, and analytical chemistry. The interest in $[Co(C_2B_9H_{11})_2]^-$ is usually explained by its solubility in water, chemical and biological stability, low toxicity, latent amphiphilic character, and similarity to the chemistry of ferrocene.

Many derivatives of $[Co(C_2B_9H_{11})_2]^-$ reported in the literature are chiral. Despite its potential use in medicinal chemistry and pharmacy, the chirality of these species has been overlooked by the scientific community. This could be partly explained by the inability to enantioseparate derivatives of $[Co(C_2B_9H_{11})_2]^-$ and their building blocks *nido*-[7,8-$C_2B_9H_{11}]^{2-}$ and their derivatives with chromatographic techniques. Thus, the pure enantiomers of these species could not be obtained on a semipreparative or preparative scale.

The dissertation presents a comprehensive picture of the chromatographic behavior of *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ and $[Co(C_2B_9H_{11})_2]^-$ derivatives on polysaccharide-based and β-cyclodextrin-based chiral stationary phases in high-performance liquid

chromatography and on polysaccharide-based chiral stationary phases in high-performance liquid chromatography and supercritical fluid chromatography. The previously observed discrepancy between the successful chiral separations of anionic derivatives of *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ in capillary electrophoresis and the unsuccessful enantioseparations in high-performance liquid chromatography is explained. As a result, the first chiral separations of four *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ and eight $[Co(C_2B_9H_{11})_2]^-$ derivatives in high-performance liquid chromatography are reported. Further, strategies and workflows for the chiral separations of anionic *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ and $[Co(C_2B_9H_{11})_2]^-$ derivatives are suggested on polysaccharide-based columns in reverse phase high-performance liquid chromatography and supercritical fluid chromatography. The chiral separations and workflows developed herein open a door to investigate the impact of chirality of studied species in multiple branches of science including medicinal chemistry and materials chemistry.

Abstrakt

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Název: Chirální separace klastrových sloučenin boru

Anorganické trojrozměrné klastrové sloučeniny boru se staly předmětem zájmu vědců zejména díky svým zajímavým vlastnostem, např. výjimečné tepelné, biologické a chemické stabilitě, neobvyklé třístředodové dvouelektronové vazbě a trojrozměrné aromaticitě. Podtřídou boranových klastrů jsou karborany, které mají v kleci inkorporován alespoň jeden atom uhlíku, čímž kombinují vlastnosti anorganických a organických látek v jedné molekule. Atom uhlíku umožňuje modifikaci klastru různými organickými substituenty, což vede k existenci nepřeberného množství derivátů. Z tisíců těchto sloučenin byly důkladně zkoumány deriváty aniontového bis(dikarbolidu) kobaltu ($[Co(C_2B_9H_{11})_2]^-$), a to zejména ve farmaceutické chemii, chemii materiálů, elektrochemii a analytické chemii. Zvýšený zájem o $[Co(C_2B_9H_{11})_2]^-$ je obvykle vysvětlován jeho rozpustností ve vodě, chemickou a biologickou stabilitou, nízkou toxicitou, skrytým amfifilním charakterem a podobností s chemií ferrocenu.

Mnoho derivátů $[Co(C_2B_9H_{11})_2]^-$ uvedených v literatuře je chirálních. Navzdory jejich potenciálnímu využití ve farmaceutické chemii a medicíně byla chiralita těchto látek vědeckou komunitou přehlížena. To lze částečně vysvětlit neúspěšnými chirálními separacemi $[Co(C_2B_9H_{11})_2]^-$, *nido*-$[7,8\text{-}C_2B_9H_{11}]^{2-}$ a jejich derivátů pomocí chromatografických technik. Z toho důvodu nebylo možné získat čisté enantiomery těchto látek v semipreparativním nebo preparativním měřítku.

Disertační práce podává ucelený obraz chromatografického chování derivátů *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ a $[Co(C_2B_9H_{11})_2]^-$ na β-cyklodextrinových chirálních stacionárních fázích ve vysokoúčinné kapalinové chromatografii a polysacharidových chirálních stacionárních fázích ve vysokoúčinné kapalinové chromatografii a superkritické fluidní chromatografii. V práci je dále objasněn rozpor mezi úspěšnými a neúspěšnými chirálními separacemi

aniontových derivátů *nido*-[7,8-$C_2B_9H_{12}$]$^-$ v kapilární elektroforéze respektive ve vysokoúčinné kapalinové chromatografii. Výsledkem jsou první chirální separace čtyř derivátů *nido*-[7,8-$C_2B_9H_{12}$]$^-$ a osmi derivátů [$Co(C_2B_9H_{11})_2$]$^-$ pomocí vysokoúčinné kapalinové chromatografie. Dále jsou navrženy strategie a pracovní postupy pro chirální separace aniontových derivátů *nido*-[7,8-$C_2B_9H_{12}$]$^-$ a [$Co(C_2B_9H_{11})_2$]$^-$ na kolonách na bázi polysacharidů ve vysokoúčinné kapalinové chromatografii na reverzních fázích a v superkritické fluidní chromatografii. Chirální separace a pracovní postupy vyvinuté v této disertační práci otevírají dveře zkoumání vlivu chirality studovaných látek v mnoha vědních oborech včetně farmaceutické chemie a chemie materiálů.

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List of abbreviations

ACN	Acetonitrile
BNCT	Boron Neutron Capture Therapy
BPR	Back Pressure Regulator
CCCC	Chiral Counter-current Chromatography
CE	Capillary Electrophoresis
CSP	Chiral Stationary Phases
HPCCCC	High-Performance Chiral Counter-current Chromatography
HPLC	High-Performance Liquid Chromatography
ICH	The International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use
LC	Liquid Chromatography
MeOH	Methanol
MES	2-(*N*-Morpholino)ethanesulfonic acid
NPLC	Normal-Phase Liquid Chromatography
POM	Polar Organic Mode
RPLC	Reversed-Phase Liquid Chromatography
SFC	Supercritical Fluid Chromatography
SMBC	Simulated Moving Bed Chromatography
SPP	Superficially Porous Particles
Tris	*tris*(hydroxymethyl)aminomethane
UHPLC	Ultra-High-Performance Liquid Chromatography

1 Introduction

Boron cluster compounds were firstly synthesized in the 1930s based on the pioneering work of Alfred Stock on boron hydrides [1]. Boron clusters possess various shapes and geometries that cover different numbers of vertices, the type and number of heteroatoms, and number of substituents resulting in the countless amount of molecules. The vastly studied group of boron clusters are carboranes that contain one or more carbon atoms embedded into the structure of a cluster cage. Carboranes have opened the door to entirely new chemistry by connecting inorganic and organic aspects in one molecule. For example, anionic carboranes are studied in medicinal chemistry [2, 3] because of the solubility of their alkali and alkaline earth metal salts in water, chemical and biological stability, and low toxicity. Despite the solubility in water, the hydrophobic character of boron hydride groups prevents the formation of a stable hydration shell. Therefore, anionic carboranes have a latent amphiphilic character that can be used for the transfer of the bioactive molecule across the blood-brain barrier.

Recently, derivatives of anionic dicarba-carboranes, i.e., 7,8-dicarba-*nido*-undecaborate(1-) (*nido*-[7,8-$C_2B_9H_{12}$]$^-$) and cobalt bis(dicarbollide) ([Co($C_2B_9H_{11}$)$_2$]$^-$), gained the attention over other carboranes due to two and four carbon atoms that can be used for further modification of the molecule. Such compounds can be investigated in different research areas including catalysis [4], synthetic chemistry [5], analytical chemistry [6, 7], medicinal chemistry [2], photochemistry [8], material chemistry [9-11], and electrochemistry [12]. The carboranes mentioned above can be chiral depending on the substitution of a molecule [13]. Nevertheless, the research on enantiomers of these compounds has not been properly investigated. This fact is surprising, especially in medicinal chemistry, where the significance of chirality cannot be neglected even in the early stages of drug development. Omitting chirality is impossible due to the substantial financial losses at best and to the losses of human lives at worst, as we know from the thalidomide affair. Therefore, the effect of the spatial arrangement of individual optical isomers on the pharmacological activity and toxicity must be investigated according to the current legislation (regulation requirements).

In this work, the primary research concerning the chromatographic behavior and chiral screening of *nido*-[7,8-$C_2B_9H_{12}$]$^-$ and [Co($C_2B_9H_{11}$)$_2$]$^-$ derivatives in high-performance liquid chromatography (HPLC) and supercritical fluid chromatography (SFC) was carried out using stationary phases based mainly on β-cyclodextrin and polysaccharides.

2 Theoretical Part

2.1 Relevance of Chirality

The importance of the chirality was shown in many research areas (**Figure 1**) including fundamental chemistry [14, 15], luminescence [16], sensors [17-19], material chemistry [20-24], catalyst chemistry [25-27], immunology [28], food chemistry [29], and most importantly medicinal chemistry [30, 31]. The relevance of chirality in medicinal chemistry is well established, e.g. the fact that the biological effect of atropine resides in only one stereoisomer is known since 1926 [32]. However, the single-enantiomer drugs were not investigated because of the lack of chiral preparative and stereospecific synthetic methods. The underdevelopment in the separations and isolations of enantiomers, together with the lack of regulatory mechanisms, resulted in an enormous tragedy named the thalidomide scandal [33] that can be perceived as a failure of a modern science.

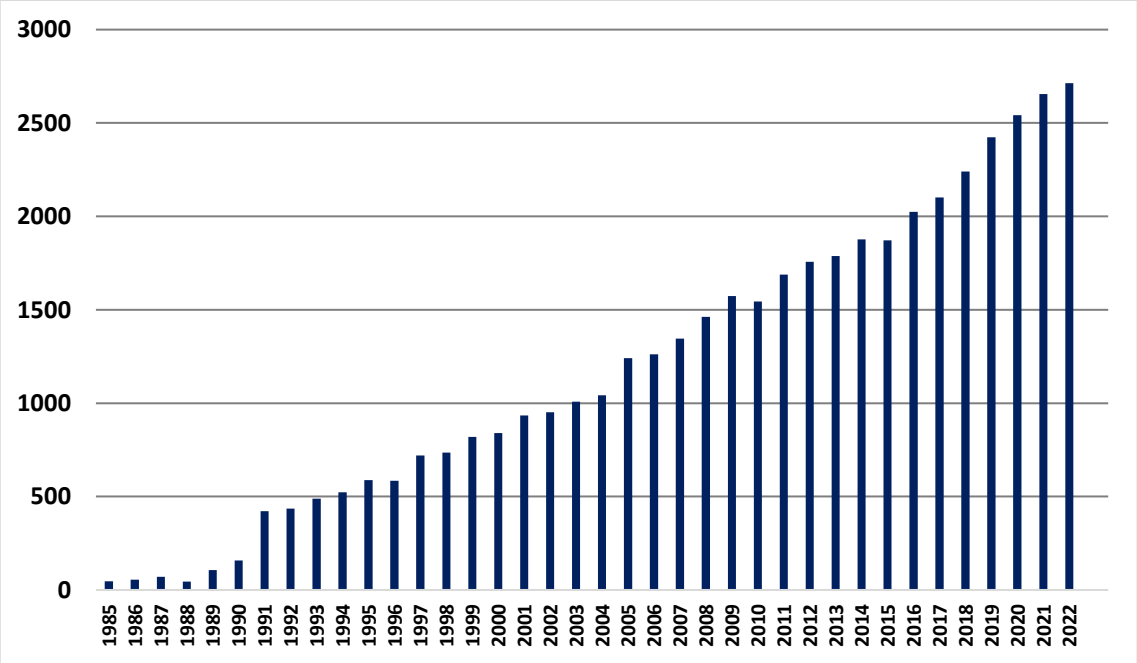

Figure 1 The number of publications on the chirality in impacted journals through the years 1985 to 2022. This time range is selected to demonstrate the rising trend of research on chirality from 1985 to present. The results were obtained by entering the key word "chirality" (All Fields) into the Web of Science database.

Especially the rapid development in chiral separation techniques (**Figure 2**) enabled the authorities to develop and regulate chirality in the pharmaceutical industry, starting with

the U.S. Food and Drug Administration guideline in 1992 [34] that was followed by the guidelines of the European Union [35] and Canada [36]. In 1999, the guidelines for the

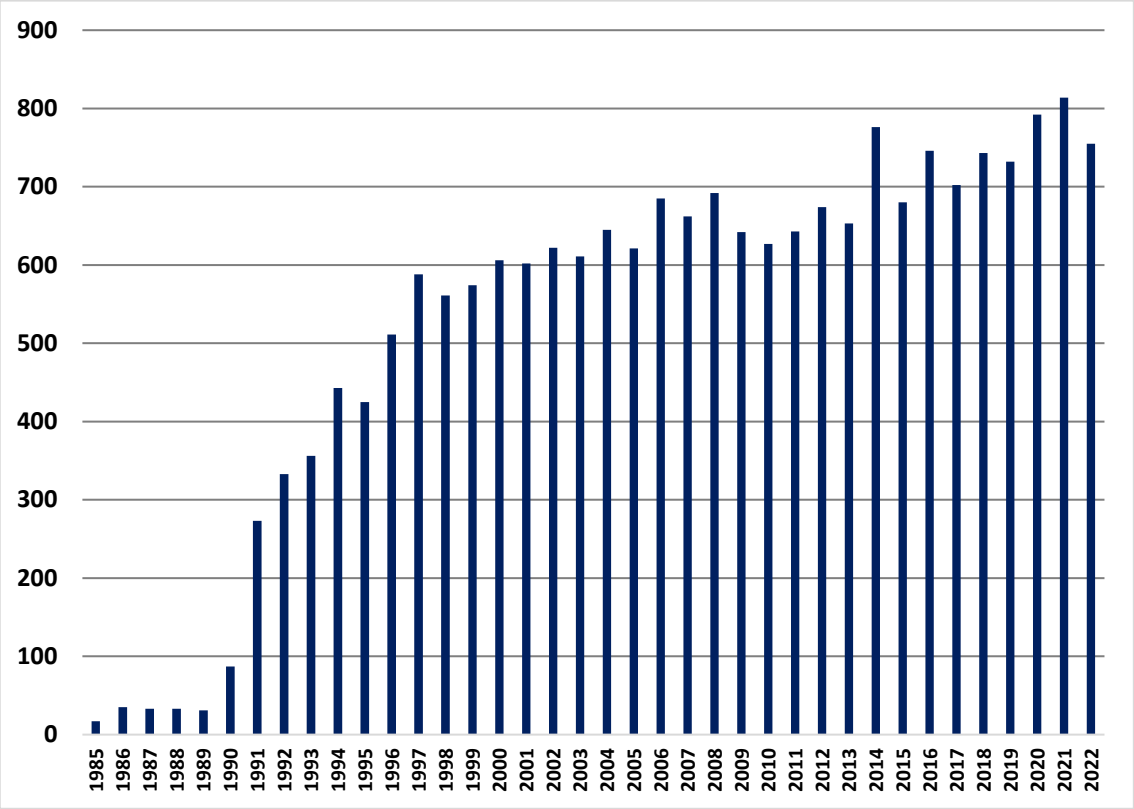

Figure 2 The number of publications on chirality in impacted journals through the years 1985 to 2022. This time range is selected to demonstrate the rising trend of research on chirality from 1985 to present. The results were obtained by entering the key word "chiral separations" (All Fields) into the Web of Science database.

registration of new drug substances and new drug products including single enantiomers, racemates, and nonracemic enantiomeric mixtures were introduced by the International Conference on Harmonization (ICH) Q6A [37]. The recommendations of ICH Q6A regarding the identification, assay, and enantiomeric purity of new chiral drug substances and products are summarized in the decision tree in **Figure 3**. Recently, the ICH Q6A guideline has been implemented by mainland China and Brazil. In contrast, the authorities in Turkey and Mexico have not implemented ICH Q6A yet [38]. Further work is necessary to ensure that the ICH Q6A guideline is implemented by the respective health agencies in the legislation of all countries.

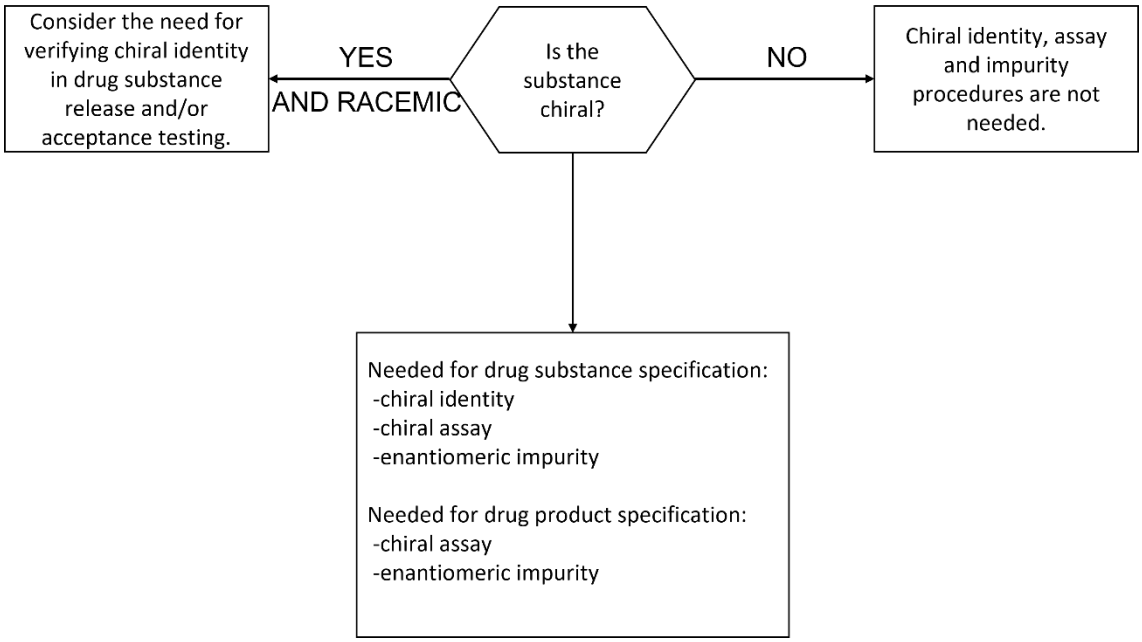

Figure 3 Decision tree for establishing identity, assay and enantiomeric purity procedures for chiral new drug substances and new drug products containing chiral drug substances according to Guideline ICH Q6A. The chiral substances of natural origin are not addressed in this guideline. Stereospecific testing of drug product may not be necessary if racemization has been demonstrated to be insignificant during drug product manufacture and during storage of the final dosage form. [37]

2.2 Chiral Separations

The evolution of chiral separation methods, asymmetric synthesis, and biotechnology greatly contributed to the drug safety by chiral switching, i.e., drug development of pure enantiomers instead of racemic mixtures. The success of chiral switching is documented by only 9 racemic drugs compared to 172 pure enantiomeric drugs approved by FDA from 2010 to 2020 [31]. To achieve successful approval of pure enantiomeric drug, reliable methods for the identification and quantitation of enantiomers and assessments of enantiomeric purity are crucial even in the early stages of drug development. Chiral analytical techniques used during the drug development are summarized in **Table 1** [39].

Moreover, the investigations and further development of chiral drugs must also be supported by the preparation of pure enantiomers. Since, the stereoselective synthesis is usually difficult to achieve, and the enantiomeric purity test is always necessary, other approaches are pursued to obtain the pure enantiomers, e.g., isolation of enantiomers by chromatography [40-42] or chiral crystallization [43]. The crucial advantage of chromatography is the possibility to use it for both analytical separations and isolation of

enantiomers. When preparative or semi-preparative chiral stationary phases (CSP) are used, the resolution between enantiomers must be high enough to entirely separate their chromatographic zones and achieve enantiomeric purity higher than 99%.

Table 1 Overview of analytical methods for chiral analysis. Reprinted and adapted with permission from [39]. Copyright (2011) Wiley.

Method	Description of Principle	Application
Chromatography -liquid -supercritical fluid	Separation of enantiomeric mixtures directly with the use of CSP or CS as a mobile phase additive.	Identification, enantiomeric purity assessment, and assay of enantiomers. Isolation of enantiomers is possible from mg to kg depending on the dimensions of the chromatographic column.
-gas	Separation of enantiomeric mixtures directly with the use of CSP.	Identification, enantiomeric purity assessment, and assay of enantiomers. Preparative separations are theoretically possible, but rarely used.
Capillary electrophoresis -aqueous	Separation of enantiomeric mixtures in aqueous environment by addition of CS, usually derivatized cyclodextrin.	Identification, enantiomeric purity assessment, and assay of enantiomers. The isolation of enantiomers in mg is possible but time-consuming and cost ineffective.
-nonaqueous	Separation of enantiomeric mixtures by addition of CS, usually derivatized cyclodextrin. Useful for compounds insoluble in aqueous environment.	
Chiral crystallization	Separation of enantiomers mostly by the formation of diastereomeric salts with the oppositely charged agent, i.e., ionizable functional group is necessary [43] and subsequent achiral separation.	Separation of enantiomers or enrichment of racemic mixture with one enantiomer enabling the identification of respective enantiomers.
X-ray crystallography **X-ray powder diffraction**	The X-ray wavelength are comparable to dimensions of crystal lattice that induces diffraction of the radiation. The structure of molecule is deduced from diffraction pattern.	X-ray crystallography in solid state is used to determine the absolute configuration of molecules and to distinguish conglomerates from racemic compounds.

(Continued on next page)

Table 1 (Continued)

Method	Description of Principle	Application
Nuclear magnetic resonance	The lanthanide shift reagents, chiral solvating agents, or chiral derivatizing agents are used to make the signals for the protons of the enantiomers nonequivalent.	It is used for assessment of the enantiomeric purity or enantiomeric composition.
Optical rotatory dispersion	It measures the change of specific rotation of an optically active compound with the wavelength of the light used	These methods are used to identify and/or quantitate enantiomers.
Circular dichroism	It measures the differential absorption of left and right circularly polarized light by an optically active compound.	
Optical rotation	Enantiomers rotate the plane of linearly polarized light in opposite directions in equal amounts.	Although this technique is widely used for identification and enantiopurity assessment, it is not very specific method for assay.
Differential scanning calorimetry	Difference in the amount of heat that is needed to be absorbed by the sample and the reference is measured as a function of temperature.	It is routinely used for polymorph assessment, that is, the melting points may be used in distinguishing enantiomers from racemate.

2.2.1 Chiral Liquid Chromatography

The success of liquid chromatography (LC) in enantioseparations stems from its universality, i.e., all compounds soluble in an organic solvent/water can be analyzed. The chiral separation with LC can be achieved directly with a chiral selector either in the mobile phase or CSP and indirectly. In this case the respective enantiomers react with a chiral agent and the resulting diastereomers are subsequently separated in an achiral system. Since the chiral separations developed in this dissertation should aid the development of the methods on a semi-preparative and preparative scale in the future, only a direct approach employing CSP will be discussed.

LC with the silica bonded CSP has been widely used for the discrimination of enantiomers since 1979 when Pirkle and House immobilized (R)-(-)-2,2,2-trifluoro-l-[9-(10-α-bromomethyl)anthryl]-ethanol onto silica gel support [44]. Soon after, the first silica-

bonded polysaccharide [45], cyclodextrin [46], protein [47], quinine [48], and macrocyclic antibiotic [49] based CSP were developed. In present, the mostly used CSP in HPLC are polysaccharides [50], followed by cyclodextrins [51] and proteins [52]. This fact was also concluded by Grybnik and Bosáková in review on chiral separations of pharmaceutically active substances by HPLC from 2018 to 2020 (**Figure 4**) [53]. The dominance of polysaccharide-based columns is based upon the commercial availability of 25 different chiral stationary phases [54], availability from different manufacturers, in multiple column lengths, diameters, particle sizes, and the highest saturation capacity of all commercially available columns [55]. The less employed CSP (presumably due to the specific range of compounds) that can be separated, are macrocyclic antibiotics, crown ethers, cyclofructans, donor-acceptor CSP, quinine/quinidine-based CSP, ligand exchangers, and synthetic polymers. The general summary of commercially available types of CSP are depicted in **Table 2**. In addition, a plethora of less employed and exotic chiral selectors are available and summarized in chapter **2.2.4**.

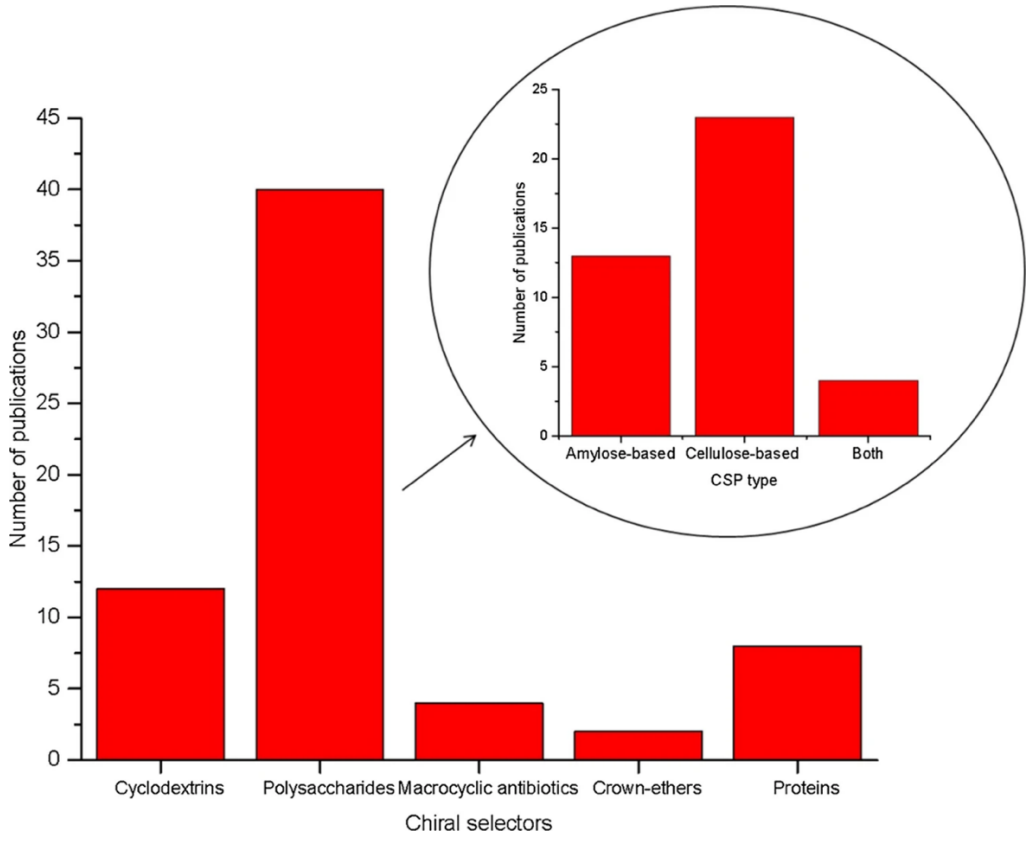

Figure 4 The published chiral separations in HPLC during the years 2018-2020. Reprinted with permission from [53]. Copyright (2021) Springer.

Table 2 General summary of commercially available CSP. Adapted from [56-58] with permission. Copyright (1997) Wiley, (2013) Human Press, and (2010) Springer.

Type of CSP	Chiral Recognition Mechanisms	Analyte Requirements	Chromatographic Modes
Polysaccharides	Inclusion complexation, steric interactions, H-bonding, π-π interactions, dipole stacking	Variable, most universal chiral selectors Steric bulk near the chiral center helps	Reversed phase, Polar organic mode, Normal phase
Cyclodextrins	Inclusion complexation, H-bonding	Polar and aromatic groups	Reversed phase, Polar organic mode, Normal phase
Proteins	Hydrophobic and electrostatic interactions	Ionizable groups Aromatic group helpful	Reversed phase
Macrocyclic glycopeptides	H-bonding, π-π interactions, dipole stacking; steric – hydrophobic pocket	Ability to form π- or H- or dipole-bond Steric bulk near chiral center assists	Reversed phase, Polar organic mode, Normal phase
Crown-ethers	Inclusion complexation, primary amino group-dipole	Primary amino group	Normal phase, Polar organic mode, Reversed phase
Donor-acceptors	H-bonding, π-π interactions, dipole stacking	Ability to form π- or H- or dipole-bond Aromatic group helpful	Reversed phase, Polar organic mode, Normal phase (recommended)
Quinine/Quinidine based	Electrostatic interactions, π-π interactions, H-bonding, dipole stacking	Acids or zwitterions Ability to form π- or H- or dipole-bond	Reversed phase, Polar organic mode
Cyclofructans	Inclusion of charged primary amino group	Primary amino group	Polar organic mode, Normal phase
Ligand-Exchangers	Coordination complexes to metals	α-Amino and α-hydroxy amino acids	Reversed phase

2.2.2 Approaches to Enantioseparations with LC

High-performance liquid chromatography (HPLC) using CSP is the most widely used liquid chromatographic technique for the separation of enantiomers. Recently, the introduction of CSP immobilized to sub-2 μm particles resulted in the need to use specialized ultra-high-performance liquid chromatography (UHPLC) systems that can withstand pressures greater than 40 MPa and possess low extra column volumes. The advantages of HPLC and UHPLC are high sensitivity (depending on the detector used) and a wide portfolio of separable analytes. The great advantage of HPLC over UHPLC is the possibility of enantioseparations on preparative scale.

The preparative separations can be also achieved by other liquid chromatographic techniques, e.g., chiral countercurrent chromatography (CCCC) and simulated moving-bed chromatography (SMBC). CCCC has been slowly adapted as a technique for chiral separations due to the high loadability, cheap liquid stationary phase, and low solvent consumption [59]. However, the development of CCCC is slower compared to that of HPLC due to the low theoretical plates and absence of highly selective chiral selectors. The separation efficiency has been recently increased by the introduction of high-performance CCCC (HPCCCC); this instrumental arrangement is today intensively studied for preparative chiral separations [59]. Moreover, multiple chiral selectors soluble in the water or organic phase have recently been investigated. In more than 50% of the applications β-cyclodextrin and its derivatives were used, followed by tartaric acid derivatives, chiral amine derivatives, and 18-crown-6-tetracarboxylic acid [59]. The significant development of HPCCCC in recent years confirms its potential to be used as a routine technique for chiral separations. However, further research addressing instrument design, chiral selectors, and knowledge of the solvent system needs to be carried out [59].

The other technique for the preparation of large quantities of pure enantiomers is simulated moving bed chromatography (SMBC) [41, 60]. Despite the high investment cost in the beginning and higher maintenance cost compared to a single column chromatography, SMBC is overall more cost-effective, especially due to the significantly lower mobile phase consumption and higher productivity. This is the reason why SMBC,

originally popular in the petrochemical industry, is an important separation technique in the modern pharmaceutical industry [41].

2.2.3 Enantioselectivity of Selected Chiral Stationary Phases in HPLC

The portfolio of separable compounds was greatly expanded since the 1980s due to the availability of multiple commercial CSP providing distinct enantioselective mechanisms. In addition to CSP, the selectivity of chiral methods is also affected by different chromatographic modes, including normal-phase liquid chromatography (NPLC), reversed-phase liquid chromatography (RPLC), and polar organic mode (POM), due to the diverse interactions that drive the chiral separation. This phenomenon is well known, especially for CSP based on cyclodextrin [61] and polysaccharides [62].

2.2.3.1 Cyclodextrin-based CSP

Cyclodextrins are naturally occurring cyclic oligosaccharides composed of D-glucopyranose units linked via α-1,4 bonds forming a shape of a truncated cone. The inner space of cyclodextrin cavity is hydrophobic due to the carbon chains of the monomers. The outer rims of the cavity are hydrophilic owing to secondary and primary hydroxy groups on the upper rim and lower rim of the cavity, respectively. Native cyclodextrins with 6, 7, and 8 monomers are named α-, β-, and γ- with the size of the cavity in diameter 0.57, 0.78, and 0.95 nm, respectively [63]. The semisynthetic cyclodextrins were prepared to enhance the selectivity of native ones by substitution of hydroxy groups on the rims of the cavity. Addition of substituents creates more interaction sites and also changes the shape and depth of the cavity [46].

The most used CSP are based on β-cyclodextrin due to the size of the cavity that can accommodate phenyl, naphthyl, and heterocyclic aromatic rings. The examples of commercially available β-cyclodextrin-based CSP bonded to fully porous particles of silica chromatographic support are Astec® CYCLOBOND™ I 2000 (native) [64], Astec® CYCLOBOND™ I 2000 RSP (*(RS)*-2-hydroxypropylether) [65], ChiraDex® LiChroCART® (native) [66], ChiraDex®HR LiChroCART® (native) [67], ReproSil Chiral-Beta-CD (native) [68], Ultron ES-CD (native) [69], and Ultron ES-PhCD-T (phenylcarbamated) [70], YMC CHIRAL *β*-CD BR (heptakis(6-bromo-6-deoxy)) [71],

Recently, CDShell-RSP (*(RS)*-2-hydroxypropyl-β-cyclodextrin bonded to SPP silica chromatographic support) has been commercialized by AZYP [72].

The mechanism of chiral recognition of cyclodextrins and their derivatives in RPLC is elucidated by the formation of a so-called inclusion complex. Its stability must be different for the respective enantiomers to achieve chiral separation (**Figure 5B)**. The inclusion complex is usually not formed in the POM, as the chiral analyte is displaced by the organic solvent (**Figure 5A**). Chiral separation in the POM is usually driven by hydrogen bonding, dipolar, and steric interactions of the enantiomer with hydroxy groups and other functional groups on the rim of native or semisynthetic cyclodextrins [61]. Furthermore, semisynthetic cyclodextrins derivatized with functional groups that can form π-π interactions, that is, acetyl, 3,5-dimethylphenyl carbamate, naphtylethyl carbamate, and *para*-tolouyl ester, were found to discriminate enantiomers in hydrophilic interaction chromatography [73], POM [73], and NPLC [46]. The groups that can form π-π interactions were found necessary for chiral separations in NPLC [74].

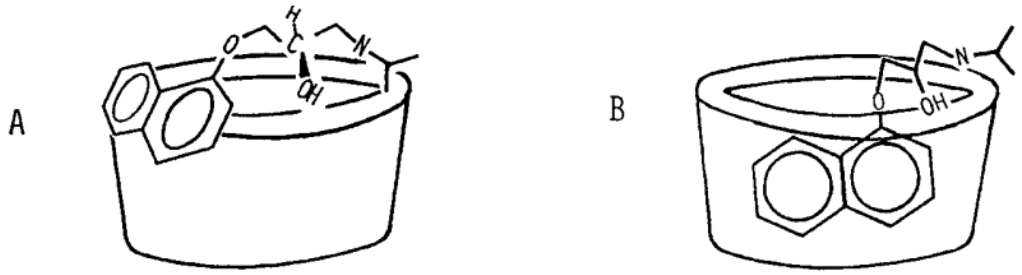

Figure 5 The mechanism of chiral recognition of propranolol on native β-cyclodextrin in the POM (A) and RPLC (B) elution mode. Reprinted with permission from [61]. Copyright (1997) Taylor and Francis.

The enantioselectivity of cyclodextrins varies with the composition of the mobile phase. Multiple studies on the effect of mobile phase additives and the type of organic solvent on enantioselectivity and peak shape in RPLC and POM were described in [74]. Briefly, organic solvents and additives can change selectivity by adjusting the type of interactions with the outer rims of cyclodextrin, thus, affect the binding affinity of the analytes to the cavity of cyclodextrin. Buffers and acidic or basic additives are commonly used for modulating the ionic state of molecules [74]. Concerning the less studied chromatographic conditions, the decrease of flow rate [75] and the decrease in

temperature [76, 77] were reported to increase enantioselectivity and peak shape of analytes on cyclodextrin-based CSP. Because the changes in temperature and flow rate have a limited effect on the enantioselectivity of only a narrow group of analytes, they are used only for the fine-tuning of a chiral method.

2.2.3.2 Polysaccharide-based CSP

Polysaccharide-based CSP are based on cellulose and amylose. Both polysaccharides are comprised of D-(+)-glucose units in chair conformation with five asymmetric carbons and three hydroxy groups in equatorial position. The different linkages of the monomers that is, β-(1,4) for cellulose and α-(1,4) for amylose result in the different higher order structures of respective polysaccharides [62].

The chiral separations on pure polysaccharides were not successful, therefore, the hydroxy groups were substituted with different groups from which the most successful are 3,5-dimethylphenylcarbamates. The structure of the semisynthetic polysaccharides was determined as 4/1 helix for amylose *tris*(3,5-dimethylphenylcarbamate) and a left-handed threefold 3/2 helix for the same derivative of cellulose [62, 78]. Although the structure of CSP is known, the separation mechanism of enantiomers on molecular level could be only estimated based on the chromatographic studies on a limited number of analytes. The interactions involved in separation mechanisms are presumed to be inclusion-complexation, steric interactions, H-bonding, π-π interactions, and dipole stacking [57, 62, 63]. The importance of respective interactions in chiral recognition is analyte dependent, therefore, the general rules are difficult to derive. Thankfully, a plethora of different polysaccharide-based columns with coated or immobilized chiral selectors for chiral screening is commercially provided by Daicel [54]. Other manufacturers have restricted number of CSP usually providing 3,5-dimethylphenylcarbamate derivatives of cellulose or amylose, and cellulose *tris*(3,5-dichlorophenylcarbamate) or another chlorinated derivative [79-83].

Similar to cyclodextrin-based CSP, polysaccharide-based CSP can be employed in NPLC, POM, hydrophilic interaction chromatographic mode, and RPLC. The enantioselectivity of the analytes can vary with the chromatographic mode employed depending on their physical and chemical properties [62]. For example, different selectivity has been recently studied for 26 acidic organic compounds on seven different

polysaccharide-based CSP in POM, RPLC, mixed POM, and NPLC [84]. The unusual hydrophilic interaction-like behavior was observed for ketoprofen on amylose *tris*(3,5-dimethylphenylcarbamate) coated on silica in mobile phase ACN-water. Similar behavior in the mobile phase containing ACN has resulted in a change in the elution order of coumatetralyl enantiomers on a column packed with cellulose *tris*(3,5-dimethyl-phenylcarbamate) coated on the silica. Furthermore, the authors tested the addition of 5%, 10%, and 14% of n-hexane to acetonitrile that allowed the increase in the enantioselectivity of coumatetralyl on amylose *tris*(3,5-dimethylphenylcarbamate) coated on the silica. On the other hand, no effect on selectivity was observed on its cellulose equivalent (**Figure 6**).

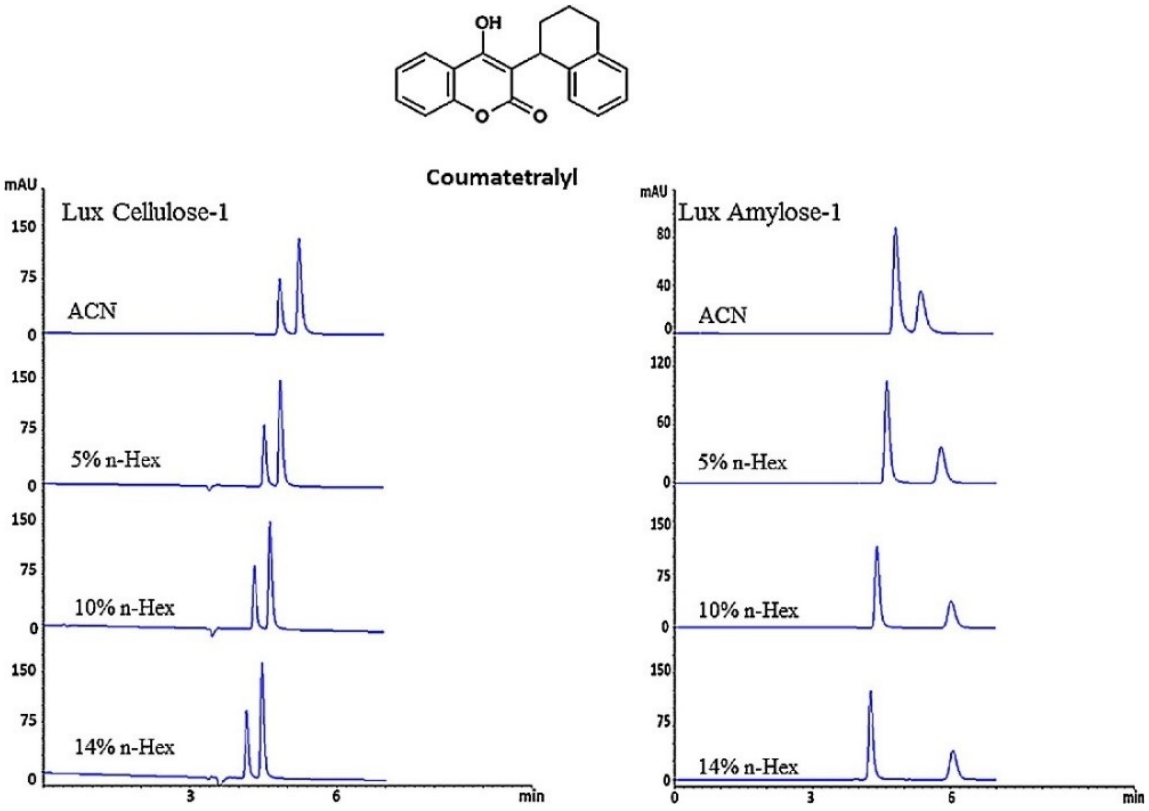

Figure 6 The effect of n-hexane addition to ACN on selectivity. Reprinted with permission from [84]. Copyright (2017) Elsevier.

The enantioselectivity of polysaccharide based CSP is also affected by the method of attaching the chiral selector on the chromatographic support, i.e., immobilization and coating. Generally, the higher enantioselectivity in LC can be achieved more often on coated CSP [62, 85]. However, the analytes separated with higher selectivity on

immobilized CSP compared to coated CSP were of course also reported [85]. The loss of enantioselectivity of immobilized phases is somewhat leveled by the possibility to use broad range of sample solvents and organic solvents in mobile phase including dimethyl sulfoxide, toluene, methyl-*tert*-butyl ether, ethyl acetate, etc. [62].

Beside the stationary phase, the mobile phase composition is well known to affect selectivity of the polysaccharide-based CSP in all chromatographic modes. More specifically, the ratio of the stronger and weaker elution solvent in the mobile phase is usually used to achieve the retention factor higher than 5 and obtain 90% of the theoretically available resolution. Subsequently, changing the type of the organic solvents [86] and the concentration and type of additives [87, 88] can significantly improve the selectivity and peak shape/resolution of the enantiomers. In NPLC and POM, the additives are usually diethyl amine, triethyl amine, trifluoroacetic acid, formic acid, and acetic acid. The acidic and basic additives are combined or used separately [88]. Probably the most known effect of additives related to polysaccharide-based columns has been shown for chaotropic salts ($NaClO_4$ and KPF_6) in RPLC [89, 90]. Chaotropic salts can improve the resolution of basic enantiomers while prolonging their retention due to the ion pairing, disruption of the solvation shell of the analytes, and adsorption of lyophilic anions on the surface of the stationary phase [91].

The effect of temperature on selectivity on polysaccharide-based columns is usually neglected; however, unlike cyclodextrin-based CSP, it should always be tested since the enormous changes in enantioselectivity resulting in reversal of elution order of enantiomers in applicable temperature range was reported several times [92-94].

2.2.4 Current Trends in Chiral HPLC

Recently, exotic CSP have been developed including molecularly imprinted polymers [95], chiral metal-organic frameworks [96, 97], aptamers [98], chiral graphene derivatives [99], and carbon nanomaterials [100]. Although high enantioselectivities for at least one compound were reported, their characteristic properties, such as low separation efficiency and high specificity, prevent from their wider use. The high specificity can be an advantage for certain applications; however, the chiral screening of multiple “universal” CSP is more cost-effective than the preparation of specific CSP for each molecule or

group of molecules. The so-called tailor-made CSP mentioned usually in relation to molecularly imprinted polymers and aptamers are still a very wild dream for which a lot of research focusing on their reproducibility and cost-effectiveness must be carried out.

In addition to chiral selectors, a chromatographic support also plays an important role during a chiral separation. It affects the stability of CSP and contributes to the unwanted achiral interactions. The common goal of chromatographic support improvements is to develop more efficient, stable, flexible, and cost-effective CSP [101]. Although other chromatographic supports are being investigated to achieve this goal, that is, metal-organic and covalent-organic frameworks, organic-polymer monoliths polymers, zirconia, and nanomaterials, silica is still the golden standard (**Figure 7**) [101]. The obvious reason for the superiority of silica is its well-known chemistry, mechanical strength, high surface area, and the known mechanisms of coating and immobilization of chiral selectors. Therefore, the developments of the chromatographic supports have been directed towards modifying silica rather than implementing entirely new chemistries. Current trends aim to enhance the pH stability of silica by introducing hybrid organosilica polymers [101, 102], increase the separation efficiency by the use of superficially porous particles, and by employment of sub-microparticles and nanoparticles [101].

The higher separation efficiencies of sub-2 μm particles compared to 5 μm particles resulted in an extended range of applications and shorter analyses times [53]. Superficially porous particles (SPP) with diameter 2.7 μm are known for similar separation efficiencies compared to sub-2 μm particles. The great advantage of SPP over sub-2 μm particles is the unnecessary use of UHPLC instrumentation. In contrast, sub-2 μm particles are considered to be "greener" due to the similar retention times and selectivity but with usually 5 to 10 times lower flow rate. Currently, the following SPP CSP are commercially available, i.e., cyclodextrin derivatives, cyclofructan derivatives, donor-acceptor CSP, macrocyclic antibiotics, maltose derivative, and quinine derivatives [72, 103, 104]. The most used polysaccharide-based CSP were firstly reported in SPP format a decade ago [105] and subsequently thoroughly studied [106-109]. However, they are still not commercially available due to the pore size and specific surface area issues [50].

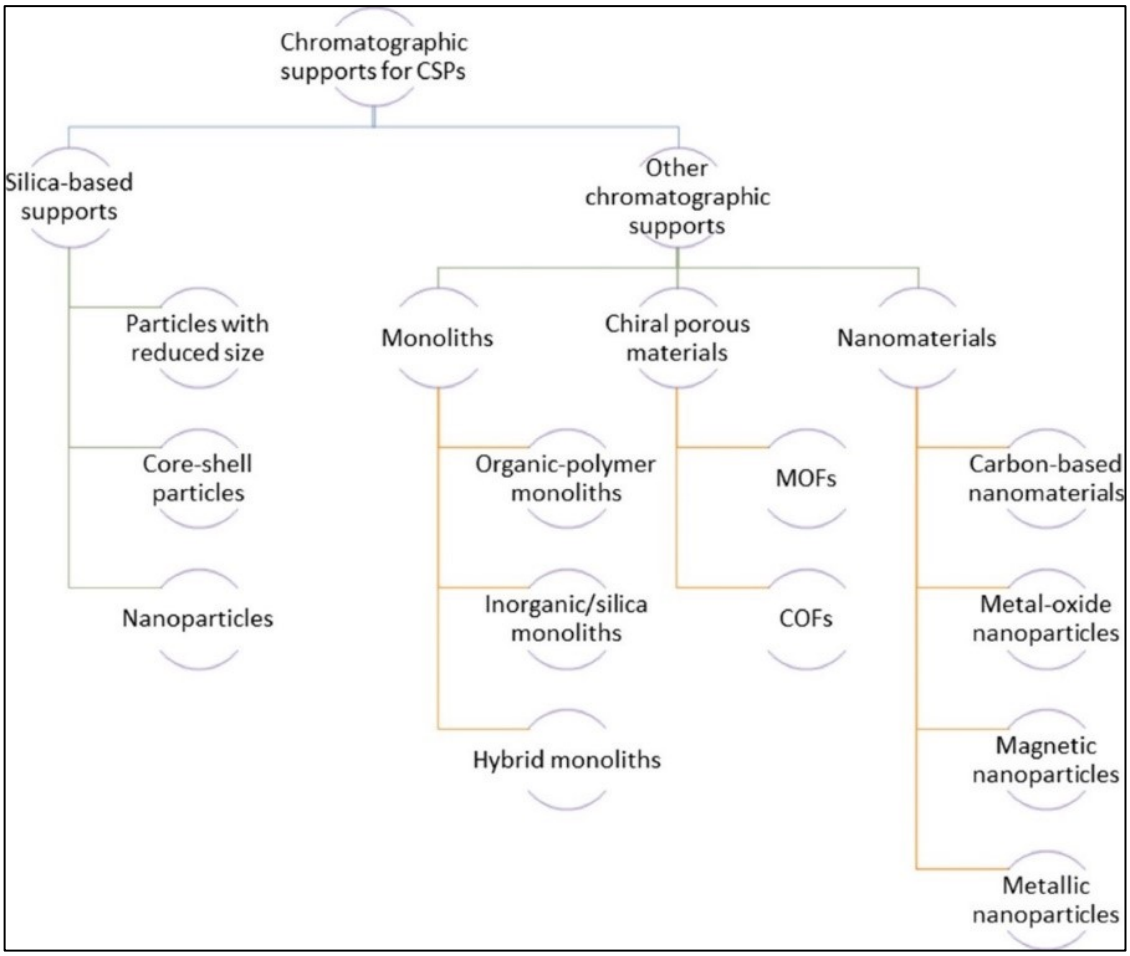

Figure 7 The developments in the chromatographic supports for CSP. Reprinted with permission from [101]. Copyright (2022) Elsevier.

2.2.5 Chiral Supercritical Fluid Chromatography

One of the first chiral separations in supercritical fluid chromatography (SFC) was carried out in 1985 on the donor-acceptor CSP (R)-*N*-(3,5-dinitrobenzoyl)phenylglycine [110]. Since then, progress in SFC instrumentation has identified this technique as the method of choice for chiral separations in the pharmaceutical industry due to the shorter analysis time, higher sample throughput, higher enantioselectivity, and use of more environmentally friendly solvents compared to NPLC [111]. The more straightforward and time efficient method development in SFC is possible due to chiral screening using steep gradients of an organic modifier with additives and flatter van Deemter curves, resulting in a very small loss of separation efficiencies at high flow rates. Rapid development in this field was possible due to the availability of a broad portfolio of CSP that changed from the dominance of donor-acceptor to polysaccharide-based CSP

(**Figure 8**) [112]. For example, 89% of all chiral separations in SFC were performed using polysaccharide-based columns from 2015 to 2022. The quinine/quinidine-based CSP were used in 5 % separations owing to the introduction of the "ZWIX" phase [113-115] that enhances the portfolio of analytes from the negatively charged compounds and acids to zwitterions. Although other types of CSP were also investigated, they cover only 6 % of chiral separations in the time range 2015 to 2022. The flourish of polysaccharide-based CSP in SFC comes from the wide use of this CSP in HPLC as discussed in **Chapter 2.2.1**. Another reason for the popularity of polysaccharide-based columns is their large versatility resulting in the high success rate of chiral separations in SFC [116].

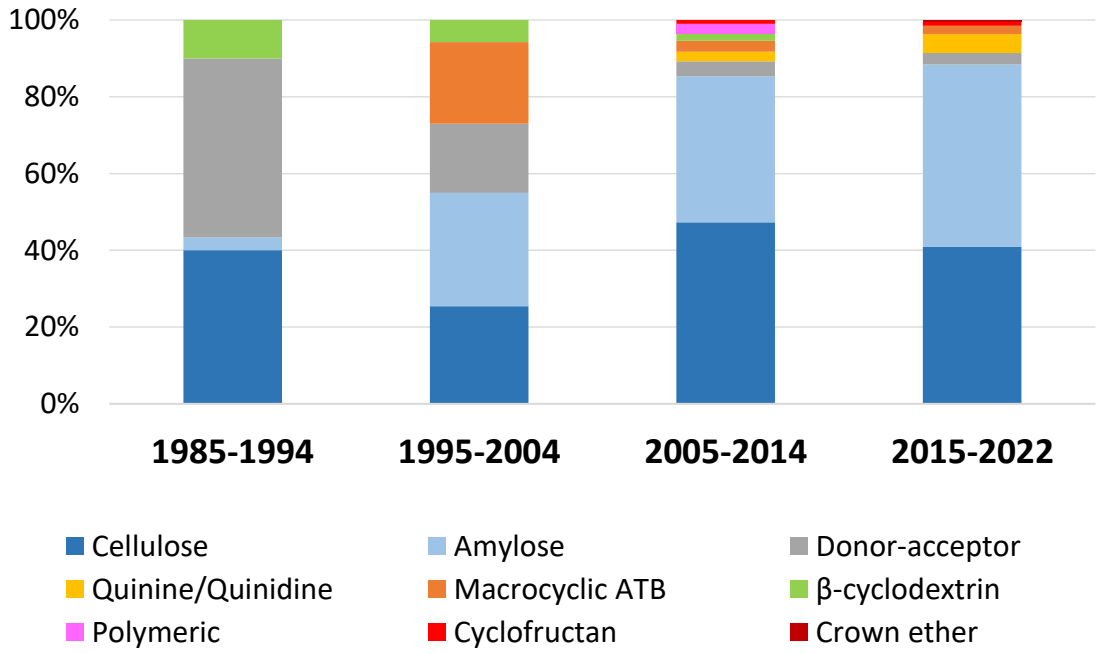

Figure 8 The employment of the chiral stationary phase in SFC from the year 1985 to 2022 [112].

2.2.6 Approaches for Enantioseparations with SFC

Today, chiral separations in SFC are commonly carried out using packed columns and a mobile phase comprised of supercritical CO_2 and a co-solvent. The strategy for chiral method development starts with the screening of multiple CSP in gradient of different co-solvents [115, 117-121] and are followed by a method optimization in an isocratic elution. The aim of gradient screening is the fast identification of the CSP and mobile phase composition with the highest selectivity toward the analyte of interest. Then, the chiral resolution is usually optimized in isocratic elution by variation of chromatographic

parameters. The main goal of the optimization step is to choose the best chromatographic conditions, i.e. concentration and type of organic modifiers and additives, temperature, back pressure regulator (BPR) pressure, and flow rate, to achieve the highest possible resolution in the determined time or to achieve the separation on the baseline in the shortest possible time [116].

Since the screening phase is a time-consuming trial-and-error approach, instruments with switching valves for at least six or eight columns and a pump with a switching valve accommodating at least four different co-solvents are a necessity. To improve the throughput, some authors reported splitting the sample into four or five parallel columns followed by four or five UV detectors [122]. This approach is time-effective but sets high demands on instrumentation that is not available for all laboratories. To overcome instrumentation demands, some authors decided to connect two or more chiral columns with complementary selectivity in series to reduce the time of chiral screening [116], e.g., a connection of donor-acceptor CSP and cellulose-based CSP [123], cellulose-based and amylose-based CSP [124], and a study using 10 different columns with 25 different column arrangements [125]. Although some investigations of two chiral columns connected in series were carried out, not all possible configurations were tested. This could be an issue, especially for the cases where the enantioselectivity for the respective analytes on two columns connected in series is not additive. The inappropriate selection, combination, and order of chiral columns can then result in the loss of enantioselectivity in comparison to the use of the single-column approach. To overcome this potential drawback, the columns connected in series need to be thoroughly studied and only the combinations of columns with no or minimal loss of selectivity for most analytes should be selected.

2.2.7 Enantioselectivity of Chiral Stationary Phases in SFC

The “carrier” of enantioselectivity is CSP. As discussed before, multiple CSP are nowadays commercially available and more exotic ones are being tested in academia. The laboratories usually have the screening set comprising of four to eight columns that ideally complement each other [116]. The screening sets usually contain only polysaccharide-based columns. More specifically, the so-called “golden four” (Chiralpak AD and AS, Chiralcel OD and OJ) was used for the chiral screening in the literature until

recently, when a new primary screening set has been introduced by Daicel [54] using four immobilized CSP, i.e., Chiralpak IA, IB (-N), IC, and IG. Polysaccharide columns are sometimes complemented with donor-acceptor CSP (Whelk O1) and macrocyclic glycopeptide-based columns (TeicoShell, TAGShell, VancoShell). Quinine/quinidine-based columns are usually implemented in chiral screening for acidic analytes and zwitterionic analytes, e.g., Chiralpak ZWIX(+) CSP has recently been employed for the chiral separations of free amino acids [115].

Together with the selection of CSP with satisfactory selectivity for the analytes during the chiral screening, the composition of the co-solvent is determined. The modifier is usually methanol, ethanol, 2-propanol, and acetonitrile [116] or mixtures of alcohols and acetonitrile. The additives are usually amines (diethylamine, triethylamine, isopropylamine, etc.), acids (trifluoroacetic acid, acetic acid, formic acid), or their mixtures at concentrations of 0.1 – 0.5%. The combined acidic and basic additives were shown to be more universal than acidic or basic additives alone [126]. Although lower enantioselectivities are generally achieved with ammonium formate and ammonium acetate, these additives are tested for MS detection to avoid the memory effect of amines and suppression of ionization by trifluoroacetic acid [126]. Their maximal concentration is of 20 mmol/L. Recently, the addition of water to the co-solvent at concentrations 1 to 10% has been shown to reduce the retention of strongly polar compounds and to improve their peak shape; therefore, even if the selectivity is slightly decreased, the improvement in peak shape can assure the similar resolution [127, 128].

The column temperature in chiral SFC is usually kept at 30 - 40 °C because the column ovens in SFC instruments are not commonly capable of holding the temperature below room temperature [116]. However, some articles showed enhanced enantioselectivity at temperatures below 25 °C; for example, the effect of temperature (in range -10 to 60°C) on enantioselectivity of metoprolol was found to be more important compared to the content of the co-solvent [129] and the higher enantioselectivity was achieved for ketamine metabolites at 15 °C compared to 50 °C [130]. Therefore, the temperature effect on the enantioselectivity should always be tested at least at room temperature and at the maximum temperature allowed by the CSP manufacturer.

The BPR pressure and flow rate rarely have an effect on the enantioselectivity. These two parameters affect retention due to changes in the density of the mobile phase [116]. In addition, chiral columns usually have low-pressure limit, i.e., 4500 PSI, which prevents testing the effect of BPR in a wide range. Even if the optimal composition of the mobile phase is identified and the pressure limit is not exceeded, the analysis is shortened by increasing the flow rate, not the setting of the BPR.

2.2.8 Current Trends in Chiral SFC

The trends in chiral SFC lead to chiral separation in the shortest time possible, introducing enantioseparations in tens of seconds using macrocyclic antibiotics-, quinine-, and cyclofructan-based chiral selectors immobilized to 2.7 μm SPP silica particles [131]. The SPP chiral columns in SFC employ only 3% of the total number of enantioseparations, and we expect that this number will increase due to the desire for fast analyses [112]. Although new stationary phases are developed every year, a dramatic change in the distribution of their employment is not expected; that is, polysaccharide-based CSP will prevail. Therefore, the improvements in polysaccharide-based CSP will determine the direction of chiral separations in SFC. The new chemistries of the cellulose and amylose derivatives are expected together with studies of the immobilization of chiral selectors on silica or other materials to maintain the enantioselectivity of the coated analogs. To reach even higher separation efficiencies and shorter time of the enantiomeric separation, the availability of more (currently 6) polysaccharide-based CSP immobilized in sub-2 μm silica particles is presumed. Another approach to achieve ultra-fast chiral separations is the use of SPP particles. Even though the prototype polysaccharide-based CSP with SPP particles was tested in HPLC [50, 106-108], the currently commercially available SPP silica materials are not optimized for polysaccharide-based chiral selectors due to a small pore size of the porous shell and low specific surface area. Once this issue is solved, the commercialization of polysaccharide-based CSP with SPP silica particles is imminent.

2.2.9 Comparison of Chiral LC to Chiral SFC

Generally, the advantage of the low viscosity of supercritical CO_2 results in higher column efficiency due to higher diffusion coefficients of analytes that are 4-10 times

higher than in liquids [116]. The 4 times lower diffusion coefficient than in liquids is given by 10% MeOH in supercritical CO_2 [132]. The upper limit is measured for pure supercritical CO_2. The lower viscosity of supercritical CO_2 connected with the shallow van Deemter curve allows higher flow rates without a significant loss of efficiency and thus faster analysis time compared to organic solvents in HPLC [116, 119, 133]. However, UHPLC enantioseparations using quinine-based columns have recently been shown to be as fast as a few seconds with separation times comparable to SFC [134]. The authors in this study used very short columns, that is, 0.5 and 3 cm, with a 0.46 cm inner diameter and 2.7 μm SPP. The extra column volume for both instruments was set as low as possible. As a result, the chiral separations achieved in SFC suffer from a larger extra-column volume compared to UHPLC, which is documented by the deformed peak shape in SFC [134]. This suggests that the SFC and now available UHPSFC instruments still have a larger extra column volume than the instrumentation dedicated to UHPLC [119]. The extra column peak broadening is always more visible in short narrow columns, which are necessary for ultrafast chiral separations. However, using conventional HPLC systems, the comparison of RP-LC and SFC resulted in faster separation times in SFC together with a higher number of separated enantiomers [135].

Comparison of SFC with LC is rather difficult, because all three chromatographic modes (RPLC, NPLC, and POM) should be tested in LC and then compared with SFC. Although this comprehensive comparison was reported in the literature [136], SFC is usually compared only to NPLC [137]. This is accounted for by the simplification that SFC is the equivalent of NPLC. Although this statement is true to some extent since NPLC and SFC have similar characteristics, these two techniques are not necessarily the same, as documented by the example of a complicated method transfer from NPLC to SFC [137]. The system of CO_2 and alcoholic co-solvent has few advantages over the NPLC, e.g., the higher stability of retention times especially in gradient elution and the high solubility of most solvents to CO_2.

The similarity between NPLC and SFC was demonstrated back in 1986 by comparing the elution strength of CO_2 - 2-propanol with hexane - 2-propanol [138]. On the contrary, sometimes different enantioselectivities for NPLC and SFC were reported [139, 140]. Apart from these differences, when the same co-solvent and similar retention factors were

used in both techniques, the elution order of the enantiomers and the enantioselectivity are identical [116]. In few cases, NPLC has been reported to have higher enantioselectivities than SFC [138, 141]; however, this is usually compensated for by higher column efficiency in SFC achieving comparable enantioresolution [141, 142]. In addition, similar retention mechanisms are observed in NPLC and SFC, although CO_2 was found to affect the enantioselectivity of 1,2-amino alcohols differently than hexane [116, 143]. It is clearly visible that the comparison of NPLC and SFC is a complex problem, and it is difficult to gain some relevant data since the methods for comparison were not always fully optimized giving the advantage to one or the other separation technique.

Although SFC and HPLC or UHPLC in different chromatographic modes are still being evaluated and compared, they should be used as complementary techniques. If the chiral separation is achieved in both LC and SFC, the separation technique should be chosen on the basis of the purpose of the developed method, economic aspects, sustainability, and the character of the analyzed compounds and matrix.

2.3 Carboranes

2.3.1 Brief History of Carboranes

The era of boron cluster compounds dates to the early 1930s when Alfred Stock and co-workers discovered polyhedral boranes [144], for example, at that time considered neutral $B_{12}H_{12}$. It took another two decades to perform the calculations [145] that showed the extra electron pair is needed to stabilize the structure of the neutral 12-vertex icosahedron to the dianion $B_{12}H_{12}^{2-}$ that was later isolated by Hawthorne and Pitochelli in 1960 [146]. These events led to a completely new chemistry that introduces incredibly thermally and chemically stable 3D boron-based compounds. [1]

The further development of boron clusters was paradoxically the result of the unsuccessful effort to use stable organic derivatives of boron hydrides as additives to aircraft and rocket fuels. After the abandonment of the project in the late 1950s and early 1960s, the large stocks of starting materials, i.e., boron hydrides B_2H_6, B_5H_9, $B_{10}H_{14}$, remained unused [1, 147]. The industrial efforts to find the broad spectrum of applications for boron hydrides led to studies of their reactivity with alkynes to get less toxic and heat-

stable products. The aim of these investigations was fulfilled by the discovery of carboranes with properties far different from organic and boron hydride precursors [1, 148], i.e., *closo*-1,2-$C_2B_{10}H_{12}$ (trivial name *o*-carborane) synthesized in 1957 at Reaction Motors, Inc. [1] and its isomers *closo*-1,7-$C_2B_{10}H_{12}$ (trivial name *m*-carborane) and *closo*-1,12-$C_2B_{10}H_{12}$ (trivial name *p*-carborane) (**Figure 9**) [1, 149].

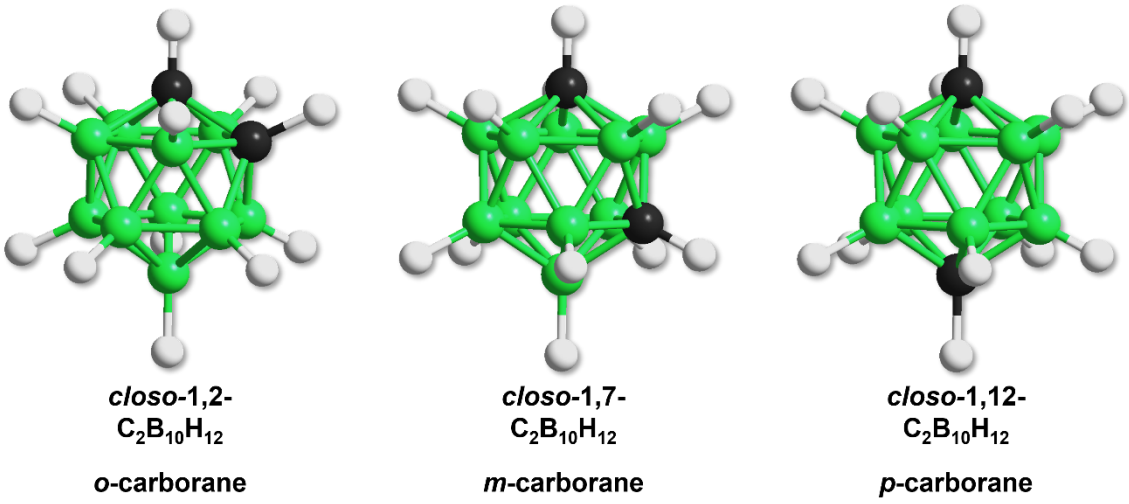

Figure 9 Models of *closo*-$C_2B_{10}H_{12}$ isomers. Atoms are color-coded as green for boron, black for carbon, and white for hydrogen.

The study of $C_2B_{10}H_{12}$ structures revealed a high polarity of C-H bonds with a partial positive charge on the hydrogen that can react with Lewis bases. As a result, C-substituted derivatives have been synthesized. Later, different synthetic mechanisms were also developed for the substitution of hydrogen at B-H vertices. The substitution of the cage at different positions with distinct organic ligands enables "tailor-made" molecules for a broad spectrum of applications in medicinal chemistry and industry [1, 2, 4, 11, 144, 148, 150]. Besides *closo*-carboranes with the general formula $C_2B_{n-2}H_n$, where n = 5-14, *nido*-, *arachno*-, *hypho*-, and *conjuncto*- carboranes were synthesized by the removal of one, two, three, or four vertices, respectively. The other variability is the number of carbon atoms. Although carboranes usually possess one or two carbons, three-, four-, five-, and six-carbon carboranes; were also synthesized [1]. The portfolio of carboranes is further extended by their coordination with metals forming metallacarboranes, e.g., synthesis of $[Co(C_2B_9H_{11})_2]^-$ (**Figure 10A**) from *nido*-$[7,8\text{-}C_2B_9H_{11}]^{2-}$ (**Figure 10B**) and cobalt chloride [151].

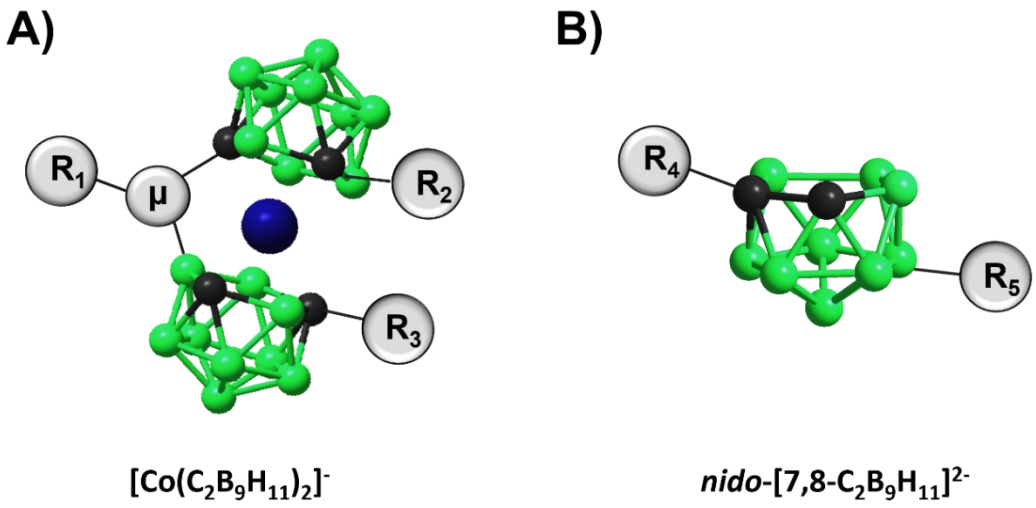

Figure 10 Models of $[Co(C_2B_9H_{11})_2]^-$ and *nido*-$[7,8\text{-}C_2B_9H_{11}]^{2-}$ with possible substitution places represented by μ (intermolecular bridge), R_1 (substitution on the bridge of $[Co(C_2B_9H_{11})_2]^-$), R_2, R_3 (substitution of carbons in $[Co(C_2B_9H_{11})_2]^-$), R_4 (substitution of carbons in *nido*-$[7,8\text{-}C_2B_9H_{11}]^{2-}$), and R_5 (substitution of boron in *nido*-$[7,8\text{-}C_2B_9H_{11}]^{2-}$). Atoms are color-coded as green for boron, black for carbon, and dark blue for cobalt. Hydrogens are omitted for clarity.

Carboranes are currently represented by several tens of thousands of compounds that have recently gained the attention of the scientific community, evidenced by the annual rise in publications (**Figure 11**). However, only around 100 carboranes have been discriminated into enantiomers and only approximately 35 papers have been dedicated to chiral carboranes over 58 years. Thus, despite recent progress in this research area, the chirality

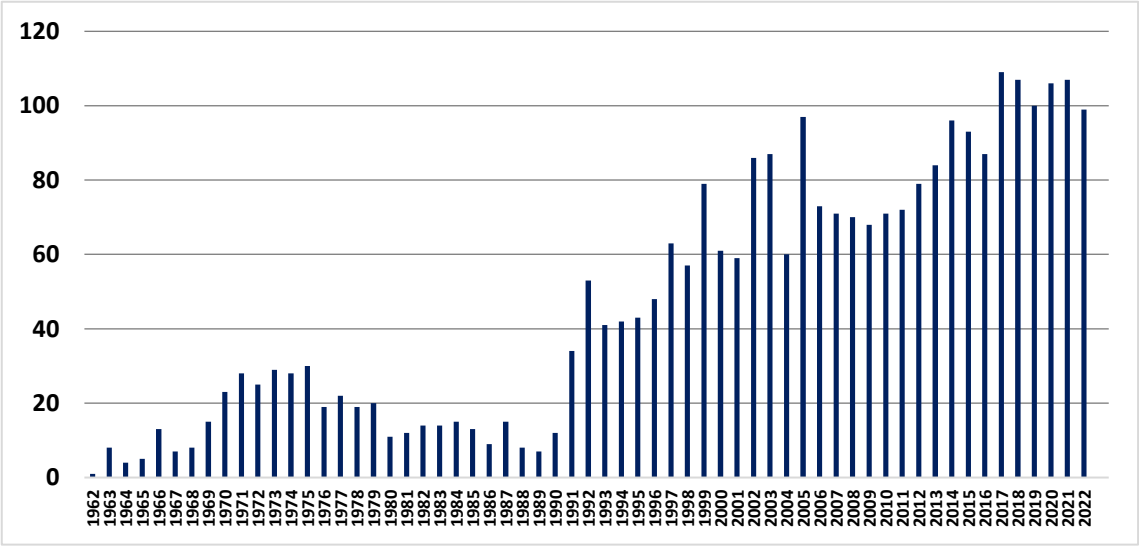

Figure 11 The number of carboranes publications in impacted journals from the year 1962 to 2023. These results were gained by entering the key word "carboranes" into the Web of Science database.

of carboranes remains mostly an uncharted territory that provides an opportunity for further systematic research.

This thesis is dedicated to $[Co(C_2B_9H_{11})_2]^-$, its starting synthetic material *nido*-[7,8-$C_2B_9H_{12}]^-$, and their derivatives. All three categories of compounds are intensively studied due to their low nucleophilicity, ionic character, high chemical and thermal stability, and solubility in various organic solvents [151]. The following chapters deal with the physical chemical properties and applications of $[Co(C_2B_9H_{11})_2]^-$, *nido*-[7,8-$C_2B_9H_{12}]^-$, and their derivatives.

2.3.2 Nomenclature and numbering

Generally, ambiguous class names, e.g., "borates" and "carboranes", are not recommended. The misleading character of these terms is demonstrated on the name borate that is a IUPAC-accepted name for trioxidoborate(3-). Term "carborane" was historically used to name *closo*-1,2-$C_2B_{10}H_{12}$. The casual approach to nomenclature resulted in the compromise of the database searches and data retrievals. Therefore, more explicit names, e.g., hydridoborates, oxidoborates, and 1,2-dicarba-dodecaboranes, are encouraged by the most recent IUPAC recommendations [152].

The anionic derivatives of *nido*-$[7,8\text{-}C_2B_9H_{11}]^{1-}$ are named additively. The cage numbering is historically [153] fixed according to **Figure 12A** with no priority given to carbon atoms. First, the name of the compounds is represented by hydrogen atoms designated as "hydrido" ligands or bridging hydrogen atoms named "μ-hydrido" ligands. Second, the type of the cage, e.g., *closo*, *nido*, etc., follows. Third, heteroatoms preceded by locants are ordered according to the atomic number with the highest atomic number given the highest priority. Fourth, the number of vertices is expressed, for example, undeca. Fifth, the suffix "ate" with the charge number in parentheses is used for the designation of anionic species, e.g., borate(2-). Since hydrogen ligands are not required for the compounds with all boron and carbon sites occupied by a hydrogen atom, *nido*-$[7,8\text{-}C_2B_9H_{11}]^{2-}$ also known as the "dicarbollide" anion is named undecahydrido-*nido*-7,8-dicarba-undecaborate(2-). The substituted species must include locants of the substituents together with locants of all hydrogen atoms -BH and -CH [1, 152].

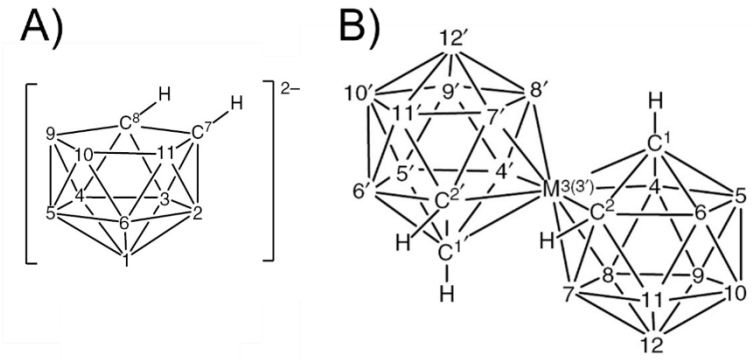

Figure 12 Numbering of cages of *nido*-[7,8-$C_2B_9H_{11}$]$^{2-}$ derivatives (A) and cobalt bis(dicarbollide) derivatives (B). Reprinted from [152].

Compound [Co($C_2B_9H_{11}$)$_2$]$^-$ and its derivatives should follow the recommendations stated in the previous paragraph and in the section BN-7.1 Conjoined cages of the IUPAC Recommendations [152]. The nomenclature of [Co($C_2B_9H_{11}$)$_2$]$^-$ is defined by the term *commo* from earlier published IUPAC Recommendations [153]. The structural term *commo* should be implemented in the compound name and surrounded by locants, e.g., ["ligand"]-3′-*commo*-3-["ligand"]. The assignment of the locants to [Co($C_2B_9H_{11}$)$_2$]$^-$ is presented in **Figure 12B** (M = Co). The structure name of [Co($C_2B_9H_{11}$)$_2$]$^-$ depends on symmetry. Symmetrical systems can be named as 3,3′-*commo*-bis[undecahydrido-*closo*-1,2-dicarba-3-cobaltadodecaborate(1-)]. However, this nomenclature cannot be used for an asymmetric system, therefore, the structure of [Co($C_2B_9H_{11}$)$_2$]$^-$ should be named [undecahydrido-*closo*-1′,2′-dicarba-3′-cobaltadodecaborate(1-)]-3′-*commo*-3-[undeca-hydrido-*closo*-1,2-dicarba-3-cobaltado-decaborate(1-)]. The recommendations towards rotamers are not strictly given. It is written: "Rotamer considerations, when relevant, are usually dealt with adequately as they arise in the scientific publications that report them; they are beyond the scope of these Recommendations."[152]. Furthermore, no recommendations are given for the naming of enantiomers.

Since the nomenclature of the optical isomers of any carboranes is far from consideration by IUPAC, Plešek et al. [13] developed a nomenclature based on their experience with chiral carboranes. According to IUPAC, both enantiomers would have to have different locants for substituents because of the rigid clockwise numbering system. Different locant numbers would implicate the pair of positional isomers instead of enantiomers.

Plešek et al. suggested to number the enantiomers clockwise and anticlockwise and then designate them σ- and ρ-, respectively [13, 154].

2.3.3 Physical chemical properties

Generally, the common bond pattern in carboranes is represented by two center two electron bonds, e.g., B-H, C-H, B-B, C-B, and C-C and three center two electron bonds, e.g., B-H-B, C-B-B, C-C-B, rarely C-H-B [155, 156]. The electron pair shared by three atoms led the scientist to designate boron clusters that include carboranes as "electron deficient" structures. However, this term should not be generally used for carboranes since there is no "deficiency" of electron density toward a more stable state, for example, *closo*-1,2-$C_2B_{10}H_{12}$ is one of the most thermodynamically stable molecules ever synthesized or discovered [157]. Furthermore, substitution of organic molecules by carboranes can have both an electron withdrawing and an electron donating effect depending on the position of substitution; e.g., the substitution of an organic molecule by bonding to carbon of *closo*-1,2-$C_2B_{10}H_{12}$ leads to the electron withdrawing effect, however, the bonding to boron atom results in the electron donating effect of the same cluster [157].

The complete delocalization of the electrons across the 3D cage of carboranes causes 3D aromaticity ("superaromaticity") [158-160]. This phenomenon employs σ-orbitals tangential to the surface of the cage and σ-orbitals directed towards the center of the cage. The three-dimensional aromaticity significantly affects the reactivity of carborane derivatives. The substituents on the carborane cage affect the electron density in a way similar to that in the two-dimensional arene chemistry [157].

Under HPLC and SFC conditions, the compounds always possess negative charges due to the strong acidity of their conjugated acids, for example, H($CHB_{11}Cl_{11}$) (so called "carborane acid") is one of the strongest Brønstad acid ever created [161]. Another important physical chemical property that can be challenging during analysis is the non-traditional solution behavior of anionic carboranes characterized by "superchaotropicity" [162, 163] and "stealth amphiphilicity" [164, 165]. These properties predetermine the anionic species in the formation of pseudo-micelles. The formation of multimolecular

complexes during the analysis can prevent the interactions with the chiral selectors, making anionic carboranes even more challenging analytes.

2.3.4 Applications of $[Co(C_2B_9H_{11})_2]^-$ and *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ in medicinal chemistry

The high thermal and chemical stability [166-169] allows *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ and $[Co(C_2B_9H_{11})_2]^-$ and their derivatives to be used in numerous applications in multiple research areas [1, 148, 151, 170], i.e. medicinal chemistry [2, 171-179], material chemistry [4, 9-11], electrochemistry [12, 180], and analytical chemistry [6, 7].

In medicinal chemistry, the *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ and $[Co(C_2B_9H_{11})_2]^-$ appear to be an auspicious research topic [2, 3] as they are not a common substrate for the human enzyme system, resistant to breakdown in biological systems, and easily penetrable in cells and tissues. The development of the carborane-based pharmaceuticals can be sorted into three categories according to the incorporation of the carborane ligand into the drug molecule, i.e., (i) substitution of the phenyl ring in the molecule of the known pharmaceuticals, (ii) synthesis of the newly designed carborane analogs of the known drugs, and (iii) conjugation with the bioactive compounds.

2.3.4.1 Nido-$[7,8\text{-}C_2B_9H_{12}]^-$ in drug design

The first approach was used for the substitution of the phenyl ring of tamoxifen [181] and isoniazid [182] by *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ reaching similar or higher pharmacological activities to the same target as parent organic pharmaceuticals. Increased activity and selectivity towards COX-2 were observed for indomethacin substituted with *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ [183]. The inhibition activity of COX-1/-2 was also observed for the *nido*-$[7,8\text{-}C_2B_9H_{11}]^-$ analog of mefenamic acid. These values were higher than that of *closo*-1,2-$C_2B_{10}H_{12}$ analogs of the same compound [184]. In contrast, the activity of *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ based potential antimalarials was found lower compared to *closo*-1,2-$C_2B_{10}H_{12}$ derivatives [185].

The second approach was used for the early investigations of the antimicrobial activity of *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ derivatives substituted with alkyl chain (C_5 to C_{17}) or *closo*-1,2-$C_2B_{10}H_{12}$ [186], antituberculotic activity of thymine modified with one or two *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$[187], and one 2'-deoxyadenosin derivative as an inhibitor of the platelet

function [188]. Recently, *nido*-[7,8-$C_2B_9H_{12}$]$^-$ derivatives substituted with sulfonamide have been studied as promising inhibitors of carbonic anhydrase IX for cancer treatment [189]. None of the compounds mentioned in this paragraph were enantioseparated, not even on analytical scale. Therefore, no activity of single enantiomers was tested.

Recently, *nido*-[7,8-$C_2B_9H_{12}$]$^-$ has been conjugated with bioactive compounds, for example, *nido*-[7,8-$C_2B_9H_{12}$]$^-$ derivatives coupled with adenine have been found to possess antiviral activity against the acyclovir-resistant strain of herpes simplex virus type I, and moderate activity against influenza viruses A and B [190]. Although the conjugates were confirmed as racemic mixtures by the chiral HPLC method using coated cellulose *tris*(3,5-dimethylphenylcarbamate) in NPLC [191], antiviral activities were carried out only for racemic mixtures.

The interest in using *nido*-[7,8-$C_2B_9H_{12}$]$^-$ as a part of bioactive compounds resulted in the synthesis and subsequent preparative HPLC isolation of enantiomerically pure amino acids based on *nido*-[7,8-$C_2B_9H_{12}$]$^-$ [192]. The pioneering work was followed by the synthesis of 17 amino acids conjugated with *nido*-[7,8-$C_2B_9H_{12}$]$^-$. In this work, two racemates of methionine and phenylalanine derivatives, either protected or the unprotected by *tert*-butyloxycarbonyl, racemate of the proline derivative protected by *tert*-butyloxycarbonyl, and racemate of unprotected tryptophan derivative were enantioseparated using chiral NPLC with amylose and cellulose *tris*(3,5-dimethylphenylcarbamate) [193]. It should be noted that these chiral separations of the racemic mixtures resulted in two peaks. However, the molecule of *nido*-[7,8-$C_2B_9H_{12}$]$^-$ based amino acids always possess at least one common stereogenic center additively to the chiral center caused by the *nido*-[7,8-$C_2B_9H_{12}$]$^-$ itself. It would be expected to have two pairs of enantiomers for all described compounds and possibly three pairs of enantiomers for hydroxyproline, isoleucine, and threonine. Therefore, more research on the chirality of *nido*-[7,8-$C_2B_9H_{12}$]$^-$ based amino acids is required to better understand the complexity of this auspicious research area. The enormous potential of *nido*-[7,8-$C_2B_9H_{12}$]$^-$ based amino acids lies in the synthesis of enantiopure peptides and proteins derivatized with boron clusters. However, if this intriguing vision is possible, will be seen in the future.

2.3.4.2 $[Co(C_2B_9H_{11})_2]^-$ in drug design

The derivatives of $[Co(C_2B_9H_{11})_2]^-$ are larger molecules compared to a phenyl ring; therefore, rather newly designed pharmaceuticals have been developed. In this way, alkoxy derivatives of [3,3'-Co(1,2-$C_2B_9H_{11})_2]^-$ with the antibacterial activity against methicillin-resistant *Staphylococcus aureus* were prepared [194]. The antimicrobial activity of alkoxy and alkylamino derivatives of [3,3'-Co(1,2-$C_2B_9H_{11})_2]^-$ was also observed against *Pseudomonas aeruginosa* (IC_{50} = less than 100 μmol/L) and *Yersinia enterocolitica* (IC_{50} = less than 10 μmol/L) [195]. The antimicrobial activity of sodium salts of alkoxy derivative of [3,3'-Co(1,2-$C_2B_9H_{11})_2]^-$, that is [3,3'-Co(8-H_3CH_2CO-$(CH_2CH_2O)_2$-1,2-$C_2B_9H_{10}$)(1',2'-$C_2B_9H_{11}$)], was found to be higher against *Streptococcus pyogenes* and *Escherichia Coli* than the commercially available broad-spectrum antibiotic thiamphenicol [196]. The same compound also had an antimicrobial activity against methicillin-resistant *Staphylococcus aureus*, multiresistant *Pseudomonas aeruginosa*, and *Candida spp.* [173, 196]. Furthermore, diethylene glycol N-alkylammonium derivatives of $[Co(C_2B_9H_{11})_2]^-$ were found to have antibacterial and antifungal activity [197]. The most active compound in this study was a non-alkylated ammonium derivative with the MIC_{80} lower than 12 μmol/L for *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, and *Trichosporon cutaneum*. Recently, a complex study on the antimicrobial and antifungal activity, water solubility, and membrane permeability of $[Co(C_2B_9H_{11})_2]^-$ derivatives *in vitro* confirmed the antimicrobial activity against multi-resistant strains of *Candida albicans*, gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*), and gram-positive (*Enterococcus faecalis*, *Staphylococcus aureus*) bacteria [198]. The authors of this study understand that antimicrobial effects determined *in vitro* must be confirmed *in vivo* to further continue in a pursuit for the $[Co(C_2B_9H_{11})_2]^-$ based antibiotics. More recently, a study has been published describing the enhancement of the antimicrobial activity of vancomycin and tetracycline against *Staphylococcus epidermidis* in the presence of sodium salt of $[Co(C_2B_9H_{11})_2]^-$ [199]. In this study, the mechanism of the better antimicrobial effect was identified as cell envelope disruption.

The 2'-deoxyadenosine modified with [3,3'-Co(1,2-$C_2B_9H_{11})_2]^-$ was tested as potential anticoagulant. The inhibition of platelet aggregation was found comparable to *nido*-[7,8-

$C_2B_9H_{12}]^-$ but also lower than adenine, 2-deoxyadenin, and significantly lower than *closo*-$[C_2B_{10}H_{12}]$ derivatives [188]. Other nucleosides decorated with $[Co(C_2B_9H_{11})_2]^-$ were tested for antiviral activity; however, 5-iodo-2'-deoxyuridine substituted with $[Co(C_2B_9H_{11})_2]^-$ has not resulted in an antiviral activity below cytotoxic concentrations [200]. In contrast, an antiretroviral activity, i.e. HIV-1, HIV-2, of $[Co(C_2B_9H_{11})_2]^-$ derivatives were expressed as inhibition constants and IC_{50} as low as 2.2 nmol/L and 0.25 μmol/L, respectively [201]. The activity of $[Co(C_2B_9H_{11})_2]^-$-based HIV-protease inhibitors was further studied on the panel of HIV-protease mutants and compared to the commercially available treatment against HIV, revealing a lower resistance of the enzyme toward $[Co(C_2B_9H_{11})_2]^-$-based HIV-protease inhibitors [202, 203]. Apart from potential antibiotic, antifungal, or antiviral treatment, $[Co(C_2B_9H_{11})_2]^-$ substituted with sulfonamides have been intensively studied as the inhibitors of cancer-associated carbonic anhydrase IX *in vitro* [204] with approximately 50 times lower inhibition constants compared to *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ and showed a significant anticancer effect in mouse models *in vivo* [166].

Conjugates of the $[Co(C_2B_9H_{11})_2]^-$ ion with naphthylimide resulted in a possible new lead structures for anthelmintics, since activities comparable with leading drugs on the market mitonafide and mebendazole were observed [205]. The authors also discuss the potential antitumor activity due to the high cytotoxicity against the HT-29 cell line [205]. The potential for antitumor activity was also tested for new conjugates of $[Co(C_2B_9H_{11})_2]^-$ and cholesterol in glioblastoma U-87 MG cells and human embryo fibroblasts FECH-15 [206]. The lower cell viability was found at a lower concentration compared to cisplatin and the negative control (1% DMSO). Cancer therapy was also investigated for the conjugates of chlorin and $[Co(C_2B_9H_{11})_2]^-$ [207]. The conjugates [205-207] could be used in the future for direct antitumor therapy together with boron neutron capture therapy to achieve the double punch effect.

2.3.4.3 Boron neutron capture therapy

Boron neutron capture therapy (BNCT) is the radiation technique for cancer treatment based on the nuclear capture of thermal neutrons by the ^{10}B core and subsequent fission reactions. The products of these reactions are high energy alpha particles and recoiling ^7Li nuclei [208]. The ideal state is when boron is administered to a patient and then

selectively introduced only to the cancer cells that are destroyed by the alfa particles with short half-life formed *in situ*. Multiple clinical studies of BNCT-treated cancer were carried out, i.e., glioblastoma, primary and recurrent head and neck cancer, melanoma, lung cancer, liver metastases of colorectal adenocarcinoma, and extramammary Paget's disease [208, 209]. The current problems of BNCT are low selectivity of boron delivery agents for cancer cells, type of delivery mode, irradiation depth of the neutron beam, and expensive neutron generating reactor [209]. All of these issues need to be resolved to make BNCT an effective tool against various types of cancer. Here, the development in the field of BNCT agents, i.e., [3,3'-Co(1,2-$C_2B_9H_{11})_2]^-$ and *nido*-[7,8-$C_2B_9H_{12}]^-$, will be shortly addressed.

Clinically tested "classical" organic BNCT agents, i.e., *para*-boron-phenylalanine and *para*-boron-phenylalanine-fructose, have a disadvantage in a very low boron load, that is, one boron atom per molecule. In contrast, sodium borocaptate ($Na_2B_{12}H_{11}SH$) has a high boron load with 12 boron atoms in the molecule, but its selectivity toward cancer cells is still not high enough. Therefore, other boron cluster molecules are being investigated in BNCT with the promise of a higher boron load and a higher selectivity toward cancer cells. Recently, [$Co(C_2B_9H_{11})_2$]Na has been reported as a candidate for the BNCT agent due to its low toxicity and its high uptake by relevant cancer cells [178]. Interestingly, authors claim that [$Co(C_2B_9H_{11})_2$]Na does not have to be synthesized from isotopically pure ^{10}B due to the high boron load that in average provides 3.6 of this isotope per molecule. Similarly, conjugates of [3,3'-Co(1,2-$C_2B_9H_{11})_2]^-$ and cholesterol [206] or chlorin [207] were proposed as promising BNCT agents.

2.3.5 Other Applications of *nido*-[7,8-$C_2B_9H_{12}]^-$ and [$Co(C_2B_9H_{11})_2]^-$

The *nido*-[7,8-$C_2B_9H_{11}]^{2-}$ ligands, its protonated form *nido*-[7,8-$C_2B_9H_{11}]^{1-}$ and their derivatives have also been investigated as modifiers to nanostructured materials and nanovehicles for drug delivery [9, 11]. The [$Co(C_2B_9H_{11})_2]^-$ and its derivatives [151] have been recently studied as radionuclide extraction agents [10], as a modulators of intermolecular interactions between peptides/proteins [177], a potential fluorescent agents for a cell tracking [167], additives to conducting polymers [210], and switchable luminescent materials [211]. Modified [$Co(C_2B_9H_{11})_2]^-$ and *nido*-[7,8-$C_2B_9H_{12}]^-$ have found application in metal-containing DNA labelling [212]. Recently, the derivatives of

$[Co(C_2B_9H_{11})_2]^-$ have been shown to form strong interactions with hydrophobic surfaces and pockets in host molecules such as cyclodextrins [162, 163, 213] and proteins [2, 166, 179, 214]. This research can aid in the elucidation of the chiral recognition mechanism and partly chromatographic behavior of $[Co(C_2B_9H_{11})_2]^-$ and *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ derivatives on cyclodextrin-based and protein-based CSP. The elucidation of interactions with proteins can be also used for the understanding of the fate and the mechanism of action of the $[Co(C_2B_9H_{11})_2]^-$ or *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ based drugs in human body.

2.3.6 Relevance of chirality for *nido*-$[7,8\text{-}C_2B_9H_{12}]^{1-}$ and $[Co(C_2B_9H_{11})_2]^-$

Due to the growing number of racemic *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ and $[Co(C_2B_9H_{11})_2]^-$ derivatives proposed and synthesized in recent literature, the aspect of chirality should be investigated in all mentioned research areas including drug discovery, pharmaceutical analysis, different properties of enantiopure materials (polymers, catalysts), enantioselective electrodes, and chiral selectors. Primarily, medicinal chemistry is the research area where the investigation of chirality of *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ and $[Co(C_2B_9H_{11})_2]^-$ is of the utmost importance. Although the racemic mixtures of some novel compounds, e.g., isoniazid [182], showed high pharmacological activity towards its targets and relatively low toxicity, no investigation was carried out for the individual enantiomers. The possibility of distinct properties resulting from different space configurations of enantiomers is not limited only to pharmaceutical research and can be applied to all the fields of science mentioned above.

The "underdevelopment" of the $[Co(C_2B_9H_{11})_2]^-$ and *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ chirality research could be explained in part by insufficient awareness of the chirality of these species and insufficient analytical methods for their chiral separation and isolation. Therefore, the future development in this area is clearly dependent on the analytical and preparative methods available for the chiral separation and/or isolation of enantiomers. The mechanistic studies of the interactions of the chiral selectors with boron clusters must be also carried out to derive at least rules of thumb for the choice of the chiral selector. Recent studies on the interactions of cyclodextrins and $[Co(C_2B_9H_{11})_2]^-$ derivatives [162, 163, 213] could help to provide a basis for understanding the chiral separation of these molecules. Unfortunately, no other interaction mechanisms with chiral selectors for the compounds of interest have been reported so far. We believe that once the chirality of

$[Co(C_2B_9H_{11})_2]^-$ and *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ is properly investigated, these compounds will certainly parallel chiral platforms that attained more attention in organometallic and organic chiral chemistry, i.e., ferrocene and BINOL.

2.3.7 Chirality of *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ and $[Co(C_2B_9H_{11})_2]^-$ derivatives

Generally, the prochirality of boron clusters is induced when three terminal hydrogen atoms are replaced by two identical and one different univalent substituent, by substituting the skeletal borons by heteroatoms, and by combining these approaches at minimally three vertices [13]. This could also be applied for *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ derivatives where the skeletal substitution of two -BH for two -CH units and one substitution in the cage result in prochiral species. The chirality of the derivatives of *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ is determined by the asymmetry caused by the introduction of the appropriate substituents in the otherwise very symmetric cage (**Figure 13**). As a result, monosubstitution, disubstitution with two identical groups, and disubstitution with two different groups lead to 4, 21, and 55 prochiral positional isomers, respectively [13]. In contrast, the chirality of the bridged $[3,3'\text{-}Co(1,2\text{-}C_2B_9H_{11})_2]^-$ is caused by the mutual helical arrangement of the *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ ligands (**Figure 13**).

The theoretical predictions of chirality of the boron cluster compounds were confirmed by the measurements of the absolute configuration of (+) enantiomer σ(+)-$BrB_{10}H_{11}[S(CH_3)_2]_2$ by X-ray crystallography in 1983 [215]. Similarly, the determination of the absolute configuration of metallacarborane $[6,6'\text{-}\mu\text{-}(CH_3)_2P\text{-}(1,7\text{-}C_2B_9H_{10})_2\text{-}2\text{-}Co]$ followed in 15 years [216] proving the chirality of these species.

2.3.8 Chiral isolations and separations of *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ and $[Co(C_2B_9H_{11})_2]^-$ derivatives

Two different approaches were used to achieve the isolation of neutral mixed sandwich 4-MeS-3-C_5H_5-1,2-$C_2CoB_9H_{10}$ and anionic *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ enantiomers, i.e., co-crystallization with chiral amine and chromatographic techniques. Probably, the first co-crystallization of *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ with chiral amine dates back in 1968 when the *d*-N,N,N-trimethyl-*α*-phenylethylammonium iodide was used to distinguish (+/-)-(3)-1-phenyl-1,2-dicarbadodecahydroundecaborate(-1), (+/-)-(3)-1-phenyl-1,7-dicarbadodeca-

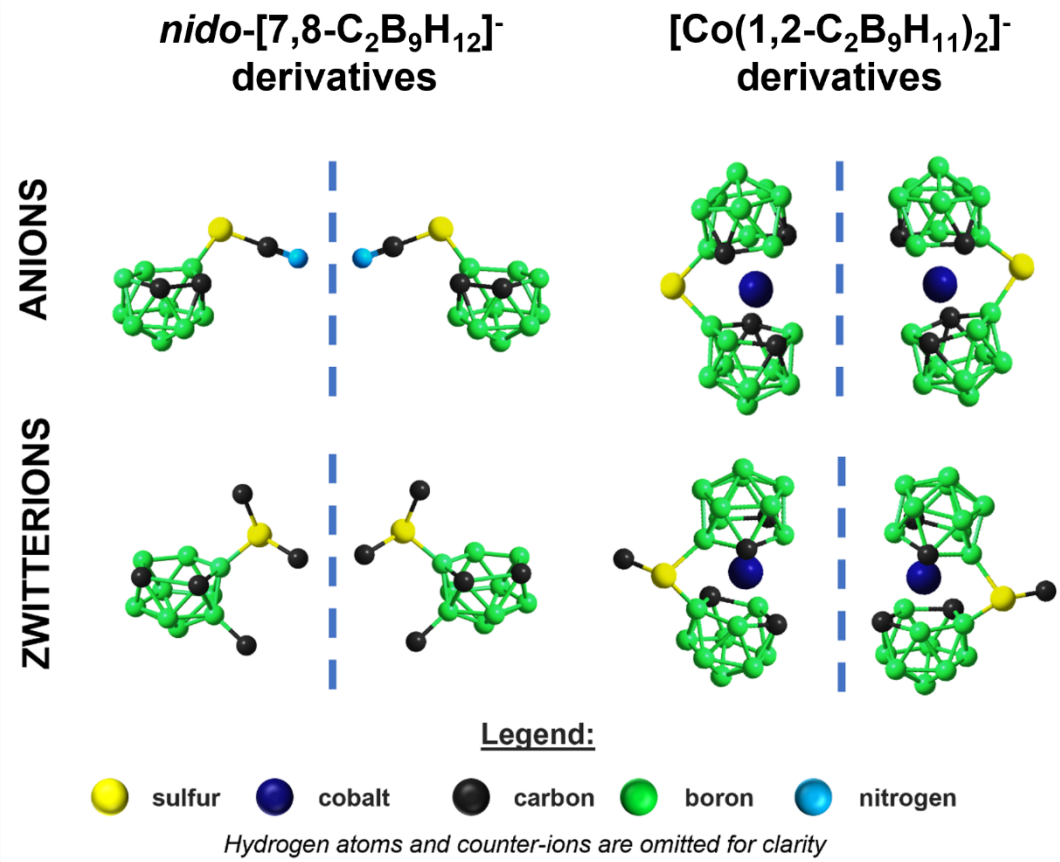

Figure 13 Illustration of the chirality of nido-$[7,8\text{-}C_2B_9H_{12}]^{2-}$ and cobalt bis(dicarbollide) derivatives. Reprinted with permission from [120]. Copyright (2022) American Chemical Society.

hydroundeca-borate(-1), and (+/-)-(3)-1-phenyl-2-methyl-1,2-dicarbadodecahydro-unde caborate(-1) [217]. The characterization of the enantiomeric purity was done by measuring the optical rotation. In 1992, the (-)-N,N,N-trimethyl-α-phenyl-ethylammonium salt was also used for the isolation of *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ enantiomers, i.e., (-) 9-CH_3S-7,8-$C_2B_9H_{11}$, with an enantiomeric excess approximately 32 % [218]. This compound was further methylated or complexed with $CoCl_2$ and $C_5H_5^-$ in concentrated ethanolic hydroxide solutions to give (-)-9-Me_2S-7,8-$C_2B_9H_{11}$ and (+)-4-CH_3S-3-C_5H_5-1,2-$C_2CoB_9H_{10}$, respectively, with similar enantiomeric excesses to the parent compound. All optically active compounds were characterized using circular dichroism. The authors claim that this was the first well-characterized optically active deltahedral sandwich complex since the enantiomers of rhodacarborane complexes published elsewhere [219] were not adequately characterized.

The chiral separation of neutral 4-CH_3S-3-C_5H_5-1,2-$C_2CoB_9H_{10}$ and zwitterionic derivatives of *nido*-[7,8-$C_2B_9H_{12}$]$^-$ was first reported in liquid chromatography on native β-cyclodextrin based columns [220]. The derivatives of [6,6'-Co(1,7-$C_2B_9H_{11}$)$_2$]$^-$ were enantioseparated next year with the majority of zwitterionic species and two anionic compounds [221], i.e., [6,6'-μ-S(1,7-$C_2B_9H_{10}$)$_2$-2-Co]Cs and [6,6'-μ-O(1,7-$C_2B_9H_{10}$)$_2$-2-Co]$^-$, on native β-cyclodextrin-based CSP directly bonded to the silica gel. Interestingly, [6,6'-μ-MeO(1,7-$C_2B_9H_{10}$)$_2$-2-Co]$^-$ was assumed to be created during the chromatographic analysis of zwitterionic 6,6'-μ-MeO(1,7-$C_2B_9H_{10}$)$_2$-2-Co. Other zwitterionic derivatives of [3,3'-Co(1,2-$C_2B_9H_{11}$)$_2$]$^-$, i.e., 8,4′-μ-H_2N-commo-(1,2-$C_2B_9H_{10}$)$_2$-3-Co and 8,4′-μ-Me_2N-commo-(1,2-$C_2B_9H_{10}$)$_2$-3-Co [221] and [6,6'-Co(1,7-$C_2B_9H_{11}$)$_2$]$^-$, i.e., [6,6′-μ-$(CH_3)_2$P-(1,7-($C_2B_9H_{10}$)$_2$)-2-Co] [216] were also enantioseparated on native β-cyclodextrin-based CSP directly bonded to the silica gel. These prospecting investigations of chiral separations of derivatives of [Co($C_2B_9H_{11}$)$_2$]$^-$ and *nido*-[7,8-$C_2B_9H_{12}$]$^-$ in liquid chromatography were reviewed by Horáková et al. [222]. All these works were carried out on the columns containing β-cyclodextrin as CSP and are characterized by the inability to separate anions except for two species. However, the early investigations of Plešek [221, 223], Grüner, and Plzák [224] defined a solid basis for the upcoming investigations in this field.

The first insight into the chiral separations of zwitterionic and challenging anionic [Co($C_2B_9H_{11}$)$_2$]$^-$ and *nido*-[7,8-$C_2B_9H_{12}$]$^-$ derivatives on polysaccharide-based columns was carried out in 2014 [225]. The enantiomers of zwitterionic compounds of both classes were separated with high selectivity. Authors also claimed that two anionic [3,3'-Co(1,2-$C_2B_9H_{11}$)$_2$]$^-$ derivatives were separated, however, the shown chromatogram of compound [8,8′-μ-S_2-Co(1,2-$C_2B_9H_{10}$)$_2$]Cs depicts two peaks with areas ratio clearly not 50:50 **(Chromatogram (f) in Figure 3 in [225])**. Therefore, the successful chiral separation of this compound is questionable. The second anion was separated with resolution 0.41 under only one chromatographic condition and the chromatogram of this separation was not shown. This is unfortunate since this anion is known to form at least three isomers that are difficult to separate and are all chiral **(not published results by our group)**. Anionic *nido*-[7,8-$C_2B_9H_{12}$]$^-$ derivatives were not chirally discriminated in this study. All chiral separations on cyclodextrin-based and polysaccharide-based columns until 2014 are summarized in **Figure 14.**

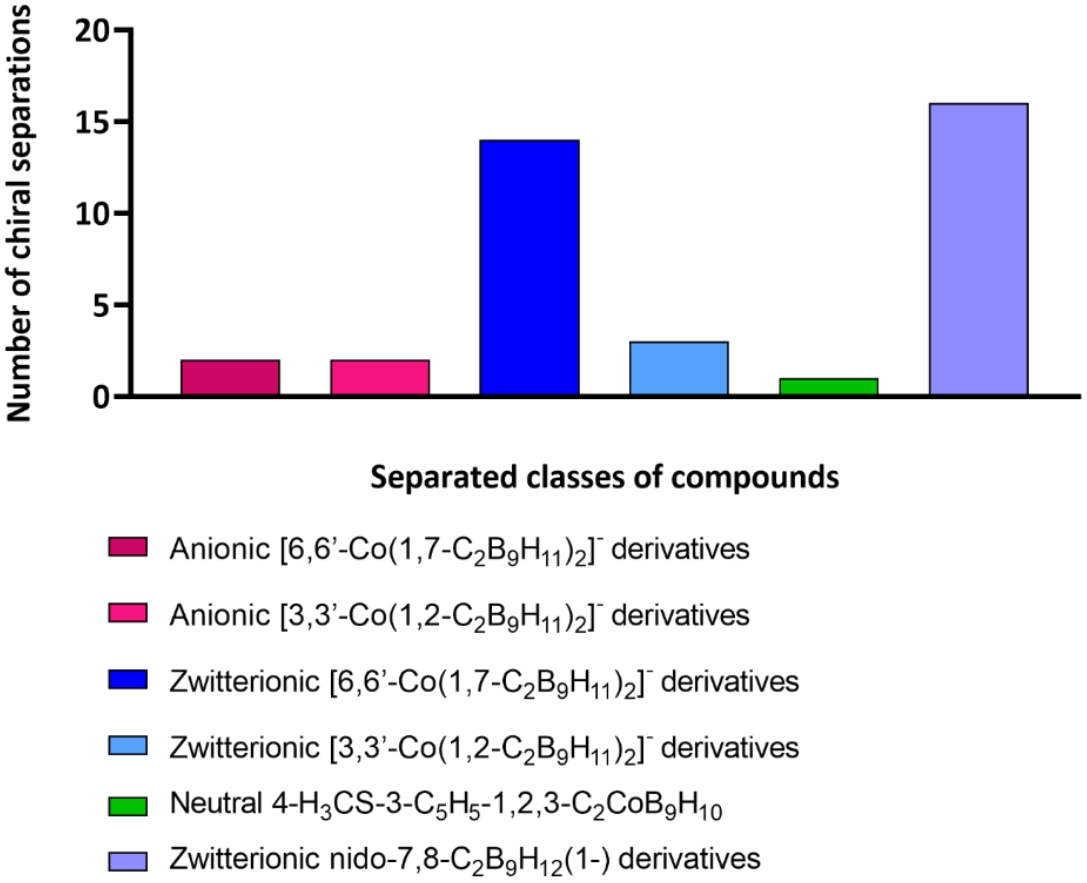

Figure 14 Chiral separations of *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ and $[Co(C_2B_9H_{11})_2]^-$ derivatives in HPLC until 2014.

Briefly, eleven, four, and one anionic derivatives of *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$, $[3,3'\text{-}Co(1,2\text{-}C_2B_9H_{11})_2]^-$, and $[6,6'\text{-}Co(1,7\text{-}C_2B_9H_{11})_2]^-$, respectively, were enantioseparated in CE [226-230] **(Figure 15)**. Generally, the separations were carried out on polyacrylamide-coated capillaries with a background electrolyte composing of borate buffer at pH 9, organic modifier, and native β-cyclodextrin for $[3,3'\text{-}Co(1,2\text{-}C_2B_9H_{11})_2]^-$ or $[6,6'\text{-}Co(1,7\text{-}C_2B_9H_{11})_2]^-$ derivatives and native α-cyclodextrin for *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ derivatives. Although the selectivity of the separation was usually high, the resolution of some enantiomers was below 1.5 especially due to the significant tailing of the peaks. Nevertheless, at least some of the developed CE methods are sufficient at least for the assessment of the enantiopurity for certain anionic compounds; however, they cannot be used for the separation of zwitterions and neutral compounds and cannot be effectively employed for the isolation of enantiomers.

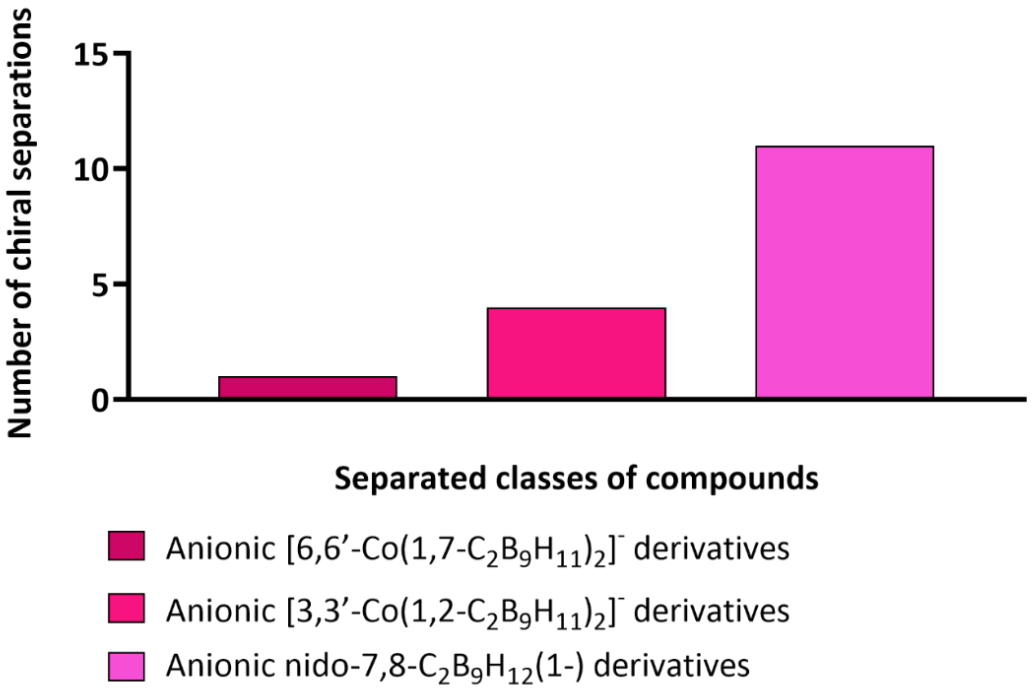

Figure 15 Chiral separations of *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ and $[Co(C_2B_9H_{11})_2]^-$ derivatives in CE by 2022.

3 Aims of the dissertation

The main aim of this dissertation is to present a comprehensive picture of the chromatographic behavior and chiral separations of *nido*-[7,8-$C_2B_9H_{12}$]$^-$ and [Co($C_2B_9H_{11}$)$_2$]$^-$ derivatives by chromatographic techniques in relation to their previously unsuccessful enantioseparations. We also intend to elucidate the discrepancy between the successful chiral separations of anionic *nido*-[7,8-$C_2B_9H_{12}$]$^-$ derivatives in CE and the unsuccessful enantioseparations in HPLC. The next objective of our work is to suggest the strategies and workflows for the chiral separations of anionic and zwitterionic *nido*-[7,8-$C_2B_9H_{12}$]$^-$ and [Co($C_2B_9H_{11}$)$_2$]$^-$ derivatives in RPLC and SFC.

4 Commentary on the published articles

Chiral separations of zwitterionic $[Co(C_2B_9H_{11})_2]^-$ and *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ were previously investigated in HPLC to some extent [216, 220-222, 225]. Nevertheless, challenging anionic $[Co(C_2B_9H_{11})_2]^-$ derivatives were enantioseparated presumably in four cases as discussed before (**see page 48**) and anionic *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ were not chirally discriminated in HPLC at all. Therefore, our research was devoted to identifying the reason for unsuccessful chiral separations of anionic species and finding chromatographic conditions for their enantioseparations in HPLC and SFC. Together with anionic analytes, zwitterions and one neutral mixed sandwich were also analyzed to compare and further widen the portfolio of available methods for chiral separation of these species (for the structures and references to the syntheses of the compounds provided by Dr. Grüner and his co-workers see **Attachement 1**).

4.1 Chiral separations of *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ derivatives

The chiral separations of *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ derivatives proved to be challenging in chromatographic techniques owing to the small size of the molecule resulting in fewer interactions with chiral selectors. Chiral separations of anionic species are even more challenging than enantioseparations of zwitterions due to the permanent negative charge that can interact with negatively charged reactive silanol groups resulting in charge exclusion.

Originally, no retention was observed between anionic *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ derivatives in reversed phase achiral systems using mobile phase MeOH-water [231]. These observations were confirmed in our work on type A silica C18 column (**Figure S1 in [232]**). We found that the retention of the anions is possible by introducing K^+ counter-ions to the mobile phase. The retention of the *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ derivatives was clearly dependent on K^+ cations and the effects of anionic co-ions on retention are negligible. The rising concentration of K^+ in the mobile phase has resulted in prolonged retention due to the suppression of the ionic diffusion layer, formation of ion-pairs and partially by salting-out effect [232]. The involvement of negatively charged reactive silanol groups is

further confirmed by at least some retention of *nido*-[7,8-$C_2B_9H_{12}$]$^-$ derivatives in mobile phase acidified with 0.1% trifluoroacetic acid (**Figure S1 in [232]**).

Enantiomers of *nido*-[7,8-$C_2B_9H_{12}$]$^-$ derivatives were previously discriminated in CE using native α- [227] and β-cyclodextrins [222, 228]. However, despite the separations in CE and the observed chromatographic behavior of *nido*-[7,8-$C_2B_9H_{12}$]$^-$ derivatives in achiral systems, no elution of these species was previously observed on native β-cyclodextrin CSP in mobile phase containing MeOH-water and ACN-water. Hence, our investigations aimed to elucidate the cause of the excessive retention. The experiments were carried out primarily on commercially available chromatographic columns containing native β-cyclodextrin and heptakis(6-bromo-6-deoxy)-β-cyclodextrin, i.e., ChiraDex® (250 × 4.0 mm; particle size 5 μm) from Merck and YMC CHIRAL CD BR (250 × 4.6 mm; particle size 5 μm) from YMC, respectively [232]. The starting mobile phase composition on column ChiraDex® was chosen based on the CE background electrolyte [226], i.e., MeOH-MES/Tris buffer (pH 7; 20 mmol/L) (90:10, v/v). Under these conditions, the elution and chiral separation of [7-CH_3-7,8-*nido*-$C_2B_9H_{11}$]$^-$ was observed but the retention time was over 120 minutes. To shorten the retention time, MeOH was substituted with ACN or THF. Although a substantial reduction of retention was observed, the chiral separation was lost. However, the addition of ACN to MeOH in ratio 1:3 (v/v) was found as a reasonable trade-off for reducing the retention with only a minor loss of selectivity and resolution.

After the first successful chiral separation of anionic *nido*-[7,8-$C_2B_9H_{12}$]$^-$ derivative, we thoroughly studied their extensive retention on ChiraDex®. The most probable cause was the strong ionic interaction with positively charged cationic metal impurities in the silica gel chromatographic support [233-235]. We investigated three different ways how to disrupt these unwanted achiral ionic interactions: (i) increasing the ionic strength of the mobile phase, (ii) decreasing pH of the mobile phase, (iii) addition of the chelating agent.

The increase of the concentration of sodium cations in mobile phase from 5 mml/L to 50 mmol/L resulted in decrease of retention factor from around 50 to 10 (**Figure 16**). The effect of pH was tested in range 5.8 to 7.4 using phosphate buffer. The substantial change in retention was observed. Considering that the analytes have a permanent negative charge, and the pH of buffer was adjusted by mixing different proportions of

Na_2HPO_4 and NaH_2PO_4; the change in retention was correlated rather to change in Na^+ concentration than to change in pH. The effect of counter-ions on the enantioseparation

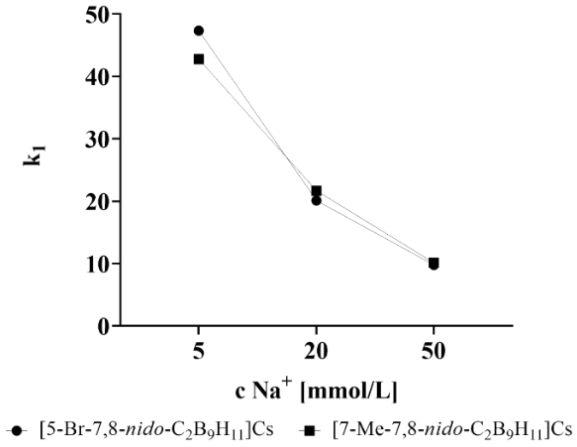

Figure 16 The effect of the concentration of sodium ions on the retention factor of two *nido*-7,8-dicarbaundecaborate derivatives. The abbreviation Me stands for methyl. Reprinted with permission from [232]. Copyright (2021) Elsevier.

of anionic *nido*-[7,8-$C_2B_9H_{12}$]$^-$ derivatives is further confirmed by testing Na^+, Tris, and morpholine. A tight ion-pair is assumed to be formed between anionic analytes and cationic counter-ions. It applies that the bigger the counter-ion, the higher retention and usually also enantioresolution (**Figure 17**)**.** A breakthrough was achieved with the addition of chelating agent, i.e., 1.25 mmol/L disodium edetate. The chelating agent reduces the retention times of studied anionic *nido*-[7,8-$C_2B_9H_{12}$]$^-$ derivatives due to the

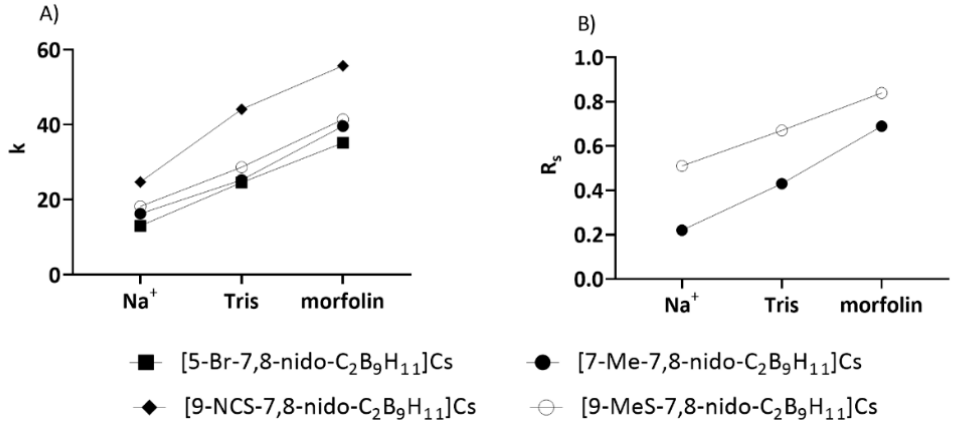

Figure 17 The effect of the type of the counter-ion on the retention factor of the first eluted enantiomer (A) and the enantioresolution (B) of anionic *nido*-7,8-dicarbaundecaborate derivatives. The abbreviation Me stands for methyl. Reprinted with permission from [232]. Copyright (2021) Elsevier.

blockage of cationic metal impurities in silica gel while preserving the resolution of enantiomers. The effect of the chelating agent on the retention of studied compounds is shown in **Figure 18**.

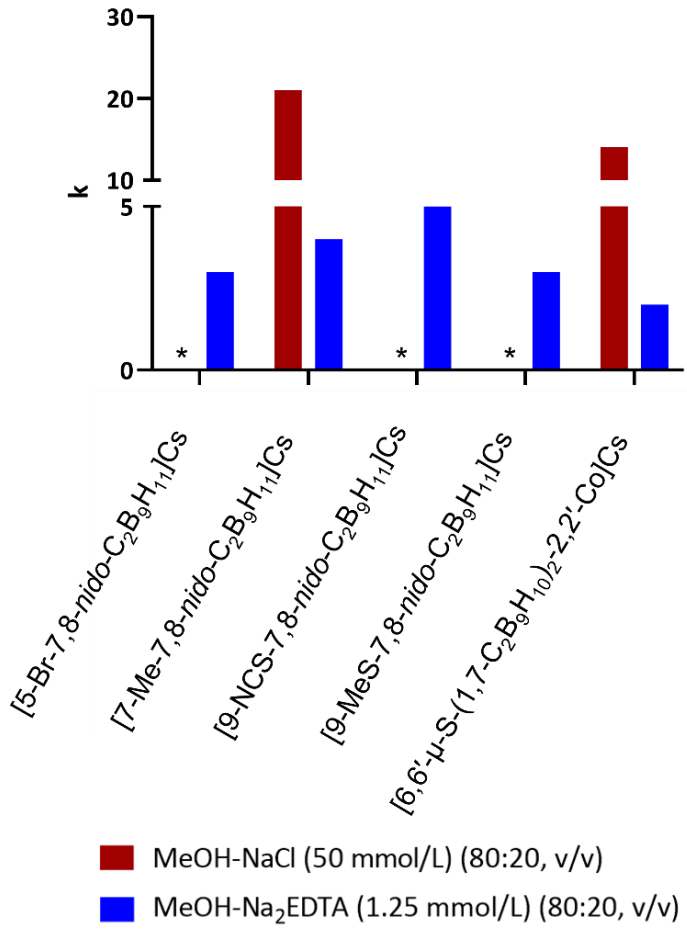

Figure 18 The effect of the mobile phase containing NaCl (red color) and Na_2EDTA (blue trace) on the retention factor of the first eluted enantiomer measured on the column ChiraDex®. The asterisks mark the retention factors higher than 30. The abbreviation Me stands for methyl.

By reducing of unwanted achiral interactions with a chelating agent, the chiral separations of two anionic *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ derivatives were achieved on ChiraDex® within 35 minutes; however, we have not observed chiral separation of other two anionic analytes. Transfer of the knowledge gained on ChiraDex® to column YMC CHIRAL CD BR resulted in chiral separations of all four anionic *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ derivatives. The chiral separations with the highest resolution are depicted in **Figure 19**.

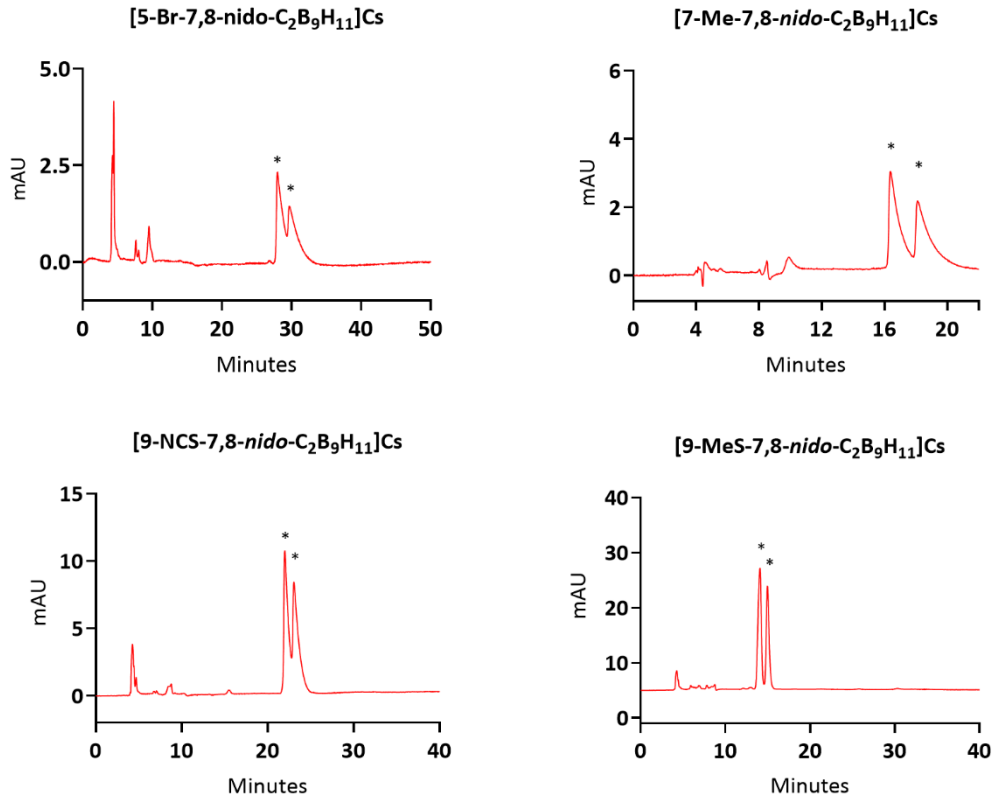

Figure 19 Chiral separations of four anionic *nido*-7,8-dicarbaundecaborate derivatives on column YMC β-CD BR with the composition of the mobile phase MeOH-ACN-Na_2EDTA (1.25 mmol/L) (75:5:20, v/v/v) for [5-Br-7,8-*nido*-$C_2B_9H_{11}$]Cs and [7-Me-7,8-*nido*-$C_2B_9H_{11}$]Cs and MeOH-ACN-Na_2EDTA (1.25 mmol/L) (65:15:20, v/v/v) for [9-NCS-7,8-*nido*-$C_2B_9H_{11}$]Cs and [9-MeS-7,8-*nido*-$C_2B_9H_{11}$]Cs. The enantiomers are marked with asterisks. The abbreviation Me stands for methyl. Reprinted and adjusted with permission from [232]. Copyright (2021) Elsevier.

Furthermore, unwanted achiral interactions were not observed for the zwitterionic *nido*-[7,8-$C_2B_9H_{12}$]$^-$ derivatives. Their chromatographic behavior is affected by the inherent intramolecular compensation of the negative charge with the positively charged functional group giving zero net charge of the molecule. Thus, chiral separations of zwitterionic *nido*-[7,8-$C_2B_9H_{12}$]$^-$ derivatives were achieved more often. These molecules were also baseline separated on amylose *tris*(3,5-dimethylphenylcarbamate) in RPLC in 30 minutes (**Figure 17**) [236] and in SFC in less than one minute (**Figure 18**) [120].

Recently, separations of anionic *nido*-[7,8-$C_2B_9H_{12}$]$^-$ derivatives has been achieved using CSP with 2-hydroxypropyl-β-cyclodextrin bonded to silica SPP (CD Shell-RSP) [237]. In this study, the anions showed a similar chromatographic behavior as on ChiraDex®

and YMC CHIRAL CD BR [232], i.e., the increase in ionic strength resulted in the shorter retention time, same as in **Figure 16**. The partial chiral separations of three anionic *nido*-[7,8-$C_2B_9H_{12}$]$^-$ derivatives were achieved in shorter time, lower selectivity, and lower resolution compared to YMC CHIRAL CD BR [237]. Moreover, the partial chiral separations of zwitterionic *nido*-[7,8-$C_2B_9H_{12}$]$^-$ compounds on TAG Shell CSP (teicoplanin aglycone bonded to SPP of silica) were also achieved. All chiral separations of zwitterions on SPP based CSP are accomplished in shorter analysis time than in our previous work on polysaccharide-based columns [236]. However, only partial resolution of enantiomers was achieved contrary to the baseline separations on polysaccharide-based columns (**Table 3 in [237]**).

It should be noted that enantioseparations are usually difficult to predict. Therefore, in addition to abovementioned β-cyclodextrin based columns, cellulose and amylose *tris*(3,5-dimethylphenylcarbamate) [236], cellulose *tris*(3,5-dichlorophenylcarbamate) [236], and TAG Shell CSP [237]; plethora of other CSP were tested by our group with no success including brominated α- and γ-cyclodextrin, native α-cyclodextrin, O-9-(tert-butylcarbamoyl) quinidine, α-1-acidic glycoprotein, and teicoplanin. In addition, commercially available CSP immobilized onto SPP silica from AZYP [72] were tested with no successful chiral separation, i.e., TeicoShell (teicoplanin based CSP), VancoShell (vancomycin based CSP), NicoShell (modified macrocyclic glycopeptide based CSP), QShell (quinine based CSP), MaltoShell (derivatized maltodextrin based CSP), LarihcShell-P (isopropyl cyclofructan-6 based CSP), and LarihcShell-RN (R-naphtylethyl cyclofructan-6 based CSP) [237].

4.2 Chiral separations of [$Co(C_2B_9H_{11})_2$]$^-$

4.2.1 Cyclodextrins

The detailed investigations in [232] revealed that anionic sulfur bridged [$Co(C_2B_9H_{11})_2$]$^-$, i.e., [6,6′-μ-S-(1,7-$C_2B_9H_{10})_2$-2,2′-Co]Cs, is separated with the high resolution on the column ChiraDex® in conditions MeOH-NaCl (50 mmol/L) and the same analyte is not separated on bromated YMC CHIRAL CD BR under any chromatographic conditions. Due to the previously mentioned findings that the chromatographic behavior of anionic sulfur bridged [$Co(C_2B_9H_{11})_2$]$^-$ is predominantly

dependent on counter-ion concentration (in this case Na^+) (**Figure 4 in [232]**), NaCl was substituted with $NaClO_4$ to avoid potential corrosive properties of NaCl. Further modifications of the method included the replacement of ChiraDex® for another native β-CD column ReproSil Chiral-Beta-CD (250 × 4.6 mm; particle size 5 μm) leading to the chiral separations of the series of oxygen-bridged $[Co(C_2B_9H_{11})_2]^-$ derivatives [238]. In this study, the CYCLOBOND™ I 2000 column with native β-cyclodextrin and CYCLOBOND™ I 2000-Acetyl column with acetylated-β-cyclodextrin were also tested. It was found that five mono-substituted compounds were enantioseparated on CYCLOBOND™ I 2000 ($Rs \leq 1$) and four di-substituted analytes with $Rs > 2.5$ on the ReproSil Chiral-Beta-CD CSP. Column CYCLOBOND™ I 2000-Acetyl enabled only partial separation of four mono- and one di-substituted analytes ($Rs < 1$).

Furthermore, we carried out the investigations of chiral separability of neutral 4-CH_3S-3-C_5H_5-Co-(1,2-$C_2B_9H_{10}$), zwitterionic, and anionic $[Co(C_2B_9H_{11})_2]^-$ derivatives on the commercially available CSP based on silica SPP in HPLC. The CD Shell-RSP was identified as the only column capable of enantioseparating all the classes of tested $[Co(C_2B_9H_{11})_2]^-$ derivatives. Enantiomers of dihydroxyalkyl derivatives of cobalt bis(dicarbollide), oxygen bridged hydroxyalkyl derivatives, and bisphenylene bridged derivative were separated in less than 5 minutes with resolution higher than 1.8 (**Figure 20**). These results were compared with our previous work. It was found out that CD Shell-RSP column can to some extent compete with the chiral separations on polysaccharide-based columns in SFC (**Table 3 in [237]**). Therefore, CD Shell-RSP column in common HPLC system can be used as an alternative to SFC for the enantioseparations of the mentioned classes of boron clusters reaching similar separation times and resolution.

4.2.2 Polysaccharides

The decision of using polysaccharide-based chiral selectors came logically based on their versatility, availability, and especially higher loadability compared to cyclodextrin-based columns. The latter property will be essential in future during the transfer of the methods from analytical to semi-preparative/preparative scale. Previously, the chiral screening of $[Co(C_2B_9H_{11})_2]^-$ derivatives was carried out on polysaccharide-based columns in NPLC and POM [225], without any mention of the applicability of RPLC or SFC.

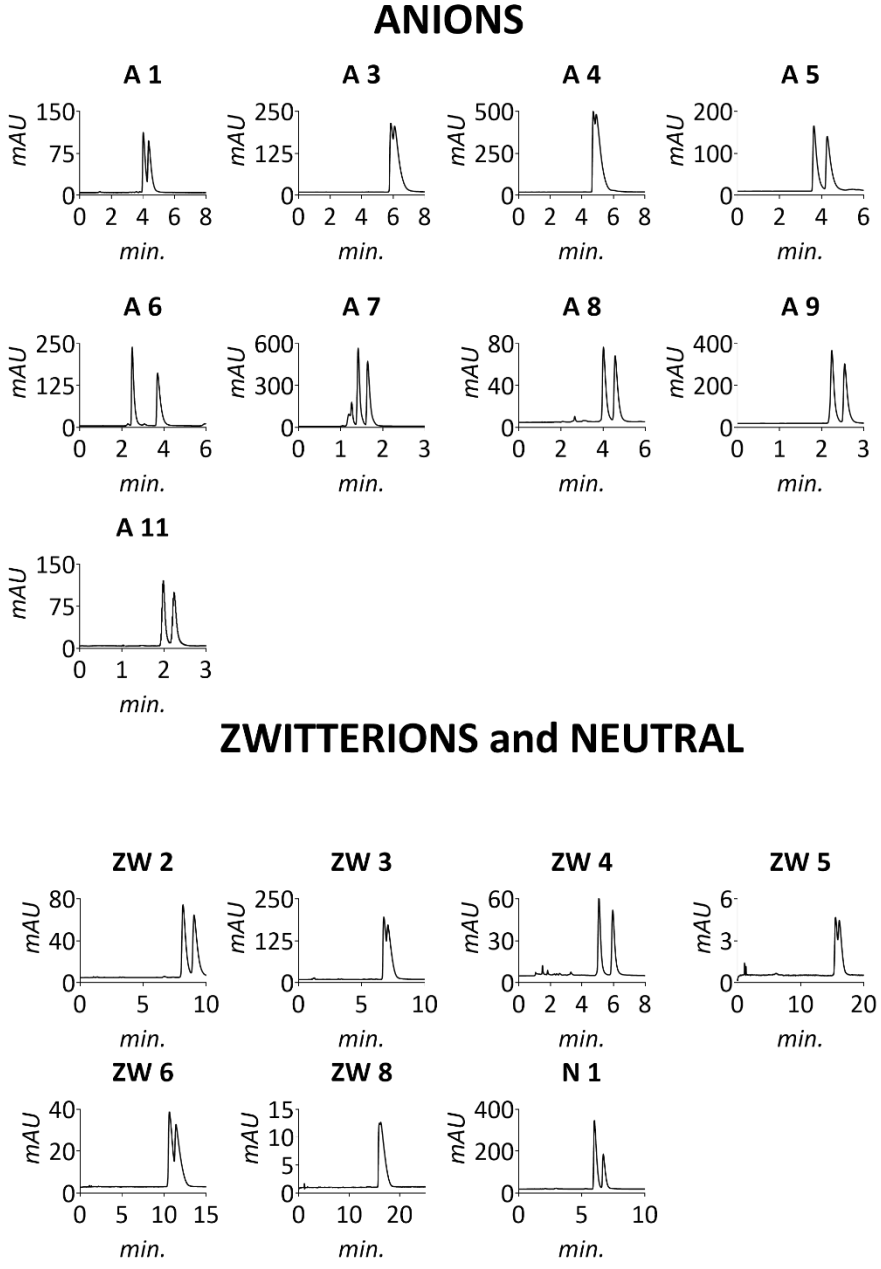

Figure 20 Chiral separations of racemic [6,6′-μ-S-(1,7-$C_2B_9H_{10})_2$-2,2′-Co]Cs (**A 1**); [1-(HOC_2H_4)-Co(1,2-$C_2B_9H_{10})_2$][$(CH_3)_3NH$] (**A 3**); [1-(HOC_3H_6)-Co(1,2-$C_2B_9H_{10})_2$][$(CH_3)_3NH$] (**A 4**); [1,1′-$(HOCH_2)_2$-Co(1,2-$C_2B_9H_{10})_2$][$(CH_3)_3NH$] (**A 5**); [1,1′-(HOC2H4)2-Co(1,2-$C_2B_9H_{10})_2$][$(CH_3)_3NH$] (**A 6**); [8,4′,8′,4-μ-bis($C_6H_4)_2$-Co(1,2-$C_2B_9H_{10})_2$]Na (**A 7**); [1-(HOC_3H_6)-8,8′-μ–O-Co(1,2-$C_2B_9H_{10})_2$][$(CH_3)_3NH$] (**A 8**); [1,1′-$(HOC_3H_6)_2$-8,8′-μ-O-Co(1,2-$C_2B_9H_{10})_2$][$(CH_3)_3NH$] (**A 9**); [1-$(H_2NC_2H_4)_2$-Co(1,2-$C_2B_9H_{10})_2$] [$(CH_3)_4N$] (**A 11**); 4,8′-μ-NH_2-Co(1,2-$C_2B_9H_{10})_2$ (**ZW 2**); 6,6′-μ-(CH_3)S-Co(1,2-$C_2B_9H_{10})_2$ (**ZW 3**); 6,6′-μ-(C_3H_5)S-Co(1,2-$C_2B_9H_{10})_2$ (**ZW 4**); 5-CH_3-11-$(CH_3)_2$S-7,8-*nido*-$C_2B_9H_{11}$ (**ZW 5**); 7-C_6H_5-9-$(CH_3)_2$S-7,8-*nido*-$C_2B_9H_{11}$ (**ZW 6**); 9-$(CH_3)_2$S-7,8-*nido*-$C_2B_9H_{11}$ (**ZW 8**); and 4-CH_3S-3-C_5H_5-Co-(1,2-$C_2B_9H_{10}$) (**N 1**). All separations were carried out in RPLC on CD Shell-RSP column. The only exception is analyte **A 11** separated with the highest resolution on VancoShell column. The zwitterionic *nido*-[7,8-$C_2B_9H_{12}]^-$ derivatives are included in the figure for comparison. Reprinted with permission from [236]. Copyright (2021) Elsevier. For further information see ref. [236].

The chromatographic behavior of the anionic analytes on the Lux® Cellulose 1 (cellulose *tris*(3,5-dimethylphenylcarbamate)) in simple water-organic mobile phase copied observations on β-cyclodextrin based columns, that is, interactions of the anionic analytes with cationic metal impurities representing strong adsorption sites. As for the β-cyclodextrin based columns, the addition of Na_2EDTA or competing counter-ion resulted in the significant reduction of retention and chiral separations (**Figure 1 in [236]**). Opposite chromatographic behavior of anionic analytes was observed on Lux® Amylose 1 (amylose *tris*(3,5-dimethylphenylcarbamate)) in the water-organic modifier mobile phase, i.e., the anions were eluted near the dead volume of the column. We assume that in this case, the helical structure of amylose hinders the contact of the anions with the silica chromatographic support and they are not retained on the inherently charge-neutral amylose *tris*(3,5-dimethylphenylcarbamate). The addition of a counter-ion prolonged the retention due to the formation of a neutral ion-pair. This phenomenon is the most significant when triethylamine was present in the mobile phase (**Figure 2 in [236]**). The increase in retention with the addition of counter-ion was also investigated on Lux® Cellulose i-5 (cellulose *tris*(3,5-dichlorophenylcarbamate (**Figure 3 in [236]**). It was found that the retention was affected in the same manner as on Lux® Cellulose 1. Our findings indicate that the chromatographic behavior of the anions is strongly associated with the type of polysaccharide.

The RPLC conditions for the isocratic chiral screening on polysaccharide-based columns were derived from enantioseparations on β-cyclodextrin based columns, i.e., MeOH or ACN-$NaClO_4$ (50 mmol/L). The screening in RPLC revealed that anionic compounds are separated in a greater number with ACN as the organic modifier on cellulose-based columns. In contrast, all zwitterionic and neutral compounds in the study were separated with ACN on amylose *tris*(3,5-dimethylphenylcarbamate).

During the optimization, the effects of the type and concentration of additives, temperature, and flow rate were tested. The highest resolution of the anions was usually reached in sodium perchlorate except for CSP with immobilized cellulose *tris*(3,5-dichlorophenylcarbamate) where the highest resolution was reached using triethylamine acetate. As a result, the recommendations (**Figure 21**) were given for the development of the chiral method. The chiral separations of eight of the ten anionic deri-

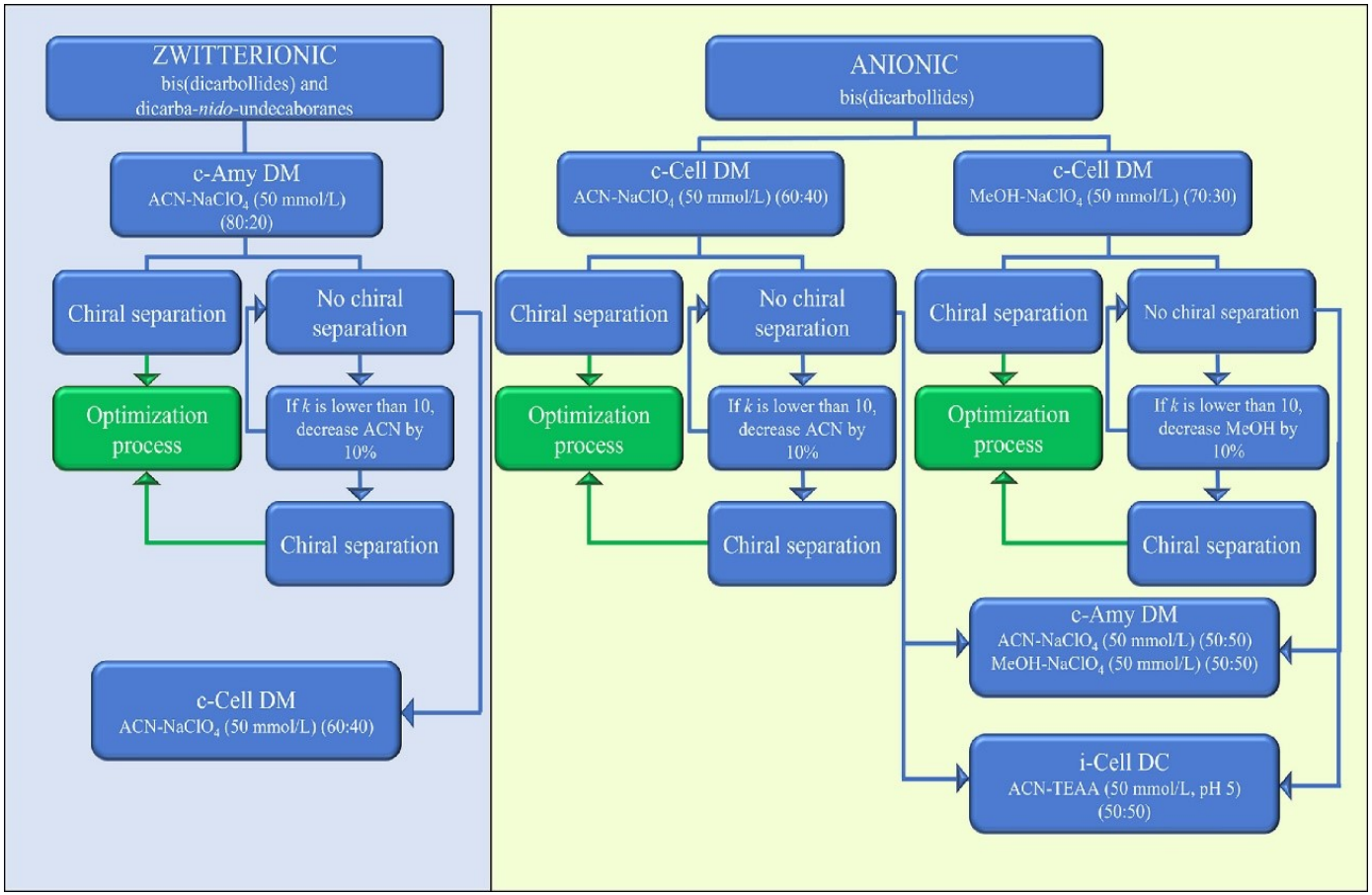

Figure 21 The recommended method development workflow for chiral separations of the anionic bis(dicarbollide) derivatives and zwitterionic bis(dicarbollide) and dicarba-*nido*-undecaborane derivatives on the studied cellulose- and amylose-based chiral selectors. Acronyms DM and DC stand for 3,5-dimethylphenylcarbamate and 3,5-dichlorophenylcarbamate. Reprinted with permission from [236]. Copyright (2021) Elsevier.

vatives of $[Co(C_2B_9H_{11})_2]^-$ were achieved with four species separated at the baseline. All four zwitterions and one neutral compound were readily separated at the baseline without the need of additives in the mobile phase (**Figure 22**).

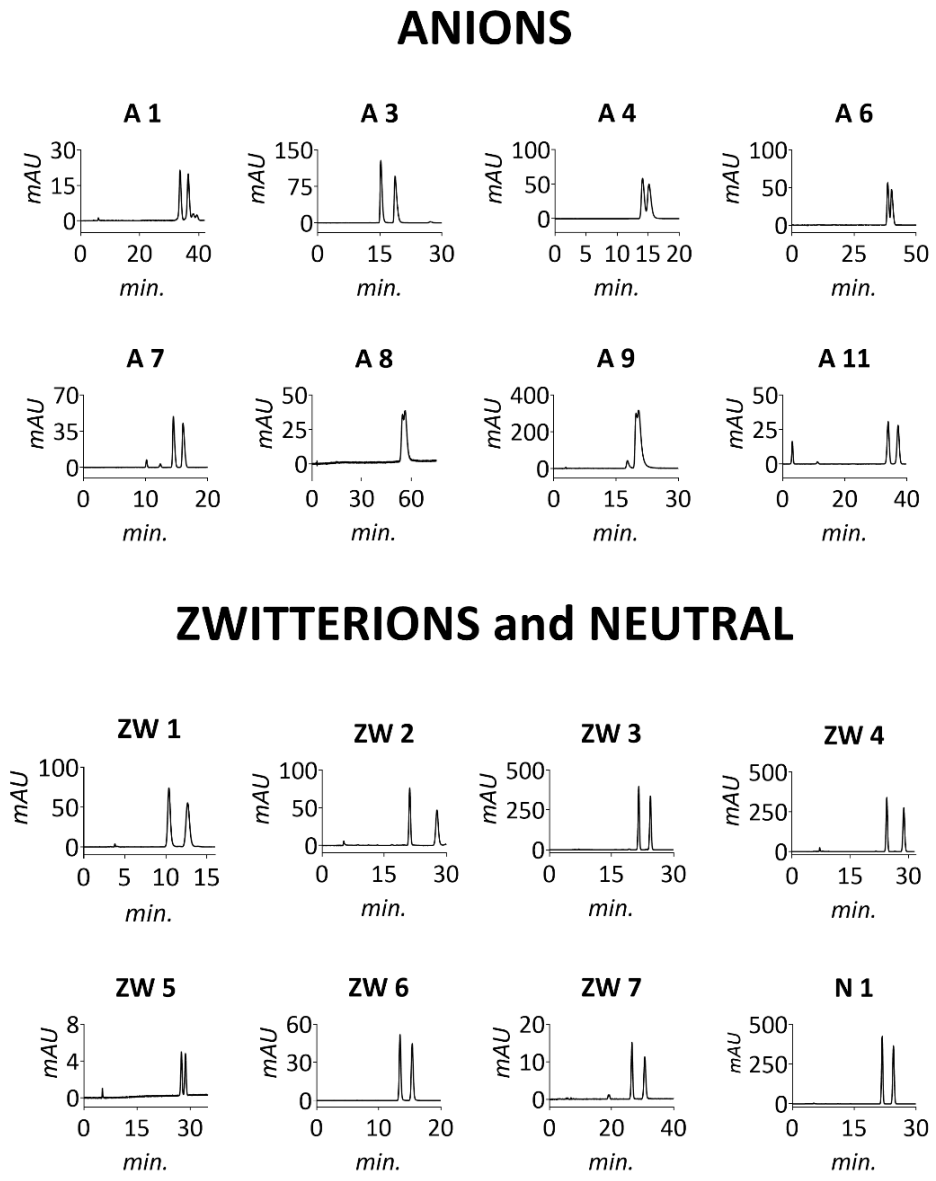

Figure 22 Chiral separations of racemic [6,6′-μ-S-(1,7-$C_2B_9H_{10})_2$-2,2′-Co]Cs (**A 1**); [1-(HOC_2H_4)-Co(1,2-$C_2B_9H_{10})_2$][$(CH_3)_3NH$] (**A 3**); [1-(HOC_3H_6)-Co(1,2-$C_2B_9H_{10})_2$][$(CH_3)_3NH$] (**A 4**); [1,1′-(HOC2H4)2-Co(1,2-$C_2B_9H_{10})_2$][$(CH_3)_3NH$] (**A 6**); [8,4′,8′,4-μ-bis($C_6H_4)_2$-Co(1,2-$C_2B_9H_{10})_2$]Na (**A 7**); [1-(HOC_3H_6)-8,8′-μ–O-Co(1,2-$C_2B_9H_{10})_2$][$(CH_3)_3NH$] (**A 8**), [1,1′-($HOC_3H_6)_2$-8,8′-μ-O-Co(1,2-$C_2B_9H_{10})_2$][$(CH_3)_3NH$] (**A 9**); [1-($H_2NC_2H_4)_2$-Co(1,2-$C_2B_9H_{10})_2$][$(CH_3)_4N$] (**A 11**); 6,6′-μ-(CH_3OCOCH_2)S-Co(1,2-$C_2B_9H_{10})_2$ (**ZW 1**); 4,8′-μ-NH_2-Co(1,2-$C_2B_9H_{10})_2$ (**ZW 2**); 6,6′-μ-(CH_3)S-Co(1,2-$C_2B_9H_{10})_2$ (**ZW 3**); 6,6′-μ-(C_3H_5)S-Co(1,2-$C_2B_9H_{10})_2$ (**ZW 4**); 5-CH_3-11-$(CH_3)_2$S-7,8-*nido*-$C_2B_9H_{11}$ (**ZW 5**); 7-C_6H_5-9-$(CH_3)_2$S-7,8-*nido*-$C_2B_9H_{11}$ (**ZW 6**); 7-C_6H_5-11-$(CH_3)_2$S-7,8-*nido*-$C_2B_9H_{11}$ (**ZW 7**); and 4-CH_3S-3-C_5H_5-Co-(1,2-$C_2B_9H_{10}$) (**N 1**) in RPLC on polysaccharide-based columns. The zwitterionic *nido*-[7,8-$C_2B_9H_{12}]^-$ derivatives are included in the figure for comparison. Reprinted with permission from [236]. Copyright (2021) Elsevier. For further information see ref. [236].

To cover all commonly used separation techniques for enantiomers, SFC was employed with a vision of fast separations of all compounds under study. Although the scientific article [120] includes only columns based on polysaccharides, the experiments were carried out employing α-, β-, γ-cyclodextrin-, teicoplanin-, teicoplanin aglycon-, vancomycin-, and quininine/quinidine-based CSP. Polysaccharide-based CSP outperformed other CSP in terms of enantioselectivity, resolution, and number of separated compounds. Thus, chiral separations using nine polysaccharide-based CSP were thoroughly investigated. Chiral screening in SFC started with the fast gradient method. All tested compounds except for [8,8′-μ-S_2-Co(1,2-$C_2B_9H_{10})_2$]Cs were at least partially enantioseparated during gradient screening (**Figure 23**). Subsequently, the gradient separations were transferred to the isocratic elution mode; and the optimization of organic modifier, the type and concentration of additives, temperature, and the flow rate were carried out. The addition of ACN, 3 or 5% of water, or a different type of an amine to the co-solvent was identified as the key tools for achieving the baseline chiral separations.

The effect of the ratios of ACN and MeOH or 2-propanol (1:0, 3:1, 1:1, 1:3, 0:1, v/v) on the chiral separation of the studied compounds was further studied. The characteristic U-shaped curves were observed for the retention factors as the function of ratios of ACN to MeOH or 2-propanol **(Figure 4 in [120])**. Although, the selectivity is usually the highest in MeOH or 2-propanol, the resolution in ACN-MeOH (2-propanol) (1:1, 3:1, v/v) mixtures is similar due to the positive effect of the ACN on the peak shape of the tested compounds. We elucidate this behavior by the different amount of adsorbed ACN and MeOH/2-propanol on the surface of CSP in their respective ratios and by different interactions offered in alcohols and ACN. The distinct amount and ratios of adsorbed organic solvents can lead to the changes of the polarity and three-dimensional structure of the polysaccharide selectors. Another explanation could be the continual change of the chiral recognition mechanism from π-π interactions driven separations in pure MeOH/2-propanol to hydrogen bonding in pure ACN. The anionic analytes may be also

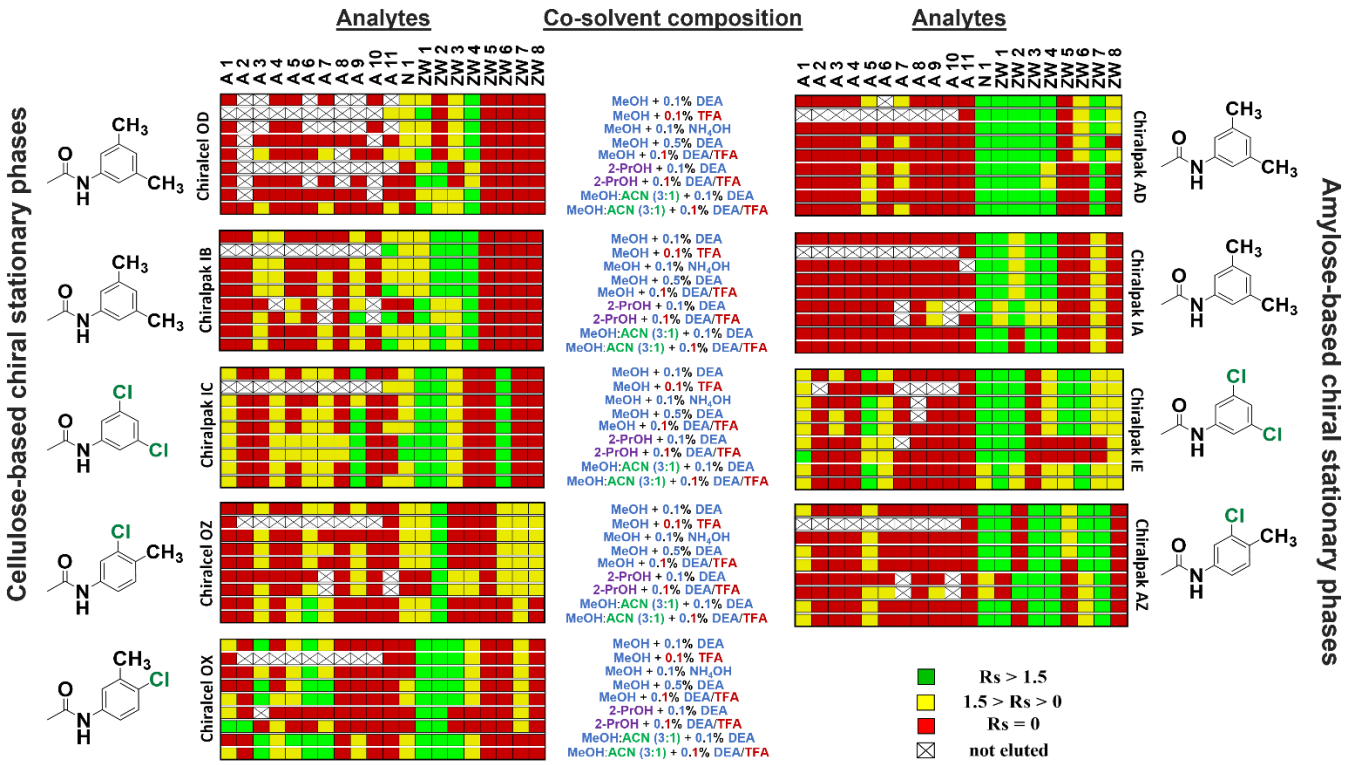

Figure 23 Heat map of the resolution achieved during the screening of polysaccharide-based chiral columns in SFC. Racemic compounds under investigation were [6,6′-μ-S-(1,7-$C_2B_9H_{10}$)$_2$-2,2′-Co]Cs (**A 1**), [1-($HOCH_2$)-Co(1,2-$C_2B_9H_{10}$)$_2$][$(CH_3)_3NH$] (**A 2**), [1-(HOC_2H_4)-Co(1,2-$C_2B_9H_{10}$)$_2$][$(CH_3)_3NH$] (**A 3**); [1-(HOC_3H_6)-Co(1,2-$C_2B_9H_{10}$)$_2$][$(CH_3)_3NH$] (**A 4**); [1,1′-($HOCH_2$)$_2$-Co(1,2-$C_2B_9H_{10}$)$_2$][$(CH_3)_3NH$] (**A 5**); [1,1′-(HOC2H4)2-Co(1,2-$C_2B_9H_{10}$)$_2$][$(CH_3)_3NH$] (**A 6**), [8,4′,8′,4-μ-bis(C_6H_4)$_2$-Co(1,2-$C_2B_9H_{10}$)$_2$]Na (**A 7**), [1-(HOC_3H_6)-8,8′-μ−O-Co(1,2-$C_2B_9H_{10}$)$_2$][$(CH_3)_3NH$] (**A 8**), [1,1′-(HOC_3H_6)$_2$-8,8′-μ-O-Co(1,2-$C_2B_9H_{10}$)$_2$][$(CH_3)_3NH$] (**A 9**); [8,8′-μ-S_2−Co(1,2-$C_2B_9H_{10}$)$_2$]Cs (**A 10**); [1-($H_2NC_2H_4$)$_2$-Co(1,2-$C_2B_9H_{10}$)$_2$][$(CH_3)_4N$] (**A 11**), 4-CH_3S-3-C_5H_5-Co-(1,2-$C_2B_9H_{10}$) (**N 1**); 6,6′-μ-(CH_3OCOCH_2)S-Co(1,2-$C_2B_9H_{10}$)$_2$ (**ZW 1**), 4,8′-μ-NH_2-Co(1,2-$C_2B_9H_{10}$)$_2$ (**ZW 2**), 6,6′-μ-(CH_3)S-Co(1,2-$C_2B_9H_{10}$)$_2$ (**ZW 3**), 6,6′-μ-(C_3H_5)S-Co(1,2-$C_2B_9H_{10}$)$_2$ (**ZW 4**); 5-CH_3-11-$(CH_3)_2$S-7,8-*nido*-$C_2B_9H_{11}$ (**ZW 5**); 7-C_6H_5-9-$(CH_3)_2$S-7,8-*nido*-$C_2B_9H_{11}$ (**ZW 6**), 7-C_6H_5-11-$(CH_3)_2$S-7,8-*nido*-$C_2B_9H_{11}$ (**ZW 7**), and 9-$(CH_3)_2$S-7,8-*nido*-$C_2B_9H_{11}$ (**ZW 8**) Reprinted with permission from [120]. Copyright (2022) American Chemical Society. For further information see ref. [120].

affected by the ability of ACN to reduce ionic interactions as was described earlier in HPLC [232]. Based on these findings, the recommendations for the chiral method development in SFC were given (**Figure 24**). In addition, baseline chiral separations of 15 out of 16 $[Co(C_2B_9H_{11})_2]^-$ derivatives were achieved. All zwitterions were separated at baseline in one minute. On the contrary, the challenging anionic species were separated at baseline in ten minutes (**Figure 25**).

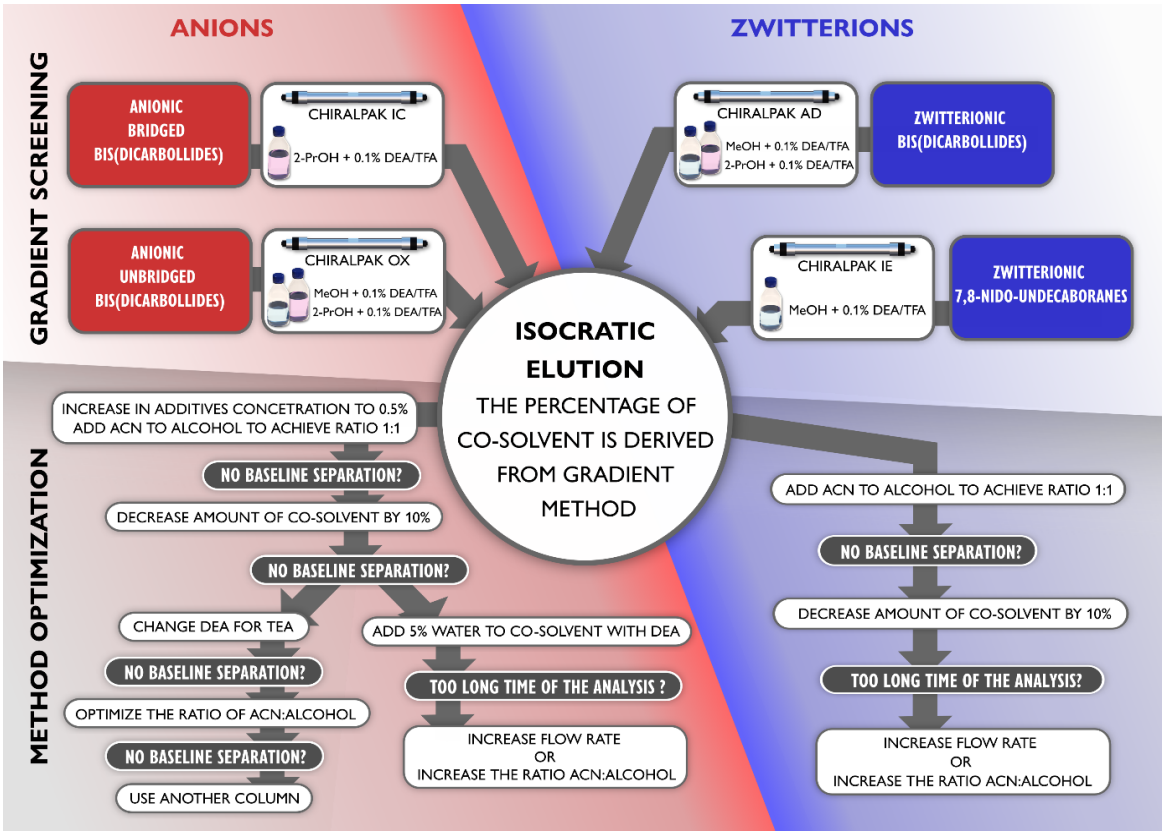

Figure 24 The diagram of the method development for chiral separations of anionic and zwitterionic boron clusters derivatives. Reprinted with permission from [120]. Copyright (2022) American Chemical Society. For further information see ref. [120].

4.2.3 Other Chiral Stationary Phases

Besides the cyclodextrin-based and polysaccharide-based CSP, chiral separations of $[1\text{-}(H_2NC_2H_4)\text{-}Co(1,2\text{-}C_2B_9H_{10})_2]^-$ possessing primary amino group were studied on the VancoShell and LarihcShell-P CSP. It should be noted that no other compound was separated on the two columns. A presence of 0.1% (v/v) trifluoroacetic acid in the mobile phase containing 0.1% (w/v) ammonium trifluoroacetate is identified as the critical parameter for decreasing retention factor and increasing separation efficiency while maintaining the resolution and selectivity on VancoShell CSP (**Figure 3 in [237]**). In case

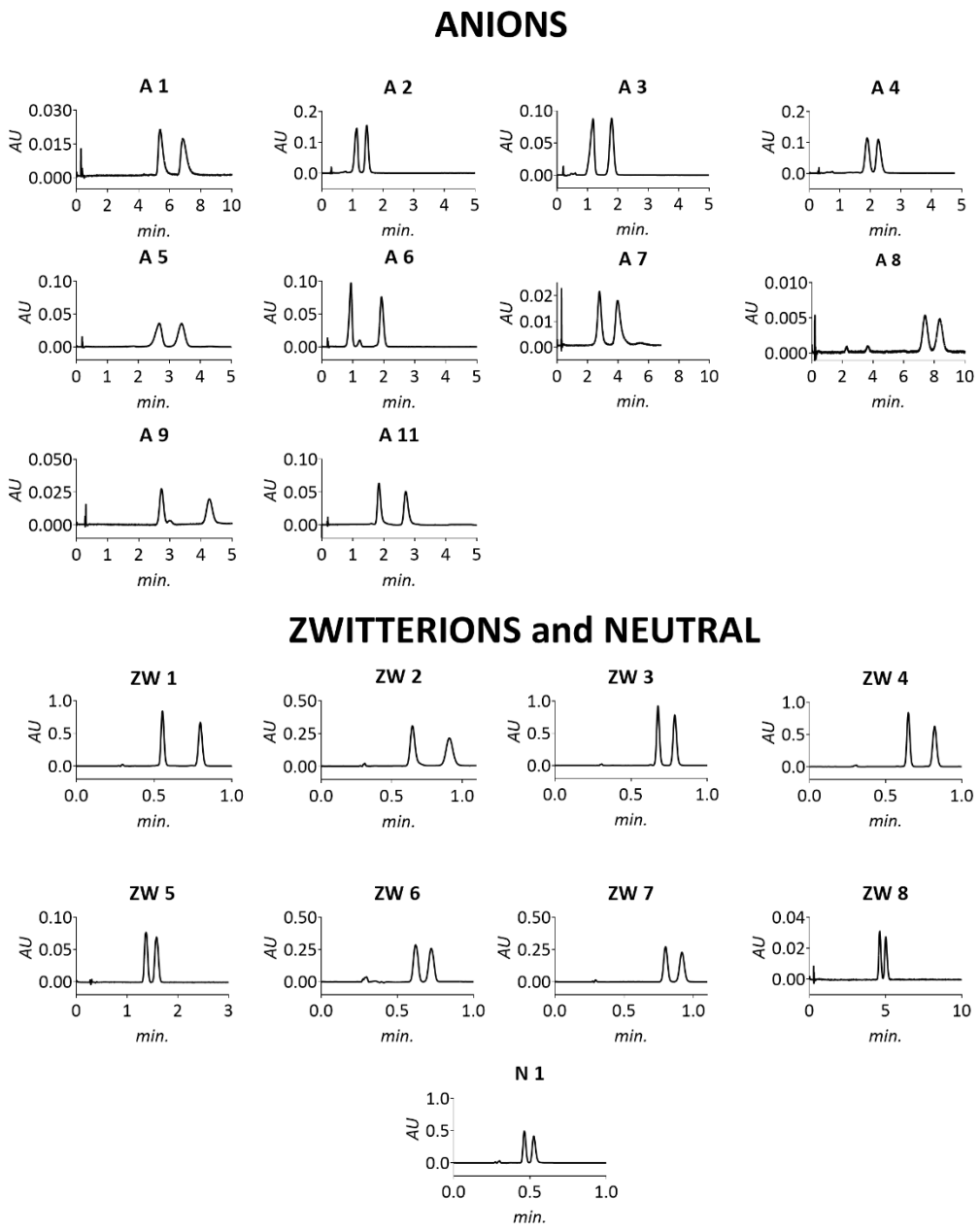

Figure 25 Chiral separations of racemic [6,6′-μ-S-(1,7-$C_2B_9H_{10})_2$-2,2′-Co]Cs (**A 1**); [1-($HOCH_2$)-Co(1,2-$C_2B_9H_{10})_2$][$(CH_3)_3NH$] (**A 2**); [1-(HOC_2H_4)-Co(1,2-$C_2B_9H_{10})_2$][$(CH_3)_3NH$] (**A 3**); [1-(HOC_3H_6)-Co(1,2-$C_2B_9H_{10})_2$][$(CH_3)_3NH$] (**A 4**); [1,1′-($HOCH_2)_2$-Co(1,2-$C_2B_9H_{10})_2$][$(CH_3)_3NH$] (**A 5**); [1,1′-(HOC2H4)2-Co(1,2-$C_2B_9H_{10})_2$][$(CH_3)_3NH$] (**A 6**); [8,4′,8′,4-μ-bis($C_6H_4)_2$-Co(1,2-$C_2B_9H_{10})_2$]Na (**A 7**); [1-(HOC_3H_6)-8,8′-μ−O-Co(1,2-$C_2B_9H_{10})_2$][$(CH_3)_3NH$] (**A 8**); [1,1′-($HOC_3H_6)_2$-8,8′-μ-O-Co(1,2-$C_2B_9H_{10})_2$][$(CH_3)_3NH$] (**A 9**); [1-($H_2NC_2H_4)_2$-Co(1,2-$C_2B_9H_{10})_2$][$(CH_3)_4N$] (**A 11**); 6,6′-μ-(CH_3OCOCH_2)S-Co(1,2-$C_2B_9H_{10})_2$ (**ZW 1**); 4,8′-μ-NH_2-Co(1,2-$C_2B_9H_{10})_2$ (**ZW 2**); 6,6′-μ-(CH_3)S-Co(1,2-$C_2B_9H_{10})_2$ (**ZW 3**); 6,6′-μ-(C_3H_5)S-Co(1,2-$C_2B_9H_{10})_2$ (**ZW 4**); 5-CH_3-11-$(CH_3)_2$S-7,8-*nido*-$C_2B_9H_{11}$ (**ZW 5**); 7-C_6H_5-9-$(CH_3)_2$S-7,8-*nido*-$C_2B_9H_{11}$ (**ZW 6**); 7-C_6H_5-11-$(CH_3)_2$S-7,8-*nido*-$C_2B_9H_{11}$ (**ZW 7**); 9-$(CH_3)_2$S-7,8-*nido*-$C_2B_9H_{11}$ (**ZW 8**); and 4-CH_3S-3-C_5H_5-Co-(1,2-$C_2B_9H_{10}$) (**N 1**) in SFC on polysaccharide-based columns. The zwitterionic *nido*-[7,8-$C_2B_9H_{12}]^-$ derivatives are included in the figure for comparison. Reprinted with permission from [120]. Copyright (2022) American Chemical Society. For further information see ref. [120].

of LarihcShell-P column, the different MeOH - ACN ratios (**Figure S4 in [237]**) and acetic acid (v/v) – triethylamine (v/v) ratios (**Figure S5 in [237]**) were tested. It was found that the [1-($H_2NC_2H_4$)-Co(1,2-$C_2B_9H_{10}$)$_2$]$^-$ was separated only in the mixtures of MeOH - ACN reaching the highest resolution in ratio 40:60 (v/v). The similar rule applies for the ratio of acetic acid and triethyl amine reaching the highest resolution in ratios 0.3-0.02 (v/v) and 0.3-0.2 (v/v). The result of these investigations is the baseline separation of [1-($H_2NC_2H_4$)-Co(1,2-$C_2B_9H_{10}$)$_2$]$^-$ on VancoShell column in 3 minutes. The analysis time of the optimized chiral separation on LarihcShell-P column was two times faster, but the maximum resolution (1.23) was insufficient for baseline separation.

The contribution results to current state of knowledge

Our work introduced a palette of methods for the chiral separations of especially anionic as well as zwitterionic *nido*-[7,8-$C_2B_9H_{12}$]$^-$ and [Co($C_2B_9H_{11}$)$_2$]$^-$ derivatives. Although the enantioseparations of zwitterions were achieved previously, the SFC methods developed herein outperforms available methods by the means of separation time. All zwitterions were separated in less than one minute. Despite the ultra-fast chiral separations of zwitterions, the essential contribution of this work lies in the first chiral separations of anionic *nido*-[7,8-$C_2B_9H_{12}$]$^-$ in HPLC [232] and anionic unbridged C-substituted derivatives of [3,3'-Co(1,2-$C_2B_9H_{11}$)$_2$]$^-$ derivatives in HPLC [236, 237] and SFC [120]. For clarity, **Figure 26** illustrates the novelty of our work in the context with previous chiral separations of anionic analytes by chromatographic techniques.

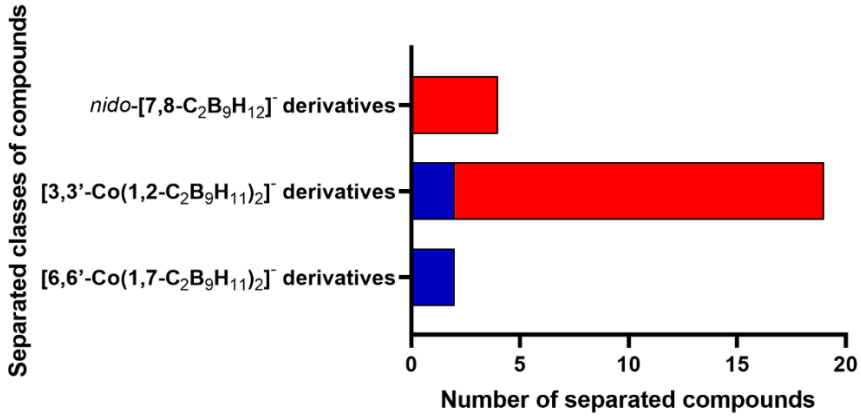

Figure 26 The HPLC/SFC chiral separations of anionic compounds obtained during the dissertation (red color) in the context with previously achieved chiral separations (blue color). Note that all compounds falling in blue columns were also separated in this dissertation in shorter time and with enhanced resolution.

5 Conclusions

Our work enabled first chiral separations of anionic *nido*-[7,8-$C_2B_9H_{12}$]$^-$ derivatives in HPLC. We elucidated up to now unsuccessful chiral separations by either the strong ionic interactions with positively charged metal impurities located on the surface of silica chromatographic support or repulsive interactions of these species with deprotonated silanols. All the negative effects of these achiral interactions were solved by adding counter-ions to the mobile phase or by adding a chelating agent (Na_2EDTA) to the mobile phase. As a result, anionic *nido*-[7,8-$C_2B_9H_{12}$]$^-$ derivatives were successfully separated on the bromated β-cyclodextrin-based CSP in HPLC. In contrast, zwitterionic *nido*-[7,8-$C_2B_9H_{12}$]$^-$ derivatives did not suffer from deteriorative achiral interactions and were enantioseparated on amylose *tris*(3,5-dimethylphenylcarbamate) in RPLC and SFC and on 2-hydroxypropyl-β-cyclodextrin bonded to silica SPP in RPLC.

Furthermore, the series of oxygen-bridged [$Co(C_2B_9H_{11})_2$]$^-$ derivatives were enantioseparated in RPLC using native β-cyclodextrin-based CSP. The panel of distinct derivatives of bridged or unbridged anionic/zwitterionic [$Co(C_2B_9H_{11})_2$]$^-$ derivatives was chirally discriminated on polysaccharide-based columns in RPLC and SFC. The judgement on which technique is more suitable for chiral separations of investigated compounds is ambiguous due to the significant bias arising from different chemistries and dimensions of the used CSP. Rather than comparing the suitability of the two techniques, the comparison of the available methods for chiral separations of investigated species is more appropriate. Generally, available SFC methods employing polysaccharide-based columns for the zwitterionic and anionic [$Co(C_2B_9H_{11})_2$]$^-$ and zwitterionic *nido*-[7,8-$C_2B_9H_{12}$]$^-$ derivatives prevail over methods in RPLC. On the contrary, RPLC using native β-cyclodextrin is the method of choice for chiral separations of anionic *nido*-[7,8-$C_2B_9H_{12}$]$^-$ derivatives. Moreover, RPLC employing 2-hydroxypropyl-β-cyclodextrin immobilized on silica SPP can be used as an alternative to SFC for fast enantioseparations of dihydroxyalkyl-, oxygen bridged hydroxyalkyl-, and bisphenylene bridged derivatives of [$Co(C_2B_9H_{11})_2$]$^-$, and structurally related compounds.

Regarding the potential use of [$Co(C_2B_9H_{11})_2$]$^-$ and *nido*-[7,8-$C_2B_9H_{12}$]$^-$ derivatives in medicinal chemistry, the results of this work are the basis for the chiral separations on a

semipreparative scale, for the control of enantiomeric purity, and for the development of required bioanalytical methods. The chiral separation methods developed in the dissertation will be employed to confirm the chirality of newly synthesized compounds and converted to a preparative scale for the isolation of pure enantiomers. Subsequently, synthesis of enantiomerically pure materials with different chiroptical properties compared to racemic materials can be carried out.

Moreover, the availability of methods for enantioseparations of *nido*-[7,8-$C_2B_9H_{12}$]$^-$ ions and [Co($C_2B_9H_{11}$)$_2$]$^-$ derivatives opens the door to investigate the impact of their chirality in other branches of science, e.g., preparation of enantioselective catalysts, chiral selectors, enantioselective electrodes, and supramolecular chemistry, where the potential of enantiopure compounds has not been investigated yet.

6 List of Publications Included in the Dissertation

1) **Horáček O.**, Papajová-Janetková M., Grüner B., Lochman L., Štěrbová-Kovaříková P., Vespalec R., Kučera R. The first chiral HPLC separation of dicarba-nido-undecarborate anions and their chromatographic behavior. *Talanta*. 2021; 222: 121652. (**IF_{2021}: 6.556, Q1; AIS_{2021}: 0.765, Q1**)

   Author's contribution: investigation, data curation, methodology, writing original draft and editing, visualization.

2) El Anwar S., Pazderová L., Bavol D., Bakardjiev M., Ruzickova Z., **Horáček O.**, Fojt L., Kucera R., Gruner B. Structurally rigidified cobalt bis(dicarbollide) derivatives, a chiral platform for labelling of biomolecules and new materials. *Chemical Communications*. 2022; 58: 2572–2575. (**IF_{2022}: 4.9, Q2; AIS_{2022}: 1.031, Q2**)

   Author's contribution: investigation – HPLC chiral separations of oxygen-bridged cobalt bis(dicarbollide) derivatives, data curation, writing the analytical part, visualization.

3) **Horáček O.**, Marvalová J., Štilcová K., Holub J., Grüner B., Kučera R. Reversed-phase chromatography as an effective tool for the chiral separation of anionic and zwitterionic carboranes using polysaccharide-based chiral selectors. *Journal of Chromatography A*. 2022; 1672 : 463051. (**IF_{2022}: 4.1, Q2; AIS_{2022}: 0.553, Q2**)

   Author's contribution: investigation, data curation, conceptualization, methodology, funding acquisition, writing original draft and editing, visualization.

4) **Horáček O.**, Nováková L., Tüzün E., Grüner B., Švec F., Kučera R. Advanced Tool for Chiral Separations of Anionic and Zwitterionic (Metalla)carboranes: Supercritical Fluid Chromatography. *Analytical Chemistry*. 2022; 94: 17551-17558. (**IF_{2022}: 7.4, D1; AIS_{2022}: 1.303, Q1**)

   Author's contribution: investigation, data curation, conceptualization, methodology, writing original draft and editing, visualization.

5) **Horáček O.,** Dhaubhadel U., Holub J., Grüner B., Arstrong D.W., Kučera R. Employment of chiral columns with superficially porous particles in chiral separations of cobalt bis(dicarbollide) and nido-7,8-$C_2B_9H_{12}$(1-) derivatives. *Chirality*. https://doi.org/10.1002/chir.23606 [published online]. (**IF_{2022}: 2.0, Q3; AIS_{2022}: 0.354, Q3)**

   Author's contribution: investigation, data curation, conceptualization, methodology, writing original draft and editing, visualization.

Note: The quartiles of Impact Factor (IF) and Article Influence Score (AIS) are stated for the category CHEMISTRY, ANALYTICAL in case of Talanta, Analytical Chemistry, Journal of Chroamotgraphy A, and Chirality and for the category CHEMISTRY, INTERDISCIPLINARY in case of Chemical Communications.

7 Other Publications Not Included in the Dissertation

1) Juhás M., Kučerová M., **Horáček O.**, Janďourek O., Kubíček V., Konečná K., Kučera R., Bárta P., Janoušek J., Paterová P., Kuneš J., Doležal M., Zitko J. N-Pyrazinoyl Substituted Amino Acids as Potential Antimycobacterial Agents-The Synthesis and Biological Evaluation of Enantiomers. *Molecules.* 2020; 25(7): 1-29. (**IF_{2020}: 4.412, Q2; AIS_{2020}: 0.694, Q2**)

Author's contribution: investigation – HPLC chiral separations of N-pyrazinoyl substituted amino acids, data curation, writing the analytical part, visualization.

2) **Horáček O.**, Portillo A.E., Dhaubhadel U., Sung Y., Readel E.R., Kučera R., Armstrong D.W. Comprehensive chiral GC-MS/MS and LC-MS/MS methods for identification and determination of N-acyl homoserine lactones. *Talanta*. 2023; 253: 123957. (**IF_{2022}: 6.1, Q1; AIS_{2022}: 0.793, Q1**)

Author's contribution: investigation, data curation, writing original draft and editing, visualization.

3) Portillo A.E., Dhaubhadel U., **Horáček O.**, Sung Y., Armstrong D.W. Investigating chirality in quorum sensing by analysis of Burkholderia cepacia and Vibrio fischeri with comprehensive chiral LC-MS/MS and GC-MS/MS methods. *FEMS Microbiology Letters*. 2023; 370: fnad011. (**IF_{2022}: 2.1, Q4; AIS_{2022}: 0.582, Q3**)

Author's contribution: investigation, data curation, original draft editing.

4) Dhaubhadel U., Portillo A.E., **Horáček O.**, Sung Y., Armstrong D.W. Unusual enantiomeric D,L-N-acyl homoserine lactones in Pectobacterium atrosepticum and Pseudomonas aeruginosa *PLoS ONE*. 2023; 18(3): 1. (**IF_{2022}: 3.7, Q2; AIS_{2022}: 0.944, Q2**)

Author's contribution: investigation, data curation, original draft editing.

5) Khalikova M., Jireš J., **Horáček O.**, Douša M., Kučera R., Nováková L. What is the role of current mass spectrometryin pharmaceutical analysis? *Mass Spectrometry Reviews.* 2023; : 1-50. (**IF_{2022}: 6.6, D1; AIS_{2022}: 1.862, Q1**)

Author's contribution: writing (sections: 2.2 MS in Pharmacopoeia 2.5 The role of MS in determination of pharmaceuticals in biological fluids) and editing original draft, proofreading

6) Plachká K., Pilařová V., Horáček O., Gazárková T., Kočová Vlčková H., Kučera R., Nováková L. Columns in analytical-scale supercritical fluid chromatography: From traditional to unconventional chemistries. *Journal of Separation Science* (Accepted for Publication). (**IF_{2022}: 3.1, Q2; AIS_{2022}: 0.371, Q3**)

   Author's contribution: comprehensive literature search, writing (section: 3. Chiral stationary phases in SFC) and editing original draft, proofreading

Note: The quartiles of Impact Factor (IF) and Article Influence Score (AIS) are stated for the category CHEMISTRY, ANALYTICAL in case of Talanta and Journal of Separation Science; for the category SPECTROSCOPY in case of Mass Spectrometry Reviews; for the category CHEMISTRY, INTERDISCIPLINARY in case of Molecules; for the category MULTIDISCIPLINARY SCIENCES in case of PLoS One; and for the category MICROBIOLOGY in case of FEMS Microbiology Letters.

8 Presentation of Results at Conferences

8.1 Oral Presentations

2023 "Comprehensive chiral GC-MS/MS and LC-MS/MS methods for identification and determination of N-acyl homoserine lactones" presented at 13th Postgraduate and Postdoc Conference at Charles University, Faculty of Pharmacy.

2021 "Enantioseparations of carboranes using chromatographic and electrophoretic techniques" Presented at the 5th STARSS Conference on Separation Science.

2021 "Chiral separations of dicarba-7,8,-nido-undecaborane and cobalt bis(dicarbollide) derivatives" Presented at the 49th "Conference Synthesis and Analysis of Drugs 2021.

2021 "Chiral separations of carboranes in supercritical fluid chromatography" Presented at the 8th Česká chromatografická škola - HPLC.cz 2021.

2021 "Chiral separations of boron cluster compounds in SFC" Presented on-line at the 11th Postgraduate and Postdoc Conference at Charles University, Faculty of Pharmacy.

2020 "Analytical study of the influence of experimental conditions on the chiral separation of boron cluster compounds" Presented at the 10th Postgraduate and Postdoc Conference at Charles University, Faculty of Pharmacy.

8.2 Poster Presentations

2023 "The Chiral Separations of Exotic Bioisosteres of Phenyl Ring: *nido*-$[7,8\text{-}C_2B_9H_{12}]^-$ and $[3,3'\text{-}Co(1,2\text{-}C_2B_9H_{12})]^{-2}$ Derivatives" presented at HPLC 2023.

2022 "Comprehensive chiral GC-MS/MS and LC-MS/MS methods for identification and determination of N-acyl homoserine lactones" presented at Chirality 2022.

2022 "Enantioseparations of cobalt bis(dicarbollide) derivatives in HPLC using chiral columns with superficially porous particles" presented at Chirality 2022.

2022 "Chiral separations of cobalt bis(dicarbollide) derivatives in supercritical fluid chromatography" presented at HPLC 2022.

9 Received Awards

2023 13th Postgraduate and Postdoc Conference at Charles University, Faculty of Pharmacy – **the award of František Švec for the best oral presentation in the section of Pharmaceutical Analysis.**

2023 Grant Agency of Charles University (168120): „ Analytical study of the influence of experimental conditions on the chiral separation of boron cluster compounds “

1) **The best project from section Přírodní vědy - Chemie (CH)**
2) **Honorary mention of the President of the Charles University for the outstanding scientific result**

2021 Conference Synthesis and Analysis of Drugs 2021 – **the 3rd place in the competition for the best presentation.**

2021 11th Postgraduate and Postdoc Conference at Charles University (on-line), Faculty of Pharmacy – **the award for the best oral presentation in the section of Pharmaceutical Analysis and Bioanalysis.**

10 Addressed Grants

10.1 Principal investigator

2020–2023 – Grant Agency of Charles University (168120): „ Analytical study of the influence of experimental conditions on the chiral separation of boron cluster compounds “.

10.2 Member of the research team

2021–**2023 –** Specialized Team for Advanced Research on Separation Science (CZ.02.1.01/0.0/0.0/15_003/0000465): „Expert in supercritical fluid chromatography and capillary electrophoresis “.

11 Internships and other professional experience

- **27. 12. 2021–23. 7. 2020** - seven-month internship at Department of Chemistry and Biochemistry, University of Texas at Arlington, United States of America

  Supervisor: prof. Daniel W. Armstrong

  Content of the internship:

  - Chiral separations of cobalt bis(dicarbollide) and *nido*-7,8-dicarbaundecaborate(1-) derivatives on chiral stationary phases immobilized on superficially porous particles
  - Development of comprehensive chiral methods using GC-MS/MS and LC-MS/MS for identification and quantitation of N-acyl homoserine lactones in bacterial media.

- **Chairperson** of the section Selective separations on the conference HPLC 2022 in San Diego
- **Member** of the Czech Pharmaceutical Society, Section of Pharmaceutical Control and Bioanalytics, 2020-present

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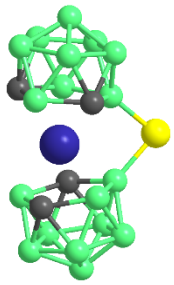
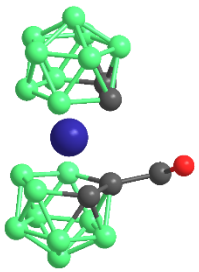
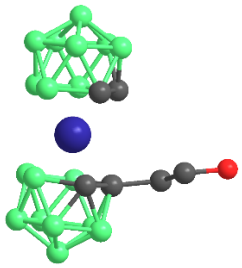
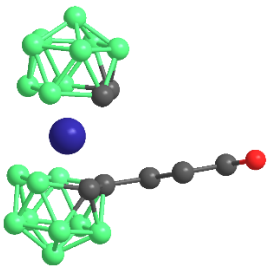
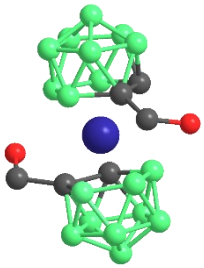
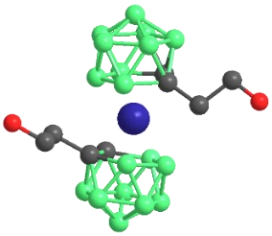
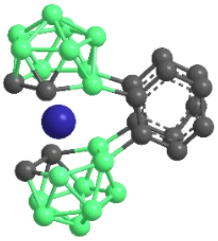
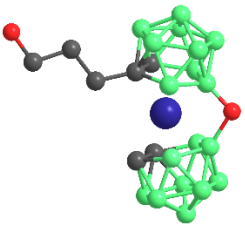
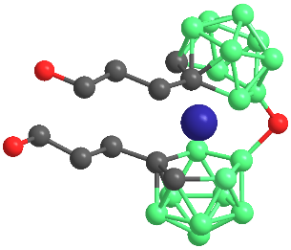
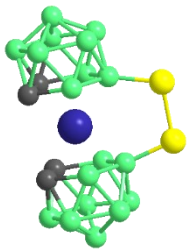
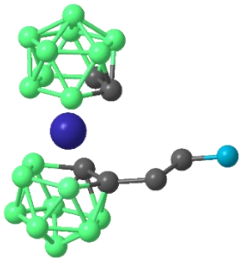
Attachements

Attachment 1

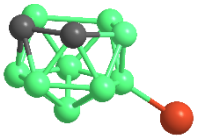
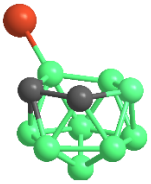
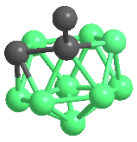
The table of the compounds analyzed in the dissertation thesis.

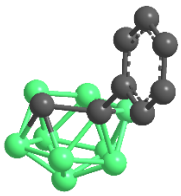
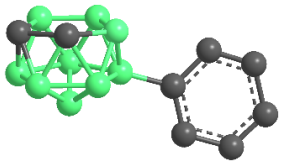
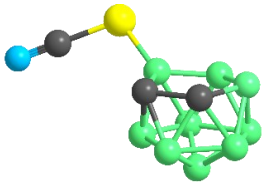
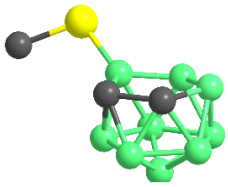
Anionic $[Co(C_2B_9H_{11})_2]^-$ derivatives		
Compound number/ reference to synthesis	**The formula of the compound**	**Model of the cluster without cation**
A 1 [1]	[6,6′-μ-S-(1,7-$C_2B_9H_{10})_2$-2,2′-Co]Cs	
A 2 [2]	[1-($HOCH_2$)-Co(1,2-$C_2B_9H_{10})_2$] [$(CH_3)_3NH$]	
A 3 [2]	[1-(HOC_2H_4)-Co(1,2-$C_2B_9H_{10})_2$] [$(CH_3)_3NH$]	
A 4 [2]	[1-(HOC_3H_6)-Co(1,2-$C_2B_9H_{10})_2$][$(CH_3)_3NH$]	

Compound number/ reference to synthesis	The formula of the compound	Model of the cluster without cation
A 5 [2]	[1,1'-$(HOCH_2)_2$-Co(1,2-$C_2B_9H_{10})_2$][$(CH_3)_3NH$]	
A 6 [2]	[1,1'-$(HOC_2H_4)_2$-Co(1,2-$C_2B_9H_{10})_2$][$(CH_3)_3NH$]	
A 7 [3]	[8,4',8',4-μ-bis$(C_6H_4)_2$-Co(1,2-$C_2B_9H_{10})_2$]Na	
A 8 [4]	[1-(HOC_3H_6)-8,8′-μ-O-Co(1,2-$C_2B_9H_{10})_2$][$(CH_3)_3NH$]	
A 9 [4]	[1,1′-$(HOC_3H_6)_2$-8,8′-μ-O-Co(1,2-$C_2B_9H_{10})_2$][$(CH_3)_3NH$]	

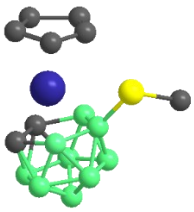
Compound number/ reference to synthesis	The formula of the compound	Model of the cluster without cation
A 10 [5]	[8,8′-μ-S_2-Co(1,2-$C_2B_9H_{10})_2$]Cs	
A 11 [6]	[1-($H_2NC_2H_4)_2$-Co(1,2-$C_2B_9H_{10})_2$] [$(CH_3)_4N$]	

Anionic *nido*-[7,8-$C_2B_9H_{12}]^-$ derivatives

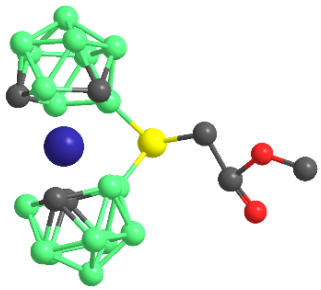
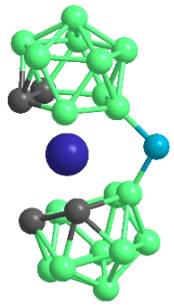
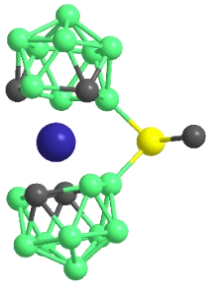
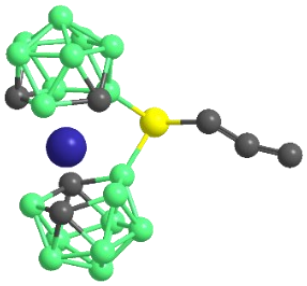
Compound number/ reference to synthesis	The formula of the compound	Model of the cluster without cation
A 12 [7]	[5-Br-7,8-*nido*-$C_2B_9H_{11}$] Cs	
A 13 [8]	[9-Br-7,8-*nido*-$C_2B_9H_{11}$] [$(CH_3)_4N$]	
A 14 [9]	[7-CH_3-7,8-*nido*-$C_2B_9H_{11}$] Cs	

Compound number/ reference to synthesis	The formula of the compound	Model of the cluster without cation
A 15 [10]	[7-C_6H_5-7,8-*nido*-$C_2B_9H_{11}$] [$(CH_3)_4N$]	
A 16 [10]	[5- C_6H_5-7,8-*nido*-$C_2B_9H_{11}$] Cs	
A 17 [11]	[9-NCS-7,8-*nido*-$C_2B_9H_{11}$] Cs	
A 18 [12]	[9-CH_3S-7,8-*nido*-$C_2B_9H_{11}$] Cs	

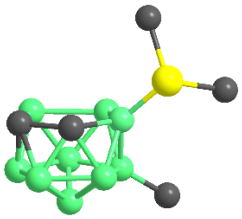
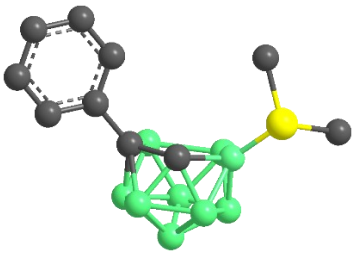
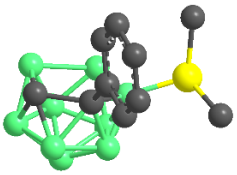
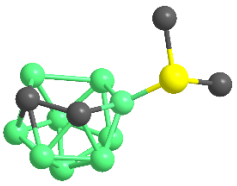
Neutral mixed sandwich 4-MeS-3-C_5H_5-Co-(1,2-$C_2B_9H_{10}$)

Abbreviation/ reference to synthesis	The formula of the compound	Model of the cluster without cation
N 1 [13]	4-CH_3S-3-C_5H_5-Co-(1,2-$C_2B_9H_{10}$)	

Zwitterionic $[Co(C_2B_9H_{11})_2]^-$ derivatives

Abbreviation/ reference to synthesis	The formula of the compound	Model of the cluster without cation
ZW 1 [3]	6,6'-μ-(CH_3OCOCH_2)S-Co(1,2-$C_2B_9H_{10}$)$_2$	
ZW 2 [14]	4,8'-μ-NH_2-Co(1,2-$C_2B_9H_{10}$)$_2$	
ZW 3 [3]	6,6'-μ-(CH_3)S-Co(1,2-$C_2B_9H_{10}$)$_2$	
ZW 4 [3]	6,6'-μ-(C_3H_5)S-Co(1,2-$C_2B_9H_{10}$)$_2$	

Zwitterionic *nido*-[7,8-$C_2B_9H_{12}$]$^-$ derivatives

Abbreviation/ reference to synthesis	The formula of the compound	Model of the cluster without cation
ZW 5 [15]	5-CH_3-11-$(CH_3)_2$S-7,8-*nido*-$C_2B_9H_{11}$	
ZW 6 [15, 16]	7- C_6H_5-9-$(CH_3)_2$S-7,8-*nido*-$C_2B_9H_{11}$	
ZW 7 [15, 16]	7-C_6H_5-11-$(CH_3)_2$S-7,8-*nido*-$C_2B_9H_{11}$	
ZW 8 [15]	9-$(CH_3)_2$S-7,8-*nido*-$C_2B_9H_{11}$	

Color coding of the atoms: boron (green), carbon (dark grey), sulphur (yellow), oxygen (red), cobalt (dark blue), nitrogren (light blue), bromie (orange). Hydrogen atoms are omitted for clarity.

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Attachment 2

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Attachment 3

El Anwar S., Pazderová L., Bavol D., Bakardjiev M., Ruzickova Z., **Horáček O.**, Fojt L., Kucera R., Gruner B. Structurally rigidified cobalt bis(dicarbollide) derivatives, a chiral platform for labelling of biomolecules and new materials. *Chemical Communications*. 2022; 58: 2572–2575. (IF_{2022}: 4.9, Q2; AIS_{2022}: 1.031, Q2)

Attachment 4

Horáček O., Marvalová J., Štilcová K., Holub J., Grüner B., Kučera R. Reversed-phase chromatography as an effective tool for the chiral separation of anionic and zwitterionic carboranes using polysaccharide-based chiral selectors. *Journal of Chromatography A*. 2022; 1672 : 463051. (**IF_{2022}: 4.1, Q2; AIS_{2022}: 0.553, Q2**)

Attachment 5

Horáček O., Nováková L., Tüzün E., Grüner B., Švec F., Kučera R. Advanced Tool for Chiral Separations of Anionic and Zwitterionic (Metalla)carboranes: Supercritical Fluid Chromatography. *Analytical Chemistry*. 2022; 94: 17551-17558. (**IF_{2022}: 7.4, D1; AIS_{2022}: 1.303, Q1**)

Attachment 6

Horáček O., Dhaubhadel U., Holub J., Grüner B., Arstrong D.W., Kučera R. Employment of chiral columns with superficially porous particles in chiral separations of cobalt bis(dicarbollide) and nido-7,8-$C_2B_9H_{12}$(1-) derivatives. *Chirality*. https://doi.org/10.1002/chir.23606 [published online]. (**IF_{2022}: 2.0, Q3; AIS_{2022}: 0.354, Q3)**