ELSEVIER

Journal of Chromatography A, 789 (1997) 497–517

JOURNAL OF CHROMATOGRAPHY A

Review

High-performance liquid chromatographic separations of boron-cluster compounds

Bohumir Grüner*, Zbyněk Plzák

Institute of Inorganic Chemistry, Academy of Sciences of the Czech Republic, 25068 Řež, Czech Republic

Abstract

A wide range of chromatographic approaches in high-performance liquid chromatography (HPLC) is available for the efficient separation and determination of different families of boron-cluster compounds. Liquid–solid, reversed-phase, ion-pair, ion and chiral liquid column separation techniques find application in boron-cluster chemistry. HPLC separations have proved to be a valuable tool for the advance of synthetic boron-cluster chemistry over the last several decades.

Keywords: Reviews; Boron clusters; Boranes; Heteroboranes; Carboranes; Metal complexes

Contents

1. Introduction

Based on the pioneering work of Alfred Stock in the first half of this century, boron-cluster chemistry has grown from several laboratory and academic curiosities to the huge family of compounds that now have its own chapter in every standard textbook on inorganic chemistry. Boron-cluster compounds repre-

*Corresponding author.

0021-9673/97/$17.00
PII S0021-9673(97)00497-4

sent a wide range of distinctive covalent species with unique molecular architecture, non-conventional cluster bonding and unusual chemical properties. The enormous scope and diversity of boron-cluster chemistry are represented by covalent polyhedral species of exceptional stability, air and moisture sensitive (even pyrophoric) open cage deltahedral boron hydride species, heteroboranes containing main-group elements in the molecular skeleton, polyhedral ions with unique hydrophobicity and acidity, metallaborane complexes and cluster boron species exhibiting chiral properties. A survey of recent developments in the field was presented recently in a series of articles in a thematic issue of *Chemical Reviews* [1–5].

The unique properties of boron-cage compounds stem from boron's electron deficiency and are demonstrated by its ability to form polyhedral skeletons composed of triangular boron facets. All electron-deficient neutral borane and carborane compounds are related to four general formulae, *closo*-$C_{0\ to\ 2}B_nH_{n+2}$, *nido*-$C_{0\ to\ 4}B_nH_{n+4}$, *arachno*-$C_{0\ to\ 6}B_nH_{n+6}$ and *hypho*-$C_{0\ to\ 8}B_nH_{n+8}$ (the subscript *n* refers to the number of boron atoms).

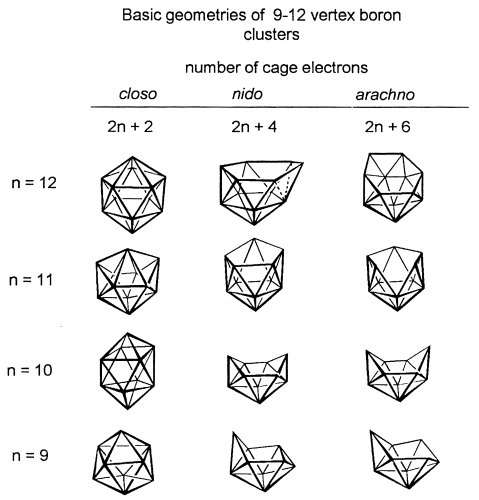
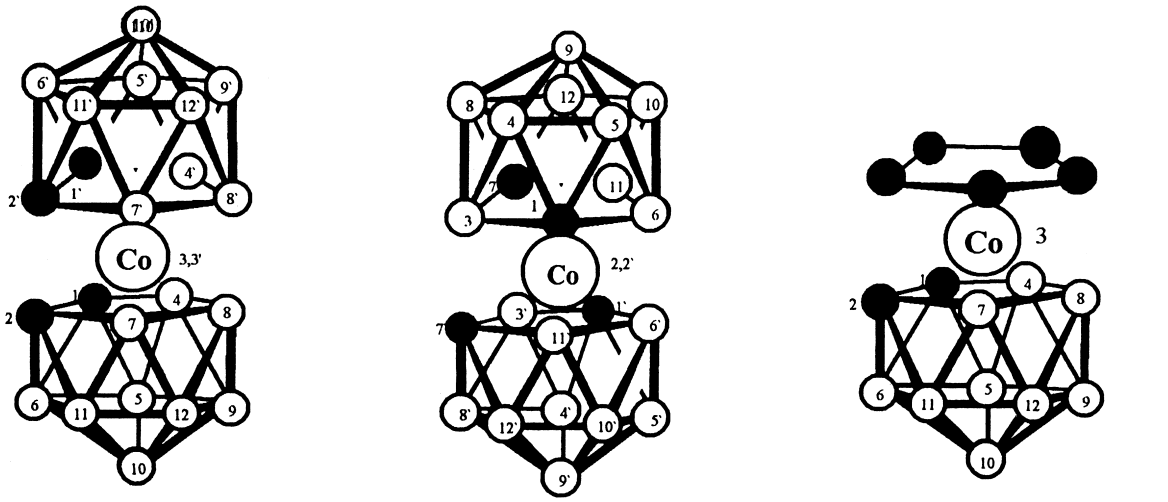


Fig. 1. Terminal and bridge hydrogen atoms are omitted.

The abstraction of one or two protons from the four general formulae produces anions and dianions. Heteroboranes, in a manner similar to carboranes, are compounds, the formulae of which can be derived by formally replacing one or more cage boron atoms by



$[(1,2\text{-}C_2B_9H_{11})_2\text{-}3\text{-}Co]^-$ $[(1,7\text{-}C_2B_9H_{11})\text{-}2\text{-}Co]^-$ $[3\text{-}C_5H_5\text{-}1,2,3\text{-}C_2CoB_9H_{10}]$

Fig. 2. Schematic structures of some sandwich type cobaltacarborane compounds. (Terminal hydrogen atoms are omitted for clarity).

other main group elements. The positions with the lowest connectivities in the skeleton are usually occupied by these heteroatoms. Many transition metals, as well as several main-group elements, form complexes with carborane ligands. The scheme in Fig. 1 exemplifies the basic geometries of medium-sized boron-clusters having nine–twelve skeletal atoms, which represent the usual application area of high-performance liquid chromatographic (HPLC) analysis in boron-cluster chemistry. In Fig. 2, an example of the structures of sandwich cobalt complexes with carborane ligands is shown.

In spite of a great amount of information being available, attempts to predict the chemical behavior of the boron-cluster compounds with sufficient reliability or to carry out controlled syntheses of desired compounds rarely meet with success. Many closely related compounds are typically obtained in reaction steps, which differ in a very subtle way. A fast and efficient analytical separation method to assay for purity, for reaction monitoring and for isolation of reaction products is thus required. In this respect, chromatographic separation techniques play a dominant role to fulfil these requirements in the field of boron-cluster chemistry. The use of classical low-pressure liquid column chromatography [6], dry column chromatography [7], thin-layer chromatography (TLC) [8] and gas chromatography [9] has been reviewed. This review is an attempt to summarize applications of high-performance liquid column chromatography from literature data and from experience gained in our laboratory for the separation, isolation and determination of boron-cluster compounds. The wide range of HPLC methods that are used corresponds to concurrent advances in HPLC separation technology and detection methods. These two aspects were used in this review to sort out developments in HPLC for both neutral and ionic boron-cluster compounds.

2. Separation of neutral solutes

Before 1975, only three liquid chromatographic studies in the area of boron-cluster compounds were reported, comprising those of Kindsvater et al. [10] on the reversed-phase separation of a series of 1,2-dicarba-*closo*-dodecaborane derivatives, Evans and Hawthorne [11], who resolved cobalt metallaboranes and Čoupek et al. [12], who investigated the separation of 1,2-dicarba-*closo*-dodecaborane derivatives using a styrene–divinylbenzene gel as the adsorbent. With the routine use of microparticulate-packed columns in HPLC, the experience gained so far in the analysis of organic compounds has spread into the field of boron-cluster compounds.

2.1. Liquid–solid systems

The first true HPLC study using microparticles of diameter <15 μm was published by our laboratory in 1978 [13]. The separation of about twenty higher boranes, heteroboranes and their derivatives was carried out on silica gel using *n*-heptane and methylene chloride as mobile phases, as illustrated in Table 1. The short time of analysis obtained proved to be a great advantage of HPLC, because the reactivity of hydride compounds with water, some solvents and oxygen was problematic. Hydrogen evolution and destruction of the adsorbent column, a phenomenon common in the application of classical liquid chromatography for the separation of these reactive species, can be suppressed. Separation of isomeric decaborane derivatives was thus achieved for the first time in this study.

It is difficult to predict the retention of borane compounds in liquid–solid chromatography systems. Due to high values of permanent dipole moment for some boron-cluster compounds, it was found in some cases that it is the dipole moment that determines the retention behavior of these compounds [12]. However, TLC can be used to obtain information on the retention of boron-cluster compounds and to provide information that enables one to choose a suitable solvent for the liquid–solid system (Tables 1 and 2).

In liquid–solid chromatography, water is usually added to the adsorbent in order to optimize the linear capacity and column efficiency. The capacity factors (k') are strongly affected by changing the water concentration in a solvent. To achieve reproducible separations, the amount of water in the stationary and mobile phases must be carefully controlled. Another type of polar compound, such as lower alcohols, e.g. isopropanol, can be used as a modifier instead of water in non-polar mobile phases. As all of these modifiers react with most of the *nido*-borane

Table 1
Chromatographic data for some heteroboranes [13]

Compound	k' (HPLC)[a]			R_F (TLC)[b]
	System I	System II	System III	
5,6-$C_2B_8H_{12}$	1.2	1.1		0.42[a]
6,8-$C_2B_7H_{13}$		5.6		0.16[a]
5-(3-F-$C_6H_4CH_2$)$B_{10}H_{13}$	19.9	1.9		0.09[a]
6-(3-F-$C_6H_4CH_2$)$B_{10}H_{13}$	25.0	2.3		0.09[a]
6,9-$C_2B_8H_{14}$		5.5		0.10[a]
6,8-SCB_7H_{11}	1.2	1.0		0.42[a]
6,8-$S_2B_7H_9$	0.3			0.71[a]
1-$SB_{11}H_{11}$	0.7			0.56[a]
1,6-(Me_2S)$_2B_{10}H_8$			2.8	0.34[b]
1,10-(Me_2S)$_2B_{10}H_8$			0.6	0.48[b]
5-Me_2S-7,8-$C_2B_9H_{11}$			6.1	0.41[c]
10-Me_2S-7,8-$C_2B_9H_{11}$			12.9	0.30[c]
9-SH-1,2-$C_2B_{10}H_{11}$			3.6	0.45[c]
4-Me_2S-B_9H_{13}			6.6	0.37[c]

[a]HPLC system I: Column 300×3.3 mm; sorbent, silica gel 13 μm; eluent, *n*-heptane; flow-rate, 0.89 ml/min; detection, UV at 254 nm (except for 1-$SB_{11}H_{11}$, refractometric detection). System II: eluent, *n*-heptane+0.4% acetonitrile; other parameters as in system I. System III: eluent, 79.6% *n*-heptane, 20% dichloromethane, 0.4% acetonitrile; flow-rate, 1.3 ml/min; other parameters as in system I.
[b]TLC: Silufol plates developed with:[a]*n*-hexane; [b]dichloromethane; [c]benzene; detection with iodine vapour and spraying with $AgNO_3$ solution.

compounds, acetonitrile at a concentration of 0.1–0.4% was found to be a convenient substitute to the modifiers mentioned above. In agreement with the results of Sauders [14], acetonitrile exhibited equal or better properties than water as far as sample capacity, plate height, convenience and equilibration time are concerned, when hexane is used as the mobile phase for the separation of reactive *nido*-boranes.

Chromatographic conditions for the separation of

Table 2
Chromatographic data for some metallacarboranes [15]

Compound	k' (HPLC)[a]				R_F (TLC)[b]
	System I	System II	System III	System IV	
1-C_5H_5Co-2,3-$C_2B_9H_{11}$	11.4	4.4	2.0	1.3	0.28
1-Et-C_5H_4Co-2,3-$C_2B_9H_{11}$	4.4	1.9	0.9		0.35
1-C_5H_5Co-2,3-$C_2B_9H_{10}$-8-F		9.0	3.9	2.2	0.22
1-C_5H_5-Co-2,3-$C_2B_9H_{10}$-8-Cl			4.6	2.7	0.16
1-C_5H_5-Co-2,3-$C_2B_9H_{10}$-8-Br		5.2		0.16	
1-C_5H_5-Co-2,3-$C_2B_9H_{10}$-8-I		5.4	3.4	0.17	
1-C_5H_5-Co-2,4-$C_2B_9H_{11}$	3.1	1.5			0.35
1-C_5H_5-Co-2,8-$C_2B_9H_{11}$	0.5	0.2		0.51	
1-C_5H_5Co-2-$SB_{10}H_{10}$	3.5	1.5		0.37	
1-C_5H_5-Fe-2,3-$C_2B_9H_{11}$	11.1		1.5		0.24
3,6-(C_5H_5-Co)$_2$-1,2-$C_2B_8H_{10}$			2.8	1.7	0.13
2,4-(C_5H_5Co)$_2$-1-SB_9H_9			5.0	3.0	0.12

[a]HPLC system I: Column 300×3.3 mm; sorbent, silica gel 13 μm; guard column, 60×3 mm packed with Corasil II; eluent, 89.8% *n*-heptane–10% dichloromethane–0.2% isopropanol; flow-rate, 1.28 ml/min; detection, UV at 254 nm. System II: eluent, 79.8% *n*-heptane–20% dichloromethane–0.2% isopropanol; other parameters as in system I. System III: eluent, 69.8% *n*-heptane–30% dichloromethane–0.2% isopropanol; other parameters as in system I. System IV: eluent, 59.8% *n*-heptane–40% dichloromethane–0.2% isopropanol; other parameters as in system I.
[b]TLC: Silufol plates developed with *n*-heptane–dichloromethane (1:1, v/v); visual detection after exposure with iodine vapour.

22 neutral metallaborane compounds were the subject of another study [15]. Mixtures of heptane, dichloromethane and isopropanol were used as a solvent system (Table 2). Again, TLC was used as a pilot technique to predict the retention behavior of compounds separated by HPLC. Using a relationship proposed by Soczewiński and Gołkiewicz [16], a good linear relationship of log k' versus R_M TLC values was obtained.

Liquid–solid chromatography on microparticular highly efficient silica gel columns is now used as a routine indispensable tool for the analysis of mixtures of positional isomers and bipolar carborane and metallaborane derivatives (Fig. 3).

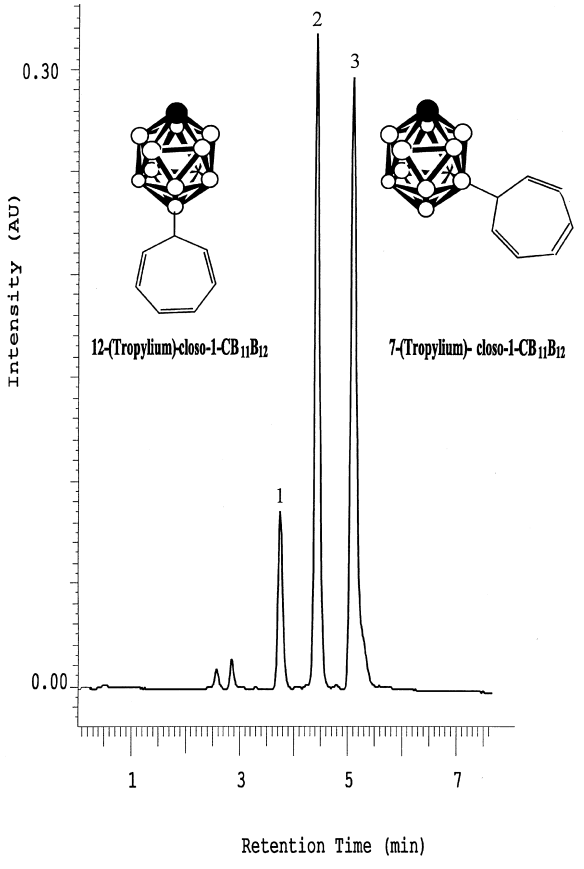


Fig. 3. Example of the routine analytical separation of a mixture of positional isomers by solid–liquid chromatography on a silica gel support. Column, Separon SGX (7 μm) glass cartridge column (300×3 mm I.D.); mobile phase, hexane–dichloromethane (25:75, v/v); flow-rate, 0.8 ml/min; detection, diode array at 210–300 nm, fixed wavelength at 260 nm. Sample, a liquid chromatographic fraction enriched with a more retained isomer. Peaks: 1=organic impurity; 2=12-tropylium-*closo*-$CB_{11}H_{11}$; 3=7-tropylium-*closo*-$CB_{11}H_{11}$. (For synthesis see [47]).

Of adsorbents other then silica, styrene–divinylbenzene gel was used for the separation of 1,2-dicarba-*closo*-dodecaborane derivatives [12] and carbon adsorbent (prepared by reduction of polytetrafluoroethylene) and it exhibited excellent separation of dicarba-*closo*-dodecaborane isomers [17]. These sorbents, namely carbon, exhibit lower reactivity towards boron species, but they never have found routine application in the HPLC separation of boron-cluster compounds, due to their commercial unavailability in the form of packed HPLC columns.

2.2. Reversed-phase systems

Reversed-phase liquid chromatography has been recognized as a dominant technique for the analyses of neutral organic compounds. In contrast, the use of reversed-phase chromatography for the analysis of neutral boron cage compounds is restricted by their reactivity towards water and protic solvents. A reversed-phase system was used to separate dicarba-*closo*-dodecaborane derivatives [12] that exhibit sufficient stability in this media. However, reversed-phase separation with counter-ions present in the mobile phase has received considerable research attention in recent years for the separation of borane cage anionic species and metallaborane anions, as will be shown in the next part of this review.

3. Separation of ionic solutes

The hydroborate anions generally exhibit unusual solution behavior, which may be regarded as borderline between that of inorganic and organic compounds. They behave simultaneously as strong electrolytes (a high degree of dissociation-free acids have pK_a values that are comparable to those of the strongest inorganic acids), but they also possess hydrophobic properties that are comparable to those of organic aromatic molecules. The relatively high solubility of lithium, sodium and potassium salts of such anions in water is therefore caused by the anionic charge.

For the most monovalent carborate and metallaborate anions, e.g., derivatives of [*nido*-$C_2B_9H_{12}$]$^-$,

[*closo*-$CB_{11}H_{12}]^-$ and [*closo*-(1,2-$C_2B_9H_{11})_2$-3-Co]$^-$, hydrophobic behavior seems to prevail. For example, alkali metal salts and conjugate acids of *closo*-anions(1−) could be extracted from water into organic solvent of medium polarity, e.g. diethylether, nitrobenzene, etc. The scope of possible methods for their separation and isolation (even by conventional methods, i.e., crystallization, extraction, and conventional adsorption and partition chromatography) is wider and seems to parallel more closely the techniques applied to strong organic acids. On the other hand, the solubility of carborate of metallaborate anion salts with bulky organic ammonium cations in polar solvents is generally much lower. The last fact should be kept on the minds of chromatographers, especially from the viewpoint of ion-pair reagent selection.

The high charge of divalent *closo*-borate anions, such as [*closo*-$B_{12}H_{12}]^{2-}$ and [*closo*-$B_{10}H_{10}]^{2-}$, makes their behavior seem closer to that of the class of so-called ''solvophobic'' inorganic anions. However, a combination of this behavior with the high hydrophobicity of the skeleton makes mixtures of such compounds almost inseparable by ordinary separation methods. This behavior also leads to problems in the development of HPLC methods, especially those with a stationary phase or ion-pair reagent selection. The separation difficulties outlined above are one of the reasons for a gap in the number of communications dealing with the syntheses and properties of $[B_{10}H_{10}]^{2-}$ and $[B_{12}H_{12}]^{2-}$ derivatives from the late sixties to the middle of the eighties. Efficient separation techniques were necessary for success, especially in the analysis of typical complex reaction mixtures of these *closo*-anion derivatives. Analysis by ^{11}B NMR spectroscopy fails to give relevant information, due to extensive peak overlapping in the narrow spectral range. During the above-mentioned time period, the number of publications was almost negligible, but it has started to grow gradually over the past decade due to the availability of separation methods.

Particular effort has been spent in this area on the development of purity assays for the $Na_2[B_{12}H_{11}SH]$ derivative (BSH), which is used widely in the boron neutron capture therapy (BNCT) of brain tumors. The main goal of these methods is to separate BSH and its two most important oxidation impurities, $Na_4[B_{12}H_{11}SSB_{12}H_{11}]$ and $Na_4[B_{12}H_{11}S(O)SB_{12}H_{11}]$, and impurities from synthesis or in BSH quantitation in biological fluids and tissues.

3.1. Reversed-phase ion-pair system

An analytical HPLC method for the separation of monovalent carborate and metallaborate species was developed more than fifteen years ago. Since then, the method has been successfully applied to the routine separation of a large variety of these species in our laboratory. The original paper [18] deals with the separation of 23 derivatives of the [*nido*-7,8-$C_2B_9H_{12}]^-$, [*nido*-$CB_{10}H_{13})^-$, [*nido*-$CB_9H_{12}]^-$ anions and [*closo*-(1,2-$C_2B_9H_{11})_2M]^-$ metallaborane anions. The structures of these species are presented on the Figs. 2 and 4. The method with direct UV detection is based on reversed-phase ion-pair chromatography on C_1-bonded phases in a water–methanol mobile phase containing primary *n*-alkylamines

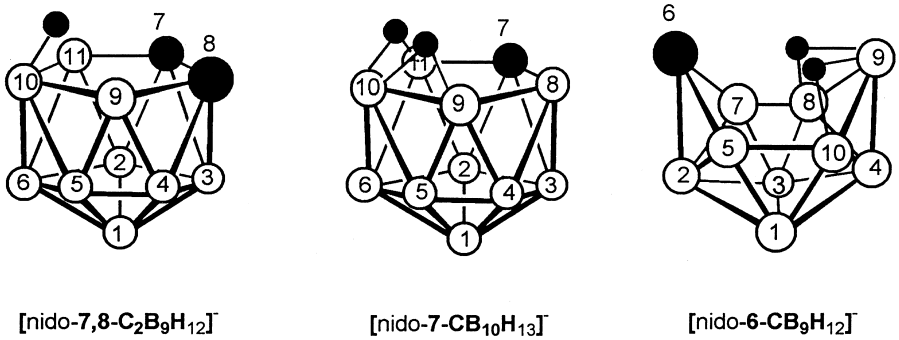


Fig. 4. Schematic structure of three eleven- and ten-vertex carborate anions [18]. (Terminal hydrogens are omitted for clarity).

with short chains, i.e., hexyl and laurylamine hydrochloride. The effects of alkyl chain length on the stationary phase and of column loading, methanol content, pH and ion-pair reagent concentration in the mobile phase, on retention and selectivity, were studied. A short chain C_1-bonded support was found to be more advantageous in this study than C_{18}-bonded phases, due to the lower methanol content required in the mobile phase and better peak shape of the strongly retained solutes. Retention data [18] obtained using this support for the above-mentioned monovalent anions with three different ion-pair systems are summarized in Table 3.

The method has proven to be a valuable tool for the analysis of sandwich cobaltacarborane [*closo*-(1,2-$C_2B_9H_{11})_2$-3-Co]$^-$ halogeno derivatives, which have found their application in the extraction of Cs^+ and Sr^{2+} in the treatment of nuclear waste [5].

A recent update of the method [19], using commercially available 7 μm C_8-bonded phases with hexylamine hydrochloride as the ion-pairing agent and acetonitrile instead of methanol, led to even better efficiencies and peak shapes. Only slightly lower acetonitrile concentrations are necessary, in comparison with methanol, to achieve similar k' values. The method allowed for a very good resolution of the series of [8,8′-μ-*R*-(1,2-$C_2B_9H_{11-n})_2$-3-Co)]$^-$ and [4,8′, 8,4′-μ-R_2-(1,2-$C_2B_9H_{11-n})_2$-3-Co)]$^-$ bridged anions, with various bridge aromatic substituents *R*, together with mono- and disubstituted cobaltacarborane anions of this type (see Fig. 5). The selectivity values for compounds differing in the

Table 3
HPLC data for some heteroborane anions [18]

Anion	Cation	k' value		
		System I	System II	System III
7,8-$C_2B_9H_{12}^-$	K^+	6.83	0.89	
7,8-$C_2B_9H_{12}^-$,	$N(CH_3)_4^+$	6.39	0.84	
7,8-$C_2B_9H_{12}^-$	Na^+		0.84	
5-SH-7,8-$C_2B_9H_{11}^-$	$N(CH_3)_4^+$		1.05	
5-iso-C_3H_7-7,8-$C_2B_9H_{11}^-$	Cs_+	16.8	1.49	
5-I-7,8-$C_2B_9H_{11}^-$	$N(CH_3)_4^+$	10.4	1.16	
5-CI-7,8-$C_2B_9H_{11}^-$	K^+		1.08	
9-CI-7,8-$C_2B_9H_{11}^-$	$N(CH_3)_4^+$		1.03	
9-I-7,8-$C_2B_9H_{11}^-$	$N(CH_3)_4^+$	13.1	1.22	
9-OH-7,8-$C_2B_9H_{11}^-$	$N(CH_3)_4^+$	12.1	1.32	
5,6-I_2-7,8-$C_2B_9H_{10}^-$	$N(CH_3)_4^+$	16.1	1.54	
5,6-CI_2-7,8-$C_2B_9H_{10}^-$	Na^+		1.11	
9,11-I_2-7,8-$C_2B_9H_{10}^-$	$N(CH_3)_4^+$		2.03	
9,11-Cl_2-7,8-$C_2B_9H_{10}^-$	$N(CH_3)_4^+$		1.22	
7,9-$C_2B_9H_{12}^-$	Cs+	7.00	0.86	
10-OH-7,9-$C_2B_9H_{11}^-$	$N(CH_3)_4^+$	2.87	0.48	
10-CH_3OH-7,9-$C_2B_9H_{11}^-$	$N(CH_3)_4^+$	6.35	0.89	
$CB_{10}H_{13}^-$	Cs^+	6.65	0.84	
$CB_9H_{12}^-$	$N(CH_3)_4^+$	6.91	0.87	
(1,2-$C_2B_9H_{11})_2Co^-$	Na^+		2.95	
(1,2-$C_2B_9H_{11})_2Co^-$ Cs^+		2.89	0.21	
(1,2-$C_2B_9H_{11})_2Fe^-$	Cs^+		4.08	
(1,2-$C_2B_9H_{11})_2Fe^-$	$N(C_4H_9)_4^+$		4.05	0.50
(1,2-$C_2B_9H_{10}I)_2Co^-$	Cs^+		22.8	1.83
(1,2-$C_2B_9H_{11})_2Ni^-$	Cs^+		4.68	
8,8′-S-(1,2-$C_2B_9H_{10})_2Co^-$	$N(CH_3)_4^+$		2.03	0.28
8,8′-C_6H_4(1,2-$C_2B_9H_{10})_2Co^-$	Cs^+		2.62	0.29

HPLC system I: Column, 150×3.3 mm; sorbent, C_1 silica gel (14.3 μm); eluent, $2.70\cdot10^{-3}$ *M* *n*-$C_{12}H_{25}NH_2$·HCl in methanol–water (6:4, v/v); flow-rate, 0.92 ml/min; detection, UV at 254 nm. System II: eluent, $2.70\cdot10_{-3}$ *M* *n*-$C_{12}H_{25}NH_2$·HCl in methanol–water (7:3, v/v); flow-rate, 1.19 ml/min; other parameters as in system I. System III: eluent, $2.70\cdot10^{-3}$ *M* *n*-$C_{12}H_{25}NH_2$·HCl in methanol–water (8:2, v/v); flow-rate, 0.93 ml/min; *n*-heptane–dichloromethane isopropanol (69.8:30:0.2, v/v/v); other parameters as in system I.

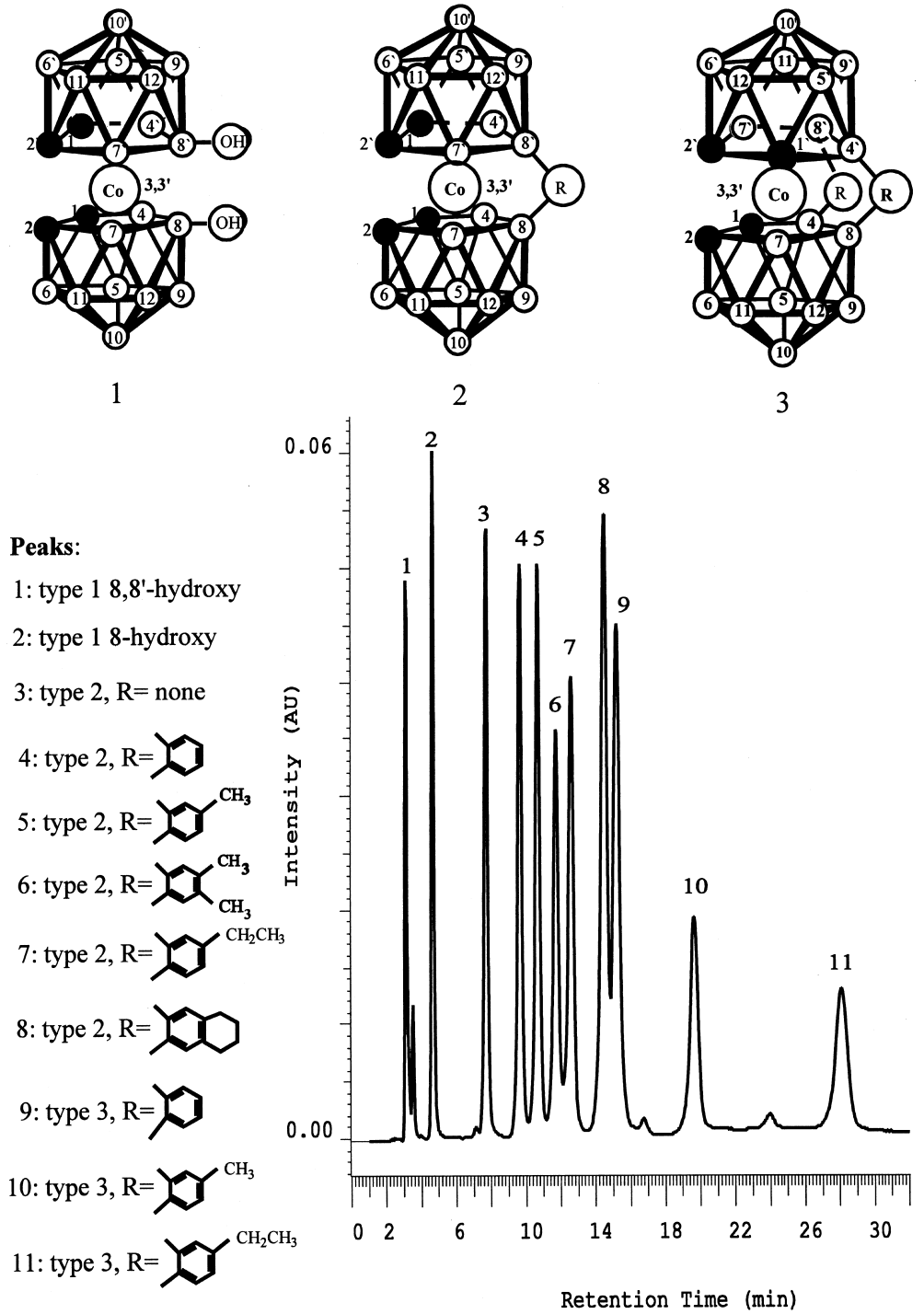


Fig. 5. Separation of sandwich complexes $(1,2\text{-}C_2B_9H_{11})_2Co^-$ bridged by various aromatic substituents. Chromatographic conditions: Column, Separon SGX octadecyl silica-bonded phase (7 μm) (250×4 mm); mobile phase, $3\cdot10^{-3}$ *M* n-$C_6H_{13}NH_2\cdot HCl$ in acetonitrile–water (57:43, v/v); flow-rate, 0.8 ml/min; detection, diode array fixed at 290 nm.

number of methyl substituents on the aromatic bridge substituent were found to be very good; lower selectivity was observed for isomeric bridge species. This trend is, however, obvious for reversed-phase methods.

A method based on ion-pair chromatography on an octadecylsilica support was reported [20] for the quantitation of BSH and its oxidation impurities. Tetrabutylammonium phosphate is used as the ion-pair reagent in a water–acetonitrile mobile phase with direct UV detection at 220 nm.

A more detailed study [21] of the influence of the organic modifier (methanol) and the ion-pair reagent [tetrabutylammonium sulfate (TBAS) or triethylamine formate (TEAF)] content in the mobile phase on the retention of BSH an its eighteen organo-substituted derivatives on a LiChrospher RP18 column has been published recently. Much lower k' values were observed for all compounds under study when TEAF was used instead of TBAS. Selection of the volatile TEAF as the ion-pair reagent along with a gradient system provided an advantage in preparative separations.

An ion-pair HPLC method [22] with UV (or fluorescent) detection and pre-column derivatization for the determination of BSH in biological fluids has been published recently. Monobromobimane, used as a pre-column derivatizing agent, was reacted with BSH prior to injection under alkaline conditions, to form a stable adduct. The derivative was separated on a Hypersil ODS column in methanolic phosphate buffer containing tetramethylammonium chloride as the ion-pair reagent. The method allowed for the specific and sensitive determination of BSH in rat plasma and urine.

3.2. Hydrophobic interaction and ion-exchange systems

The separation of monosubstituted derivatives of the *closo*-dodecahydrododecaborate anion $[B_{12}H_{12}]^{2-}$, and particularly of halogen derivatives (CI, Br, I) and their positional isomers, on Separon HEMA (hydroxyethylmethacrylate) sorbents was reported [23]. The effects of parameters, including the presence of cations and anions in the mobile phase, the nature and the concentration of the electrolyte in the eluent, pH, the addition of organic modifier, and of the separation temperature, on the separation process were studied. The separation mechanism is governed by the hydrophobic interaction of the *closo*-$[B_{12}H_{12}]^{2-}$ framework and its substituted groups with a polymer surface in the presence of a strong electrolyte in the mobile phase. Optimum conditions for the separation were accomplished with Separon HEMA BIO 300 and Separon HEMA BIO 1000 sorbents, using 0.1–0.5 *M* $NaClO_4$ solutions in 0.01 *M* phosphate buffer (pH 8.5) as the mobile phase. The retention of strongly retained species can be adjusted either by increasing the separation temperature or by varying the acetonitrile content in the mobile phase. The compounds were detected using direct UV detection in the range of 200–210 nm. The method is suitable for the sepa-

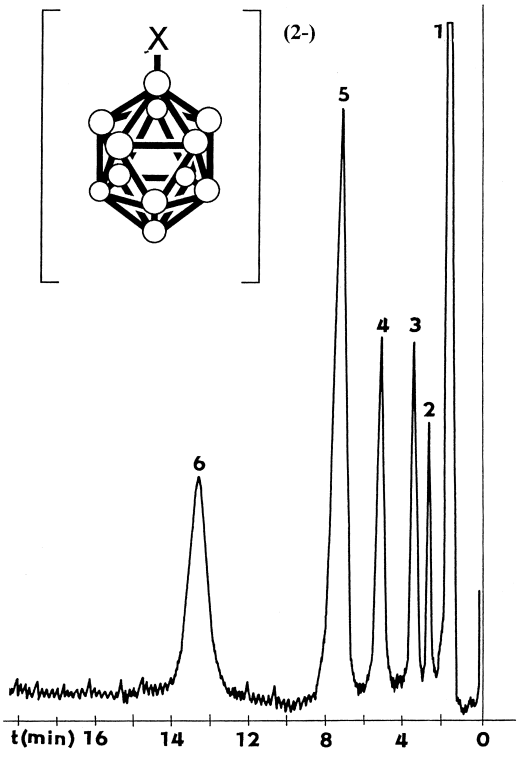


Fig. 6. Separation of *closo*-$[B_{12}H_{12}]^{2-}$ anion and some of its monosubstituted derivatives. Column: CGC, 150×3.3 mm I.D. Separon HEMA-BIO 300 12 μm; mobile phase, 0.1 *M* $NaClO_4$ in 0.01 *M* phosphate buffer (pH 8.5); flow-rate, 0.5 ml/min; detection, UV at 205 nm; sensitivity, 0.16 AUFS; injection volume, 20 μl; temperature of the separation, 40°C. Peaks: 1=$[B_{12}H_{11}OH]^{2-}$; 2=$[B_{12}H_{12}]^{2-}$; 3=$[B_{12}H_{11}SH]^{2-}$; 4=$[B_{12}H_{11}Cl]^{2-}$; 5=$[B_{12}H_{11}Br]^{2-}$ and 6=$[B_{12}H_{11}I]^{2-}$ [24].

ration of a wide spectrum of *closo*-$[B_{12}H_{12}]^{2-}$ derivatives and their positional isomers. An example is given in Fig. 6.

Ion-exchange chromatography on materials modified with quarternary ammonium groups (Separon HEMA-S 1000 Q-L) was shown to be less suitable for the separation of halogen derivatives [23]. Very strong retention and the involvement of a mixed separation mechanism that caused peak tailing were usually observed. In contrast, such a method has been found to be more suitable than hydrophobic interaction chromatography for the separation of anions with more polar substituents. For example, hydroxy- and fluoro-derivatives of the $[B_{12}H_{12}]^{2-}$ anion, along with the unsubstituted anions $[B_{12}H_{12}]^{2-}$, $[B_{10}H_{10}]^{2-}$, could be separated using this approach. The above methods seem to offer a very simple and powerful tool for the analysis of mixtures of *closo*-hydroborate anions arising from synthetic work, and for assaying the purity of *closo*-hydroborate species.

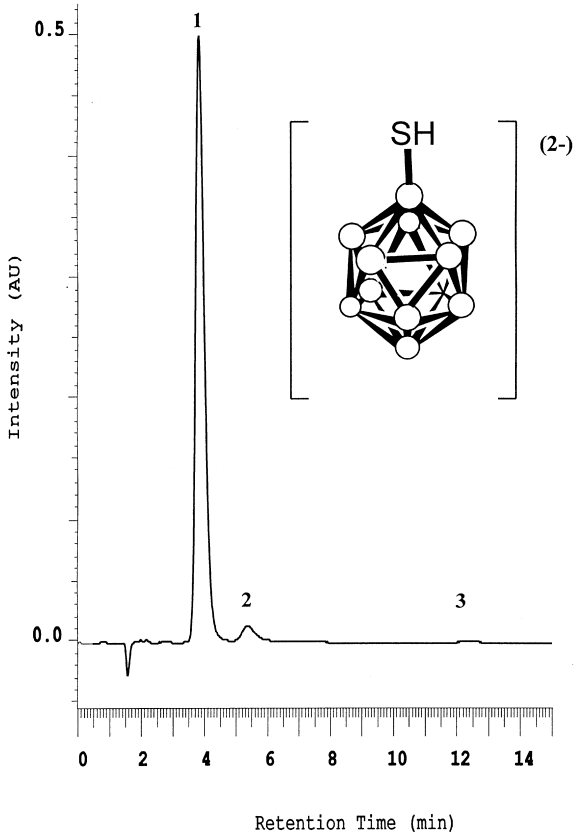


Fig. 7. Separation of a slightly oxidized sample of $Na_2B_{12}H_{11}SH$. Pure dry substance was exposed to air for eleven days (the concentration of impurities II and III were 1.2 and less than 0.2%, respectively). Column: Separon HEMA-BIO 300 12 μm; eluent, 0.1 *M* $NaClO_4$ in 0.01 *M* phosphate buffer, pH 8.5; flow-rate, 0.5 ml/min; detection, UV at 204 nm; temperature of separation, 40°C; injection volume, 20 μl. Concentration of the sample, 0.4 mg/ml; for analysis, the sample was diluted in Milli-Q water. Peaks: 1=$[B_{12}H_{11}SH]^{2-}$ (I); 2=$[B_{12}H_{11}SSB_{12}H_{11}]^{4-}$ (II) and 3=$[B_{12}H_{11}S(O)SB_{12}H_{11}]^{4-}$ (III).

A method based on a modification of the above HPLC techniques, using Separon HEMA-BIO 300 12 μm sorbent, has been developed to assay the purity of, and to quantitate, $Na_2B_{12}H_{11}SH$ [24]. Optimum separation was achieved using 0.1 *M* aqueous $NaClO_4$ in 0.01 *M* phosphate buffer (pH 8.5) as the mobile phase (See Fig. 7). Direct UV detection at 204 nm, where BSH absorbs maximally, was employed. The advantage of the method is the separation of all impurities and on-line sensitive detection and quantitation of the most significant oxidation impurities, $Na_4B_{12}H_{11}SSB_{12}H_{11}$ and $Na_4B_{12}H_{11}S(O)SB_{12}H_{11}$. The minimum amounts detectable were $8.6\cdot10^{-8}$, $8.8\cdot10^{-9}$ and $6.4\cdot10^{-8}$ g for the sodium salts of BSH, $[B_{12}H_{11}SSB_{12}H_{11}]^{4-}$ and $[B_{12}H_{11}S(O)SB_{12}H_{11}]^{4-}$ anions, respectively.

4. Chiral separations

More than 50 chiral boranes and metallaboranes have been resolved into enantiomers during the past five years, using HPLC on chiral stationary phases (CSPs). To date, all successful separations were performed on β-cyclodextrin (β-CD) CSPs, either native or modified, in the reversed-phase mode [25–31], and some of them were separated with high enantioselectivity and resolution values.

The β-CD CSPs are widely used supports for the resolution of a variety of organic chiral compounds, and this area has been the subject of several reviews [32,33]. The advantage of these CSPs in organic chemistry is the large variety of enantioselectivities and the easy accessibility of β-CD columns. A slight disadvantage is that the separation mechanism is complex. The reason lies in the fact that the β-CD cone, as the chiral selector, provides a large number

of chiral centers and many hydrogen bonding sites are available for interaction.

It should be pointed out that chiral cage carborane and metallaborane species differ generally from chiral organic molecules. In their three-dimensional structures, only the general asymmetry of the molecules usually accounts for chirality, and it is not associated with the presence of distinct monatomic chiral centers. On the other hand, the three-dimensional structures of carboranes and metallaboranes are probably responsible for the fact that β-CD supports were so useful in their enantiomeric discrimination. The internal diameter of the β-CD cone (7.8 Å) [32], which is composed of seven glucose units, is apparently well suited to tightly fit carborane or borane cages of medium size. Furthermore, the extremely high capability of boron-clusters to repulse water molecules from their surface helps the carborane part of the molecule to enter the relatively non-polar interior of the CD cavity, to form a strong intercalation complex. Additional interactions of the cage substituents with the upper rim β-CD hydroxyls are responsible for appropriate orientation of the solute in the chiral cavity and account for enantiomeric discrimination. The latter assumption is supported by the fact that unsubstituted boranes, carboranes and metallaboranes have not been resolved yet.

An intercalation mechanism on native β-CD columns is workable only in strongly polar media, under reversed-phase conditions in aqueous organic mobile phases. The reversed-phase separation conditions seem to still be a limiting factor for carborane and metallaborane separations, with only hydrolytically stable species from these series being capable of being resolved into enantiomers using this approach.

4.1. Chiral carboranes

The retention data for the enantiomeric resolution of 29 chiral carboranes are summarized in Table 4 Table 5 Table 6 (compounds 1–29).

The resolution of eleven-vertex disubstituted zwitterionic species *R,L*-7,8-*nido*-$C_2B_9H_{11}$ (Table 4) was reported [25] as the first study in this field describing the retention behavior of fourteen enantiomeric pairs. The structural requirements for the resolution of enantiomers of these species were studied. Also, the effects of the content of organic

Table 4
Optimum separation conditions of enantiomers of isomeric compounds of the *R-L*-$C_2B_9H_{10}$ type on a directly bonded β-CD columns A and B

Compound	No.	[a]k_1'	α	R_s	%O.M.[c]
9-Me_2S-CH_2-7,8-$C_2B_9H_{11}$	1	11.5	1.04	0.55	28 A[d]
9-Me_2S-CH_2-7,8-$C_2B_9H_{11}$	2	10.4	1.08	0.88	60 M[e]
7-Py-CH_2-7,8-$C_2B_9H_{11}$	3	7.45	1.09	0.6	57 M
9-Ph-11-Me_2S-7,8-$C_2B_9H_{10}$	4	5.84	1.04	0.5	27 A
7-Ph-9-Me_2S-7,8-$C_2B_9H_{10}$	5	4.50	1.09	1.12	58 M
7-Ph-11-Py-7,8-$C_2B_9H_{10}$	6	3.88	1.09	0.65	55 M
7-Ph-9-Py-7,8-$C_2B_9H_{10}$	7	7.06	1.09	1.06	27 A
7-Me-11-Me_2S-7,8-$C_2B_9H_{10}$	8	6.60	1.06	0.60	28 A
7-Me-9-Me_2S-7,8-$C_2B_9H_{10}$	9	5.69	1.08	0.75	28 A
7-Me-11-Py-7,8-$C_2B_9H_{10}$	10	3.94	1.0	NR[b]	58 M
7-Me-9-Py-7,8-$C_2B_9H_{10}$	11	4.17	1.07	0.7	28 A
3-Me-9-Me_2S-7,8-$C_2B_9H_{10}$	12	8.09	1.06	0.6	28 A
4-Me-9-Me_2S-7,8-$C_2B_9H_{10}$	13	2.80	1.16	1.5	60 M
9-Me-11-Py-7,8-$C_2B_9H_{10}$	14	9.63	1.08	1.20	27 A
5-Me-11-Me_2S-7,8-$C_2B_9H_{10}$	15	6.14	1.06	0.95	78 M[f]
5-Br-11-Me_2S-7,8-$C_2B_9H_{10}$	16	5.54	1.06	1.05	78M[f]

(A) 250×4 mm I.D. medium CD loading column. (B) semi-preparative 250×8 mm, directly bonded β-CD column with high β-CD loading; aqueous methanolic or aqueous acetonitrile mobile phase; flow-rate, 0.8 ml/min [25].
[a]k_1'=Capacity factor of the first-eluting enantiomers, [b]NR=not resolved, [c]O.M.=organic modifier content, [d]A=acetonitrile, [e]M=methanol, [f]Column B

Table 5
Enantiomeric separation of the eleven-vertex isomeric compounds of the *R-L*-$C_2B_8H_{10}$ type on column C

Compound	No.	[a]k_1'	α	R_s	%MeOH
9-Me_2S-CH_2-7,8-$C_2B_9H_{11}$	1	3.09	1.0	NR[b]	50
7-Py-CH_2-7,8-$C_2B_9H_{11}$	3	3.85	1.13	1.06	45
9-Ph-11-Me_2S-7,8-$C_2B_9H_{10}$	4	3.99	1.14	2.21	45
7-Ph-9-Me_2S-7,8-$C_2B_9H_{10}$	5	5.45	1.08	1.23	45
7-Ph-11-Py-7,8-$C_2B_9H_{10}$	6	3.39	1.14	1.80	45
7-Ph-9-Py-7,8-$C_2B_9H_{10}$	7	3.88	1.11	1.30	45
7-Me-11-Me_2S-7,8-$C_2B_9H_{10}$	8	2.74	1.07	0.85	45
7-Me-9-Me_2S-7,8-$C_2B_9H_{10}$	9	4.20	1.03	0.5	45
7-Me-11-Py-7,8-$C_2B_9H_{10}$	10	4.21	1.04	0.6	45
7-Me-9-Py-7,8-$C_2B_9H_{10}$	11	2.63	1.08	1.0	45
3-Me-9-Me_2S-7,8-$C_2B_9H_{10}$	12	3.28	1.10	1.10	45
4-Me-9-Me_2S-7,8-$C_2B_9H_{10}$	13	3.61	1.08	0.8	45
9-Me-11-Py-7,8-$C_2B_9H_{10}$	14	5.18	1.12	2.0	45

Column C Astec CYCLOBOND I β-CD, 250×4 mm I.D. in aqueous methanolic mobile phases; flow-rate, 0.8 ml/min [25].
[a]k_1'=Capacity factor of the first-eluting enantiomers, [b]NR=not resolved.

modifier in the mobile phase, the flow-rate and the separation temperature on the retention, enantioselectivity and resolution on two different β-CD CSPs were described. Several factors important for understanding the separation mechanism were pointed out: The compounds always enter the β-CD cavity by their more hydrophobic carborane part. One substituent on the carborane is sufficient for successful enantiomeric discrimination. The second substituent (including Ph, Me) interacts (steric, hydrogen bonding) with hydroxyls on the edge of the β-CD cone and are responsible for the enhancement of resolution. An example is given in Fig. 8.

Significant differences in the selectivities of two native β-CD supports, directly bonded β-CD CSPs (columns A and B) and Cyclobond I (bonded via a

Table 6
Chromatographic data for the optimum enantiomeric separation of two isomeric series of the L-*arachno*-$C_2B_8H_{12}$ compounds on directly bonded β-CD CSPs in aqueous methanolic mobile phases [26]

Compound	No.	[a]k_1'	α	R_s	Column	% MeOH
6-Et_3N-5,1O-$C_2B_8H_{12}$	17	2.58	1.12	1.35	B	100
6-isoquinoline-5,10-$C_2B_8H_{12}$	18	4.55	1.07	1.0	B	100
6-isoquinoline-5,10-$C_2B_8H_{12}$	18	6.8	1.17	1.30	D	66
6-urotropine-5,10-$C_2B_8H_{12}$	19	9.89	1.10	1.20	B	95
6-urotropine-5,10-$C_2B_8H_{12}$	19	4.11	1.30	1.21	D	70
9-Piperidine-5,6-$C_2B_8H_{12}$	20	6.63	1.24	0.95	C	50
9-Piperidine-5,6-$C_2B_8H_{12}$	20	6.64	1.31	2.10	D	55
9-H_3N-5,6-$C_2B_8H_{12}$	21	4.36	1.13	1.33	D	55
9-MeH_2N-5,6-$C_2B_8H_{12}$	22	6.16	1.23	1.18	D	50
9-PrH_2N-5,6-$C_2B_8H_{12}$	23	5.26	1.25	1.65	D	55
9-BuH_2N-5,6-$C_2B_8H_{12}$	24	6.64	1.21	1.38	D	55
9-t-BuH_2N-5,6-$C_2B_8H_{12}$	25	5.20	2.28	5.32	D	55
9-Et_2HN-5,6-$C_2B_8H_{12}$	26	8.88	1.61	2.09	D	50
6-Et_2HN-5,10-$C_2B_8H_{12}$	27	6.85	1.21	1.36	D	55
9-Bu_2HN-5,6-$C_2B_8H_{12}$	28	5.99	1.21	1.12	D	55
6-Bu_2HN-5,10-$C_2B_8H_{12}$	29	6.47	1.09	0.65	D	55

[a]k_1'=Capacity factor of the first-eluting enantiomers, [b]NR=not resolved.
Columns: B=directly bonded native β-CD column, 250×8 mm with high β-CD capacity; flow-rate, 0.8 ml/min; C=Cyclobond I, β-CD column, 250×4.6 mm; flow-rate, 0.8 ml/min; D=directly bonded acetyl-β-CD column, 125×4 mm; flow-rate, 0.4 ml/min.

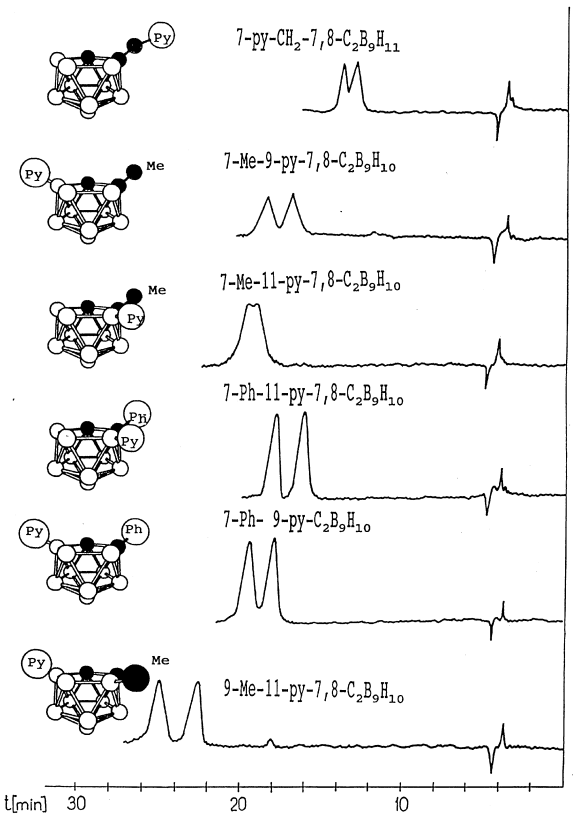


Fig. 8. Separation of the enantiomers of pyridine-substituted zwitterionic species of the R-py-$C_2B_9H_{10}$ type. Column, β-CD Cyclobond I (250×4.6 mm I.D.); mobile phase, 45% aqueous methanol; flow-rate, 0.8 ml/min; detection, UV at 254 nm; sensitivity, 0.02 AUFS [25].

six–eight atom spacer) (column C), were observed. While the former gave good resolution values only for ''meta'' substituted species, the latter material was far more efficient for the separation of almost all enantiomeric pairs investigated (for a comparison of retention data, selectivity and resolution values, see Tables 4 and 5).

The separation of amino-substituted zwitterionic *arachno*-compounds of exceptional stability, in the ten-vertex series will be the subject of another detailed communication [26]. Three compounds of this series, 6-Et_3N-5,10-$C_2B_8H_{12}$ (17), 6-(urotropine)-5,10-$C_2B_8H_{12}$ (18) and 9-(piperidine)-5,6-$C_2B_8H_{12}$ (19), all having tertiary amino groups, were resolved on native β-CD supports, with only Cyclobond I CSPs (column C) being effective in the enantiomeric separation of the compound (19). Ten other compounds (20–29) from this series were resolved, generally with high selectivity and R_s values, using a modified acetyl-β-CD support (column D) (see Fig. 9). The enantioselectivity on both native and acetyl-bonded β-CD columns is almost complementary, i.e., species resolvable on the acetyl-β-CD column cannot be resolved on native β-CD CSP columns and vice versa. Only two exceptions from this rule were observed, for 9-(piperidine)-5,6-$C_2B_8H_{12}$ and 6-(urotropine)-5,10-$C_2B_8H_{12}$, which were resolved on both materials. Native β-CD was generally more selective for the carborane compounds with tertiary amino substituents, whereas acetyl-β-CD was proved to be very valuable in the

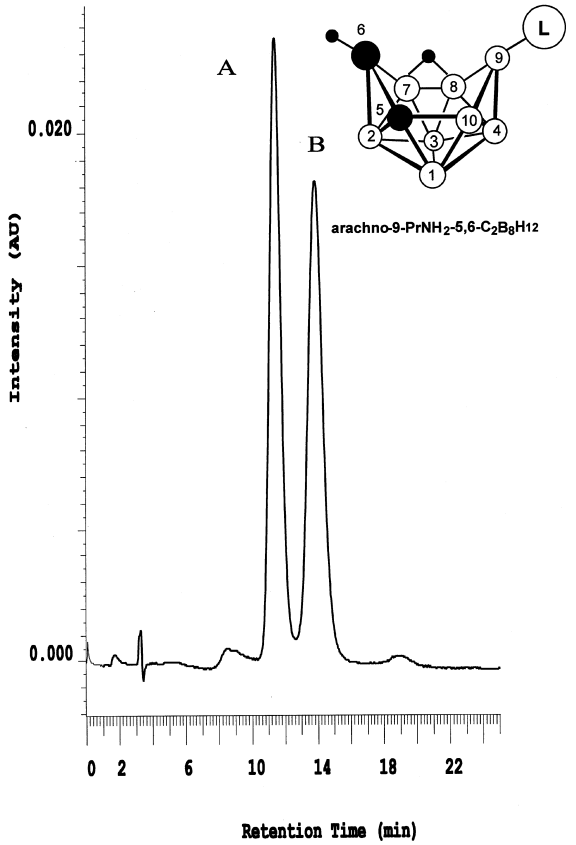


Fig. 9. Separation of the enantiomers of 9-$PrNH_2$-5,6-$C_2B_8H_{10}$. Column: Directly bonded acetyl β-CD (column D, Tables 6 and 8) (125×4 mm I.D.); mobile phase, 50% aqueous methanol; flow-rate, 0.4 ml/min; detection, diode array at 200–280 nm, fixed at 254 nm.

Table 7
Chromatographic data for the enantiomeric separation of sandwich metallaboranes on directly bonded β-CD CSPs [28–31,44]

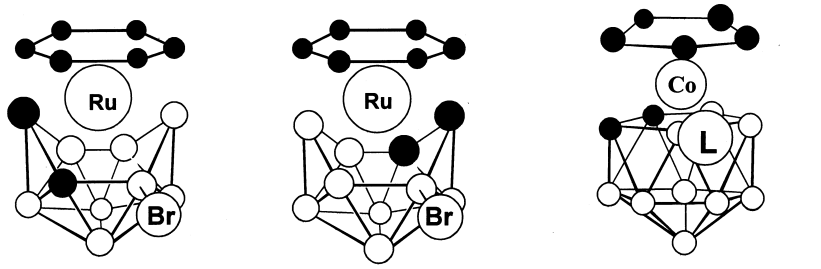
Compound	No,	[a]k_1'	α	R_s	Column	% MeOH
4-MeS-3-C_5H_5Co-1,2,-$C_2CoB_9H_{10}$	1	3.17	1.09	0.94	A	60
1-C_6Me_6Ru-7-Br-2,7-$C_2B_8H_9$	2	4.33	1.08	0.75	B	68
1-C_6Me_6Ru-5-Br-2,7-$C_2B_8H_9$	3	3.27	1.12	1.02	B	68
4-Me_2S-4′-MeS-(1,2-$C_2B_9H_{10})_2$-3-Co	4	3.29	1.08	0.55	A	60
6-PMe_3(1,7-$C_2B_9H_{10}$)(1,7-$C_2B_9H_{11}$)-2-Co	5	5.71	1.10	1.00	B	78
[6,6′-μ-S-(1,7-$C_2B_9H_{10})_2$-2-Co]$^-$	6	8.0	1.37	0.85	B	55
6,6′-μ-MeS-(1,7-$C_2B_9H_{10})_2$-2-Co	7	7.50	1.17	1.08	B	85
6,6′-μ-EtS-(1,7-$C_2B_9H_{10})_2$-2-Co	8	12.0	1.31	2.06	B	78
6,6′-μ-PrS-(1,7-$C_2B_9H_{10})_2$-2-Co	9	7.89	1.16	1.47	B	78
6,6′-μ-i-PrS-(1,7-$C_2B_9H_{10})_2$-2-Co	10	7.06	1.17	1.50	B	78
6,6′-μ-AllylS-(1,7-$C_2B_9H_{10})_2$-2-Co	11	7.72	1.15	1.42	B	78
6,6′-μ-BuS-(1,7-$C_2B_9H_{10})_2$-2-Co	12	6.50	1.09	1.13	B	78
6,6′-μ-HexS-(1,7-$C_2B_9H_{10})_2$-2-Co	13	5.85	1.07	0.7	B	78
6,6′-μ-$MeOCOCH_2$S-(1,7-$C_2B_9H_{10})_2$-2-Co	14	7.67	1.13	1.22	B	78
6,6′-μ-MeO-(1,7-$C_2B_9H_{10})_2$-2-Co	15	4.0	1.72	1.30	B	56[b]
6,6′-μ-H_2N-(1,7-$C_2B_9H_{10})_2$-2-Co	16	7.33	1.12	0.95	B	75
6,6′-μ-MeHN-(1,7-$C_2B_9H_{10})_2$-2-Co	17	7.83	1.16	1.06	B	78
6,6′-μ-Me_2P-(1,7-$C_2B_9H_{10})_2$-2-Co	18	7.67	1.23	1.47	B	85
6,6′-μ-Me_2P-(1,7-$C_2B_9H_{10})_2$-2-Co	19	6.11	1.14	1.32	B	80
4,8′-μ-H_2N-(1,2-$C_2B_9H_{10})_2$-3-Co	20	4.77	1.46	3.80	B	70
4,8′-μ-Me_2N-(1,2-$C_2B_9H_{10})_2$-3-Co	21	2.33	1.19	2.00	B	100

[a]k_1'=Capacity factor of the first-eluting enantiomers, [b]Me group is split upon dissolution in methanol.

resolution of primary and secondary amino-substituted species (for retention, enantioselectivity and resolution values, see Table 6).

4.2. Chiral metallaboranes

About twenty pairs of chiral metallaborane species have been resolved by HPLC on various bonded β-CD CSPs. Retention, selectivity and resolution data of all reported compounds (1–21) are summarized in Table 7. As with carborane series, only compounds that had an exo-skeletal substituent could be resolved. Good resolution was achieved on the directly bonded CD-CSPs for both mixed sandwich series having eleven- or ten-vertex carborane ligands (1–3, Table 7) (Fig. 10) [25,27,31,44]. On the other hand, deterioration or remarkable decreases in the resolution were usually observed in the series of compounds with two carborane ligands, in comparison with similar mixed sandwich species. This



[7-Br-1-$(CH_3)_6C_6$Ru-2,7-$C_2B_8H_9$] [5-Br-1-$(CH_3)_6C_6$Ru-2,7-$C_2B_8H_9$] [4-MeS-3-C_5H_5-1,2,3-$C_2CoB_9H_{10}$]

Fig. 10. Schematic structures of three mixed-sandwich compounds resolved into enantiomers by HPLC on β-CD CSPs. (Terminal hydrogen atoms are omitted for clarity).

loss of enantioselectivity is probably a consequence of averaging the interactions with both carborane parts of these molecules, combined with free rotation of ligands in solution around the central atom. Only two compounds (4,5) of this series that were substituted by bulky groups were successfully resolved, the groups probably having prevented the averaging or the rotation.

Good resolution of thirteen enantiomeric pairs of the cobaltacarboranes series [6,6′-μ-R_nE (1,7-$C_2B_9H_{10})_2$-2-Co] (E=S<, *R*=none, Me, Et, Pr, i-Pr, Bu, Hex, CH_3OOCCH_2; *n*=1, E=O<, *R*=none; E=N<, *R*=H, Me, *n*=2) (6–18, Table 7) with monatomic bridge and helically twisted structures was achieved on native directly bonded β-CD CSPs [28,29,31]. The rigid structures with completely hindered rotation of ligands around the central cobalt atom are undoubtedly responsible for the good enantiomeric discrimination. The resolution values on native directly bonded β-CD were found to decrease with increasing size of the bridge substituent (see Table 7 and Fig. 11 for example), showing almost no dependence on substituent polarity. However, for substituent sizes up to *n*-butyl, nearly baseline resolutions can be obtained. Noteworthy is the identical orientation of Cotton curves in circular dichroism spectra of the first- and second-eluting enantiomers in the whole series of these compounds [28]. Very recently, the HPLC separation of enantiomers of the last synthesized member of this family [34], [6,6′-μ-Me_2P(1,7-

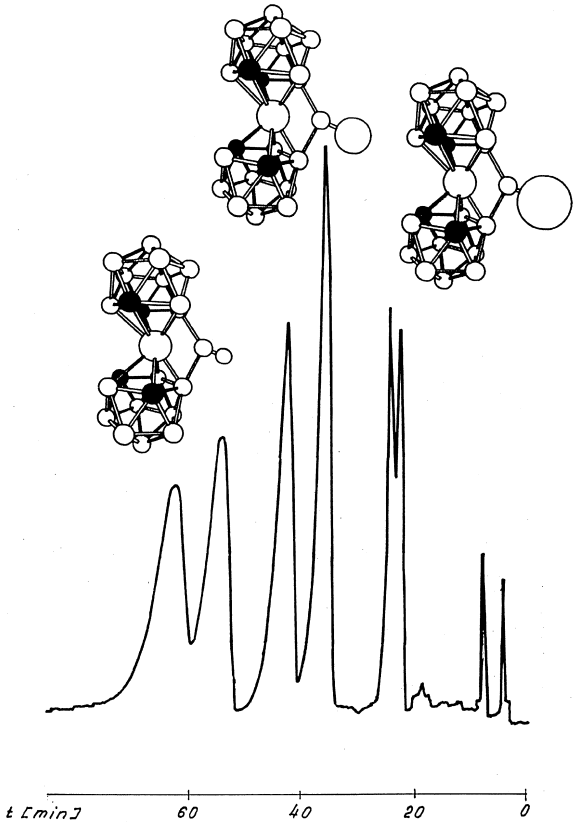


Fig. 11. Separation of the three pairs of enantiomers of the zwitterionic species of the 6,6′-μ-*R*-*S*(1,7-$C_2B_9H_{10})_2$-2-Co type with Bu-S<, Et-S< and MeS< bridges (see 7, 8 and 12 in Table 7). Column B, directly bonded β-cyclodextrin (250×8 mm I.D.); mobile phase, 80% aqueous methanol; flow-rate, 1.2 ml/min; detection, UV at 290 nm; sensitivity, 0.04 AUFS [28].

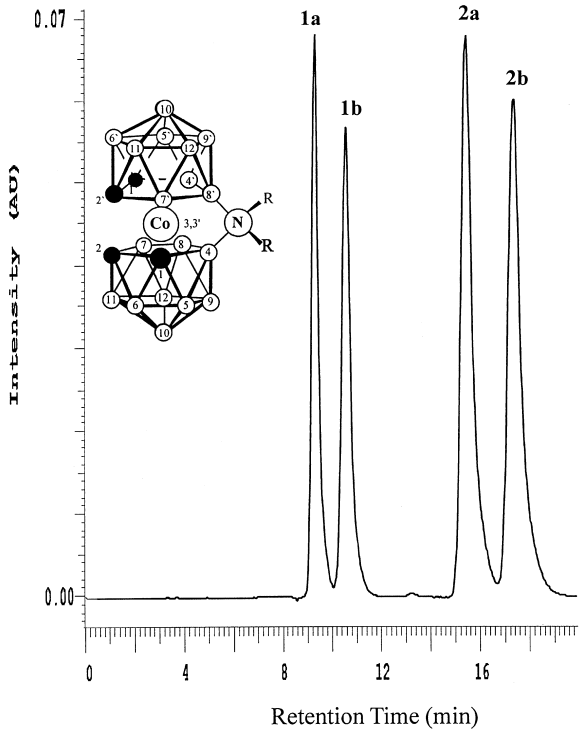


Fig. 12. HPLC separation of the enantiomers of compounds 20 and 21 (see Table 7) on 250×8 mm I.D. directly bonded β-cyclodextrin column B (UOCHAB AS CR, Prague, Czech Republic); mobile phase, 85% aqueous methanol; flow-rate, 1.6 ml/min; detection, UV at 280 nm; injection: 2 μl of a mixture of the methanolic solutions of compounds IIa and IIb (concentration, 0.5 mg/ml). Peaks 1 a,b=enantiomers of 20; 2 a,b=enantiomers of 21. [30].

$C_2B_9H_{10})_2$-2-Co] (19), was achieved, even on a semi-preparative scale. The absolute configuration of both enantiomers of (19) was successfully determined by X-ray diffraction (34), which should be identical for the whole series (6–19) of compounds. A manuscript [35] describing this study is being prepared.

Also, the enantiomeric separation of three closely related compounds [4,8-μ-R_2N(1,2-$C_2B_9H_{10})_2$-3-Co] (R=H, Me) (20,21), differing in the position of the cluster carbon atoms [30], has been published (see Fig. 12 and Table 7). The higher dipole moments of the compounds have been suspected to account for the enhancement in resolution compared with that of the former cobaltacarborane family.

In contrast to carborane derivatives, directly bonded β-CD material has been found to be more efficient for the separation of metallaboranes than Cyclobond I columns (31), which failed in the separation of the above-mentioned mixed sandwiches (6–19). Also, in this case, only methyl- and ethyl-substituted sulfur bridged compounds (7,8) of this series could be resolved on Cyclobond I CSP and only compounds (16) with a nitrogen bridge.

A study of the separation of bridged metallaboranes on a directly bonded acetyl-β-CD column has shown that compounds from the above series containing bulkier alkyl groups also remained unresolved. In contrast, acetyl-β-CD CSP exhibited strong enhancement of the resolution for compounds with a polar carboxymethyl substituent (14). Data for successfully resolved species are presented in Table 8 (last two columns). Bridge metallaboranes (20 and 21), were baseline separated on all three β-CD supports. Because of their high stability and high absorbances in the UV–Vis region, these species are used as standards for testing column efficiency, resolution, and β-CD loading in our laboratory. A comparison of retention, selectivity and resolution data can be made from Tables 7 and 8.

5. Semi-preparative applications

In synthetic chemistry, analytical information on the composition of the reaction mixture or the purity of the product isolated is often only the first step to be achieved. The final goal is usually to obtain, in sufficient quantity, a material for complete structural characterization by other methods and/or a workable amount of the starting material of required purity for the next reaction step. For mixtures containing neutral borane species successfully resolved by liquid–solid chromatography, the analytical proce-

Table 8
Chromatographic data for the successful[a] enantiomeric separation of sandwich metallaboranes on a Cyclobond I column (C, 250×4.6 mm) and on a directly bonded β-CD acetyl column (D; 125×4 mm)

Compound	No.	[b]k_1'	α	R_s	Column	% MeOH
4-MeS-3-C_5HCo-1,2-$C_2CoB_9H_{10}$	1	3.50	1.07	0.70	C	51
6,6′-μ-MeS-(1,7-$C_2B_9H_{10})_2$-2-Co	7	10.6	1.17	0.80	C	50
6,6′-μ-MeS-(1,7-$C_2B_9H_{10})_2$-2-Co	7	6.60	1.17	1.07	D	66
6,6′-μ-EtS-(1,7-$C_2B_9H_{10})_2$-2-Co	8	1.18	1.15	1.00	C	52
6,6′-μ-EtS-(1,7-$C_2B_9H_{10})_2$-2-Co	8	10.1	1.21	0.95	C	50
6,6′-μ-MeOCOCH_2S-(1,7-$C_2B_9H_{10})_2$-2-Co	14	7.30	1.25	1.82	D	66
6,6′-μ-H_2N-(1,7-$C_2B_9H_{10})_2$-2-Co	16	3.45	1.06	0.6	C	48
6,6′-μ-H_2N-(1,7-$C_2B_9H_{10})_2$-2-Co	16	5.20	1.15	1.63	D	66
6,6′-μ-MeHN-(1,7-$C_2B_9H_{10})_2$-2-Co	17	5.30	1.16	1.30	D	66
6,6′-μ-Me_2N-(1,7-$C_2B_9H_{10})_2$-2-Co	18	5.60	1.17	1.54	D	66
4,8′-μ-H_2N-(1,2-$C_2B_9H_{10})_2$-3-Co	20	4.09	1.57	2.56	C	48
4,8′-μ-H_2N-(1,2-$C_2B_9H_{10})_2$-3-Co	20	9.01	1.19	1.50	D	62
4,8′-μ-Me_2N-(1,2-$C_2B_9H_{10})_2$-3-Co	21	5.91	1.26	1.35	C	48
4,8′-μ-Me_2N-(1,2-$C_2B_9H_{10})_2$-3-Co	21	9.70	1.20	1.82	D	62

Flow-rate, 0.8 ml/min [34].
[a]With the exception of compounds 5 and 19 from this series, which were not studied, none of the other compounds summarized in Table 7 was resolved on these two columns. [b]k_1'=Capacity factor of the first-eluting enantiomers.

dure used may be scaled up easily, so that required amounts of a pure material may be isolated. This approach, described by Plešek et al. [46] is illustrated in Fig. 13 Table 9. The liquid–solid system used here can be scaled up to a preparative level to isolate up to gram amounts of pure B-alkyl-*o*-carboranes. Totally porous 29 μm silica gel seems to be the best adsorbent in cases where the highest separation efficiency is not required. For highly reactive compounds, the time of separation should be kept to a minimum and this limiting factor dictates the use of short, highly efficient preparative columns packed with microparticulate sorbent. Stainless steel 7 μm silica gel HPLC preparative columns are routinely used for such separations in our laboratory (Fig. 14 Table 10). Wide bore preparative columns, with internal diameters of 17 or 25 mm, are necessary the isolation of the 100 mg–1 g of material required for a single injection. An example showing the separation of 500 mg of an isomeric mixture on a 25-mm bore column is given in Fig. 15.

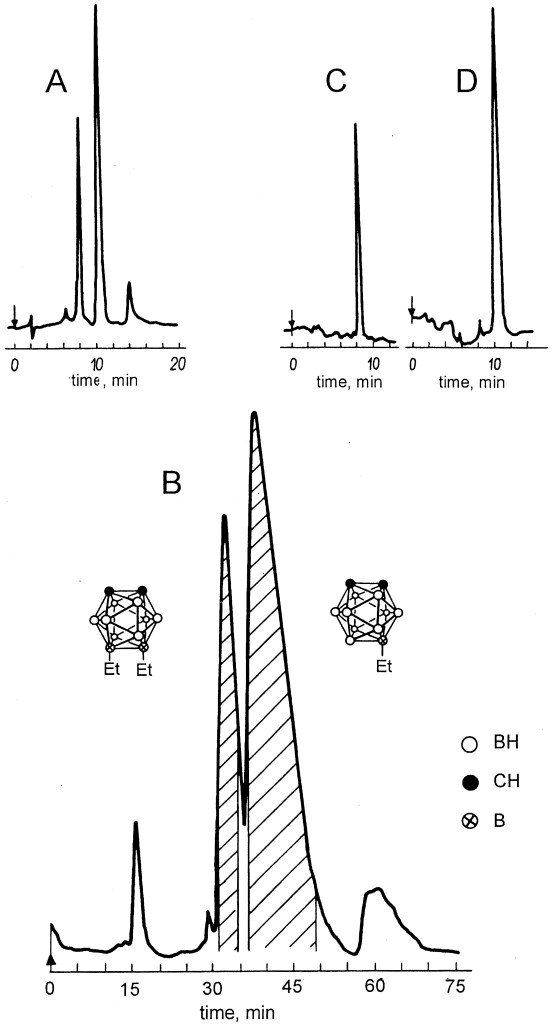


Fig. 13. Preparative isolation of alkylcarboranes: A, analysis of the reaction mixture, 9,12-diethyl-*o*-carborane (t_R=7.9 min), 9-ethyl-*o*-carborane (t_R=10.2 min), *o*-carborane (t_R=14 min). C, D, analysis of isolated sample. Chromatograms A, C and D: Column, 300×3.7 mm, silica gel 13 μm; eluent, heptane; flow-rate, 1.4 ml/min; inlet pressure, 3.0 MPa; RI detection. Chromatographic conditions for B, see Table 9.

To obtain 5–50 mg amounts of material, semi-preparative columns with internal diameters of about 8 mm are more convenient. These columns are cheaper, use less solvent during equilibration and, due to the lower flow-rate, do not need special equipment, e.g., a preparative cell or a solvent splitter.

In reversed-phase mode, preparative separations of chiral carboranes and metallaboranes were reported [25–31] on a 25 cm×8 mm bonded β-CD column with high CD loading. Pure enantiomers were obtained after several injections in 1–10 mg quantities, which are necessary for circular dichroism measurements and for crystal growth.

Unfortunately, there is still a lack of reliable preparative HPLC methods in the area of ionic boron-cage species. Therefore, other methods are usually used for separation on a preparative scale and the analytical HPLC methods described in Section 3 might only be used for monitoring the purity.

Only one example has been reported of the use of a HPLC method for the preparative separation of borate anions [36], based on partition chromatography on silica. Separation of the unsubstituted anions $[B_{10}H_{10}]^{2-}$ and $[B_{12}H_{12}]^{2-}$ with conductivity detection is described in this article. However, the potential of this approach does not seem to be sufficiently powerful to separate complex mixtures of derivatives and isomers originating from many substitution reactions of the above-mentioned anions.

In this area, ion-exchange methods on amino-derivatized cellulose supports are noteworthy. However, these supports are not pressure resistant and could not be used for true HPLC separations. On the other hand, this is the system that is known to be

Table 9
Parameters for the preparative isolation of alkylcarboranes [46]

Parameter	Value
Sorbent/particle size (μm)	Silica gel 29 μm
Column (L×I.D., mm)	500×17
Efficiency, theoretical plates	2000
Solvent	2% Dichloromethane in pentane
Pressure drop (MPa)	0.6
Linear flow-rate (cm/s)	0.07
Amount of sample (mg)	140
Sample size (μl)	1400
Time of the separation run (min)	65
Yield of pure compound (mg/min)	1.5
Detection	Refractometric

suitable for the successful separation of a large variety of derivatives and isomers of stable *closo*-borate anions(2−). A hydrophilic cellulose support does not interfere with hydrophobic anions and the separation mechanism is based on true ion-exchange. The method provides good selectivity, even for positional isomers, and could be scaled up easily. Preparative separations of $[B_{10}H_{10}]^{2-}$ and the $[B_{12}H_{12}]^{2-}$ halogen derivatives [37,38] and rhodano derivatives [39] have been reported using liquid column chromatography on DEAE–cellulose with 0.5–1 *M* aqueous solutions of NaCl as the eluent. Even better results were obtained in the separation of $[B_{12}H_{12}]^{2-}$ halogen derivatives when a pearl macroporous DEAE support (100 μm) with lower capacity was used. NH_4NO_3 or NH_4Cl salts in aqueous or water–acetonitrile mobile phases were used [40,41]. A better mass transfer on this support afforded fast elution of anions from the column, good selectivity and easy isolation of the species by precipitation as Bu_4N^+ salts. Mobile phase strength can be approximately adjusted according to R_F values obtained from a modified TLC method [42] on DEAE–cellulose plates. When 1 to 2 *M* aqueous NH_4Cl was used as the mobile phase, species eluted from the column were detected directly by analytical HPLC, using the HPLC method discussed above with HEMA material [23,24].

5.1. Detection methods

The virtually three-dimensional, pseudoaromatic character of bonding in open-cage polyhedral borane and heteroborane clusters is reflected in their chromophoric properties in the region of approx. 220–260 nm. In contrast, almost all unsubstituted twelve-vertex *closo*-hetero-boranes exhibit no UV absorption down to about 210 nm. Sandwich complexes based on borane ligands form colored solutions and exhibit maximum absorption at 340–300 and 280–260 nm.

Direct UV absorbance detection is thus the most straightforward and dominant method for the detection of boron-cluster compounds. UV detectors with variable wavelength, or even fixed-wavelength, UV detectors, working at 254 nm, can be used for the detection of almost all *nido*-boranes, *nido*-heteroboranes and metal complexes with heteroborane ligands. Working with a fixed-wavelength detector, only 6,9-dicarba-*arachno*-decaborane ($C_2B_8H_{14}$) derivatives and some tricarba- and tetracarbaboranes are transparent at this wavelength. The minimum detectable amount (the amount of substance that gives a detector signal equal to twice the noise level), of the order of 10^{-7} to 10^{-9} g, can be routinely achieved with a fixed-wavelength UV detector of a relatively simple design for this group of compounds [13,14]. Direct UV detection of *closo*- $[B_{12}H_{12}]^{2-}$ derivatives often requires work in the UV region below 210 nm and care must be taken to choose sufficiently transparent reagents for the preparation of the mobile phase.

Indirect UV detection, used for measurements with ion-exchange systems [43], using eluents containing salts of aromatic acids, such as sodium sulfosalicylate or potassium terephthalate can also be

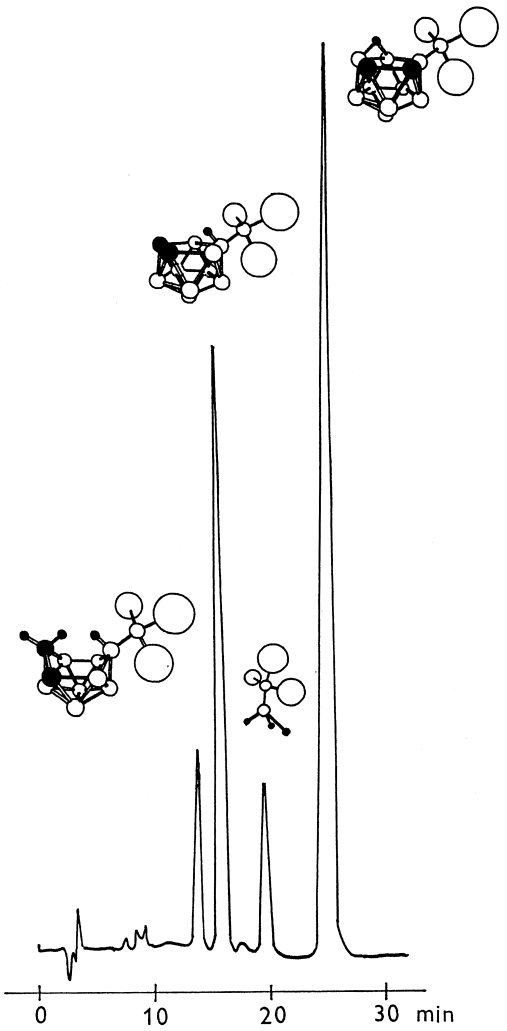


Fig. 14. Example of the preparative separation of some *nido*- and *arachno*-triphenylphosphine derivatives; a mixture of products from the reaction of *nido*-5,6-$C_2B_8H_{13}$ with a PPh_3 column: Silica gel column, Separon SGX 7 μm (250×25 mm I.D.) (Tessek, Prague, Czech Republic); mobile phase, hexane–dichloromethane (8:2, v/v); flow-rate, 30 ml/min; detection, UV at 254 nm. Peaks: 1=*arachno*-9-PPh_3-5,6-$C_2B_9H_{11}$, 2=*nido*-10-PPh_3-7,8-$C_2B_9H_{11}$, 3=PPh_3-BH_3, 4=*nido*-9-PPh_3-7,8-$C_2B_9H_{11}$

Table 10
Chromatographic data on the preparative separation of some zwiterionic derivatives of $[C_2B_9H_{12}]^-$ anion

Compound	k'	α	R_s	Eluent (% CH_2Cl_2)
7-Me-9-Py-7,8-$C_2B_9H_{10}$	2.47		65	
7-Me-11-Py-7,8-$C_2B_9H_{10}$	3.06	1.24	2.25	65
10-Me_2S-7,8-$C_2B_9H_{10}$	3.36			40
9-Me_2S-7,8-$C_2B_9H_{10}$	4.64	1.38	2.05	40
1-Me-9-Me_2S-7,8-$C_2B_9H_{10}$	5.36			30
2-Me-9-Me_2S-7,8-$C_2B_9H_{10}$	6.37	1.19	2.42	30
10-Me-9-Me_2S-7,8-$C_2B_9H_{10}$	6.84	1.07	1.17	30
3-Me-9-Me_2S-7,8-$C_2B_9H_{10}$	7.31	1.07	1.15	30
4-Me-9-Me_2S-7,8-$C_2B_9H_{10}$	7.72	1.06	0.95	30
9-Me_2S-7,8-$C_2B_9H_{10}$	8.62	1.11	1.83	30
7-Me-9-Me_2S-7,8-$C_2B_9H_{10}$	6.42			30
7-Me-11-Me_2S-7,8-$C_2B_9H_{10}$	6.94	1.08	1.05	30
10-$PPh_3$7,8-$C_2B_9H_{10}$	3.06			25
9-PPh_3-7,8-$C_2B_9H_{10}$	3.56	1.16	1.65	25

Each section of this table represents a particular separation of a real reaction mixture composed of the presented $[C_2B_9H_{12}]^-$ anion derivatives.
Chromatographic conditions: Column, 250×25 mm; sorbent, silica gel Separon SGX (7 μm); eluent, hexane–dichloromethane mixture (see table); flow-rate, 30 ml/min; detection, UV at 225 or 254 nm.

used for the analysis of boron-cluster compounds [23,43]. This method is subject to many restrictions in the experimental set-up and the results are strongly dependent on the performance characteristics of the detector used [43].

A general approach to the detection of UV-transparent borane compounds is based on using non-specific refractometric detection. The minimum detectable amounts are in the order of 10^{-6} to 10^{-7} g and are generally two orders of magnitude worse than those obtained with UV detection under comparable conditions. The thermal stability of refractometric detectors is a problem recognized by a number of workers [45]. This restricts the practical application of the refractometric detection method to UV-transparent neutral dicarba-*closo*-dodecaborane derivatives [12,13].

The use of other detection systems, like conductivity detection for borane anions [36] and selective electrochemical amperometric detection for thio derivatives of $[B_{12}H_{12}]^{2-}$, is relative scarce and is applicable only to specific compounds. All detection methods used are inextricably linked to the separation method used, as the eluent must be used with both the separation and detection methods in mind. In the analysis of boron-cluster compounds, these experimental requirements are often achieved using a

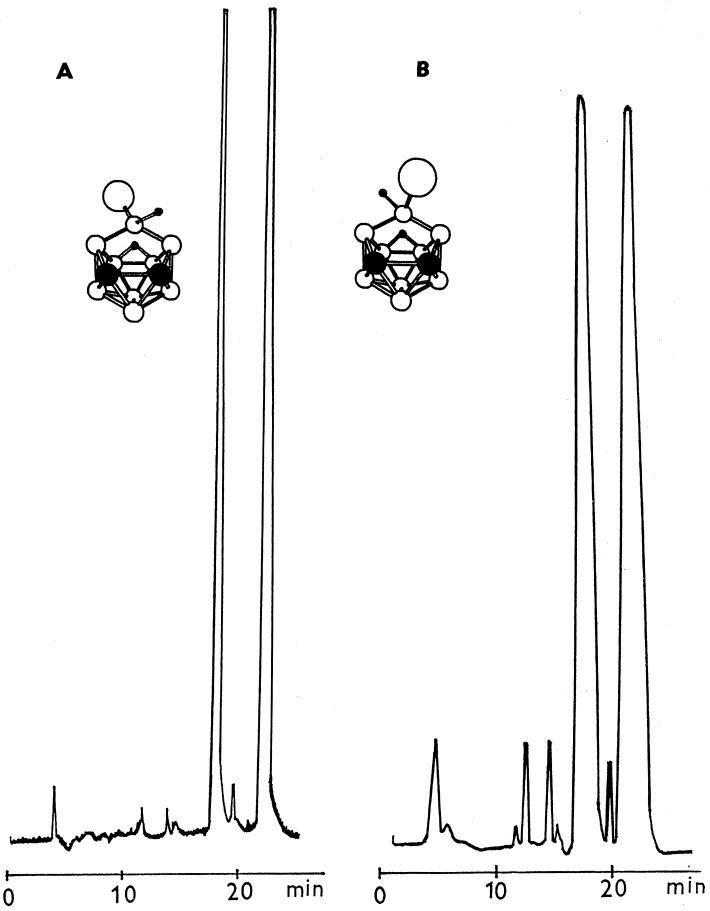


Fig. 15. Demonstration of the scaling up of the amount of sample to be separated by adsorption chromatography. Example shows the separation of the *syn-* and *anti-* isomers of 6-μ-9-μ-*tert.*-BuNH-$C_2B_8H_{11}$ (for synthesis see [48]). A: Column, semi-preparative Separon SGX 7 μm (250×8 mm I.D.) (Tessek); mobile phase, hexane–dichloromethane (95:5, v/v); flow-rate, 3 ml/min; detection, UV at 230 nm; sensitivity, 0.08 AUFS; injection volume, 20 μl. B: Column, preparative Separon SGX 7 μm (250×25 mm I.D.) (Tessek); flow-rate, 30 ml/min; detection, UV at 254 nm; preparative cell, injection of 5 ml of a 0.1 g/ml solution.

diode array or variable-wavelength UV detector in the simplest experimental set-up and the most convenient way.

Acknowledgements

The area of boron-cage compounds is entirely man-made chemistry, all compounds mentioned in this study had to be synthesized before they could be submitted to systematic HPLC analysis. We are deeply indebted to Prof. J. Plešek, Drs. B. Štíbr, J. Holub, Z. Janoušek, T. Jelínek, A. Franken and M. Brown for preparing most of the compounds described in this article and for valuable discussion of their properties.

A large number of the anion separations and the chiral separations were achieved thanks to Drs. I. Vinš and T. Vaněk, who kindly provided us with a series of HPLC columns.

We thank Drs. B. štíbr and Z. Janoušek for helpful discussion of the text.

The authors acknowledge the partial financial support of this work by the Grant Agency of the

Academy of Sciences of the Czech Republic (Grant No. 432402), the Grant Agency of the Czech Republic (Grant Nos. 203 09 84, 203 03 62 and 203 97 0060) and of the European Community (CIPA-CT93 01 33 Project).

References

[1] R.E. Williams, Chem. Rev. 92 (1992) 177.
[2] V.I. Bregadze, Chem. Rev. 92 (1992) 209.
[3] B. Štíbr, Chem. Rev. 92 (1992) 225.
[4] R.N. Grimes, Chem. Rev. 92 (1992) 251.
[5] J. Plešek, Chem. Rev. 92 (1992) 269.
[6] S. Heřmánek, in Z. Deyl, K. Macek and J. Janák (Editors), Liquid Column Chromatography: A Survey of Modern Techniques and Applications, Elsevier, Amsterdam, 1975, pp. 945–951.
[7] Z. Plzák, Chem. Listy 70 (1976) 879.
[8] S. Heřmánek, J. Plešek, Collect. Czech. Chem. Commun. 31 (1966) 1975.
[9] T.R. Crompton, Gas Chromatography of Organometallic Compounds, Plenum Press, New York, 1982, pp. 74–113.
[10] J.H. Kindsvater, P.H. Weiner, T.J. Klingten, Anal. Chem. 46 (1974) 892.
[11] W.J. Evans, M.F. Hawthorne, J. Chromatogr. 88 (1974) 187.
[12] J. Čoupek, S. Pokorný, V. Gregor, V. Jehlička, J. Chromatogr., 88 (1974) 323.
[13] Z. Plzák, B. Štíbr, J. Chromatogr. 151 (1978) 363.
[14] D.L. Sauders, J. Chromatogr. 125 (1976) 163.
[15] Z. Plzák, J. Plešek, B. Štíbr, J. Chromatogr. 168 (1979) 280.
[16] E. Soczewiński, W. Gołkiewicz, J. Chromatogr. 118 (1976) 91.
[17] Z. Plzák, F.P. Doušek, J. Jansta, J. Chromatogr. 147 (1978) 137.
[18] Z. Plzák, J. Plešek, B. Štíbr, J. Chromatogr. 212 (1981) 283.
[19] B. Grüner, Z. Plzák, J. Plešek, S. Heřmánek, manuscript in preparation.
[20] A.K. Gianotto, W.F. Bauer, in A.H. Soloway, R.F. Barth, D.E. Carpenter (Editors), Advances in Neutron Capture Therapy, Plenum Press, New York, 1993, p. 459.
[21] S. Harfst, D. Moller, H. Ketz, J. Rösler, D. Gabel, J. Chromatogr. A 678 (1994) 41.
[22] K. Abu-Izza, D.R. Lu, J. Chromatogr. B 660 (1994) 347.
[23] B. Grüner, Z. Plzák, I. Vinš, J. Chromatogr. 588 (1991) 201.
[24] B. Grüner, Z. Plzák, I. Vinš, J. Chromatogr. 595 (1991) 169.
[25] J. Plešek, B. Grüner, P. Maloň, J. Chromatogr. 626 (1992) 197.
[26] B. Grüner, J. Holub, J. Plešek, T. Vaněk, H. Votavová, J. Chromatogr., in press.
[27] J. Plešek, B. Grüner, P. Maloň, Collect. Czech. Chem. Commun. 58 (1993) 1087.
[28] J. Plešek, B. Grüner, T. Vaněk, H. Votavová, J. Chromatogr. 633 (1993) 73.
[29] J. Plešek, B. Grüner, J. Fusek, H. Votavová, Collect. Czech. Chem. Commun. 58 (1993) 2936.
[30] J. Plešek, B. Grüner, S. Heřmánek, J. Fusek, H. Votavová, Collect. Czech. Chem. Commun. 59 (1994) 374.
[31] B. Grüner, J. Plešek, G.W. Kabalka (Editors), Current Topics in the Chemistry of Boron, Royal Society of Chemistry, Cambridge, 1994, pp. 371–374.
[32] R.A. Menges, D. W. Armstrong, in S. Ahuja (Editor), Chiral Separations by Liquid Chromatography, American Chemical Society, Washington, DC, 1991, p. 67; and references cited therein.
[33] D.W. Armstrong, A.M. Stalcup, M.L. Hilton, J.D. Duncan, J.R. Faulkner, S.C. Chang, Anal. Chem. 62 (1990) 1610.
[34] A. Franken, J. Plešek, J. Fusek, M. Semvan, Collect. Czech. Chem. Commun. 62 (1997) 1070.
[35] B. Grüner, V. Císařová, A. Franken, J. Plesek, unpublished results.
[36] E.M. Colombier, B. Bonnetot, H. Mongeot, Bull. Soc. Chim. Fr., (1988) 844.
[37] H.G. Srebny, W. Preetz, H.C. Marsmann, Z. Naturforsch. 39 (1984) 6.
[38] W. Preetz, H.G. Srebny, H.C. Marsmann, Z. Naturforsch. 39 (1984) 189.
[39] H.G. Srebny, W. Preetz, Z. Anorg. Allg. Chem. 513 (1984) 7.
[40] B.Grüner, Thesis, Institute of Inorganic Chemistry, Czechoslovak Academy of Sciences, Prague, 1990.
[41] B. Grüner, S. Heřmánek, Z. Plzák, Proc. Int. Meeting Boron Chemistry, Torun, Poland, July 30–August 3 1990, Nicolai Copernicus Univ., Torun, 1990, Abstr. Pos. 5.
[42] G.R. Wellum, E.I. Tolpin, L.P. Andersen, R. Sneath, J. Chromatogr. 103 (1975) 153.
[43] P.R. Haddad, A.L. Heckenberg, J. Chromatogr. 300 (1984) 357.
[44] M. Bown, B. Štíbr, B. Grüner, X.L.R. Fontaine, T. Jelínek, J. Holub, J.D. Kennedy, M. Thornton-Pett, J. Chem. Soc., Dalton Trans. submitted for publication.
[45] H. Engelhardt, Hochdruck-Flüssigkeits-Chromatographie, Springer-Verlag, Berlin, 1975, p. 60.
[46] J. Plešek, Z. Plzák, J. Stuchlík, S. Heřmánek, Collect. Czech. Chem. Commun. 46 (1981) 1748.
[47] B. Grüner, J. Michl, Z. Janoušek, Proceeding of the IX International Meeting on Boron Chemistry, July 14–18 1996, Ruprecht-Karls University Heidelberg, Germany, abstr. No. P 56, p. 149.
[48] Z. Janoušek, J. Fusek, B. Štíbr, J. Chem. Soc., Dalton Trans., (1992) 2649.