CHARLES UNIVERSITY IN PRAGUE

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Synthesis of new carborane and metallacarborane building blocks applicable in design of biologically active compounds

Syntéza nových karboranových a metallakarboranových strukturních bloků pro vývoj biologicky aktivních látek

Diploma Thesis

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PRONOUNCEMENT

I thereby declare that this thesis was written independently and under supervising by RNDr. Bohumír Grüner, CSc. I cited all used information sources and literature. This thesis or its any part was not introduced to claim another or same academic title.

In Prague 13th May 2014

Jan Nekvinda

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ABSTRACT

Compounds with carboxylic and amidic functions belong to basic structural blocks, which are used for construction of functional molecules in organic, organometallic and also in carborane chemistry. However, considering cobalt bis(dicarbollide)(1-) ion, the synthetic ways to these derivatives have been virtually unknown. A published procedure on lithiation in THF and reaction with CO_2 leading to mono- and dicarboxylic acids had failed in our hands. Nevertheless, a detailed revision of the experimental conditions provided finally good yields of mixture of both acids, which could be separated by chromatography and crystallization, and compound of general formulation $[(1\text{-}HOOC\text{-}1,2\text{-}C_2B_9H_{10})(1',2'\text{-}C_2B_9H_{10})\text{-}3,3'\text{-}Co(III)]^-$ and stereoisomeric mixture of $[(HOOC)_2\text{-}(1,2\text{-}C_2B_9H_{10})_2\text{-}3,3'\text{-}Co(III)]^-$ were characterized for the first time by combination of NMR, MS and HPLC. Also, the carboxylic acid derivatives with methylene and ethylene connectors of the general formula $[(1\text{-}HOOC\text{-}(CH_2)_n\text{-}1,2\text{-}C_2B_9H_{10})(1',2'\text{-}C_2B_9H_{10})\text{-}3,3'\text{-}Co(III)]^-$ were prepared by lithiation of Cs**1** in DME at low temperatures followed by reaction with $BrCH_2COOEt$ and subsequent hydrolysis of the resulting ester or by oxidation of the respective propylhydroxy derivative. The acids were converted to reactive *p*-nitrophenyl esters $[1\text{-}(1,4\text{-}NO_2C_6H_4OOC\text{-}(CH_2)_n\text{-}1,2\text{-}C_2B_9H_{10})(1',2'\text{-}C_2B_9H_{10})\text{-}3,3'\text{-}Co(III)]^-$, which readily reacts with various amines under mild conditions with formation of amidic bonds. The synthetic ways to these compounds open new possibilities in design of biologically active metallacarboranes addressing various therapeutic targets. Indeed, syntheses of new covalently bonded compounds were performed and research on biologically active derivatives is still in the progress.

CONTENT

LIST OF ABBREVIATIONS

6-APA	6-Aminopenicillanic acid
BNCT	Boron Neutron Capture Therapy
BPA	1-*p*-Hydroxyborylphenylalanine
COSAN	Cobalt Sandwich Anion
DCC	Dicyclohexylcarbodiimide
DFT	Density Functional Theory
DME	Dimethoxymethane
DMF	Dimethylformamid
DMS	Dimethyl sulphate
DMT	Dimethoxytrityl
ESI	Electrospray Ionisation
GIAO	Gauge Including/Invariant Atomic Orbitals
HIV	Human Immunodeficiency Virus
IC_{50}	Inhibition Concentration by 50%
IOCB	Institute of Organic Chemistry and Biochemistry AS CR, v.v.i.
KDN	2-Keto-3-deoxynonic acid
MO	Molecular Orbitals
Neu5Ac	*N*-Acetylneuraminic acid
Neu5Gc	*N*-Glycolylneuraminic acid
NHS	*N*-Hydroxysuccimide
NMR	Nuclear Magnetic Resonance
NSAID	Non-Steroidal Anti-Inflammatory Drug
PEG	Polyethylene glycol
PDT	Photodynamic Therapy
SAR	Structure-Activity Relationship
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography

1. INTRODUCTION

1.1 CARBORANES

Carboranes ("carbaboranes" according to IUPAC nomenclature recommendations) are polyhedral boron-carbon molecular clusters that are stabilized by electron-delocalized covalent bonding in the skeletal framework. Carbon atoms represented in carboranes by {CH} groups notionally replace isolobal $\{BH\}^-$ and are typically located sites possessing lowest connectivity in the cluster and in general contribute positively to the stability of the molecule.

Carboranes are known for more than half a century, but most of this time they had been the subject of main interest of mainly boron chemists (new skeletal types of reactions and transformations, isomerism, spectroscopy), theoreticians (structure and bonding) and small group of industrial researchers due to their potential to be included in heat-stable copolymers. This picture has changed in recent years, when carboranes have been experiencing a major surge in interest across a wide spectrum of emerging technologies, such as developing applications in medicine, nanoscale engineering, catalysis, metal recovery from radioactive waste and others. Exploring carboranes electronic properties, geometry and versatility leads to recognition, that carboranes chemistry affords a whole new realm of possibilities that outreached conventional organic or organometallic synthesis.[1]

Carboranes had been presented by Lipscomb and Hoffman from theoretical consideration[2,3] before first reports of the synthesis of any such compounds appeared. Much of the interest is focused on the icosahedron, a polyhedral with 12-vertex and 20-sided facets. As icosahedral B_{12} clusters and their fragments have been found in all known forms of elemental boron, metal borides and several boron hydrides (e.g. $B_{10}H_{14}$),[4] it was assumed that an icosahedral $B_{12}H_{12}$ hydride might exist. In 1955 it was calculated by Longuet-Higgins that two additional electrons would be required, stabilizing the icosahedron as a $B_{12}H_{12}^{2-}$ dianion.[5] Soon after the salts of $B_{12}H_{12}^{2-}$ were isolated by Pitochelli and Hawthorne[6] and have been found to be incredibly stable, withstanding temperature above 800 °C and inert towards most of reagents. Salts of this ion thus belong between the most stable molecules known to science. This was closely paralleled by discovery of two carbon insertion into open cage $L_2B_{10}H_{12}$ (L = Lewis base), which led to the first synthesis of neutral $C_2B_{10}H_{12}$ clusters, isoelectronic analogues of $B_{12}H_{12}^{2-}$ where two BH^- units are replaced by isoelectronic CH group whose carbon atoms are seemingly six-coordinated (quite surprising in 1960).

It is interesting that the first icosahedral carboranes had been prepared in industrial laboratories in the 1950s, but had been kept classified and thus remained unreported until 1963. These compounds were obtained in the course of an industrial effort to synthesize stable organic derivatives of boron hydrides under a post-World War II United States government program "Hermes". Their purpose were to develop practical borane-based aircraft and rocket fuels that could embrace much higher energies generated by combustion of boron hydrides compared to hydrocarbons.[7] In this content various hydrides were prepared (e.g. B_2H_6) and used to prepare a variety of alkylated derivatives to be tested as fuel additives. These projects were later abandoned because of products of combustion (such as solid boron oxide and boron nitride) and their effects on jet and rocket engines.

The icosahedral carboranes are only part of the story, as was discovered in laboratories around the globe. The three known $C_2B_{10}H_{12}$ isomers (Figure 1) are members of *closo*-carborane family (general formula $C_2B_{n-2}H_n$, n = 5-14). An isoelectronic monocarborane series of *closo*-$CB_{n-1}H_n$- anions (n = 6-12) also exists.

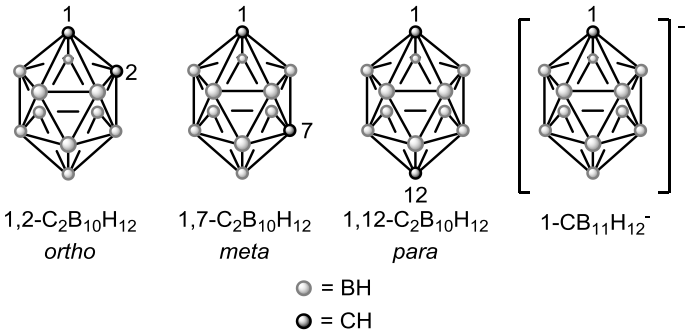


Figure 1: *The isomers of 12 vertex closo carborane.*

In addition, there is 10 vertex *closo* series and a plenty of open-cage carboranes whose cage structure are usually fragments of polyhedra. These include *nido* (Greek for nest), *arachno* (web) and *hypho* (net) clusters that are formally derived from *closo* frameworks by removal of one, two, or three vertexes (Figure 2). The isomerism in the location of carbon atoms is often observed.[1]

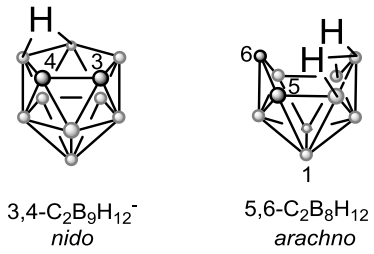


Figure 2: *Open frameworks of 12 vertex carborane.*

Finally, boranes and carboranes are able to incorporate almost every element in the Periodic Table (except Noble Gases and extremely electronegative or electropositive elements). There are phospha-, thia-, azacarboranes, etc. and metallacarboranes involving essentially all of the transition and lanthanide elements.[8,9]

The extension of electron-counting rules has been developed for multi-cluster systems featuring linked or fused polyhedral, in which two or more cluster units share vertexes, edges, or faces. In this context, the *mno* rule[10] has been consolidating: in a system having *m* polyhedral, *n* vertexes and *o* shared vertexes, the required number of electron pairs will be the sum of *m*, *n* and *o*. This rule is essentially confined to metal-containing clusters, like *commo*-metallacarboranes in which a metal atom occupies a vertex common to two polyhedral cages as in $Ni(C_2B_9H_{11})_2$.[11] Applying the *mno* rule to this nickel compound, we have $m = 2$, $n = 23$ and $o = 1$, so that 26 electron pairs are needed; these are supplied by the 4 CH, 18 BH and Ni units, which provide 12, 36 and 4 electrons.

The discovery of the extraordinarily robust $B_{10}H_{10}^{2-}$ and $B_{12}H_{12}^{2-}$ ions and their *closo*-carborane counterparts led to comparisons with aromatic hydrocarbons. Icosahedral $B_{12}H_{12}^{2-}$ in particular is often described as a three-dimensional inorganic benzene analogue in which 26 skeletal electrons occupy 13 filled bonding MOs; sometimes this is labelled "superaromatic".[12,13] In contrast to aromatic hydrocarbons, to which feature belong delocalized C-C π-bonding system, in polyhedral boron clusters the aromaticity is caused by delocalized system of three center bonds. Aromaticity in polyhedral boranes and carboranes has been widely explored theoretically[2,12] and is supported experimentally by their high stability, magnetic properties, NMR behaviour and susceptibility to electrophilic reactions.[13]

1.2 COBALT BIS(1,2-DICARBOLLIDE)

1.2.1 INTRODUCTION AND SYNTHESIS

The first synthesis of metallacarboranes was reported in 1965.[14] Two isomeric 18-electron low-spin d^6 cobalt bis(1,2- and 1,7-dicarbollides) [*commo*-3,3´-Co(III)(1,2-$C_2B_9H_{11})_2]^-$ (**1**) and [*commo*-2,2´-Co(III)(1,7-$C_2B_9H_{11})_2]^-$ (**2**) sandwiches were among the very first metallacarboranes synthesized (Figure 3).[15] The official IUPAC name of the compound 3,3-*commo*-3,3´-cobalta-bis(1,2-dicarba-*closo*-dodekabora)(1-)ate is often replaced in the literature by *semi*-trivial cobalt bis(1,2-dicarbollide)(1-) or trivial abbreviation COSAN, which stands for CObalt Sandwich Anion. Due to its high stability and diamagnetic character, chemistry of this ion is the most elaborated among the metallacarboranes.

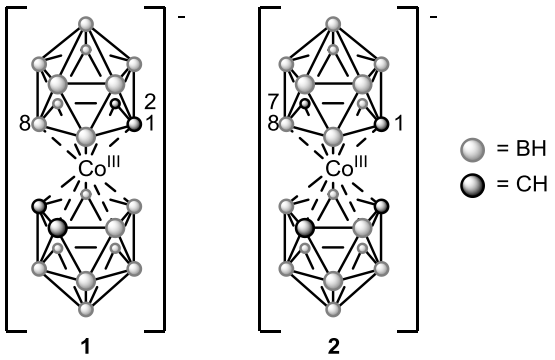


Figure 3: *Structure of cobalt bis(dicarbollide) anions.*

Crystal structure of Cs[3,3´-Co(1,2-$C_2B_9H_{11})_2$] was determined in 1967;[16] however, position of the carbon atoms were not refined with certainty. The first crystal and molecular structures of Et_3NH^+ and Cs^+ salts were determined soon after synthesis of this cobalt sandwich ion.[16] The recent crystallographic results report the hydrated sodium salt [3,3´-Co(1,2-$C_2B_9H_{11})_2$]Na·4 H_2O (Figure 4).[17] In this structure comprising the parent ion, sodium atoms with coordinated water, form infinite chains surrounded by watts where cobalt bis(dicarbollide) ions are located.[17]

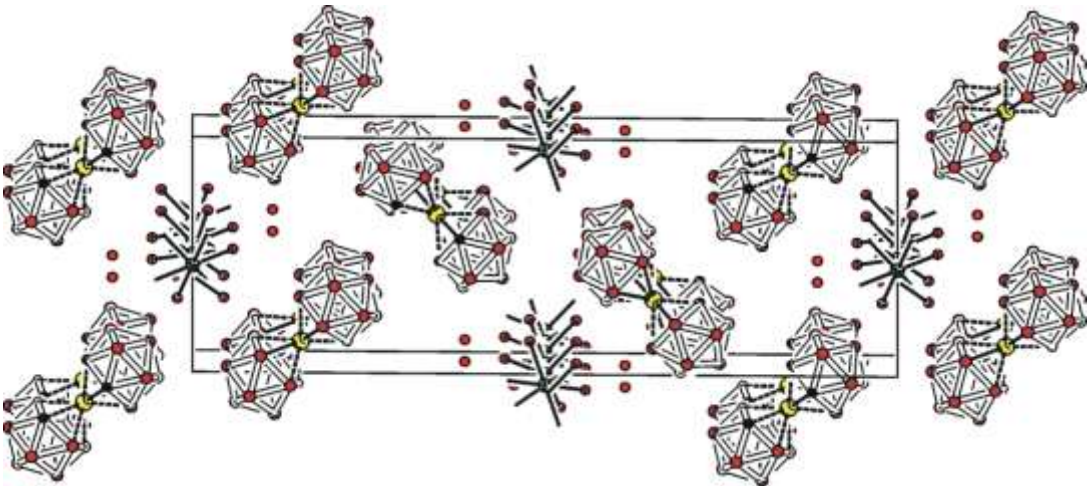
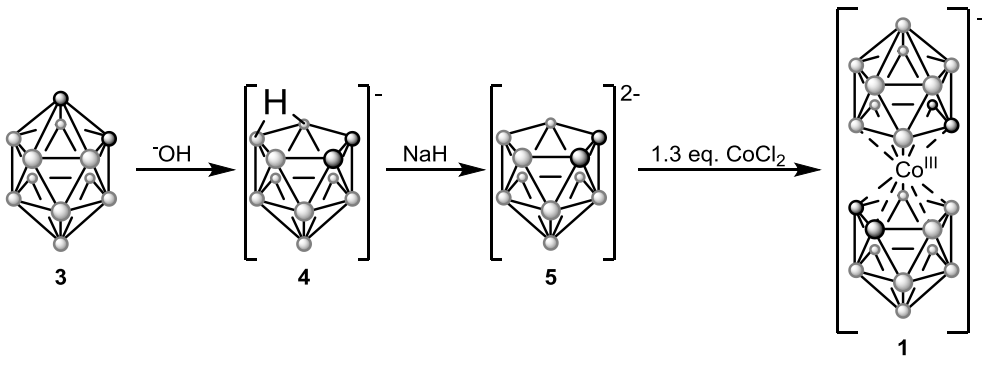


Figure 4: *X-ray structure of Na[3,3′-Co(1,2-$C_2B_9H_{11}$)$_2$]. Hydrogen atoms are omitted for clarity.*

The anion **1**$^-$ consists of two $(C_2B_9H_{11})^{2-}$ (dicarbollide) units sandwiched around a formally trivalent Co(III) ion. The vector distances from cobalt to the C_2B_9 planes are almost identical (1.466 and 1.476 Å), and the metal is approximately equidistant from the facial boron and carbon atoms (Co-C 2.046, Co-B 2.097 Å). The two $(C_2B_9H_{11})^{2-}$ ligands freely rotate in solution but three distinct thermodynamic minima have been found, the lowest corresponds to their mutual rotation by approx. 37° (*cisoid* conformation) found in majority of crystal structures. This cause that molecule has approximate overall C_2 symmetry.

Also, the anion has been characterized by the methods of UV,[18] IR,[19] Raman,[20] NMR,[21] X-ray photoelectron[22] and X-ray spectroscopy.[23]

The classical approach to the **1** consist in the partial degradation of *o*-carborane (**3**) with base into *nido*-[7,8-$C_2B_9H_{12}$]$^-$ (**4**) followed by its deprotonation to the *nido*-[7,8-$C_2B_9H_{12}$]$^{2-}$ (**5**) and insertion of metal *via* reaction with $CoCl_2$ (Scheme 1).[24]

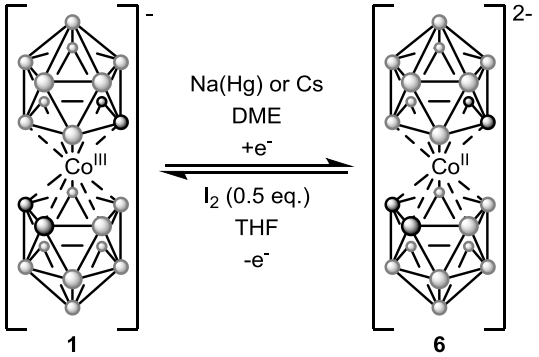


Scheme 1: *The synthesis route leading to COSAN.*

1.2.2 CHEMISTRY

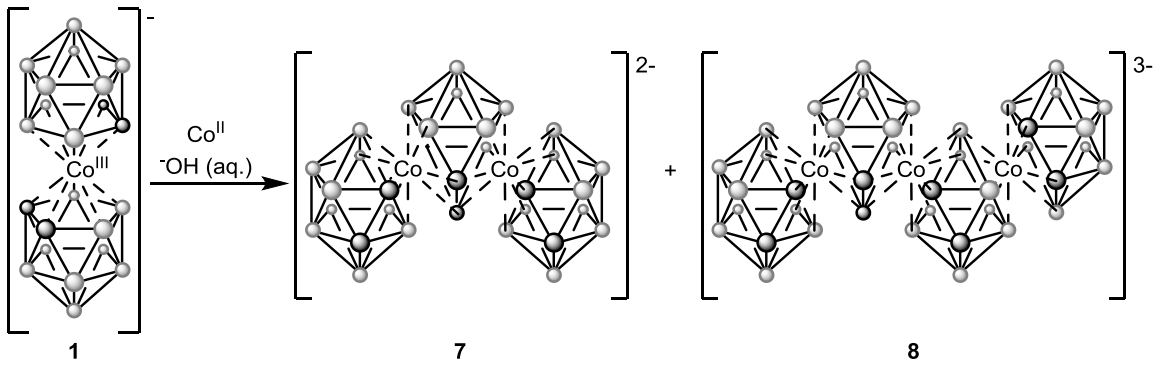
In the following text, I would like to mention same basic chemistry of COSAN (**1**) at its two specific reaction centres; at the carbon atom (C1) and the boron atom at the opposite side of cage (B8). Also, there is a plenty of reductive/oxidative reaction and reaction leading to the three or four carbollide clusters.

Reduction of **1** with sodium amalgam or caesium metal in DME forms the brown cobalt(II) dianion (**6**), which reverts back to yellow-orange **1** upon action of iodine in THF (Scheme 2).[25]



Scheme 2: *Redox reaction affects COSAN (**1**).*

Refluxing **1** with $CoCl_2$ in 30% aqueous alkali results in cobalt-linked multicluster species $Co_2(C_2B_9H_{11})_3^{2-}$ (**7**) and $Co_3(C_2B_9H_{11})_4^{3-}$ (**8**) containing one boron degraded central parts corresponding to the so called "canastide" ion (from Spanish word for basket) (Scheme 3).[26,27]



Scheme 3: *Preparation of multi-cluster compounds **7** and **8**.*

Until recently, a general approach to synthesize C-substituted derivatives of COSAN (**1**) consisted in preparation of the corresponding substituted *o*-carboranes, their degradation into the *nido*-7,8-dicarbaundecaborates, followed by deprotonation and reaction with $CoCl_2$.

A mixture of two geometric isomers (a racemic mixture and a *meso* form) arises from initial carboranes with one substituent.[28] This approach was used by Hawthorne *et al.*[29] to synthesize metallacarboranes with C-bridged dicarbollide ligands (Figure 5).

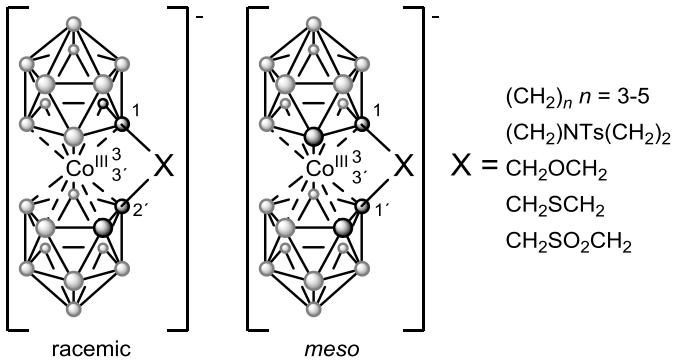
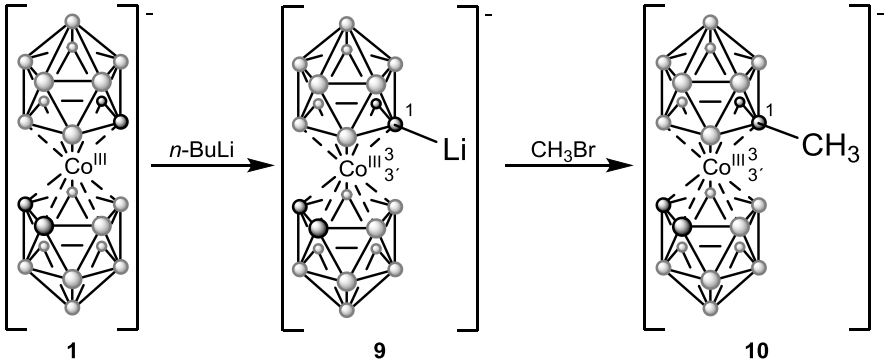


Figure 5: *C-bridged dicarbollide ligands of **1**.*

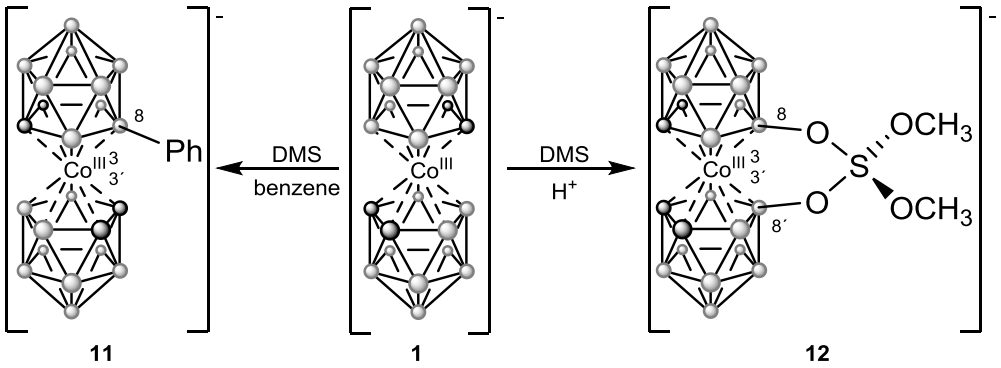
Another way how to prepare C-substituted derivatives of **1** is treatment with *n*-butyllithium (**9**) followed by the reaction with alkyl halides (**10**) (Scheme 4).[25] Due to the ratio of *n*-BuLi, mono- (1 eq.) or disubstituted (2 eq.) derivatives are obtained. Nevertheless, due to impossibility to reproduce these syntheses under describes reaction conditions, this approach had remained abandoned until recently. The interest about these pathways was further promoted by recent report on synthesis of bridged silyl and phosphine derivatives using modified procedure.[30-35]



Scheme 4: *The alkylation of COSAN (**1**) via lithiation followed by the reaction with alkyl halide.*

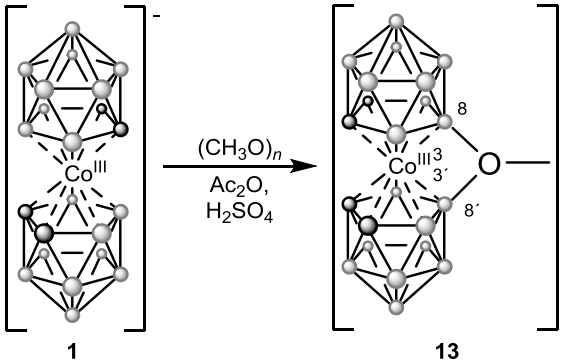
Until recently, substitutions at boron atoms of **1** have been much more studied as the only direct ways how to modify the COSAN. As a rule, the substitution proceeds at the boron atoms with maximum electron density.[15] These substituting reactions proceed due to reactivity toward nucleophiles in the presence of strong electrophile to form B(8)-substituted and B(8,8′)-disubstituted derivatives (such as **11** and **12**) (Scheme 5).[26] In these reactions, the

attacking electrophile (very often proton) removes the hydridic B(8)-hydrogen, creating a vacancy that is filled by the electron donor. It is possible that in some instances this substitution proceeds by electrophilic mechanism. Some examples are shown below.



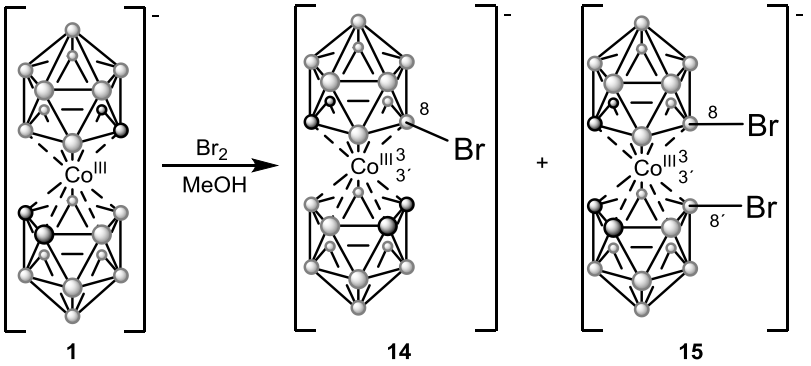
Scheme 5: *Deprotonation of COSAN (**1**) followed by reactions with various reagents leads to mono- (**11**) and disubstituted (**12**) products.*

Heating **1** with paraformaldehyde in acetic anhydride in the presence of sulfuric acid results in the formation of bridged oxonium derivative [8,8′-μ-(CH_3O)-3,3′-Co(1,2-$C_2B_9H_{10}$)$_2$] (**13**) (Scheme 6).[25]



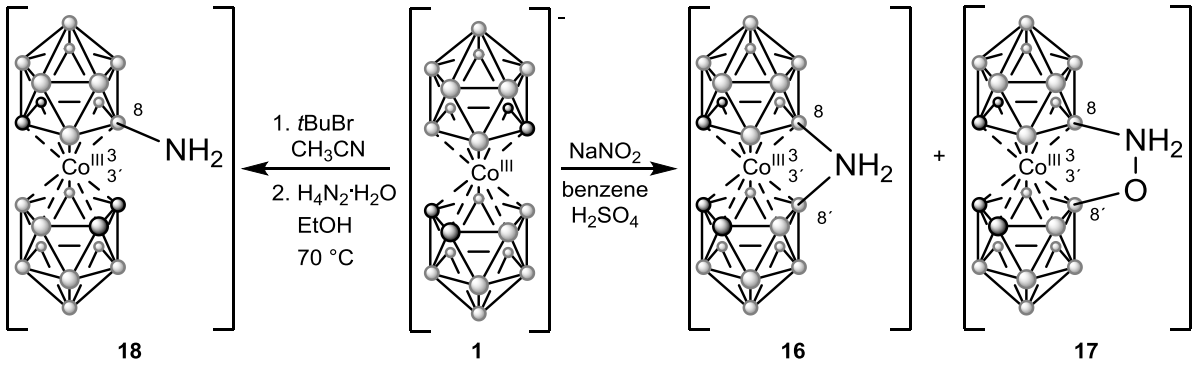
Scheme 6: *The oxonium bridged product **13** after reacting COSAN (**1**) with paraformaldehyde in acidic conditions.*

The halogenation of **1** is also known to chemists. For example, the reaction with bromine in methanol at the room temperature (Scheme 7) was explored. Resulting product **14** or **15** depends on the ratio of the reagents.[36,37]



Scheme 7: *Formation of mono- (**14**) and dibromo (**15**) derivatives of **1**.*

Also, the amination of **1** was developed. Treatment **1** with $NaNO_2$ in a mixture of benzene and sulfuric acid[38,39] produces mixture of products [8,8′-μ-(NH_2)-3,3′-Co(1,2-$C_2B_9H_{10})_2$] (**16**) and [8,8′-μ-{H_2NO-*N*,*O*}-3,3′-Co(1,2-$C_2B_9H_{10})_2$] (**17**) and the insertion of nitrile and subsequent reduction[40] gives product [8-(NH_3-1,2-$C_2B_9H_{10}$)-(1′,2′-$C_2B_9H_{11}$)-3,3′-Co] (**18**) (Scheme 8).



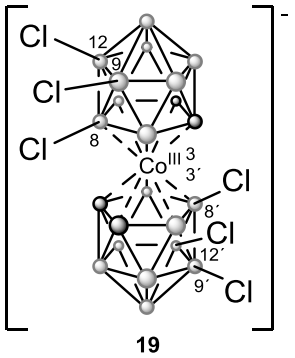
Scheme 8: *The synthesis of two zwitterionic bridged aminoderivatives of COSAN (**1**) **16, 17** and ammonium derivative **18**.*

1.2.3 USE OF COBALT BIS(1,2-DICARBOLLIDE)

Carboranes and metallacarboranes are widely used in fields such as polymer science, catalysis and medicine. In these fields, the properly designed carborane derivatives can provide advanced solutions and properties that are superior to these provided by conventional organic and inorganic chemistries.[55] Because the medicinal use will be mentioned later and polymer science and catalysis are out of range of this thesis, only few, but rather important, uses will be mentioned in next lines.

Substituted derivatives of COSAN (**1**) are the effective agents to recovery metals from radioactive waste, such as ^{90}Sr (half-life 29 years) and ^{137}Cs (30) along with lanthanides and

actinides, and has been demonstrated at industrial scale in Russia and USA. The whole area was recently reviewed in two book chapters.[1] The solution properties of this hydrophobic sandwich complex is extraordinary: it is capable to quantitatively transfer almost all monovalent ions from a 0.5 M aqueous solution of diethyl ether on shaking with an equal volume of the latter solvent.[41] The anion **1** is often employed as a nearly noncoordinating counterion as in study of dendritic species in a water-dichloromethane system.[42] In 1974 the Czech chemists as first applied parent COSAN (**1**) and its derivatives, namely hexachloro derivative 3-Co(1,2-$C_2B_9H_8Cl_3)_2$ (**19**) (Figure 6), to solvent extraction of radionuclides. It was found, that derivative **19** is stable toward 10M HNO_3 (while the parent ion **1** is not), allowing its use in industrial-scale removal of nuclides such as $^{137}Cs^+$ and $^{90}Sr^{2+}$.[15,41,43-45]



19

Figure 6: *Hexachloro derivative (**19**) of the cobalt bis(1,2-dicarbollide) (**1**) used for removal of nuclides from radioactive waste.*

The liquid-liquid extraction of cations of Group 1, 2, lanthanide and actinide elements, as well as Pb, Zn, Ag, and other metals, employing **19** and its derivative anions together with crown ethers, calix[*n*]arenes, polyethers as synergic agents has been extensively investigated.[46-57] A favoured solvent is nitrobenzene because it promotes selectively toward desired metal cations.[28,60,61] However, this is ecologically unfriendly. The radionuclide-extraction properties of analogous bis(dicarbollyl) complexes of other metals including Fe and Ni[28,61] have been explored, as well as functionalized derivatives of parent anion **1** having aromatic, ether or other substituents on the cage.[28,61]

Also, the metallacarboranes-based ionic liquids have been described, but until now only in several sole examples. As the example could be mentioned 1-alkyl-3-methyl-imidazolium salts of cobalt bis(1,2-dicarbollide) (**1**), which are liquids at room temperature and have very low glass transition points; the low melting points in these salts are attributed to poor crystal packing.[62]

There have been proposed many newly emerging applications of carboranes and metallacarboranes in several other fields, such as nonlinear optical materials, electroactive systems, films and monolayers, biomaterials or carborane-based ceramics.[1]

1.3 CARBORANES AND MEDICINAL CHEMISTRY

The applications of carboranes in medicinal area form promising concept, which is currently far from being explored, but there has been considerable movement in this direction. In recent years, a number of reviews have been published on various topics in this area. They mainly cover the use of icosahedral carboranes as hydrophobic pharmacophores replacing phenyl rings in a variety of existing drugs.[63-68]

Pioneering research in this area of biologically active carboranes reported an introduction of phenylalanine analogue *closo*-L-carboranylalanine into the peptide inhibitors of chymotripsin. Later, carboranes were employed in ligands for receptors in peptide hormone like bradykinin, angiotensin II and enkefalin.[69]

Most advanced have been the studies of $C_2B_{10}H_{12}$ analogues of steroids. Endo`s group carried out over last 15 years and leading to novel ligands for estrogen, androgen and vitamine D receptors. Systematic exploration of SAR led to development of both estrogen agonists and antagonists; some of them selective for individual estrogen receptor isoforms. Icosahedral carborane estrogen agonists were active in mouse model of uterus atrophy and osteoporosis.[70-72] Carborane analogue of selective estrogen receptor modulator tamoxifen was also prepared.[69] Other drugs with $C_2B_{10}H_{12}$ moiety inhibit retinoid[74] and adenosine[75] receptors. Enzyme targets of $C_2B_{10}H_{12}$ inhibitors include carbonic anhydrase and aldoketoreductase[73] (asborine – an analogue of aspirin).

An interesting case is the development of $C_2B_{10}H_{12}$ derivatives for the treatment of *transthyretin amyloidosis*.[76] NSAIDs were reported to bind hydrophobic pockets of the protein and prevent its misfolding and aggregation. This showed for the first time that replacement of phenyl ring by $C_2B_{10}H_{12}$ can provide compounds with similar effect but without unwanted inhibitory activity against cycloxygenase.

Charged *nido*-carborane $C_2B_9H_{12}^-$ derivatives were studied for example as inhibitors of carbonic anyhydrase[77] and estrogen receptor (analogue of tamoxifen, boroxifen).[73]

Anyway, this area is too large and in the following text I would like to mention only some features which are most important or correspond to topics of my thesis.

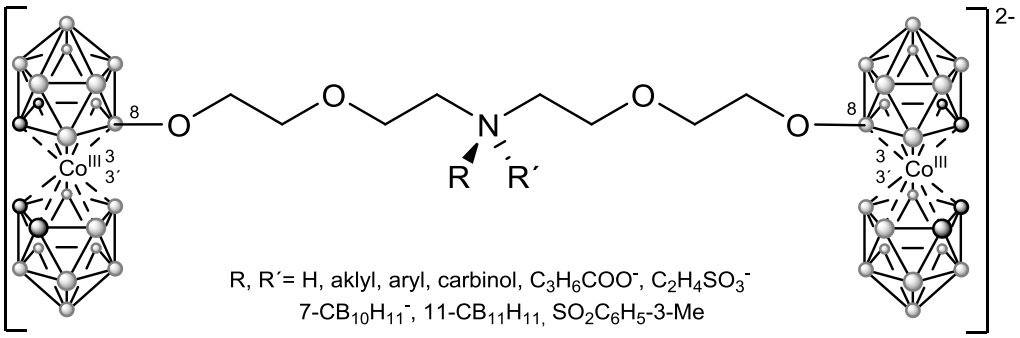
A number of general properties of carboranes lend themselves to exploitation in medicine:

- thermal and chemical stability in a variety of environments without degradation, low toxicity;[1]

- lipophilic and hydrophobic character arising from the hydridic nature of the BH bond is advantage in various of biomedical applications;[1]
- high boron content affords efficient delivery of ^{10}B isotope to target cells for boron neutron capture therapy (BNCT);[1]
- significant pharmacological activity has been observed in a number of designed carborane and metallacarboranes derivatives as a way to create of novel and revolutionary classes of drugs.[1]

1.3.1 HIV PROTEASE INHIBITORS

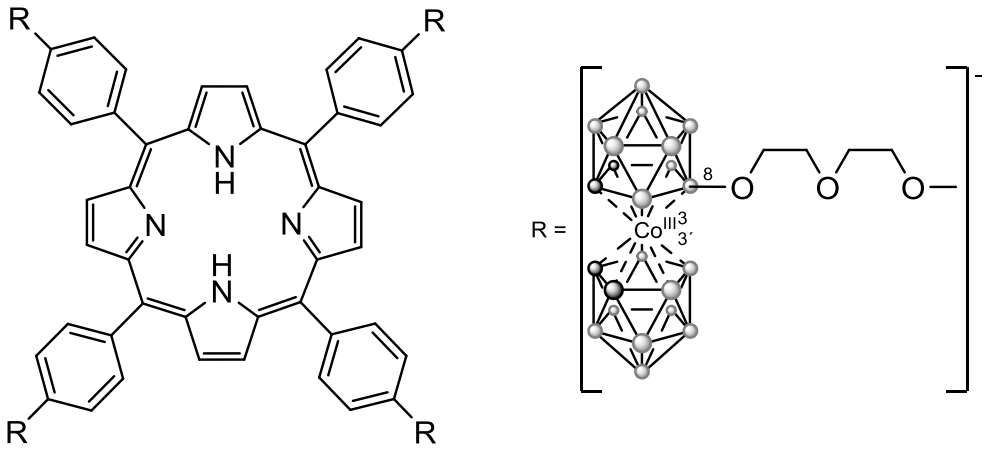
A potentially important biomedical application in metallacarborane chemistry has emerged from the observations that certain functionalized derivatives of the cobalt bis(1,2-dicarbollide) anion (**1**) can act as specific and efficient inhibitors of HIV-1 protease.[78-86] Current anti-HIV treatment using organic site active drugs, based on mimicking the neutral substrate, faces to a serious problem connected with side effects and a resistance development and thus, boron cluster compounds offer another alternative due to abiotic concept and distinctly different mechanism of their action. Cooperation between several laboratories in Czech Republic has demonstrated that sodium salt of water-soluble anions containing pairs of linked bis(dicarbollyl) sandwiches, such as **20** and related species are effective variants of inhibitors of HIV-1 protease that are resistant to conventional drugs. As was told above, metallacarboranes salts show relatively low toxicity, very good bioavailability and high stability in biological systems. Crystallographic data on the complex formed by the parent anion (R = R´= H) and the HIV protease revealed the novel mode of binding of the anion to hydrophobic pockets formed by side chains of the HIV protease. On the basis of this information combined with computational studies,[79,80] the hydrophobic interaction was increased further by substitution on the cages, resulting in even higher levels of selectivity and inhibition (Figure 7).[84]



20

Figure 7: *The cobalt bis(1,2-dicarbollide) sandwich anions (**20**) linked via ether chain with different substitution at nitrogen atom show as effective inhibitors of HIV-1 protease.*

A closely related, but different class of HIV-1 inhibitor contain tetraphenylporphyrin cores with one or four cobalt bis(1,2-dicarbollide) (**1**) anionic substituents (Figure 8).[87] This compounds show interesting properties in solution, being monomeric in methanol and forming triplet states and singlet oxygen on excitation, but quickly forming aggregates in aqueous media. Both compounds are effective noncompetitive HIV-1 protease inhibitors with high specificity; in addition, they may find possible use in boron neutron capture and photodynamic therapies (BNCT and PDT).



21

Figure 8: *Tetraphenylporphyrine core with cobalt bis(1,2-dicarbollide) substituent (**21**).*

1.3.2 BORON NEUTRON CAPTURE THERAPY

For many decades, application of boron chemistry in medicine was aimed only on the BNCT approach for cancer treatment, which exploits a remarkable and unique property of the ^{10}B nucleus. This isotope (~20% of boron in nature) is itself nonradioactive but combines with

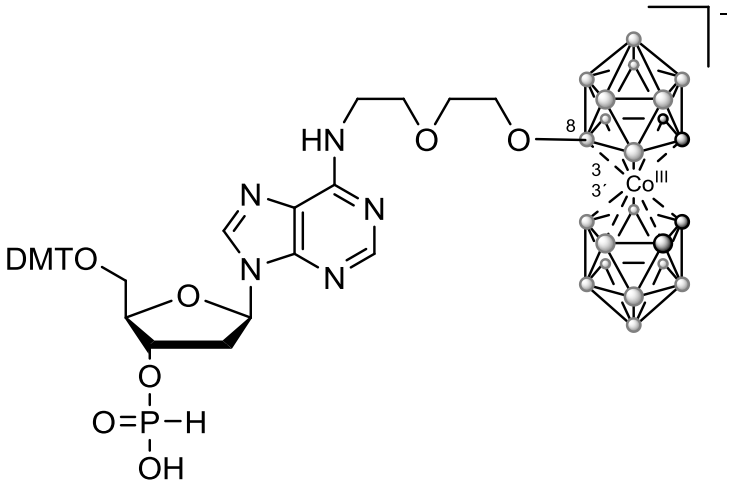
a large cross-section for low-energy neutrons to generate high-energy α particles that are capable of destroying cellular constituents within a radius corresponding to approximately one cell diameter (5 - 9 μm).[1] This reaction, first outlined in 1935,[88] soon came to the attention of physician G. L. Locher,[89] who recognized its potential to be a treatment for inoperable tumors in which two apparently benign agents, boron and slow neutrons, are brought together within tumor cells that are then selectively destroyed while leaving neighbouring healthy tissue intact. However, at that time and for years thereafter, boron compounds with the required combination of low toxicity, solubility, stability in aqueous media and high boron content did not exist until the discovery of the extraordinary stable polyhedral $B_{12}H_{12}^{2-}$ anion by Hawthorne and Pitochelli in 1960.[6]

With the availability of these ions along with appropriately functionalized carborane derivatives and other boron compounds, a serious research effort targeting BNCT as a viable treatment for cancer was launched in the United States and other countries and has continued to the present time. Unfortunately, the BNCT has yet to receive U.S.'s FDA or European authority's approval for general clinical application. Some success was reported in the treatment of brain cancer patients in Japan by H. D. Hatanaka, who has access to a nuclear reactor and employed BNCT using the mercapto-substituted anion $B_{12}H_{11}SH^{2-}$ against surgically exposed gliomas.[90,91] However, some other experimental clinical studies carried out in Europe and US suggested that the survival rate was not significantly different from that expected for untreated patients.[92]

Larger scale clinical trials in the U.S., Japan and Europe have provided results that are comparable to those achieved by using conventional radiation therapy.[64] It should be noted, that these studies have been grossly limited in both, in their extent and in the selection of compounds that have been applied. First, they have been largely confined to patients with high-grade brain tumors such as *glioblastoma multiforme*, an invariably fatal cancer for which no effective treatment is known; BNCT remains essentially untested clinically against most other forms of cancer. Second, BNCT trials on human patients have been restricted to just three compounds dating to the 1960s or earlier, none specifically designed for use against the tumors of interest, consisting of a simple boron species, 1-*p*-hydroxyborylphenylalanine (BPA), and the disodium salts of $B_{12}H_{11}SH^{2-}$ and $B_{10}H_{10}^{2-}$. While the first two show some selectivity toward tumor cells and, in case of BPA, an ability to pass the blood-brain barrier, in truth these compounds are relics of an earlier, primitive era in BNCT research. Despite the current availability of wide library of carborane and metallacarboranes derivatives designed

specifically for BNCT application, some of which have shown very persuading results in animal studies, none has reached the stages of approval for studies in human yet.[1]

As an example I would like to mention nucleoside-metallacarborane derivative presented by Lesnikowski[93], which could serve as BNCT candidate; namely bis(dicarbollyl)cobalt deoxyadenosine derivative (**22**) that can be converted to DNA-dinucelotides (Figure 9). This approach was developed by J. Plešek and co-workers for polyhedral borane derivatives and consists of cyclic ether ring-opening reactions.[94-96]



22

Figure 9: *Bis(dicarbollyl) deoxyadenosine (**22**) conjugate targeted to BNCT.*

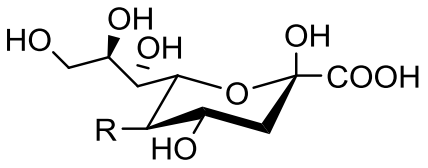
1.4 ORGANIC COMPOUNDS AS POTENTIAL AMINO GROUP DONORS

The living world is based on plenty of interesting organic compounds and many of them are in the spotlight of the medicinal chemistry due to specific interactions with various receptors. This field is one of the most competitive and dynamically developing in the last decades. The drug development of the organic compounds and their derivatives is the main way how to achieve successful drug design. In the following text, I would like to present two very important biologically active organic groups, which form currently the main targets of our research, i.e. *N*-acetylneuraminic acid and 6-aminopenicillanic acid for considerations of their cross-combinations with cluster molecules.

1.4.1 *N*-ACETYLNEURAMINIC ACID

N-Acetylneuraminic acid (Neu5Ac) is a part of sialic acid family. Sialic acid is a term established by Swedish biochemist G. Blix in 1952 and it stands for saliva in Greek. It is compound with the most complicated molecule structure among the monosaccharides. Sialic acids are found widely distributed in animal tissues and also in other organisms, such as plants, fungi and even bacteria. They are mostly in glycoproteins and gangliosides.[97] In humans, the highest sialic acid concentration is in brain and they have an important role in neural transmission and ganglioside structure in synaptogenesis.[97]

Primarily, it is deoxy uronic acid consisting of the nine carbons and appears in the 43 *N*- or *O*- substituted derivatives. They unusually appear free in nature, but as components of oligosaccharide chains of mucins, glycoproteins and glycolipids in external and internal membrane areas.[97] The most common sialic acids are Neu5Ac (**23**), *N*-glycolylneuraminic acid (Neu5Gc) (**24**) and deamino derivative 2-keto-3-deoxynonic acid (KDN) (**25**). These derivatives are present in solution mostly as β-anomer (90%) (Figure 10).[98]



23-25

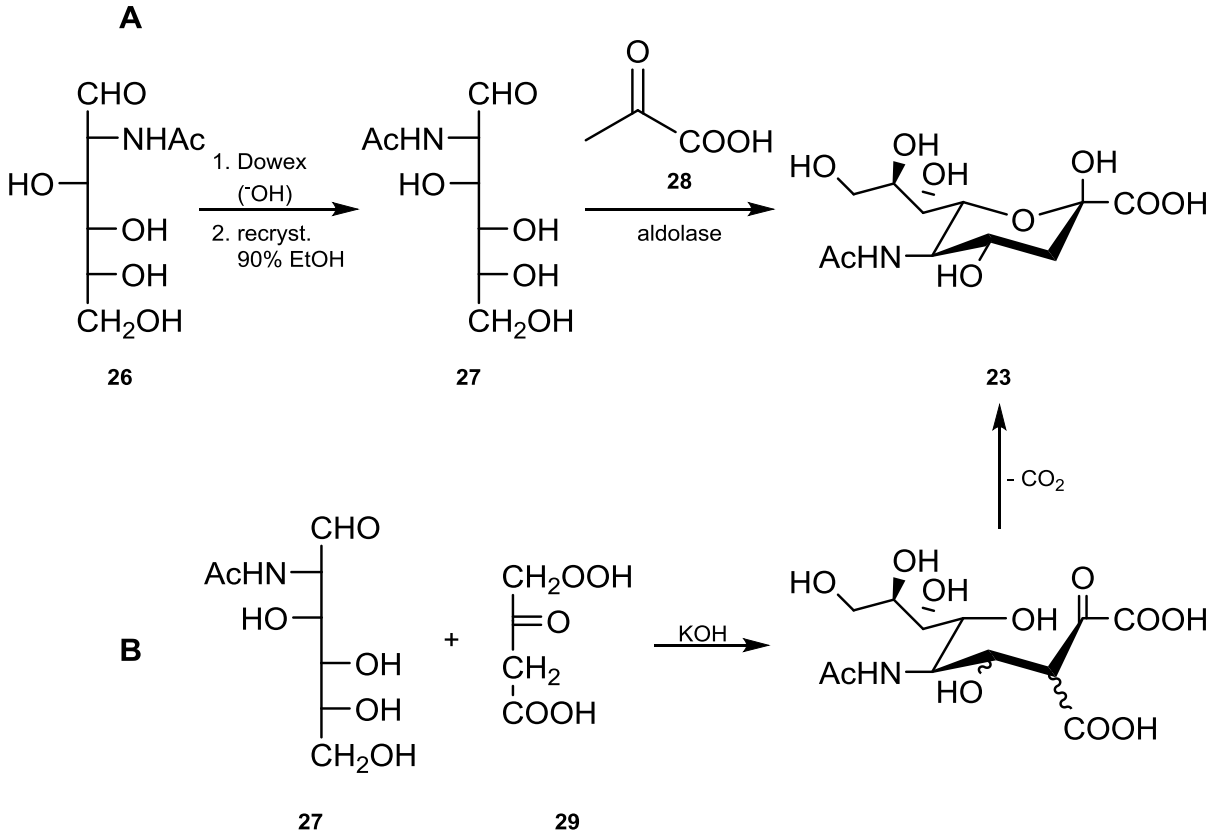
23 Neu5Ac NHAc
24 Neu5Gc R = $NHCOCH_2OH$
25 KDN OH

Figure 10: *The most common derivatives of sialic acid **23-25** presented in β-anomer form.*

N-Acetylneuraminic acid can be prepared both, the enzymatic (**A**) or chemical (**B**) synthesis.

The enzymatic way consists from epimerization of *N*-acetylglucosamine (**26**) to *N*-acetylmannosamine (**27**) and aldol condensation with pyruvate (**28**) (Scheme 9).[99-104]

Oxalacetic acid (**29**) with greater nucleophilicity is used instead of pyruvic acid in chemical synthesis and then aldol condensation with *N*-acetylmannosamine (**27**) under the basic condition and Neu5Ac (**23**) is formed *via* decarboxylation (Scheme 9).[105-108]



Scheme 9: *The enzymatic (**A**) and chemical (**B**) synthesis of Neu5Ac (**23**).*

N-Acetylneuraminic acid is involved in preventing infections (mucus membranes in mouth, nose, respiratory tract, etc.) and also acts as a receptor for influenza viruses. It is mediated by allowing attachment to mucous cells *via* hemagglutinin (an early step in acquiring influenza virus infection). [109,110]

1.4.2 6-AMINOPENICILLANIC ACID

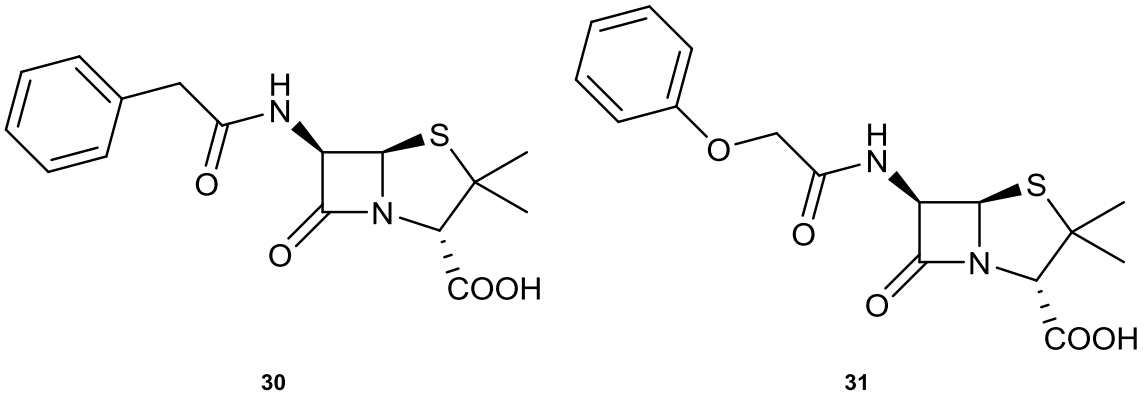
Until the 1957 only two types of compounds was known, penicillin G and penicillin V. The development of industrial processes to cleavage the active part off the molecules, the 6-APA from the products of fermentation, enable wide possibilities to semi-synthetize more than 20 different approved penicillins that became one of the most important groups of

antibiotics in clinical practice. Also, it enhanced development of other types of β-lactam antibiotics. This discovery saved probably millions of lives and a massive reduction in morbidity from infection.[111]

The β-lactam antibiotics as a group are active against the majority of bacteria causing infections in all systems of the body.[111]

Research on the preparation of new penicillins that might have advantageous properties has quite a long history. In the early work on penicillin fermentation in the 1940s it was found that the mould *Penicillinum chrysogenum* produced not just one penicillin, but a small family of penicillins differing only in the nature of an acyl side chain attached to a fused β-lactam thiazolidine ring system, which has become known as the penicillin nucleus. These penicillins are designated by letters, e.g. G (**30**), V (**31**), X.[111]

It was then discovered that addition of phenylacetic acid to the fermentation medium resulted in the preferential formation of penicillin G, the phenylacetic acid being accepted by a mould for the particular side chain present in penicillin G (**30**), namely benzyl group (Figure 11).[111]



30 **31**

Figure 11: *Penicillin G (**30**) and V (**31**) and their different acylamino-group attached to β-lactam thiazolidine ring system.*

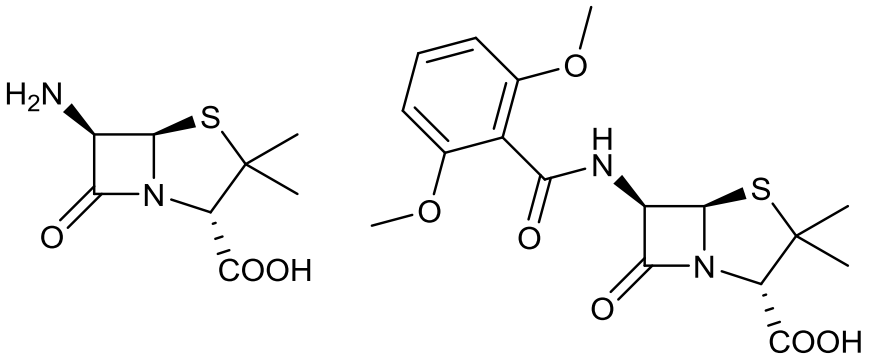
Research showed that a great part of new penicillins could be produced if the desired side chain structure was provided in the fermanetation medium in the form of precursor.[112]

The studies to prepare new derivatives of penicillins were carried out with the possibility of chemical modification in *p*-aminobenzoyl penicillin. The amino group at the *para* position provides important point through which these modifications could be realized.[113] The *p*-aminobenzyl penicillin itself was prepared by fermentation using *p*-aminophenylacetic acid as the precursor and the production of his penicillin was followed using two different assay procedures. One was the conventional cup-plate bioassay and the

other was a chemical method not depending on the antibacterial activity of the penicillin. It was found that when the side chain precursor was present these two assays gave very similar results, but when the side chain precursor was absent the chemical way gave a very much higher value than the bioassay.[114] Interpretation of these results was that under fermentation conditions in which there was a lack of available side chain structures, the nucleus of the penicillin molecule was produced without any side chain attached. Such a compound could be expected to be accessible by the chemical way. This interpretation proved to be correct and the compound was identified as 6-APA (**32**) (Figure 12).[115]

In 1957 was found that a penicillin deacylase was produced by a number of actinomycetes as well as certain filamentous fungi. This deacylase split penicillin G (**30**) very slowly, but penicillin V (**31**) was deacetylated very readily.[116] A process for 6-APA production was developed using a deacylase from *Streptomyces lavendulae* and penicillin V as a substrate. Subsequently, a deacylase of bacterial origin was discovered[116-119] penicillin G was deacetylated very readily. Later, a process for chemical deacetylation of penicillin was developed.

The first semi-synthetic penicillin stable to staphylococcal penicicillinase and clinically effective against penicillin-resistant *Staphylococcus aureus* was methicillin (**33**) (Figure 12).[120]



32 **33**

Figure 12: *The structure of 6-APA (**32**) and first effective penicillin against the staphylococcus infection, methicillin (**33**).*

2. AIMS OF THE THESIS

We tried to develop new synthetic pathways for preparation of various acids derived from cobalt bis(1,2-dicarbollide). Subsequently, we focused on functionalization of these acids into the active esters. Another task consisted in explorations of model reactions with various amines leading to formation of previously inaccessible amidic bond between metallacarborane cluster and aliphatic, aromatic or others moieties, such as derivatives of Neu5Ac or 6-APA. This could be summarised:

1. Development of new effective ways to prepare set of carboxylic acids of cobalt bis(dicarbollide) with variable length of aliphatic chain.
2. Esterification of the respective acids by easily leaving residues resulting in active esters capable to react under mild conditions with amino functions.
3. Evaluation of the scope of amidic bonds formation using active esters derived from cobalt bis(1,2-dicarbollide) and various amines.

3. RESULTS

As was reported in Chapter 1.2.2, alkylation at the carbon atom of the cobalt bis(1,2-dicarbollide) (**1**) has been described.[25] In analogy with main known synthetic modifications on carboranes this procedure consisted in the lithiation of the C-atom vertexes followed by the reaction with alkylating agent. Nevertheless, direct lithiation reactions on cobalt bis(dicarbollide) do not proceed so easily and are rather sensitive to reaction conditions. This is apparently why the described results of alkyations could not be later reproduced by numerous examiners. However, the recently published study has shown that under strictly controlled conditions C-C bond formation using lithiated COSAN **1** and highly reactive *para*-formaldehyde, oxirane or trimethylene oxide is feasible producing various alkylhydroxy derivatives.[121] This procedure depends on the lithiation of the C-atom followed by the reaction with alkylating agent. This method was used in this research to insert carboxyl function group into the metallacarborane structure. Also, this method was used 15 years ago the carboxylations *via* the lithiation of COSAN in THF followed by reaction with CO_2 led to mono- and dicarboxylic acid and acid chlorides of the COSAN **1**.[122] Surprisingly, this paper was published without any details on the characterisation of these compounds with expectation of their IR spectra, which however are insufficient in case of cluster species. Despite we initially failed to reproduce these results, detailed revisions of the reaction conditions led to good yield isolations of mono- and dicaboxylic acids their full NMR and MS characterisation.[123]

In addition, two new acids were prepared and characterized and synthesis of their *p*-nitrophenyl group based active esters is described. The chemistry of active esters has been developing in organic chemistry over last years as the tool for clean and quantitative introduction of functional groups, often on multiple sites available in the same molecule, e.g. at the calix[4]arene platform.[124] However, no analogy on use of such derivatizations could be found in boron cluster chemistry. We developed synthetic procedures which made readily available active esters based on cobalt bis(dicarbollide) in good yields. Furthermore, reaction between active esters and various amines under the mild conditions were elaborated. This led to synthesis of a series of metallacarborane based amides and dicluster molecules tested in vitro as HIV-PR inhibitors at IOCB.[123] The compounds have shown IC_{50} values 79±2 nM (**XIII**) and 82±2 nM (**XIV**), which is comparable to values of previously published inhibitors.[78,81]

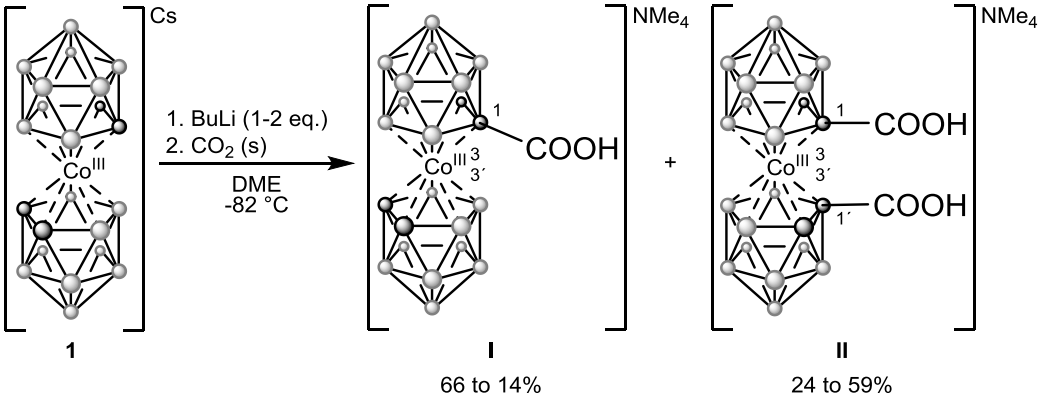
Attempts to react active esters of cobalt bis(dicarbollide) with biologically active *N*-acetylneuraminic acid and 6-aminopenicillanic acid have been performed. 6-APA is basically penicillin nucleus and coupling it with biologically stable metallacarborane might provide assumed enhanced activity and better stability towards β-lactamase enzyme and thus towards bacterial strains resistant to antibiotics. Unfortunately, this intention was not carried to successful stages of compound's isolation yet. Despite of MS observation of the almost quantitative formation of the assumed product in the reaction mixture, its separation from organic and inorganic species became rather difficult. This can be due to hydrolytic instability of the linker or the β-lactam ring. Therefore, further time consuming developments of the different linker or improvements of milder isolation procedures would be necessary. The assumed formation of amidic bond between anion **1** and amino derivative of Neu5Ac was precluded by serious problems in preparing sufficient amount of amine derived from Neu5Ac, where the final step in conversion to the amine precursor failed. Solving this issue is still in progress.

The detailed procedures, methods and problems will be discussed in the following text.

3.1 ACIDS

3.1.1 MONO- AND DISUBSTITUTED CARBOXYLIC ACIDS OF COBALT BIS(DICARBOLLIDE)

As was mentioned in the previous text, the reaction to direct carboxylation of the anion **1** was revised and finally provide good yields of mixture of both, mono- (**I**) and dicarboxylic (**II**) acid of the formulations [1-(HOOC-1,2-$C_2B_9H_{10}$)-(1′,2′-$C_2B_9H_{11}$)-3,3′-Co]$^-$, [1,1′-(HOOC)$_2$-(1,2-$C_2B_9H_{10}$)$_2$-3,3′-Co]$^-$ respectively. The reaction was carried out at the low temperature (-82 °C) in DME with carefully dried caesium salt Cs**1** (12 h at 180°C) and CO_2 (s) (Scheme 10). The amount of lithiating agent, namely butyl lithium, determined the ratio between **I** and **II**. With only one equivalent reaction affords 66% of **I** and 24% of **II**. The reaction with two equivalents of BuLi reaction resulted in 14% of **I** and 59% of **II**. Following this trend, formation of tri- or tetrasubstituted derivatives could be anticipated, but MS analysis revealed no presence of such products even using molar higher ratios of BuLi.



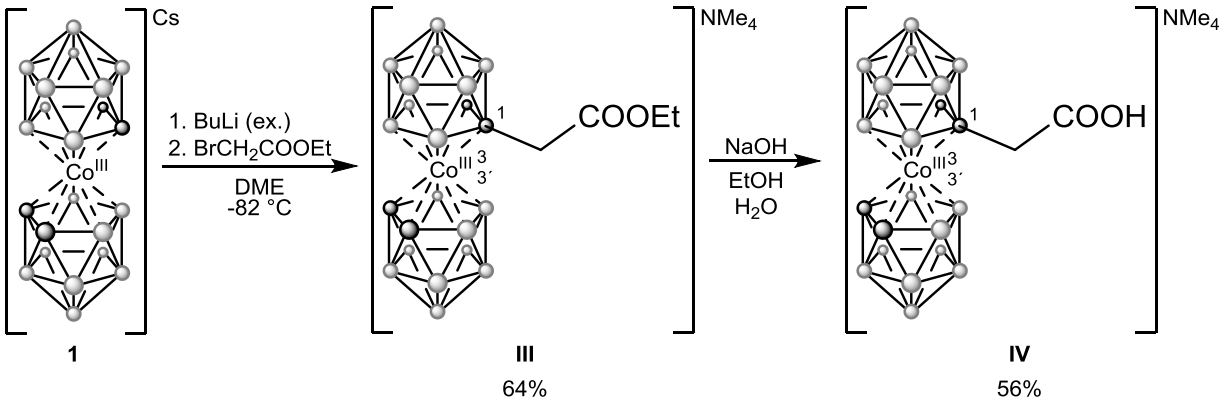
Scheme 10: *Direct carboxylation of Cs**1** results in mixture of mono- and disubstituted products **I** and **II**.*

Products **I** and **II** were purified by chromatography and crystallization and were isolated in pure form. Noteworthy is that the acid **II** can in principle comprise three diastereoisomeric forms, nevertheless only the 1,1′-isomer with *anti*-position of the substituted carbon atoms could be isolated as the highly prevailing species. Both, the mono- and dicarboxylic acids were precipitated as tetramethylamonium salt and then characterised by NMR, HPLC and MS, and also with DFT calculations of optimized geometries and NMR shifts, the latter was performed at the GIAO-DFT level.[123]

3.1.2 METHYLENE CARBOXYLIC ACID OF COBALT BIS(DICARBOLLIDE)

To proceed in the assigned task, the direct alkylation of lithiated **1** followed by reaction with $BrCH_2COOEt$ resulted in the ethyl ester **III** with a good yield of 67% after precipitation as tetramethylamonium salt. It should be noted, no disubstituted product formed during this reaction.

Subsequent alkaline hydrolysis was performed furnishing acid **IV** with a formula [1-(HOOC-CH_2-1,2-$C_2B_9H_{10}$)-(1′,2′-$C_2B_9H_{11}$)-3,3′-Co]$^-$ (Scheme 11). This hydrolysis yielded 56% after purification of crude product by chromatography and precipitation as tetramethylamonium salt.

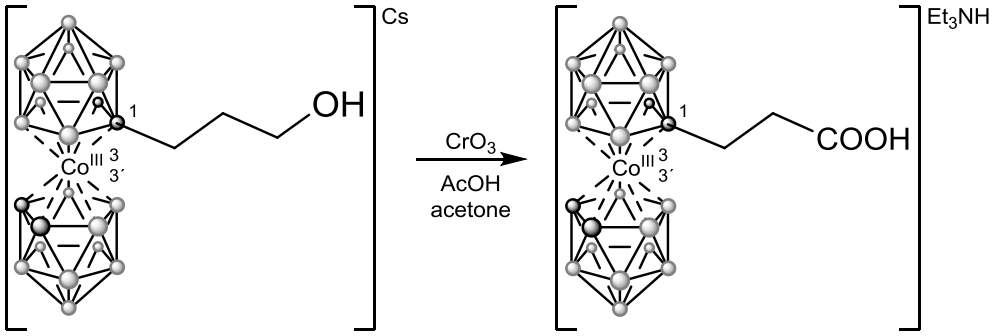


Scheme 11: *Alkylation of the parent ion **1** and hydrolysis of resulted ethyl ester **III** leads to a methylene acid **IV**.*

It should be mentioned that the alkylation proceed without formation of side products. In contrast, the procedure using instead $BrCH_2COO^tBu$, performed in parallel, resulted in a mixture of products, which was difficult to separate and the yield was thus not satisfactory.

3.1.3 1-ETHYLENE CARBOXYLIC ACID OF COBALT BIS(DICARBOLLIDE)

After having good results with bromoethyl acetate and subsequent alkaline hydrolysis, analogous reaction with bromoethyl propionate $BrCH_2CH_2COOEt$ was performed. Unfortunately, as expected, a significant decrease in the reactivity of the bromoalkyl end in the propionate compared to bromo acetate was observed. This reaction resulted only in low conversion to expected product even after prolonged reaction time and showed real limit of the lithiated cobalt bis(1,2-dicarbolide) (**1**) to act as nucleophile. Therefore, this method was abandoned and replaced by alternative approach. This pathway consisted in Jones oxidation[125] of hydroxypropyl derivative[121] of the COSAN (**34**) (Scheme 12).



34 **V**

46%

Scheme 12: *Oxidation of the hydroxy derivative (**34**) leads to the carboxylic acid **V** with a longer linker.*

This reaction proceeded smoothly and gave the expected product of the formula [1-(HOOC-$(CH_2)_2$-1,2-$C_2B_9H_{10}$)-(1´,2´-$C_2B_9H_{11}$)-3,3´-Co]$^-$ in good yield. This compound was purified by chromatography and precipitation as triethylamonium salt, nevertheless attempts at obtaining a crystalline product was not successful.

3.1.4 pK_A

As long as we talk about carboxylic acid, it should be mention its acidity. The pK_a values were determined by pH-metric titration of the Me_4N^+ salts. It means that in the titration curve we should observe only point of equivalence for COO^- (and no point of equivalence for the parent ion **1**$^-$). Indeed, we observed only points of eq. for COO^- around the value 5. These results are summarised in Table 1 and shows assumed trend in decreasing acidity with longer linker. Also, values of *m*-carborane carboxylic acid and benzoic acid are presented with their tabulated[71] and experimentally measured values, for comparison.

Table 1: *pK_a values of prepared acids **I**, **II**, **IV** and **V** compared with their close analogues and theirs tabulated values.*

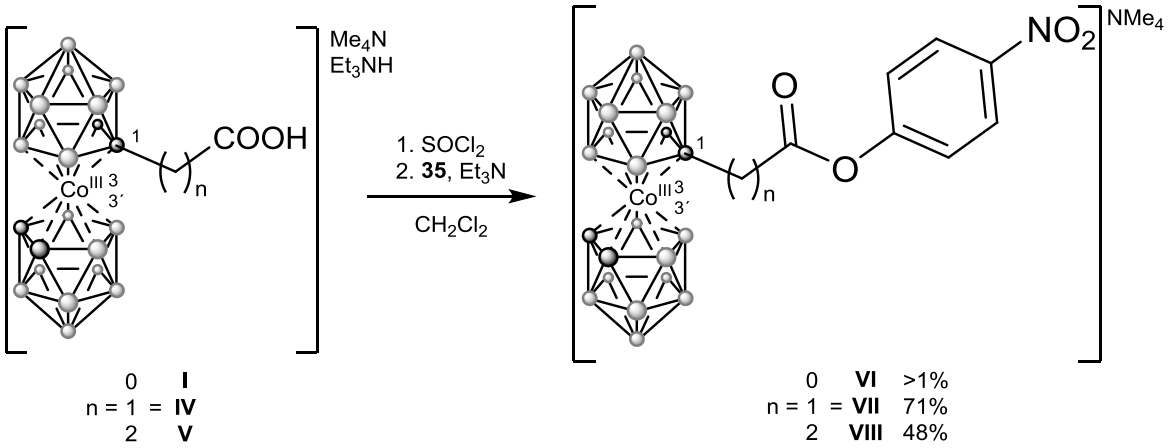
	pK_a values	
	R-COOH in 50% ethanol in water	
R	experimental	tabulated
m-$C_2B_{10}H_{11}$	3.76	3.34
C_6H_5	5.47	5.76
I	4.74	
II	5.39	
IV	5.65	
V	5.95	

The value of pK_a of the compound **II** was quite unexpected. In respect to the fact that we deal with dicarboxylic acid, we observed only one point of equivalence shifted to higher values of pK_a. This could be result of fast proton exchange or electron distribution over the metallacarborane cage.

3.2 ACTIVE ESTERS

3.2.1 GENERAL PROCEDURE

From several possible options how to activate the respective metallacarborane carboxylic acids for reactions with amines, *p*-nitrophenol (**35**) esters were selected over other ester groups (e.g. perfluorophenol) or chloride derivatives. The decision was simple, phenolate anion is very readily leaving group and thus very reactive. This task consists of the chlorination of the individual acids **I**, **IV** and **V** and immediate subsequent reaction with *p*-nitrophenol (**35**) (Scheme 13).



Scheme 13: *Chlorination followed by reaction with p-nitrophenol (**35**) results in active esters **VI** and **VII** of the original acids **IV** and **V**.*

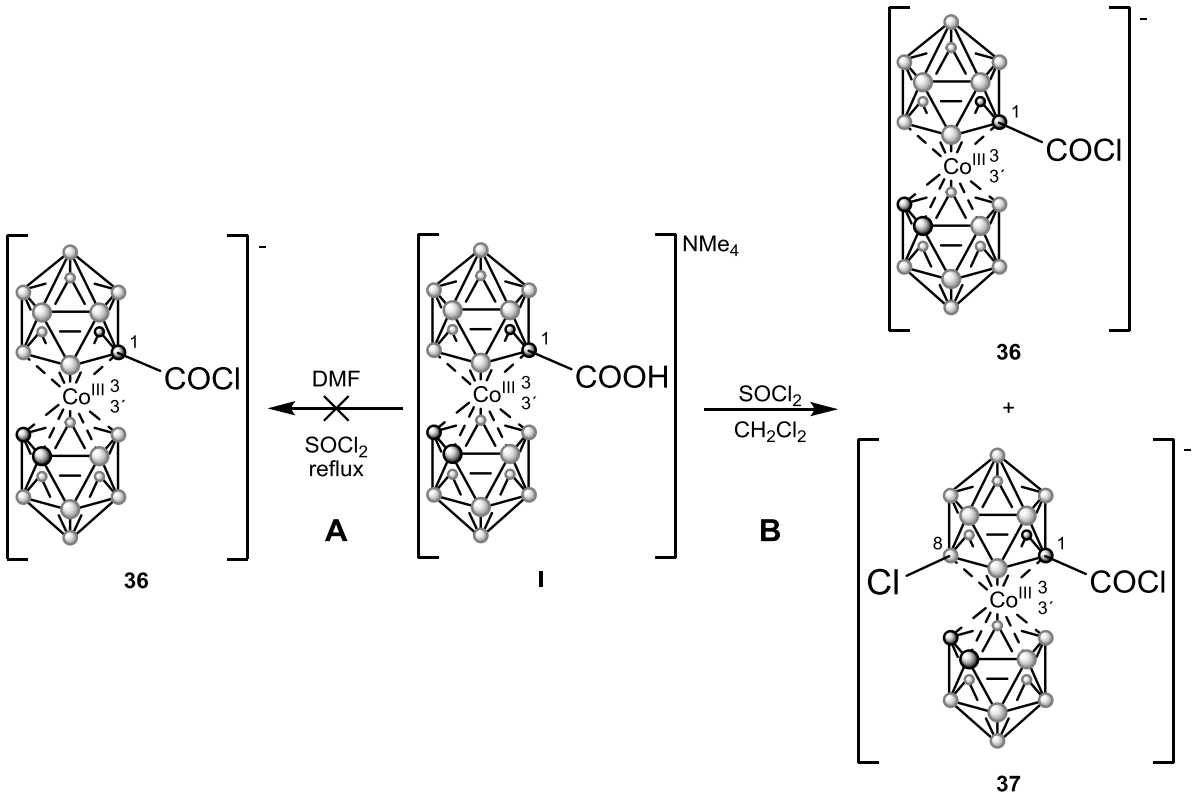
Chlorination was carried out at room temperature with excess of the $SOCl_2$ and after stirring for 1 h, almost complete conversion to the respective chloride was achieved (MS analysis). The unreacted residue $SOCl_2$ was then removed in vacuum and the product was immediately dissolved in CH_2Cl_2 and reaction was continued with addition of *p*-nitrophenol under basic catalysis (traces of Et_3N).

A significant advantage of this reaction pathway consists in its clean process without formation of any side product, and thus no subsequent chromatography is necessary for purification of the resulting ester.

3.2.2 INDIVIDUAL STEPS

As was mentioned above, the preparation of monocarboxylic acid and its chlorination was reported.[122] After careful revision of this reaction, we achieved good yields and

performed complete spectral characterization of **I**. We tried to apply the described reaction conditions (Scheme 14, **A**) to prepare acyl chloride (compound **36** in Scheme 14), but this was unsuccessful. Under reaction conditions depicted in Scheme 14, **B** the product formed, but the conversion was small and product composition was complicated by presence of chlorinated derivative **37**. This could not been separated from the assumed chloride and the unreacted acid. This problem seemed not crucial, because linker in the derivative **36** will be too short and could lead to the appreciable steric tension between COSAN polyhedra and the assumed functional groups.



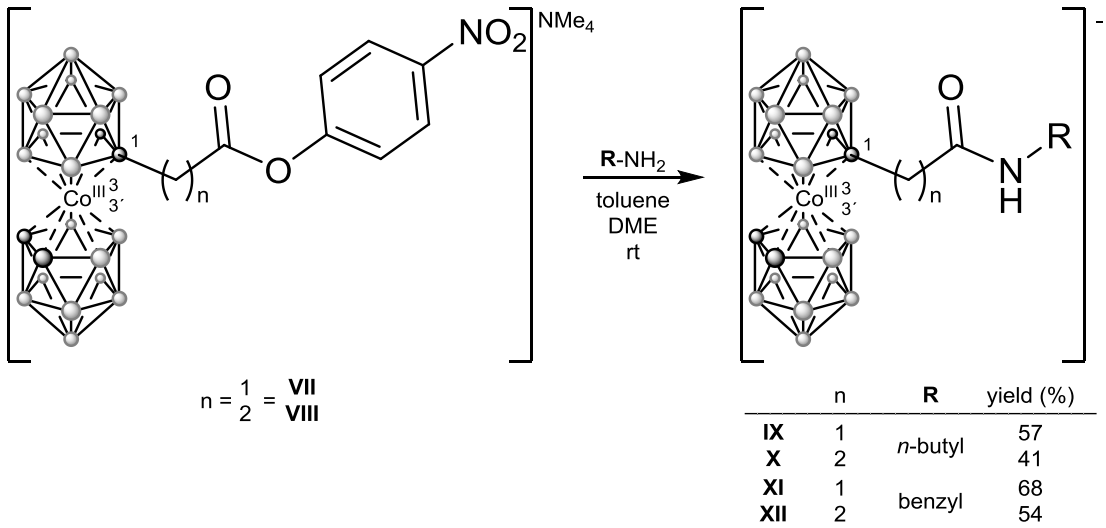
Scheme 14: *Two possible ways, how prepare acid chloride **36**. None of them was satisfying.*

At the other hand, reaction shown in Scheme 13 with acids **IV** and **V** proceeded smoothly and with good yields of the esters, which and after precipitation with tetramethylamonium chloride provided their respective tetramethylamonium slats **VII** [1-(1,4-$NO_2C_6H_4OOC$-CH_2-1,2-$C_2B_9H_{10}$)-(1´,2´-$C_2B_9H_{11}$)-3,3´-Co]Me_4N and **VIII** [1-(1,4-$NO_2C_6H_4OOC$-$(CH_2)_2$-1,2-$C_2B_9H_{10}$)-(1´,2´-$C_2B_9H_{11}$)-3,3´-Co]Me_4N as sufficiently stable solids, which were widely used in various subsequent synthesis.

3.3 AMIDES

According to previous experience of colleagues from Boron Group in Řež, formation of amidic bond from acid chlorides and cluster amines required harsh conditions, addition of an activator[47,126] or often completely failed. In following text, clean and high yield covalent bonding of the targeted molecule is presented.

At the start, we performed some model reactions with aliphatic and aromatic amines, namely *n*-butylamine and benzylamine. These reactions proceed very smoothly and quickly under mild conditions in quite good yields. In the mixture of the toluene and DME were prepared amides from the active esters **VII** and **VIII** (Scheme 15). The compounds **IX - XIII** could be easily separated and purified by chromatography.

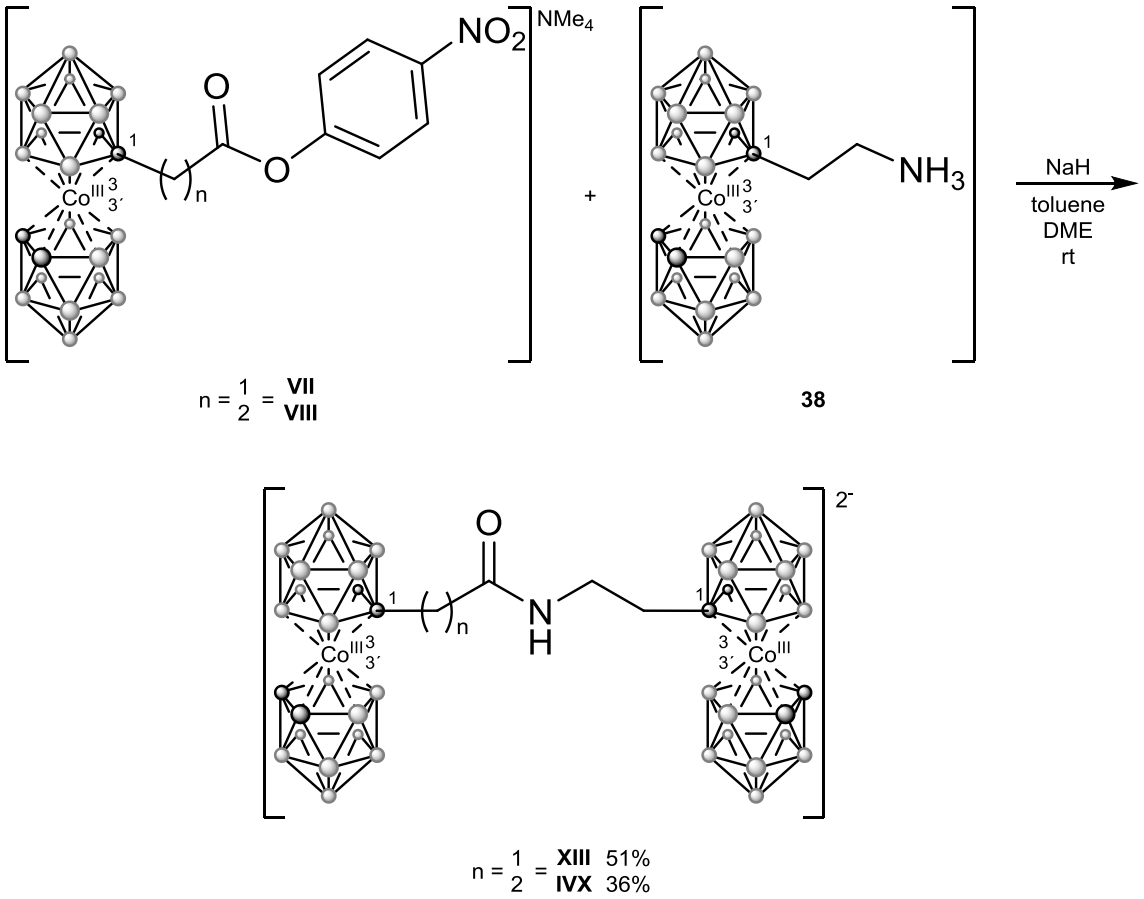


	n	R	yield (%)
IX	1	*n*-butyl	57
X	2		41
XI	1	benzyl	68
XII	2		54

Scheme 15: *Performing of an amidic bond with aliphatic or aromatic amines gives good results even without any catalyst needed.*

From previous research on metallacarborane HIV-PR inhibitors[78,81] followed by that the connection of several clusters is required to attain high efficiency and specific action. Our research was conducted with the aim to introduce new type of bonding between two metallacarboranes clusters *via* amidic bond. Unlike the previous reaction with amines, in this case the presence of the basic catalyst was required. As proper base, the strongly basic and low nucleophilic sodium hydride was chosen. This acted as a catalyst and also as for trapping the strongly acidic phenolate anion. Under these conditions, the reactions between active esters **VII** and **VIII** and aminoethyl derivative of cobalt bis(1,2-dicarbollide) (**38**)[127] were

performed and dicluster molecules **XIII** and **IVX** were obtained in relatively good yields after precipitation as tetramethylamonium salt. (Scheme 16).



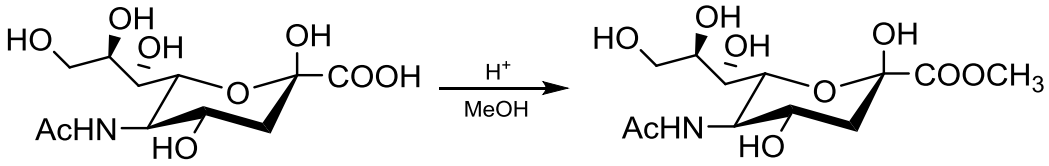
Scheme 16: *Formation of the dicluster molecules **XIII** and **IVX** connected via amidic bond.*

Because of the biological testing and its necessity of the biologically tolerable cation, the cation metathesis was accomplished after washing up the dicluster molecule in ether repeatedly with 3M HCl, water and subsequently with potassium carbonate. Using these conditions for cation metathesis, the tetramethylamonium cation was removed and the potassium salt was obtained.

3.4 *N*-ACETYLNEURAMINIC ACID

In the respect to the biological activity of the Neu5Ac (**23**) at the cell surface and the chemical properties of the COSAN (**1**) mentioned previously, their connection through the amidic bond could potentially enable some interesting properties. The respective building blocks based on the metallacarborane clusters (**VII** and **VIII**) were prepared successfully and their model reactions were successfully carried out verifying viability of this concept (Chapter 3.3). It therefore remains prepare appropriate amino derivative of the Neu5Ac.

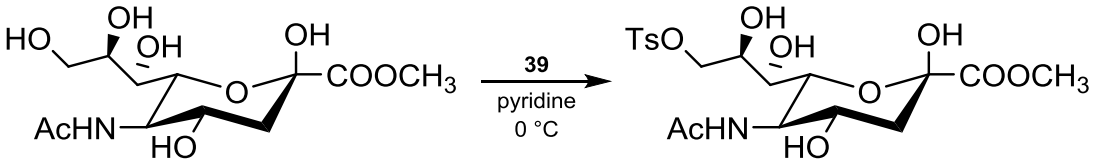
The first step consisted in protecting the carboxylic group. This was accomplished by an easy solvolytic reaction[128] of Neu5Ac (**23**) in methanol catalysed by acidic resin (Amberlite™ IR 120 Na) (Scheme 17).



23 **XV** 84%

Scheme 17: *Acidic-catalysed esterification of the Neu5Ac (**23**) results in high yields of the methyl ester **39**.*

Isolated methyl ester Neu5Ac-1Me (**XV**) with a primary hydroxyl group at carbon 9 was subsequently subjected to reaction with *p*-toluenesulfonyl chloride (**39**) under basic conditions giving the expected 9-*O*-tosyl derivative (**XVI**).[129] The reaction was carried out only for 12 h at 0 °C in contrast to 24 h described in reference[115] and probably due to this, the conversion and yield did not correspond to the indicated in the literature. However, yields around 50% seemed still acceptable for this second reaction step in the synthetic pathway (Scheme 18). The introduction of the easily-leaving ester group was necessary for the further reactions.



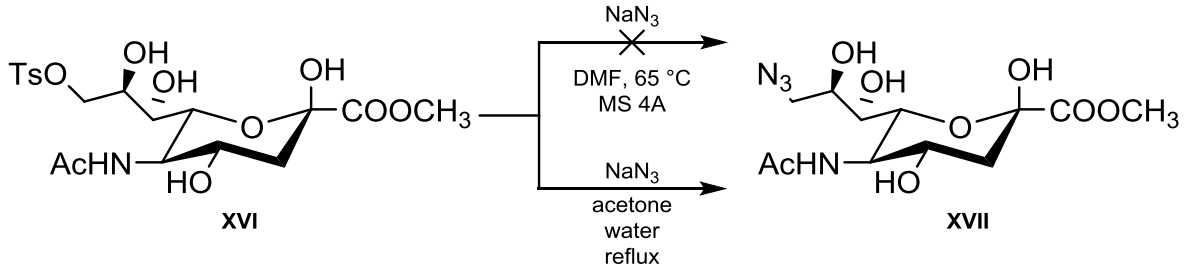
XV **XVI** 50%

Scheme 18: *Tosylation of the primary hydroxy group of the Neu5Ac-1Me (**XV**) in the pyridine gives pure product **XVI**.*

To follow next steps in the synthesis of the amino derivative, the amino functional group needs to be introduced into the structure. This has been achieved in literature[130] by S_N2 reaction with sodium azide and its reduction into the amine. Unfortunately, insertion of the azide has represented a major problem in the whole synthesis and unfortunately failed in our hand.

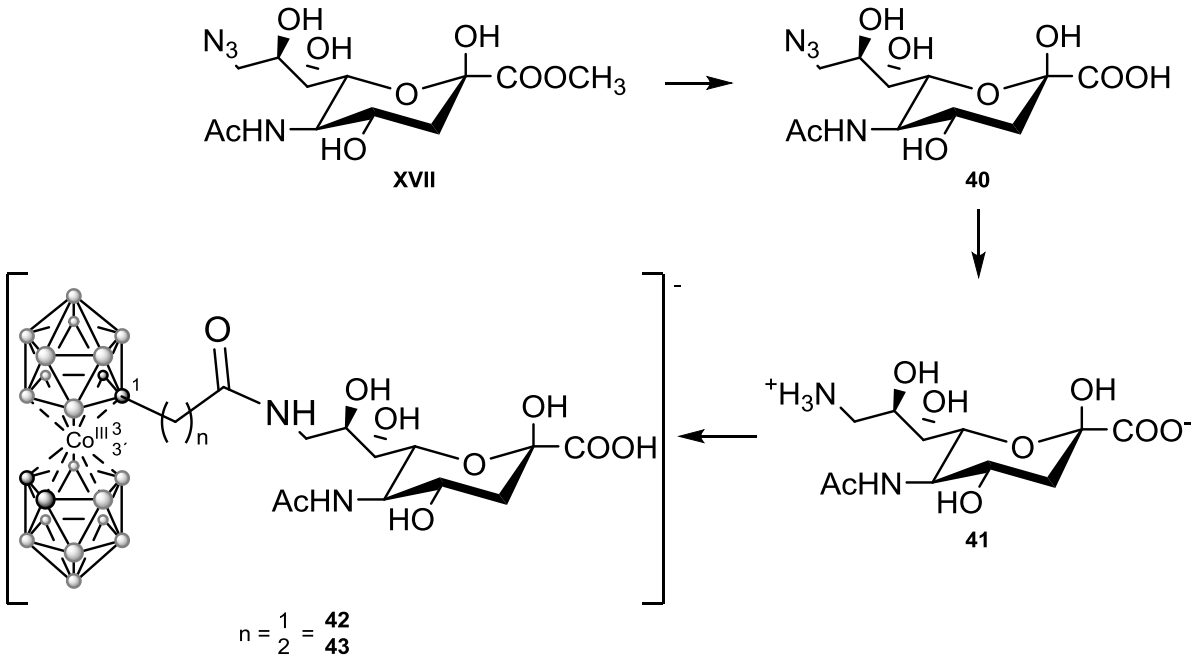
Two described methods have been tested. The first one[130] consisted in heating of the mixture of **XVI**, NaN_3 and molecular sieves in DMF at the exact temperature 65 °C. This temperature is important because of a limited thermal stability of **XVI**. But following precisely the same conditions as is in the original paper,[130] a decomposition of the starting material was observed during several individual repeating of the procedure (by TLC) and this was also the reason, why we turned to the another available process.

The second method[131] also uses NaN_3 as the azide group donor, but aqueous acetone was used as a solvent. As in the first method, the reaction required prolonged heating, for this time under reflux. In this case, the expected product apparently formed according to TLC (Scheme 19). Unfortunately, during the isolation and purifying, the major part of the product decomposed and even the NMR analysis did not proved the presence of the desired azide **XVII**.



Scheme 19: *Substituting of the tosylate group for the azide group under different conditions.*

Difficulties in the preparation of the respective amino derivative **XVII** led to the delay of the assumed synthesis of the cluster derivative. We hope that further detailed revision of the reaction conditions (temperature, solvent) or applying azide with a suitable organic cation would lead to successful synthesis of the intermediate **40**. When this compound is available, subsequent saponification of the methyl ester should afford the free acid **40** and the reduction of the azide in the last step should provide zwitterionic structure **41**. This will be tested in reactions with active esters, which would finally result in target molecules **42**, **43** respectively (Scheme 20).



Scheme 20: *Continuation of the work on the preparation of the new compounds derived from cobalt bis(1,2-dicarbollide) and N-acetylneuraminic acid.*

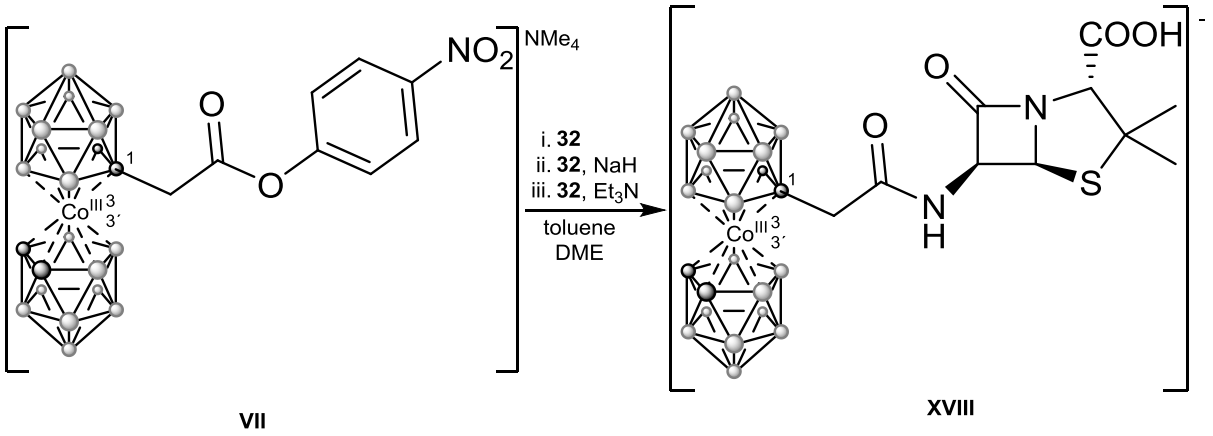
3.5 METALLACARBORANE ANALOGUES OF PENICILLIN

The unique properties of the anion **1**$^-$ and the positive results from previous research in the field of compounds active against resistant viral HIV strains prompted our interest in the field of boron antibiotics. Here, a simplest model was followed based on trials to modify aminofunction of 6-APA by metallacarborane cages using reactions described in previous part of this Diploma thesis.

The previous chapter 1.4.2 is devoted to chemistry of 6-APA describing a background relating to isolation and fundamental reactions that lead to the broad family of organic penicillins. A report on successful bonding of ferrocene carboxylic units[132] to 6-APA *via* amidic bonds was also described. Due to similarity of the species we started from conditions described in this paper to develop the chemistry based on cobalt bis(dicarbollides).

Using experience gained in the easy formation of amidic bonds between active esters **VII** and **VIII** and various model amines, we applied the same starting material for reaction with 6-APA (**32**).

At first, the simplest reaction at the room temperature without any catalyst was performed despite the bad solubility of the 6-APA with unprotected carboxyl group. After stirring for 3 days no reaction occurred (TLC, MS), then solid NaH was added as a base and the stirring was continued. Even with hydride, no reaction was detected but some decomposition of the starting material could be seen after another 2 days. The same reaction was carried out under conditions described in the literature[132] using Et_3N as a base, which forms triethylamonium salt with the carboxylic acid group of 6-APA. After stirring for 4 days, the reaction mixture contained the compound with mass corresponding to formula **XVIII** as the main product (MS check) (Scheme 21).

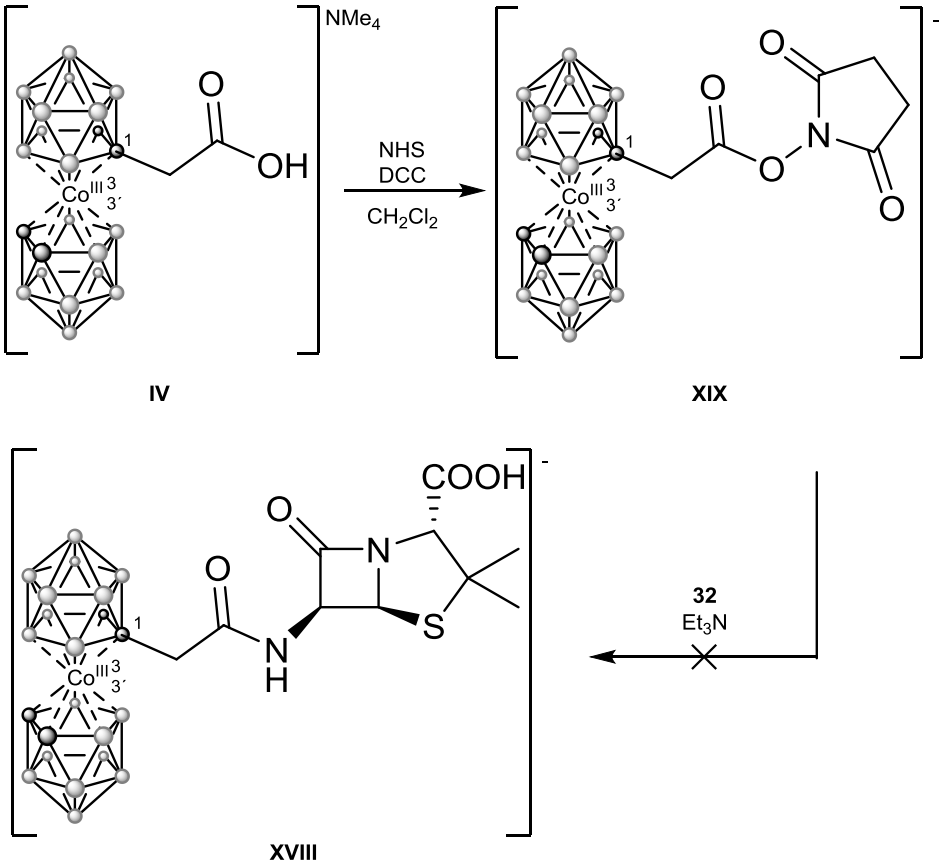


VII XVIII

Scheme 21: *Synthetic routes **i** and **ii** did not works at all, but experiment **iii** shows appearance of the product **XVIII**.*

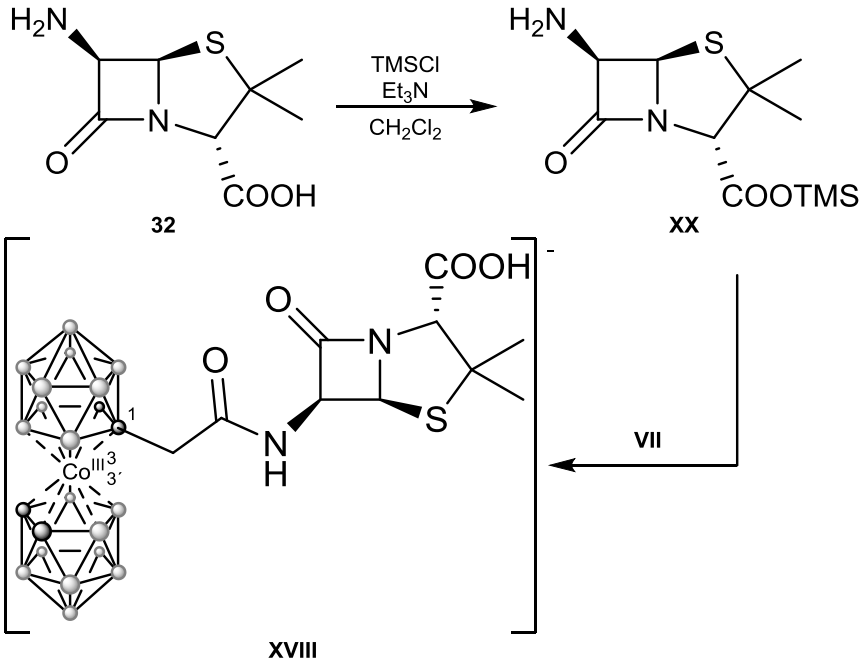
Unfortunately, the pure metallacarborane product could not be either isolated or measured by NMR, because of Et_3NH^+ salts of the phenol and others as impurities which could not been removed by other ways than using chromatography on silica gel. Due to strong retention of the product, mobile phases containing methanol had to be used to effect elution of the coloured bands from the column. A decomposition of **XVIII** was observed under these conditions and also, the remaining not-decomposed material was probably transformed into the methyl ester by solvolysis on silica (MS). Also, the inclination of the β-lactam ring to be opened by strong nucleophile present in the reaction mixture (nitrophenolate anion) indicates this approach is far to be ideal.

Thus, we tried to perform one-pot reaction with different active ester used in the studies on ferrocene,[133] which would not affect the reaction conditions with acidic products. Reaction between acid **IV** and *N*-hydroxysuccimide (NHS) and dicyclohexylcarbodiimide (DCC) were carried out and analysed by MS. When all the starting material disappeared, the 6-APA (**32**) along with Et_3N was added to the mixture and the mixture was stirred overnight (Scheme 22) and analysed by MS. But this analysis showed no conversion even after heating for long reaction time (2 days).



Scheme 22: *One-pot reaction creating active ester **XIX** and subsequent transformation into the amide **XVIII** was not successful after all.*

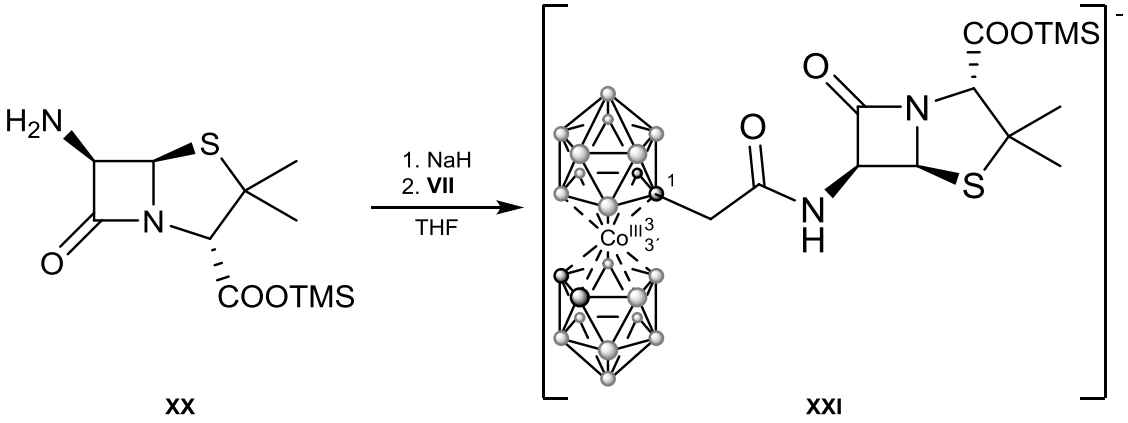
Then we turned our focus back on optimizing conditions for the reaction of the ester **VII** with 6-APA (**32**). This acid suffers from poor solubility in almost all anhydrous solvents of medium polarity (ethers). However, it can be solubilized by formation of its respective triethylamonium salt, either directly in the reaction mixture or before the reaction. Therefore, reactions with this salt were attempted. With the aim to increase the solubility and to limit interferences with the free carboxylic group, we also tried to prepare and employ derivative of 6-APA with trimethylsilyl-protected carboxylic group **XX**. This derivative was subsequently reacted with active ester **VII** following similar conditions described in literature[134,135] (Scheme 23).



Scheme 23: *Silylation of the 6-APA (**32**) and immediate reaction of its product **XX** with p-nitro phenolate ester **VII** leads to formation of amide **XVIII**.*

The one-pot reaction shown in Scheme 23 proved to be much faster than the same reaction carried out without silyl protection of the carboxylic group. Progress of the reaction was monitored by MS and a formation of the amide **XVIII** was observed. This means that desilylation of the starting silylated product proceeds simultaneously under applied reaction conditions. This can be probably ascribed to presence of the nucleophilic phenolate anion. However, the same problems with isolation and purification of the product have been encountered. All attempts to purify the product by chromatography failed. Again, the use of methanol was necessary to elute a metallacarborane containing band from the column. Analysing the eluted mixture revealed presence of decomposition products. The composition of the product mixture consisted from acid **IV**, expected product **XVIII** and a major part of another compounds, which mass corresponded apparently to methyl ester of **XVIII**. Another attempt to remove inorganic and water soluble organic impurities from the product consisted from shaking the reaction mixture between water and diethyl ether or ethyl acetate. Unfortunately, using this procedure, the product **XVIII** decomposed completely. This seems to indicate hydrolytic instability of the compound **XVIII**. In general, antibiotics should withstand aqueous conditions during tests of their biological activity. Therefore, further modifications of the linker would be probably necessary.

The so far last experiment was connected with the aim to avoid the deleterious effect of the presence Et_3NH^+ cation on product isolation. Reaction conditions depicted in Scheme 23 were applied using the silyl ester of the acid but without presence of triethylamine. The solid silylated derivative **XX** which was prepared and isolated earliest was dissolved in THF, solid NaH was added and after stirring the slurry for 1 h, the active ester **VII** was added. Concept of this method was based on filtration of solid sodium phenolate, which would precipitate off form the THF solution and only silylated product **XXI** would remain dissolved in the organic phase (Scheme 24).



XX **XXI**

Scheme 24: *Alternative reaction conditions to form amidic bond between 6-APA derivative **XX** and active ester **VII**.*

This reaction was monitored periodically by MS and increasing height of the peak corresponding to the desilylated product **XVIII** was observed. Also, the assumed precipitation of a solid from the mixture seemed promising. This monitoring was continued for 4 days and only before the quenching of the reaction, the observed peak of **XVIII** disappeared and the product was reverted back to the starting acid **IV**. This could be due to traces of humidity which could penetrate into the flask. This reaction will be repeated after preparing additional quantities of the starting ester. But it seems again, the linkage between the anionic cluster unit and the β-lactam ring seem quite labile towards hydrolysis.

The synthesis of the new amide **XVIII** was only partially successful as consequence of apparently poor hydrolytic stability of the amidic bond due to length of the connecting arm. This caused difficulties in the isolations and in the process of preparing pure product that should contain biocompatible cations. This should be carried out only using suitable methods of metathesis in aqueous solutions. Therefore, despite some evidence about feasibility of the overall concept for the formation of the amidic bonds has been gained, pure product could not be isolated. We believe, these problems can be bypassed by introducing additional atom or

heteroatom (e.g. oxygen, nitrogen) into the linker connecting the metallacarborane cluster with the terminal carboxylic acid. These studies are currently in progress.

4. EXPERIMENTAL PART

General

The caesium salt of cobalt bis(1,2-dicarbollide) (Cs**1**) was purchased from Katchem Ltd, Czech Republic. This salt was crystallized from hot aqueous ethanol (60%) and dried in vacuum for 4 h at 120 °C and then 12 h at 180 °C prior to use. Solvents, i.e. toluene and DME, were dried and distilled. Other chemicals and solvents were purchased from Aldrich and Lachner, Czech Republic, respectively, and were used without purification. Analytical TLC was carried out on Silufol® (silica gel on aluminium foil, starch as the binder, Kavalier, Czech Republic) and Merck 60 F_{254}. Unless otherwise specified, column chromatography was performed on a high-purity silica gel (Merck Grade, Type 7754, 70–230 mesh, 60 Å). All the reactions were performed using the standard vacuum or inert-atmosphere techniques under high-purity argon or nitrogen (99.999%) as described,[136] although some operations, such as flash chromatography and crystallisation, were carried out in the air. Melting points were determined in sealed capillaries on the BŰCHI Melting Point B-545 apparatus and are uncorrected. The majority of the new derivatives was precipitated in the form of the respective Me_4N^+, Me_3NH^+ or Et_3NH^+ salts that are not hygroscopic and do not contain any residual water or solvents. Before measurements of NMR spectra, melting points and elemental analyse the salts were carefully dried in vacuum at 60 °C, which ensured consistently good results. The identity of all the reported compounds was also unambiguously proven by their spectral data. All the carbon mono- and disubstituted species exhibit the respective molecular *m/z* base peaks $[M]^-$ in their electrospray ionisation (ESI^-) mass spectra and $[M]^{2-}$ base peak was observed for the dianionic species. For each particular boron cluster compound, the experimental and calculated isotopic patterns were in agreement with the calculated ones (using the mass spectrometric software EXcalibur).

Instrumental techniques

^1H, ^{11}B and ^{13}C NMR spectroscopy was performed on a Varian Mercury 400Plus Instrument. ^1H (400 MHz) and ^{13}C NMR (100 MHz) chemical shifts are referred to the residual ^1H signal(s) of a deuterated solvent used and are given in ppm. ^1H NMR chemical shifts $\delta(^1H)$ are given in ppm, coupling constants *J* (H,H) in Hz. ^{11}B NMR (128 MHz) chemical shifts are given in ppm to high-frequency (low field) and to $Et_2O{\cdot}F_3B$ as the external

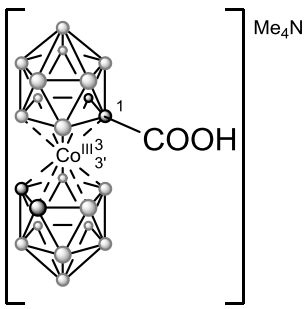
reference. Coupling constants *J* (^{11}B–^{1}H) were measured by resolution-enhanced ^{11}B spectra with a digital resolution of 2 Hz and are given in Hz. The ^{11}B NMR data are presented in the text below in the following format: ^{11}B chemical shifts δ(^{11}B) (ppm), multiplicity, and coupling *J* (^{11}B–^{1}H) constants are given in Hz. The peak assignment is based on {^{11}B–^{11}B} COSY NMR spectroscopy and compared with the spectrum of the parent salt Cs**1** (for assignment of the unsubstituted ligand). Only ^{13}C{1H} NMR resonances are listed; the peak assignment is in full agreement with signal multiplicities observed in coupled ^{13}C NMR experiments.

Mass spectrometry measurements were performed on a Thermo-Finnigan LCQ-Fleet Ion Trap instrument using electrospray ionisation (ESI). Negative ions were detected. Symplex dissolved in acetonitrile (concentrations approx. 100 ng mL^{-1}) were introduced to the ion source by infusion of 5 μL min^{-1}, source voltage 5.57 kV, tube lens voltage 49.8 V, capillary voltage 10.0 V, drying temperature at 188 °C, drying gas flow 8 L min^{-1}, and auxiliary gas pressure 6 Bar. In all cases negative ions corresponding to the molecular ion were observed with 100% abundance for the highest peak in the isotopic distribution plot. The experimental and calculated isotopic distribution pattern was in full agreement for all these compounds. The isotopic distribution in the boron plot of all peaks agrees perfectly with the calculated spectral pattern. The data are presented for the most abundant mass in the boron distribution plot (100%) and for the peak corresponding to the highest *m/z* value.

p*K*a values were determined after potentiometric titration using pH-meter Radelkis OP-208/1. 0.1 M aqueous solution of NaOH was used along with the 50% aqueous ethanol as solvent for measured acids.

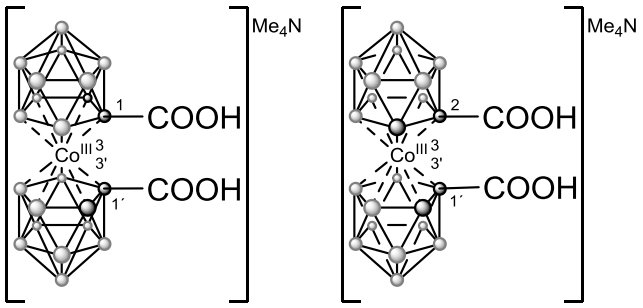
Synthetic methods and characterization of acids

Monocarboxylic acid of Cs1 (I) and isomers of dicarboxylic acid (IIa) and Et_3NH(*anti*-IIa)



*Synthesis of monocarboxylic acid **I** by reaction in Cs**1** to BuLi ratio 1:1.25.* Cs**1** (4.56 g, 10.0 mmol) was dissolved in dry DME (150.0 mL) under an inert atmosphere (Ar). An *n*-BuLi (2.5 M in hexane, 5.0 mL, 12.5 mmol) was added to the solution at the -82 °C. The reaction mixture went dark while stirring for 1 h at this temperature, then it was warmed up to room temperature and cooled again to the -82 °C and the CO_2 (s) (1.2 g, 27.5 mmol) was added. The reaction mixture was stirred overnight at room temperature. The mixture went dark red and the reaction was quenched by

adding 1.0 mL of MeOH and few drops of AcOH (conc.). The solvents were removed in vacuum. Water (20.0 mL) was added to the red residue followed by diluted HCl (3M, 30.0 mL) and the products were extracted into a mixture of ether and ethyl acetate (1:1, 75.0 mL). The organic layer was separated and washed with two additional portions of HCl (3M, 50 mL). The organic layer was separated, water (50 mL) was added and the anions were precipitated by an excess of aqueous solution of Me_4NCl and let stand for 2 hours. The precipitate was filtered, washed with water, hexane and dried in vacuum. The product was purified by column chromatography on silica gel (CH_2Cl_2-CH_3CN 5:1 + 1% AcOH) to obtain 2.95 g (yield 66%) of the compound **I** and 1.22 g (yield 24%) of the compound **II**. The starting salt Cs**1** (0.39 g, yield 7%) was obtained as well and was recycled as caesium salt. Found: R_f (CH_2Cl_2-CH_3CN 4:1) 0.10; ^{1}H NMR δ_H(400 MHz; CD_3CN) 3.67 (2H, br.s., C*H* carborane); ^{11}B NMR δ_B(128 MHz; CD_3CN; $Et_2O{\cdot}BF_3$) 8.67 (2B, d, *J* = 150, B8, 8'), 2.06 (2B, d, *J* = 140, B10, 10'), -5.57 (8B, d, *J* = 147, B4, 4', 7, 7', 9, 9', 12, 12'), -14.05 (1B, d, *J* = 150, B5), -17.84 (3B, d, *J* = 153, B5', 11, 11'), -19.23 (2B, d, *J* = 171, B6, B6'); ^{13}C δ_C (100 MHz; acetone-d_6) 169.77 (1C, *C*OOH), 55.97 (4C, $(CH_3)_4N^+$), 55.31 (1C, *C* caborane), 54.31 (1C, *C* caborane), 50.98 (1C, *C* caborane); *m/z* (ESI$^-$) 371.42 (M^-, 9%), 368.40 (100), calc. 371.26 and 368.76; m.p. 282 °C; pK_a = 4.74.

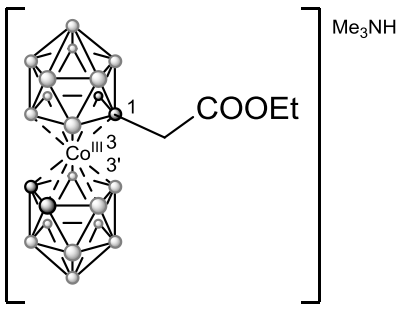


IIa 1,1´-*anti* **IIb** 2,1´-*syn*

*Synthesis of isomeric dicarboxylic acid **IIa,b** by reaction of Cs**1** to BuLi in ratio 1:2.* Cs**1** (0.69 mg, 1.5 mmol) dissolved in DME (30 mL) was reacted with *n*-BuLi (1.6 M in hexane, 2.0 mL, 3.2 mmol) and CO_2 (s) (0.6 g, 13.8 mmol) under identical conditions as these describes above. The product after the initial work up was precipitated by aqueous solution of CsCl. The semi-solid residue was decanted, dried in vacuum and crystallized from Et_2O (with few drops of MeOH for dissolution) – hexane. The solids were dissolved and subjected to chromatography on a silica gel column in CH_2Cl_2-CH_3CN mixture 3: 1 and then 2:1. The orange front band contained Cs**1** (105 mg, yield 14%). The red band was collected containing a mixture of two diastereoisomers of Cs salts of **IIa** and **IIb**, overall yield 0.48 g (59%). The *anti*-**IIa**$^-$ (55 mg) was isolated in pure form by chromatography in mixture of Et_2O-*i*PrOH 2:1 as front part of a red band and the product was precipitated as Et_3NH salt. Additional amount of Me_4N^+ salt of **IIa**$^-$ (35 mg) used for analysis

was obtained by chromatography of mother liquors, dissolving the solid residue in water and precipitation by aqueous Me_4NCl, washing with water and drying. Found for Me_4N**IIa**: R_f 0.05; ^{1}H NMR δ_H (400 MHz; acetone-d6) 3.93 (2H, br. s., C*H* carborane), 3.45 (12H, s, $(CH_3)_4N^+$); ^{11}B NMR δ_B(128 MHz; acetone-d6; $Et_2O{\cdot}BF_3$) 8.51 (2B, d, *J* = 159, B8, 8'), 1.54 (2B, d, *J* = 140, B10, 10'), -4.31, 5.90 (8B, 2d, *J* = 162, B4, 4', 7, 7', 9, 9', 12, 12'), -13.20 (1B, d, *J* = 162, B5, 5'), -16.99 (2B, d, *J* = 158, B11, 11'), -19.81 (2B, d, *J* = 180, B6, 6'); Cs**IIa** ^{13}C δ_C (100 MHz; acetone-d6) 169.16 (1C, *C*OOH), 66.17 (2C, *C* carborane), 55.31 (2C, *C*H carborane); *m/z* (ESI^- 412.33 (100), calc. 415.25 and 412.26. *Individual signals of Me_4N salt of **IIb** substracted from the spectra of the isomeric mixture:* ^{11}B NMR δ_B(128 MHz; acetone-d6; $Et_2O{\cdot}BF_3$) 7.32 (2B, d, *J* = 159, B8, 8'), other boron signals overlap with **IIa**$^-$ due to coincidence; ^{1}H NMR δ_H(400 MHz; acetone-d6), 4.48 (2H, br. s., C*H* carborane); Cs**IIb** ^{13}C δ_C (100 MHz; acetone-d6) 168.4 (1C, *C*OOH), 64.43 (2C, *C* carborane), 52.54 (2C, *C*H carborane); m.p. 102 °C; pK_a = 5.39.

[1-(EtOOC-CH_2-1,2-$C_2B_9H_{10}$)-(1′,2′-$C_2B_9H_{11}$)-3,3′-Co](Me_3NH) (III)

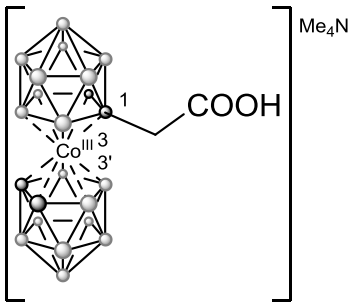


III

The salt Cs**1** (4.95 g, 10.9 mmol) was dissolved in dry DME (130 mL) under an inert atmosphere (Ar). An *n*-BuLi (2.5 M in hexane, 17.4 mL, 43.4 mmol) was added to the solution at the -82 °C. The reaction mixture went dark while stirring for 1 h at this temperature then was warmed up to room temperature and cooled again to the -82 °C and the bromic ester (6.1 mL, 54.5 mmol) was added dropwise. The reaction mixture was stirred overnight at room temperature. The mixture went dark red and the reaction was quenched by the addition of 1.0 mL of MeOH and few drops of AcOH (conc.). According to MS, the reaction mixture contained almost no disubstituted product. The product was purified by column chromatography on silica gel (CH_2Cl_2-CH_3CN 4:1) and precipitated by an excess of aqueous solution of $Me_3N{\cdot}HCl$, filtered and washed with water and hexane. The filtrate was dried to obtain 3.1 g (yield 67%) of the product **III**. Found: R_f (CH_2Cl_2-CH_3CN 4:1) 0.41; ^{1}H NMR δ_H(400 MHz; acetone-d_6) 4.46 (1H, br. s., C*H* carborane), 4.23 (2H, m, OCH_2CH_3), 4.08 (1H, br. s., C*H* carborane), 3.45 (2H, d, *J* = 6.0, CCH_2COOEt), 3.00 (9H, s, $(CH_3)_3N$), 1.35 (3H, t, *J* = 7.2, OCH_2CH_3); ^{11}B NMR δ_B(128 MHz; acetone-d_6; $Et_2O{\cdot}BF_3$) 7.20 (2B, d, *J* = 137, B8, 8'), 1.33 (2B, d, *J* = 88, B10, 10'), -5.55 (8B, d, *J* = 143, B4, 4', 7, 7', 9, 9', 12, 12'), -11.83 (1B, d, *J* = 156, B5), -16.11 (3B, d, *J* = 162, B5', 11, 11'), -19.98 (1B, d, *J* = 180, B6), -22.98 (1B, d, *J* = 174, B6'); ^{13}C δ_C (100

MHz; acetone-d_6) 171.06 (1C, *C*OOH), 62.64 (1C, O*C*H_2CH$_3$), 56.21 (4C, (*C*H_3)$_4$N$^+$), 54.07 (2C, *C* caborane), 52.15 (2C, *C* caborane), 45.47 (1C, *C*H_2CO), 14.50 (1C, OCH$_2$*C*H$_3$); *m/z* (ESI$^-$) 413.42 (M$^-$, 11%), 410.50 (100), calc. 413.31 and 410.32; m.p. 198 °C.

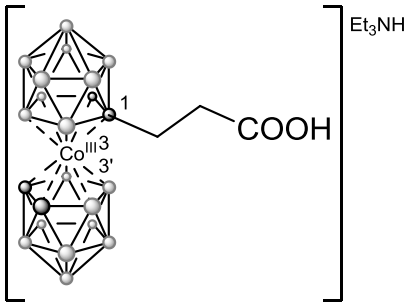
[1-(HOOC-CH$_2$-1,2-C$_2$B$_9$H$_{10}$)-(1′,2′-C$_2$B$_9$H$_{11}$)-3,3′-Co](Me$_4$N) (IV)



IV

The compound **III** (4.0 g, 9.76 mmol) was dissolved in 140 mL of 60% aqueous EtOH and 40 mL of 10% NaOH. This mixture was refluxed overnight. After that, 150 mL of water was added and mixture was cooled down to the room temperature. The mixture was shaken up to the Et_2O (3×40 mL). Organic phase was evaporated and crude product was precipitated by an excess of aq. solution of Me_4NCl. The product was filtered, washed with water and hexane and dried in vacuum. The product **IV** was obtained (2.1 g, yield 56%). Found: R_f (CH_2Cl_2-CH_3CN 4:1) 0.32; ^{1}H NMR δ_H(400 MHz; acetone-d_6) 4.48 (1H, br. s., C*H* carborane), 3.96 (1H, br. s., C*H* carborane), 3.33 (2H, d, *J* = 6.8, CC*H*$_2$COOH), 3.46 (12H, s, (C*H*$_3$)$_4$N); ^{11}B NMR δ_B(128 MHz; acetone-d_6; $Et_2O{\cdot}BF_3$) 7.01 (2B, d, *J* = 137, B8, 8’), 1.11 (2B, d, *J* = 134, B10, 10’), -5.64 (8B, d, *J* = 140, B4, 4’, 7, 7’, 9, 9’, 12, 12’), -11.51 (1B, d, *J* = 146, B5), -17.58 (3B, d, *J* = 162, B5’, 11, 11’), -19.92 (1B, d, overlap, B6), -23.09 (1B, d, *J* = 128, B6’); ^{13}C δ_C (100 MHz; acetone-d_6) 172.25 (1C, *C*OOH), 56.07 (4C, (*C*H_3)$_4$N$^+$), 52.98 (2C, *C* caborane), 50.99 (2C, *C* caborane), 44.20 (1C, *C*H_2CO); *m/z* (ESI$^-$) 385.33 (M$^-$, 10%), 382.42 (100), calc. 385.28 and 382.29; m.p. 144 °C; pK_a = 5.65.

[1-(HOOC-C$_2$H$_4$-1,2-C$_2$B$_9$H$_{10}$)-(1′,2′-C$_2$B$_9$H$_{11}$)-3,3′-Co](Et$_3$NH) (V)



V

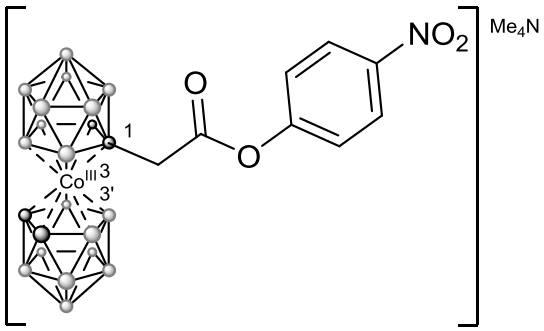
The caesium salt of 3-hydroxypropyl cobalt bis(1,2-dicarbollide) (**34)** (1.0 g, 2.6 mmol) was dissolved under an inert atmosphere (N_2) in acetone and this solution was cooled down to 0 °C. Then, the Jones reagent (CrO_3 + AcOH + H_2O; 1.6 g, 16.2 mmol + 14.0 mL + 18.0 mL) was added *via* syringe. The reaction mixture was stirred overnight at room temperature. Then, water (18.0 mL) was added and the solution was shaken up to Et_2O (2×20 mL). The organic phase was evaporated and purified by chromatography on silica gel (CH_2Cl_2-CH_3CN 3:1 + 1% AcOH). The product was precipitated by an excess of aq. solution of Et_3NHCl. The product was filtered, washed with water and hexane and dried in vacuum. It was obtained 0.6 g (yield 46%) of **V**. Found: R_f

(CH_2Cl_2-CH_3CN 4:1) 0.13; ^{1}H NMR δ_H(400 MHz; acetone-d_6) 4.21 (1H, br. s., C*H* carborane), 3.88 (1H, br. s., C*H* carborane), 3.81 (1H, br. s., C*H* carborane), 3.50 (9H, t, J = 6.8, (CH_3CH_2)$_3$NH), 3.13 (2H, dd, J_{12} = 7.2, J_{13} = 14.4, CH_2 CH_2CO), 2.78 (2H, dd, J_{12} = 7.2, J_{13} = 14.0, CH_2 CH_2CO), 3.50 (6H, q, J = 13.2, (CH_3CH_2)$_3$NH); ^{11}B NMR δ_B(128 MHz; acetone-d_6; $Et_2O{\cdot}BF_3$) 6.56 (2B, d, J = 134, B8, 8'), 0.92 (2B, d, J = 137, B10, 10'), -5.79 (8B, d, J = 140, B4, 4', 7, 7', 9, 9', 12, 12'), -15.23 (1B, d, overlap, B5), -17.72 (3B, d, J = 162, B5', 11, 11'), -19.53 (1B, d, overlap, B6), -23.05 (1B, d, J = 153, B6'); ^{13}C δ_C (100 MHz; acetone-d_6) 174.12 (1C, *C*OOH), 69.05 (1C, *C* caborane), 57.71 (1C, *C* caborane), 54.00 (1C, *C* caborane), 51.98 (1C, *C* caborane), 46.84 (3C, (CH_3CH_2)$_3$NH), 34.67 (1C, CH_2 CH_2CO), 34.64 (1C, CH_2 CH_2CO), 8.48 (3C, (CH_3CH_2)$_3$NH); *m/z* (ESI$^-$) 399.33 (M^-, 11%), 396.42 (100), calc. 399.30 and 396.31; m.p. 30 °C; pK_a = 5.95.

Synthetic methods and characterization of active esters

The salt of active ester derivative (1 eq.) was dissolved in dry CH_2Cl_2 (25 mL per 1 mmol) under an inert atmosphere (N_2), $SOCl_2$ (10 eq.) was added at one portion and the reaction mixture was stirred at room temperature for 1 h. The reaction was checked by MS to the total conversion of the starting material. The content of the flask was evaporated and co-distilled with dry CH_2Cl_2 (4×30 mL). The crude product of chlorination was dissolved in dry CH_2Cl_2 (21 mL per 1 mmol) under an inert atm. Then, *p*-nitrophenol (1 eq.) was dissolved in dry CH_2Cl_2 (same volume as chlorinated derivative) and it was added *via* syringe to the solution. At least, Et_3N (1.2 eq.) was added. The reaction mixture was stirred at room temperature for 1 h. Then it was shaken up with H_2O (3×), 1M HCl (1×) and brine (4×). Organic phase was dried over Na_2SO_4, filtered and evaporated. The product was dissolved in 30% ethanol, precipitated by an excess of aqueous Me_4NCl, filtered, washed with water and hexane and dried in vacuum.

[1-(4-NO_2-C_6H_4-1-O-)OC-CH_2-1,2-$C_2B_9H_{10}$)-(1′,2′-$C_2B_9H_{11}$)-3,3′-Co](Me_4N) (VII)

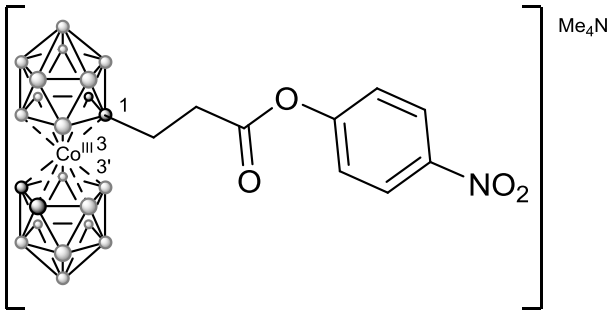


VII

The compound **IV** (1.58 g, 3.5 mmol), CH_2Cl_2 (90 mL) and $SOCl_2$ (2.5 mL, 35.0 mmol) was used for chlorination. The crude product of chlorination in CH_2Cl_2 (75 mL), *p*-nitrophenol (0.48 g, 3.5 mmol) in CH_2Cl_2 (75 mL) and Et_3N (0.6 mL, 4.2 mmol) was used. It was obtained 1.2 g, (yield 71%) of **VII**. Found: R_f (CH_2Cl_2-CH_3CN

4:1) 0.53; ^{1}H NMR δ_H(400 MHz; acetone-d_6) 8.34 (2H, d, *J* = 8.8, Ar*H*), 7.48 2H, d, *J* = 8.8, Ar*H*), 4.38 (2H, br. s., C*H* carborane), 4.07 (1H, d, *J* = 15.2, CCH_{2a}CO), 3.98 (2H, br. s., C*H* carborane), 3.87 (1H, d, *J* = 14.8, CCH_{2b}COOEt), 3.46 (12H, s, (CH_3)$_4$N); ^{11}B NMR δ_B(128 MHz; acetone-d_6; $Et_2O{\cdot}BF_3$) 7.37 (2B, d, *J* = 143, B8, 8'), 1.37 (2B, d, *J* = 131, B10, 10'), -5.38 (8B, d, *J* = 139, B4, 4', 7, 7', 9, 9', 12, 12'), -12.73 (1B, d, *J* = 140, B5), -17.39 (3B, d, *J* = 162, B5', 11, 11'), -19.60 (1B, d, overlap, B6), -22.95 (1B, d, *J* = 153, B6'); ^{13}C δ_C (100 MHz; acetone-d_6) 168.48 (1C, *C*OOH), 126.05 (2C, Ar), 123.82 (2C, Ar), 69.38 (2C, *C* caborane), 66.45 (2C, *C* caborane), 56.03 (4C, (*C*H_3)$_4$N$^+$) 46.07 (1C, *C*H_2CO); *m/z* (ESI$^-$) 506.42 (M$^-$, 12%), 503.50 (100), calc. 506.30 and 503.30; m.p. 97 °C.

[1-(4-NO_2-C_6H_4-1-O-)OC-C_2H_4-1,2-$C_2B_9H_{10}$)-(1′,2′-$C_2B_9H_{11}$)-3,3′-Co](Me$_4$N) (VIII)



VIII

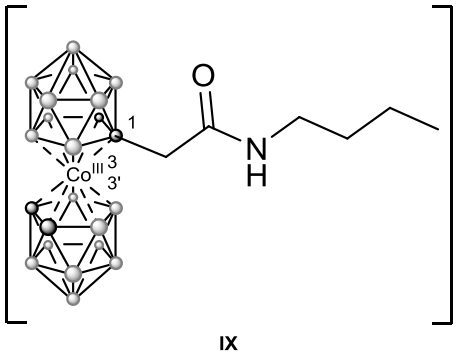
The compound **V** (350 mg, 0.7 mmol), 0.5 mL (7.0 mmol) of $SOCl_2$, 117 mg (0.84 mmol) of *p*-nitrophenol, 1μL (0.07 mmol) of Et_3N and 60 mL of CH_2Cl_2 was used. It was obtained 200 mg (yield 48%) of **VIII** salt. Found: R_f (CH_2Cl_2-CH_3CN 4:1) 0.50; Found: ^{1}H NMR δ_H(400 MHz; acetone-d_6) 8.38 (2H, d, *J* = 8.8, Ar*H*), 7.48 2H, d, *J* = 9.2, Ar*H*), 4.20 (2H, br. s., C*H* carborane), 4.15 (2H, br. s., C*H* carborane), 3.48 (12H, s, (CH_3)$_4$N), 3.01 (2H, t, J = 4.8, CH_2CH_2CO), 1.46 (2H, t, J = 6.4, CH_2CH_2CO); ^{11}B NMR δ_B(128 MHz; acetone-d_6; $Et_2O{\cdot}BF_3$) 6.63 (2B, d, *J* = 134, B8, 8'), 0.95 (2B, d, *J* = 134, B10, 10'), -5.81 (8B, d, *J* = 137, B4, 4', 7, 7', 9, 9', 12, 12'), -15.39 (1B,d, overlap, B5), -17.70 (3B, d, *J* = 162, B5', 11, 11'), -19.50 (1B, d, overlap, B6), -23.07 (1B, d, *J* = 146, B6'); ^{13}C δ_C (100 MHz; acetone-d_6) 174.10 (1C, *C*OOH), 156.75 (1C, ArO), 146.33 (1C, ArNO_2),126.04 (2C, Ar), 123.89 (2C, Ar), 58.02 (1C, *C* caborane), 56.12 (4C, (*C*H_3)$_4$N$^+$), 54.07 (1C, *C* caborane), 52.02 (1C, *C* caborane), 46.78 (1C, CH_2*C*H_2CO), 9.10 (1C, *C*H_2CH_2CO); *m/z* (ESI$^-$) 520.42 (M$^-$, 10%), 517.42 (100), calc. 520.31 and 517.32; m.p. 165 °C.

General procedure for amides

The respective salt of active ester (1 eq.) was dissolved under an inert atm. (N_2) in mixture of dry toluene and DME (1:1, 29 mL per 1 mmol) and benzylamine or *n*-butylamine (1.1 eq.) was added *via* syringe. After stirring for 1 h at room temperature, starting material almost disappeared (TLC). The reaction mixture was evaporated, dissolved in CH_2Cl_2 and

separated with cold 5% aq. $NaHCO_3$, water and brine to neutral reaction. Organic phase was concentrated and purified by column chromatography on silica gel (CH_2Cl_2-CH_3CN 4:1). The product was dissolved in aqueous methanol and precipitated by an excess of aq. Me_4NCl or Me_3NH, washed with water and hexane and dried in vacuum.

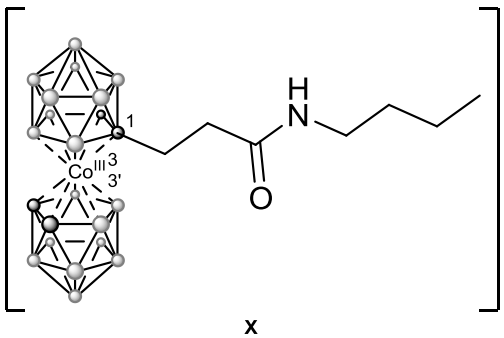
[1-(*n*BuNHOC-CH_2-1,2-$C_2B_9H_{10}$)-(1′,2′-$C_2B_9H_{11}$)-3,3′-Co](Me$_4$N) (IX)



IX

The compound **VII** (80 mg, 0.14 mmol), *n*-butylamine (0.02 mL, 0.15 mmol) in 12.0 mL was used. It was obtained 40 mg (yield 57%) of the **IX**. Found: R_f (CH_2Cl_2-CH_3CN 4:1) 0.45; ^{1}H NMR δ_H(400 MHz; acetone-d_6) 7.52 (1H, br. s., N*H*), 4.84 (1H, br. s., C*H* carborane), 4.30 (1H, br. s., C*H* carborane), 4.02 (1H, br. s., C*H* carborane), 3.47 (12H, s, $(CH_3)_4N^+$), 3.63 (2H, d, *J* = 15.6, CH_2CO), 3.23 (2H, q, *J* = 6.8, $NHCH_2CH_2CH_2CH_3$), 1.47 (2H, m, $NHCH_2CH_2CH_2CH_3$), 1.35 (2H, m, $NHCH_2CH_2CH_2CH_3$), 0.91 (3H, t, $NHCH_2CH_2CH_2CH_3$);^{11}B NMR δ_B(128 MHz; acetone-d_6; $Et_2O{\cdot}BF_3$) 6.77 (2B, d, *J* = 150, B8, 8’), 0.95 (2B, d, *J* = 112, B10, 10’), -5.86 (8B, d, *J* = 122, B4, 4’, 7, 7’, 9, 9’, 12, 12’), -11.40 (1B, d, *J* = 145, B5), -16.49 (1B, d, overlap, B5), -17.87 (3B, d, *J* = 146, B5’, 11, 11’), -20.58 (1B, d, overlap, B6), -23.43 (1B, d, *J* = 189, B6’); ^{13}C δ_C (100 MHz; acetone-d_6) 170.01 (1C, *C*OOH), 55.98 (4C, $(CH_3)_4N^+$), 54.87 (1C, *C* caborane) 53.8 (1C, *C* caborane) 52.58 (2C, *C* caborane), 46.07 (1C, CH_2CO), 39.64 (1C, $NHCH_2CH_2CH_2CH_3$), 32.15 (1C, $NHCH_2CH_2CH_2CH_3$), 20.74 (1C, $NHCH_2CH_2CH_2CH_3$), 14.01 (1C, $NHCH_2CH_2CH_2CH_3$); *m/z* (ESI$^-$) 440.62 (M^-, 12%), 437.67 (100), calc. 440.36 and 437.37; m.p. 204 °C.

[1-(*n*BuNHOC-C_2H_4-1,2-$C_2B_9H_{10}$)-(1′,2′-$C_2B_9H_{11}$)-3,3′-Co](Me$_4$N) (X)

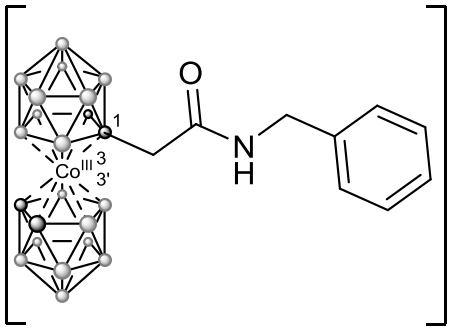


X

The compound **VIII** (30.0 mg, 50.8 μmol), *n*-butylamine (0.01 mL, 55.1 μmol) in (8.0 mL) was used. It was obtained 12 mg (41%) of **X** salt. Found: R_f (CH_2Cl_2-CH_3CN 4:1) 0.35; ^{1}H NMR δ_H(400 MHz; acetone-d_6) 7.09 (1H, br. s., N*H*), 4.07 (1H, br. s., C*H* carborane), 3.81 (1H, br. s., C*H* carborane), 3.72 (1H, br. s., C*H* carborane), 3.47 (12H, s, $(CH_3)_4N^+$), 3.17 (2H, q, *J* = 6.8, $NHCH_2CH_2CH_2CH_3$), 2.71 (2H, m,

CH_2CH_2CO), 2.39 (2H, m, CH_2CH_2CO), 1.47 (2H, m, $NHCH_2CH_2CH_2CH_3$), 1.40 (2H, m, $NHCH_2CH_2CH_2CH_3$), 0.95 (3H, t, $NHCH_2CH_2CH_2CH_3$); ^{11}B NMR δ_B(128 MHz; acetone-d_6; $Et_2O{\cdot}BF_3$) 6.27 (2B, d, J = 143, B8, 8'), 0.66 (2B, d, J = 143, B10, 10'), -5.98 (8B, d, J = 140, B4, 4', 7, 7', 9, 9', 12, 12'), -15.23 (1B, d, overlap, B5), -18.08 (3B, d, J = 153, B5', 11, 11'), -19.65 (1B, d, overlap, B6), -23.73 (1B, d, J = 147, B6'); ^{13}C δ_C (100 MHz; acetone-d_6) 171.74 (1C, *C*OOH), 60.13 (1C, *C* caborane), 55.93 (4C, $(CH_3)_4N^+$), 50.25 (2C, *C* caborane), 38.51 (1C, CH_2CH_2CO), 36.58 (1C, $NHCH_2CH_2CH_2CH_3$), 35.18 (1C, $NHCH_2CH_2CH_2CH_3$), 31.60 (1C, CH_2CH_2CO), 19.77 (1C, $NHCH_2CH_2CH_2CH_3$), 13.19 (1C, $NHCH_2CH_2CH_2CH_3$); *m/z* (ESI$^-$) 454.62 (M^-, 11%), 451.67 (100), calc. 454.38 and 451.38; m.p. 173 °C.

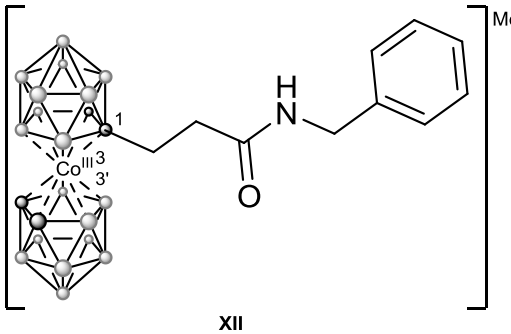
[1-(1-$C_6H_5CH_2$-NHOC-CH_2-1,2-$C_2B_9H_{10}$)-(1′,2′-$C_2B_9H_{11}$)-3,3′-Co](Me_3NH) (XI)



XI

The compound **VII** (200 mg, 0.35 mmol), mixture of solvents (10.0 mL) and benzylamine (0.04 mL, 0.38 mmol) was used. It was obtained 112 mg (yield 68%) of **XI**. Found: R_f (CH_2Cl_2-CH_3CN 4:1) 0.38; ^{1}H NMR δ_H(400 MHz; acetone-d_6) 7.67 (1H, s, N*H*), 7.43 (3H, m, Ar*H*), 7.26 (2H, d, J = 6.9, Ar*H*), 4.52 (2H, br. s., C*H* carborane), 4.32 (2H, s, CH_2CO), 3.60 (2H, br. s., C*H* carborane), 3.42 (9H, s, $(CH_3)_3NH$), 2.06 (2H, s, $NHCH_2Ar$); ^{11}B NMR δ_B(128 MHz; acetone-d_6; $Et_2O{\cdot}BF_3$) 6.75 (2B, d, J = 113, B8, 8'), 0.95 (2B, d, J = 122, B10, 10'), -5.79 (8B, d, J = 131, B4, 4', 7, 7', 9, 9', 12, 12'), -11.45 (1B, d, J = 125, B5), -17.65 (3B, d, J = 156, B5', 11, 11'), -20.17 (1B, d, overlap, B6), -23.10 (1B, d, J = 162, B6'); ^{13}C δ_C (100 MHz; acetone-d_6) 170.24 (1C, *C*OOH), 139.80 (1C, Ar*C*H_2), 129.25 (2C, Ar), 128.56 (2C, Ar), 127.91 (1C, Ar*C*H_2) 64.27 (1C, *C* carborane), 55.97 (3C, $(CH_3)_3NH$), 54.93 (1C, *C* caborane), 53.95 (1C, *C* caborane), 52.57 (1C, *C* caborane), 46.00 (1C, CH_2CO), 43.69 (1C, CH_2Ar); *m/z* (ESI$^-$) 474.50 (M^-, 13%), 471.58 (100), calc. 474.32 and 471.35; m.p. 151 °C.

[1-(1-$C_6H_5CH_2$-NHOC-C_2H_4-1,2-$C_2B_9H_{10}$)-(1′,2′-$C_2B_9H_{11}$)-3,3′-Co](Me_3NH) (XII)

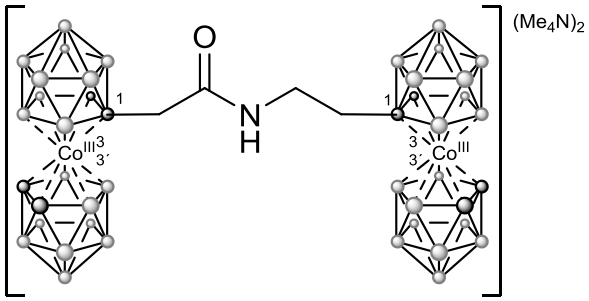


XII

The compound **VIII** (30 mg, 0.05 mmol)**,** benzylamine (0.01 mL, 0.06 mmol) in 8.0 mL was used. It was obtained 15 mg (yield 54%) of the **XII**. Found: R_f (CH_2Cl_2-CH_3CN 4:1) 0.40; ^{1}H

NMR δ_H(400 MHz; acetone-d_6) 7.60 (1H, s, N*H*), 7.28 (3H, m, Ar*H*), 7.23 (2H, m, Ar*H*), 4.38 (2H, d, *J* = 6.0, NCH_2Ar), 4.09 (1H, br. s., C*H* carborane), 3.82 (1H, br. s., C*H* carborane), 3.75 (1H, br. s., C*H* carborane), 3.46 (12H, s, (CH_3)$_4$N), 2.99 (2H, m, CH_2CH_2CO), 2.78 (2H, m, CH_2CH_2CO); ^{11}B NMR δ_B(128 MHz; acetone-d_6; $Et_2O{\cdot}BF_3$) 6.41 (2B, d, *J* = 1413, B8, 8'), 0.78 (2B, d, *J* = 140, B10, 10'), -5.86 (8B, d, *J* = 140, B4, 4', 7, 7', 9, 9', 12, 12'), -15.11 (1B, d, overlap, B5), -17.91 (3B, d, *J* = 177, B5', 11, 11'), -19.72 (1B, d, overlap, B6), -23.19 (1B, d, *J* = 162, B6'); ^{13}C δ_C (100 MHz; acetone-d_6) 171.98 (1C, *C*OOH), 140.58 (1C, $ArCH_2$), 129.14 (2C, Ar), 128.196 (2C, Ar), 127.60 (1C, $ArCH_2$) 57.39 (1C, *C* carborane), 55.97 (4C, (*C*H_3)$_4$N), 54.09 (1C, *C* caborane), 52.00 (1C, *C* caborane), 43.37 (1C, CH_2CH_2CO), 37.48 (1C, NH*C*H_2Ar), 36.07 (1C, *C*H_2CH_2CO); *m/z* (ESI$^-$) 488.42 (M^-, 10%), 485.50 (100), calc. 488.36 and 485.37; m.p. 240 °C.

Dicluster compound XIII

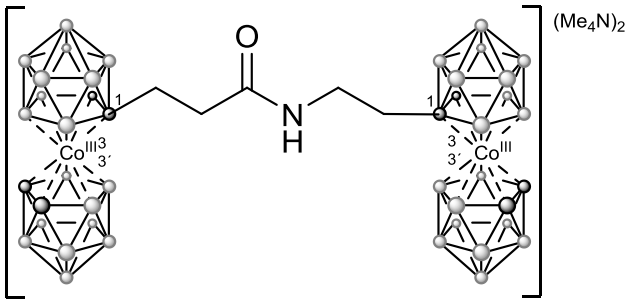


XIII

The dicluster compound **XIII** was prepared by reaction of the active ester **VII** (217 mg, 0.38 mmol) and [(1-NH_3-C_2H_4-1,2-$C_2B_9H_{10}$)(1´,2´-$C_2B_9H_{11}$)-3,3´-Co] (**38**)[127] (115 mg, 0.38 mmol) in mixture of toluene and DME (8 + 8 mL). The reaction conditions were almost the same as described above, but addition of solid NaH (18 mg, 0.76 mmol, 60% min. oil) was crucial to achieve reasonable conversion. The reaction mixture was evaporated and the residue was dissolved in EtOAc and the solution was shaken with cold $NaHCO_3$ (5% aq.), water and brine. The organic layer was evaporated and purified on silica gel (CH_2Cl_2-CH_3CN 3:1). The product was dissolved in aqueous methanol and precipitated by Me_4NCl. The solid was filtered, washed with water and hexane and dried in vacuum. It was obtained 140 mg (yield 51%) of **XIII**. Found: R_f (CH_2Cl_2-CH_3CN 4:1) 0.15; ^{1}H NMR δ_H(400 MHz; acetone-d_6) 7.64 (1H, br. s., N*H*), 4.41 (1H, br. s., C*H* carborane), 4.36 (1H, br. s., C*H* carborane) 4.23 (1H, br. s., C*H* carborane), 4.15 (1H, br. s., C*H* carborane), 4.03 (2H, br. s., C*H* carborane), 3.63 (2H, m, CONHCH_2CH_2), 3.46 (24H, s, (CH_3)$_4$N$^+$), 3.11 (1H, d, *J* = 16.6, CH_2CONH), 2.65 (1H, d, *J* = 16.4, CH_2CONH), 2.21 (2H, m, $CONHCH_2CH_2$); ^{11}B NMR δ_B(128 MHz; acetone-d_6; $Et_2O{\cdot}BF_3$) 6.51 (2B, d, *J* = 116, B8, 8'), 1.06 (2B, d, *J* = 122, B10, 10'), -5.86 (8B, d, *J* = 73, B4, 4', 7, 7', 9, 9', 12, 12'), -17.53 (5B, d, *J* = 153, B5', 5, 6, 11, 11'), -23.03 (1B, d, *J* = 165, B6'); ^{13}C δ_C (100 MHz; acetone-d_6), 169.37 (1C, *C*ONH), 55.97 (4C, (*C*H_3)$_4$N$^+$), 50.95 (2C, *C* caborane), 32.63 (1C, *C*H_2CO),

22.93 (1C, CONH*C*H$_2$CH$_2$), 14.37 (1C, CONHCH$_2$*C*H$_2$); *m/z* (ESI$^-$) 368.00 (M^{2-}, 11%), 365.58 (100), calc. 367.80 and 365.30; mp's 126 °C; IC_{50} = 79±2 nM.

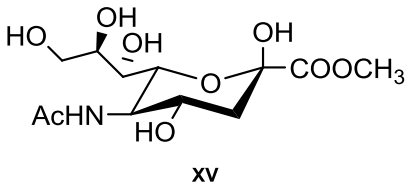
Dicluster compound IVX



IVX

The dicluster compound **IVX** was prepared by the same reaction as the **XIII**. 50 mg (0.09 mmol) of **VIII**, 37 mg (0.10 mmol) of **38**, 4.1 mg (0.17 mmol, 60% min. oil) of NaH in the mixture of toluene and DME (3 + 3 mL) was used. It was obtained 45 mg (yield 60%) of **IVX**. Found: R_f (CH_2Cl_2-CH_3CN 4:1) 0.13; ^{1}H NMR δ_H(400 MHz; acetone-d_6) 7.16 (1H, br. s., N*H*), 4.22 (2H, br. s., C*H* carborane), 3.78 (1H, br. s., C*H* carborane) 3.69 (2H, br. s., C*H* carborane), 3.46 (24H, s, (C*H*$_3$)$_4$N$^+$), 3.41 (1H, br. s., C*H* carborane), 3.23 (2H, m, CONHC*H*$_2$CH$_2$), 2.49 (2H, m, C*H*$_2$CONH), 2.46 (2H, m, CONHCH$_2$C*H*$_2$); 0.96 (2H, t, J = 7.6, C*H*$_2$CH$_2$CONH); ^{11}B NMR δ_B(128 MHz; acetone-d_6; $Et_2O{\cdot}BF_3$) 6.41 (2B, d, *J* = 129, B8, 8'), 0.78 (2B, d, *J* = 137, B10, 10'), -5.95 (8B, d, *J* = 125, B4, 4', 7, 7', 9, 9', 12, 12'), -17.84 (5B, d, *J* = 164, B5', 5, 6, 11, 11'), -22.93 (1B, d, *J* = 168, B6'); ^{13}C δ_C (100 MHz; acetone-d_6), 171.94 (1C, *C*ONH), 57.37 (2C, *C* carborane), 56.02 (8C, (*C*H$_3$)$_4$N$^+$), 54.17 (1C, *C* carborane), 54.02 (1C, *C* carborane), 51.98 (2C, *C* caborane), 40.88 (1C, CONH*C*H$_2$CH$_2$), 39.72 (1C, CH$_2$*C*H$_2$CO), 37.45 (1C, CONHCH$_2$*C*H$_2$), 36.00 (1C, *C*H$_2$CH$_2$CO); *m/z* (ESI$^-$) 372.42 (M^{2-}, 11%), 370.42 (100), calc. 372.80 and 370.80; mp's 142 °C; IC_{50} = 82±2 nM.

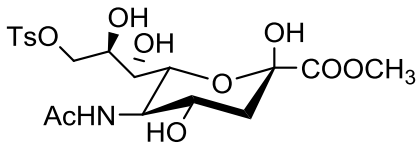
Methyl 5-acetamido-3,5-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosylonate (XV)



XV

Neu5Ac (**23**) (5.0 g, 0.02 mol) was suspended in 170 mL of methanol under an inert atmosphere (N_2) and molecular sieves (4 Å) were added. The reaction mixture was stirred at room temperature overnight. The suspension had dissolved completely after 2 days and the reaction mixture was filtered and concentrated to volume ~5 mL. Ether was added to turbidity and **XV** crystallized as a white solid on standing. The product **XV** was dried in vacuum to obtain 4.4 g (yield 84%). ^{1}H NMR corresponded to the literature[128] and product was used without any further purification to other reaction.

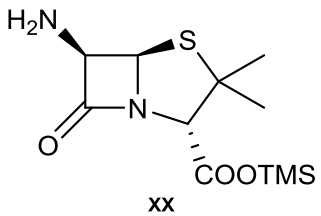
Methyl 5-acetamido-9-*O*-tosyl-3,5-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosylonate (XVI)



XVI

The methyl ester **XV** (1.0 g, 3.0 mmol) was dissolved in pyridine (15 mL) twice and evaporated. Then it was dissolved again in pyridine (20 mL) under an inert atmosphere (N_2) and cooled down to 0 °C. The *p*-TsCl (0.67 g, 3.5 mmol.) were added in portions during 1 h and the reaction mixture was let to stir overnight at 0 °C. The solvent was removed under vacuum and the residue was applied to a column chromatography (CH_2Cl_2-MeOH 10:1). Obtained yellow syrup was co-distilled with acetone till the white foam appeared. The product was dried and it was obtained 0.83 g (yield 58%). Found: R_f: 0.72 (EtOAc–MeOH 4:1); ^{1}H NMR corresponded to the literature.[129]

6-Aminopenicillanic acid trimethylsilyl ester (XX)



XX

6-APA (**32**) (1.0 g, 4.6 mmol) was suspended in CH_2Cl_2 (20.0 mL) and Et_3N (1.3 mL, 9.3 mmol) under an inert (N_2) was added. After 30 min of stirring the 6-APA had dissolved and Me_3SiCl (1.0 g, 9.3 mmol) was added. Precipitated solid was filtered, filtrate was evaporated and co-distilled in CH_2Cl_2 (3×20mL) and dried. It was obtained 0.81 g (yield 62%) of slightly yellow solid **XX**. Found: ^{1}H NMR δ_H(400 MHz; acetonitrile-d_3), 1.57 (3H, s, CH_3), 1.51 (3H, s, CH_3), 0.04 (9H, s, Si(CH_3)$_3$).

5. CONCLUSION

We successfully prepared and fully characterised C-substituted carboxylic acids **I**, **II**, **IV** and **V** of the cobalt bis(1,2-dicarbollide) (**1**). These compounds were subsequently transformed into the *p*-nitrophenolate active esters **VII** and **VIII**. This leads to the possibility of formation stabile amidic bond with various amines (**IX**–**IVX**). These results were published in *Dalton Transactions* in 2014.[123]

Synthetic ways of those compounds open new possibilities in the design of biologically active metallacarboranes. This effort addressing various therapeutic targets is currently under study in our laboratories. For example, tests are in progress on the inhibition of HIV-1-PR enzyme with the compounds **XIII** and **IVX**.

The attempt of preparation of the amides with organic compounds such as 6-aminopenicillanic acid led to difficulties with isolations of the products. It can say, that we have identified problem, which probably consisted in the particular selection of the length of the spacer between the boron cage and the β-lactam ring. This could be revealed only experimentally and solution based on creating the active molecules on different building blocks seems feasible. Nevertheless, synthesis of such blocks would require prolonged time. Therefore, this area of research is still in progress.

The derivatization of the *N*-Acetylneuraminic acid fails unexpectedly in the intermediate step of the synthesis. The literature reaction conditions and purity of the reagents must be adequately improved before continuing of this study.

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