# An Insight into Derivatization of *closo*-Dodecaborate

*And*

# Synthesis and Application of Indocyanine Green Dye

*by*

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of the requirements for the degree of

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# in Chemistry

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*To my beloved Parents*

*“Life is like riding a bicycle. To keep your balance, you must keep moving”*

*Albert Einstein*

# Abstract

The content of this Ph.D. thesis is divided into two main topics. The goal of the first chapter is the development of novel, robust, simple, broad scope, and economic methodologies for derivatization of dodecahydro-*closo*-dodecaborate anion $[B_{12}H_{12}]^{2-}$. The goal of the second chapter is the specific synthetic modulation of heptamethine indocyanine green dyes, and exploring their staining ability, toxicity, and stability in aqueous solution, thus broadening the applicability in cataract surgery.

The first part of the thesis, chapter one, presents a novel synthesis of dodecahydro-*closo*-dodecaborate anion $[B_{12}H_{12}]^{2-}$ derivatives. Nowadays, boron-rich dodecahydro-*closo*-dodecaborate anion $[B_{12}H_{12}]^{2-}$ has gained wide interest and providing great opportunities in designing of various geometric and electronic structures with promising properties useful for modern host-guest chemistry and organoelement chemistry, in addition to its wide application in boron neutron capture and radionuclide diagnostics therapy. Herein, we report our efforts on the development of a novel synthesis of cyclic oxonium derivatives of $[B_{12}H_{12}]^{2-}$ and effective approach to mono-halogenation $[B_{12}H_{11}X]^{2-}$ (X= I, Br, Cl) of dodecahydro-*closo* dodecaborate anion, the procedure yields therefore much purer material than any of the literature procedures, the yields are very high, and work-up is very straight-forward. Additionally, an attractive methodology for construction of B-N bond has been established. In general, derivatization of the dodecahydro-*closo*-dodecaborate dianion $[B_{12}H_{12}]^{2-}$ suffers from the lack of efficient methodology for introducing N-containing organic moieties to boron cage, thus, we have developed an approach based on the palladium-catalyzed cross-coupling amination of $[B_{12}H_{11}I]^{2-}$. The new methodology provides broad scope of $B_{12}$-N-containing organic moieties in good to excellent yield.

The second chapter highlights the outcomes in the synthesis and application of heptamethine cyanine green dyes. In details, synthesis of heptamethine indocyanine green dyes by addressing different positions in the chemical structure of the cyanine dye skeleton with the aim to generate green dye with optimal aqueous solubility and fewer tendencies to form aggregation. The staining ability of dye, toxicity, and stability in aqueous solution have been investigated. Two of the new substances stained the model for inner limiting membrane (ILM) in green and showed good stability in aqueous solution.

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## An Insight into Derivatization of *closo*-Dodecaborate

# Chapter 1

# Introduction

For many years, polyhedral boron hydrides including the polyhedral boranes, heteroboranes, and their metallic derivatives have attracted the attention of researchers. Boron hydrides are boron-rich molecular clusters which are described as three-dimensional (3D) analogue to benzene. Boron hydrides illustrates an electron-deficient structure, which means there are few valence electrons for bonding to be described exclusively in terms of two-center- two-electron bonds. Electron-deficient structures represent the aggregation of atoms to form unusual three-center-two-electrons bonds, which mainly result in the formation of trigonal faces and hyper-coordination.

Additionally, the low electron density in skeletal bonds would provide a compensation of the high connectivity of atoms in a cluster. The discovery of formation of three-centre two-electron bonds made a revolution in the theory of chemical bonding. William Lipscomb was awarded the Nobel Prize in 1976 "for his studies on the structure of boranes illuminating problems of chemical bonding". The new concept of three-dimensional aromaticity was developed and becoming attractive for chemists because of wide diversity of structural types and unusual nature of their bonds.[1]

The polyhedral boron hydrides are slightly stable in most of the known chemical reactions that have been published so far. The intensive studies of the nature of polyhedral boron hydrides demonstrated the aromaticity of such compounds as well as highlight the first example of nonplanar three-dimensional aromatic compounds and resulted in the development of the concept of three-dimensional aromaticity that is generally accepted at present.[2] Their unique delocalized 3D aromatic bonding, high stability, and potential for site-selective functionalization make them attractive building blocks for variety of pharmacophores, unique ligand scaffolds, and building blocks for materials applications , in addition, these advantages open numerous perspectives to their practical use in; boron neutron capture therapy[3], the carborane-based superacid[4], as components of rocket propellant, various pyrotechnic mixtures[5],  potential sources of hydrogen[6] and as carriers of radioactive labels in radionuclide imaging.[7] Therefore, active development of polyhedral boron hydrides chemistry has been provoked due to their interests in the practical application. Moreover, synthesis of boron compounds for medical

applications has been one of the main driving forces for the development of boron hydride chemistry for the last twenty-five years. Recently, researchers demonstrated that the ionic boron cluster can interact very strongly with hydrophobic surfaces such as the interior of cyclodextrins [8] or the exterior of cucurbiturils. [9] This unexpected behavior stems in part from the weak coordination of water around the boron clusters. [10] This effect has been named superchaotropic. [8b, 11]

## 1.1 Chemical Structure of Polyhedral Boron Hydrides

$BH_3$ is known as the simplest borane, it is existing in the form of a dimer $(BH_3)_2$. The higher boranes result from the clustering of boranes $B_mH_{m+2}$ ($m$ = 1, 2, 3 etc.) and such boranes do not exist in pure form. Generally, higher boranes chemical formulas follow the molecular formula $B_nH_{n+4}$ ($n$ = 2, 5, 6, 8, 10, 11, 12, 14, 16, 18) and $B_nH_{n+6}$ ($n$ = 4, 5, 6, 7, 8, 9, 10, 13, 14, 15, 20), but the boranes $B_nH_{n+8}$ ($n$ = 8, 10, 14, 15, 30) and $B_nH_{n+10}$ ($n$ = 8, 26, 40) also exist. In polyboranes the boron atoms are connected to cage-like structures, polyhedrons, means each boron atom is located on a polyhedron vertex (**Figure 1**). The chemical and electronic structures had been predicted by the rule of Wade. A *closo*-structure polyhedron with n edges has the formula $B_nH_{n+2}$, while the *nido*-structures $B_nH_{n+4}$ result in a polyhedron with n+1 vertex where one vertex is not occupied. In *arachno*-polyhedrons $B_nH_{n+6}$ two vertices are not filled from a total of n+2 and three vertices are not filled in *hypho*polyhedrons $B_nH_{n+8}$ with n+3 vertices.[12] Exchange of a BH unit with a heteroatom is possible, e.g., the substitution of BH versus CH leads to the formation of carboranes (**Figure 2**). Existence of carbon atom offer the possibility of normal organic chemistry in view of the C-C-bond formation. Carboranes such as dicarba-o-dodecaborane (1,2-$C_2B_{10}H_{12}$) and dicarba-p-dodecaborane (1,12-$C_2B_{10}H_{12}$) are characteristic of high biological and chemical stability and exceptional hydrophobic nature. [13]
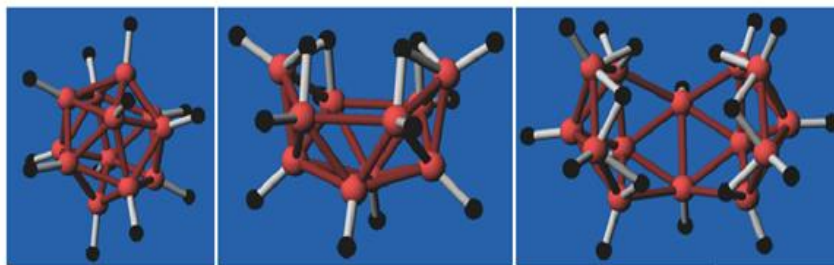


**Figure 1.** Polyhedrons: *closo*-structure (left), *nido*-structure (central) and *arachno*-structure (right). Adapted from (http://ruby.chemie.uni-freiburg.de).

| • C |
| • B |

ortho-carborane
1,2-$C_2B_{10}H_{12}$

meta-carborane
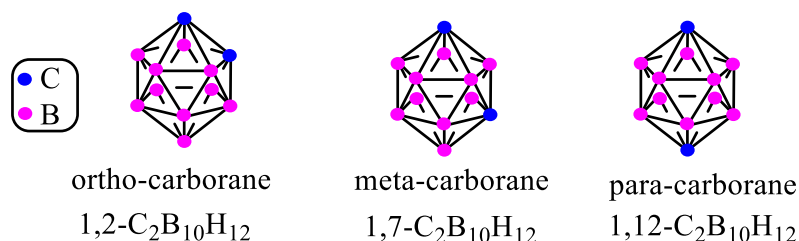1,7-$C_2B_{10}H_{12}$

para-carborane
1,12-$C_2B_{10}H_{12}$

**Figure 2**. Common structures of carboranes.

## 1.2 Electronic Structure Polyhedral Boron Hydrides

A broad investigation had been carried by the researcher to recognise the electronic structure of boron compounds, because the nature and reactivity of such compounds are not developed as the understanding of organic compounds, and their reactivity are not familiar. later, electron distribution in electron deficient molecules was described using resonance structures in which the valence electrons were assigned to localized 2-center BH and BB and delocalized 3-center BHB and BBB bonds. The discovery of this phenomena inspired Lipscomb to describe bonding in several boranes and carboranes. Since the distribution of the BB and BBB bonds over the 3n-6 edges and 2n-4 faces of the polyhedron does not accurately describe, this model suffers from several disadvantage.[14] Furthermore, as the number of vertices increases, the number of canonical resonance structures needed to describe the electronic structure accurately becomes staggering.[15]

In 1971, Wade *et al*. developed an alternative approach, known as Wade's rules for electron counting, the new rule focused on assigning the electrons to the vertices of the polyhedron, not to edges or faces.[16] New approach demonstrated that each BH vertex contributes two electrons to the polyhedral cage because boron has three valence electrons and one hydrogen, and two of these four valence electrons are needed to make an ordinary sigma BH bond. For $[B_{12}H_{12}]^{2-}$, 26 electrons are available for cage bonding; 12 electron pairs provided by the 12 BH vertices and a 13th electron pair, which originates in the double negative charge. If a BH is replaced with a CH vertex, as in $[CB_{11}H_{12}]^-$, there are still 26 cage electrons but the dianion becomes a monoanion since a carbon nucleus carries 6 positive charges as opposed to boron's 5. The carboranes bearing two carbon vertices, as in $[C_2B_{10}H_{12}]$, classified as electroneutral. Molecular orbital (MO) calculations provided quantitative description of bonding in boron clusters. Each boron

and carbon donate three hybrid or atomic valence orbitals to the polyhedral cage; therefore, the fourth valence orbital is involved in a 2-center-2-electron exoskeletal bond to the substituent. Developed methods such as MNDO, EHMO, and AM1, have been reviewed by Ŝtíbr, [17] and the methods were useful to be used in qualitative studies.[18] They offer an improved description of the electronic structure but still did not provide instinctive insight into the electron counting rules proposed by Wade. Such vision resulted from Stone's treatment of the problem using tensor surface harmonics.[19] This model is a three-dimensional analogue of the perimeter model used by Platt [20] and Moffitt [21] to account for the optical properties of planar aromatics.

## 1.3 Classification of Polyhedral Boron Hydrides

Polyhedral boron hydrides are classified as neutral and anionic. The neutral group includes *ortho meta*-, and para-isomers of icosahedral dicarbadodecaboranes [$C_2B_{10}H_{12}$], due to the hydridic character of the BH groups, such compounds are a highly hydrophobic, which are not allow them to form a classical hydrogen bonds with water. Moreover, because the CH groups in carboranes are weakly acidic, normal organic chemistry can be carried out for such compounds.[22] As a result, functionalization of carborane cages and incorporated into organic structures has be achieved, and syntheses of a wide range of various carborane-containing analogues of biomolecules have been reported.[23] Formation of anionic 7,8-dicarba-*nido*-undecaborate(nido-carborane): [$7,8-C_2B_9H_{12}$]$^-$ can be achieved by removal of one of the boron atoms adjacent to the two carbon atoms of *ortho*-carborane by action of base. This approach is used to increase the water solubility of carborane-containing biomolecules. [23c] Nowadays, an efficient method for the direct functionalization of the parent *nido*-carborane has been intensively described.[24]

The decahydro-*closo*-decaborate anion, [$B_{10}H_{10}$]$^{2-}$, is a member of the [$B_nH_n$]$^{2-}$ family, as well as it is classified as anionic polyhedral boron hydrides. The sodium salt of the parent *closo*-decaborate, $Na_2$[$B_{10}H_{10}$] (GB-10), is approved for human use by the US Food and Drug Administration (FDA). It should be noted that the chemistry of *closo*-decaborate is still much less studied than that of *closo*-dodecaborate.[25] It should be noted that only two reports on the medical application of *closo*-decaborate derivatives have been published for the last years.[26]

The cobalt bis(dicarbollide) anion, [$3,3-Co(1,2-C_2B_9H_{11})_2$]$^-$, is a boron compound that has been extensively used in medicinal chemistry. The cobalt atom in this unusually stable complex is clasped between two η5-bonding [$C_2B_9H_{11}$]$^{2-}$ ligands derived from nido-carborane.[27] The

sodium salt of this metallacarborane cluster show good solubility in water; however, the anion itself is rather lipophilic, which is bring high attention in medical applications. The synthesis of mono-substituted functional derivatives of cobalt bis(dicarbollide) was developed by Sivaev *et al*. by synthesizing 1,4-dioxane oxonium derivative which allowed a wide scope of nucleophilic opening reactions and a widely used for the synthesis of various functional derivatives of cobaltbis(dicarbollide).[28] Nowadays, Pd-catalyzed cross-coupling of the 8-iodo derivative is well known as powerful approach for other functionalization. [29]

Dodecahydro-*closo*-dodecaborate anion $[B_{12}H_{12}]^{2-}$ is known as the isoelectronic and isostructural analogue of carboranes, it is a typical representative of anionic polyhedral boron hydrides. Because of good solubility in water od its sodium salts of the parent *closo*-dodecaborate and their derivatives, it becomes an important precursor for many medical applications. The most important advantage of using the sodium salt of the parent anion, $Na_2[B_{12}H_{12}]$ is their very low toxicity with an approximate lethal dose forrats 7.5 g/kg of body weight, which is roughly comparable to that of sodium chloride.[30] However, due to the absence of a clear active reaction centre, functionalization of this inorganic system has been problematic and challenging up to recent time. To the extent of our knowledge,  two effective approaches to the synthesis of functional derivatives of *closo*-dodecaborate have been known.[28] The first approach represents the introduction of a primary substituent (-OH, -SH, -NH$_2$) followed by modification of using standard methods of organic chemistry.[31] The second approach is the preparation of cyclic oxonium derivatives of *closo*-dodecaborate followed by ring opening with various nucleophilic reagents.[16, 31d] This method is very efficient for the synthesis of derivatives with pendant functional groups connected to the boron cage through flexible spacers of 5–6 atoms and can be successfully applied to other types of polyhedral boron hydrides.[31d]

The carba-*closo*-dodecaborate anion $[CB_{11}H_{12}]^{-}$ has advantages like good solubility in water as sodium salt and the presence of a carbon atom available for functionalization by standard methods of organic chemistry. It should be noted that the applications of of the carba-*closo*-dodecaborate anion were very limited until Franken *et al*.  developed a convenient preparative method for the parent cluster and their C-phenyl derivatives in 2001. [32]

**1.4 Synthetic Approaches of *closo*-Dodecaborate [B$_{12}$H$_{12}$]$^{2-}$**

Boron hydride compounds have been synthesized and used in biomedical applications, among them, one of central places belongs to the dodecahydro-*closo*-dodecaborate anion [B$_{12}$H$_{12}$]$^{2-}$. It has a structure of a regular icosahedron and represent high simplicity and high symmetry, see (**Figure 3**). Calculated structures (based on X-ray crystal structures) and representative molecular orbitals of A$_{1g}$ symmetry, demonstrating delocalization of the electron density in *closo*-[B$_{12}$H$_{12}$]$^{2-}$, are also described in (**Figure 3**).



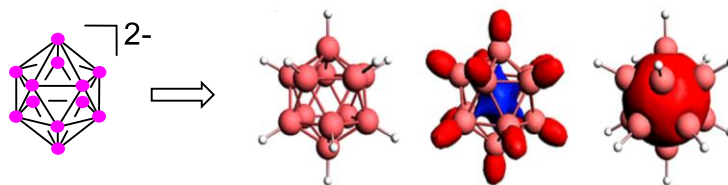**Figure 3.** Structures (based on X-ray crystal structures) and representative molecular orbitals of *closo*-dodecaborate dianion [B$_{12}$H$_{12}$] $^{2-}$. Adapted from ref [33]

The chemistry of dodecahydro-*closo*-dodecaborate anion developed after 1955 when Longuet-Higgins and Roberts reported its electronic structure based on MO-LCAO calculations, they expected that such icosahedral borane would be stable only as dianion [B$_{12}$H$_{12}$]$^{2-}$.[34] Five years later, Longuet-Higgins and Roberts expectation was verified experimentally by Hawthorne and Pitochelli in 1960 when the *closo*-dodecaborate anion was prepared for the first time as a side-product of the reaction of 2-iododecaborane and triethylamine in refluxing benzene.[35] The isolated yield of [B$_{12}$H$_{12}$]$^{2-}$ in this reaction was less than 4%. However, a few years later several effecient methods of synthesis of the [B$_{12}$H$_{12}$]$^{2-}$ anion were proposed by different research groups.

The first reported synthetic method includes reactions of various boranes with H$_3$B·L complexes (L is Lewis's base, usually NEt$_3$). High yield dodecaborate product [B$_{12}$H$_{12}$]$^{2-}$ has been achieved in two basic reactions: (1) a hydride ion source and a boron hydride, e.g., NaBH$_4$ with B$_2$H$_6$; and (2) an organic Lewis base and a boron hydride. The reactions of triethylamine-borane with diborane at 180 °C and that of triethylamine-borane with pentaborane B$_5$H$_9$ at 125 °C both produce the [B$_{12}$H$_{12}$]$^{2-}$ anion in 90% yield. The reaction of the coupled pentaborane

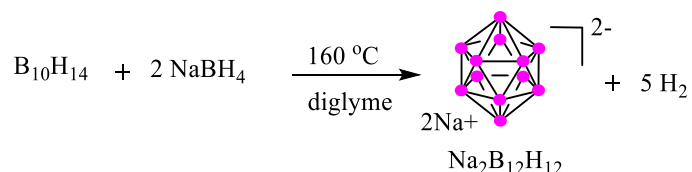dimer 1:2-$[B_5H_8]^{2-}$ with triethylamine-borane in decane at 100 °C produces the $[B_{12}H_{12}]^{2-}$ anion in 59% yield. Availability of pentaborane makes these reaction sequencies attractive routes.[36]

Another convenient method of synthesis of the *closo*-dodecaborate anion reported by Greenwood *et al.* the approach was based on the pyrolysis of triethylamine-borane with decaborane in ultrasonic at 190 °C, the desired product was achieved in 92% yield. (**Scheme 1**).[37]

$$B_{10}H_{14} \ + \ 2 \ Et_3NH.BH_3 \xrightarrow{\ 190\ ^oC\ } \left[ \quad \right]^{2-} + \ 3H_2$$

$$2(Et_3N)^+$$
$$(Et_3NH)_2[B_{12}H_{12}]$$
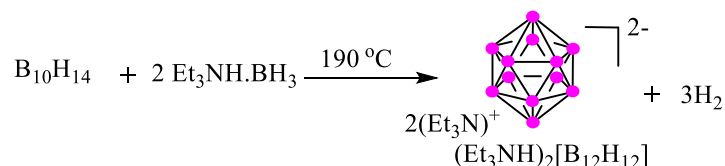
**Scheme 1.** Pyrolysis of triethylamine-borane with decaborane.

Another widely used practice method of synthesis the $[B_{12}H_{12}]^{2-}$ anion is the reaction of sodium tetrahydroborate with decaborane in refluxing diglyme, this efficient method giving the target product in 91% yield (**Scheme 2**).[38] [39]

$$B_{10}H_{14} \ + \ 2 \ NaBH_4 \xrightarrow[\text{diglyme}]{\ 160\ ^oC\ } \left[ \quad \right]^{2-} + \ 5 \ H_2$$

$$2Na+$$
$$Na_2B_{12}H_{12}$$

**Scheme 2.** Reaction of sodium tetrahydroborate with decaborane in refluxing diglyme.

The reactions of $NaBH_4$ or $KBH_4$ with trimethyl- and triethylamineborane in high-boiling alkanes (dodecane, hexadecane) at 200–250 °C result in the *closo*-dodecaborate anion in up to 95% yield.[40]

Over the last fifty years the synthesis of $[B_{12}H_{12}]^{2-}$ was improved and nowadays it is best synthesized from the cheap starting materials $NaBH_4$ and $I_2$. Komura *et al.* reported for the first time the sufficient approach to synthesize $[B_{12}H_{12}]^{2-}$ using $NaBH_4$ and $I_2$, the reaction carried out at high temperature for several hours, the desired product was collected in 53% yield.[41] In 2009, Knapp *et al.* published a modified procedure that provides a high yield from Komura synthetic approach, the reaction is carried out in two steps in a one pot reaction. First the

formation of $[B_3H_8]^-$ occurs followed by its thermal decomposition to $[B_{12}H_{12}]^{2-}$. The following (**scheme 3**) illustrated the Knapp approach in diglyme as solvent. [42]

$$NaBH_4 \xrightarrow[\text{95-100 }^o\text{C, 2h}]{\text{I}_2\text{, diglyme}} NaB_3H_8 \xrightarrow[\text{Et}_3\text{N, NaOH, 35h}]{\text{H}_2\text{O, conc. HCl}} [B_{12}H_{12}]^{2-} \quad 2Na+$$
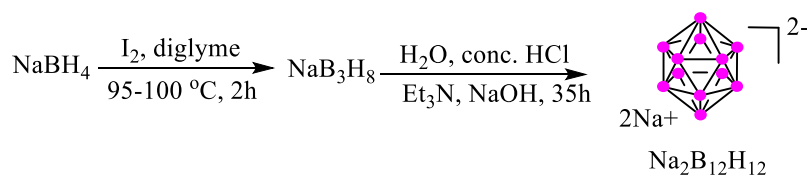
$$Na_2B_{12}H_{12}$$

**Scheme 3.** Synthesis of $Na_2B_{12}H_{12}$.

Starting from the sodium salt of $[B_{12}H_{12}]^{2-}$ other alkali metal salts and trialkylammonium salts are obtained easily. Simple metathesis reaction with the amine hydrochloride in water leads to the precipitation of the desired product. For the generation of the alkali metal salts the trialkylammonium salt $[R_3NH]_2[B_{12}H_{12}]$ again is reacted with the corresponding MOH (M = Li,Cs) in water under liberation of the amine.

$$Na_2B_{12}H_{12} + 2\ Et_3N.HCl \xrightarrow{\text{H}_2\text{O}} [Et_3NH]_2[B_{12}H_{12}] + 2\ NaCl$$

$$[Et_3N]_2[B_{12}H_{12}] + 2\ MOH \xrightarrow[\text{Heat}]{\text{H}_2\text{O}} M_2[B_{12}H_{12}] + 2\ Et_3N + 2\ H_2O$$
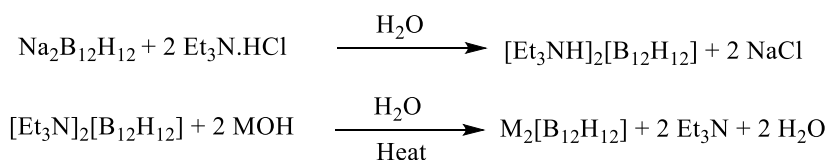
**Equation 1.** Obtaining alkali metal and trialkylammonium salts from sodium salt of $[B_{12}H_{12}]^{2-}$.

Cation exchange reactions are a powerful well-known approach of dodecahydro-*closo*-dodecaborate (2–). Cesium salts of the boron cluster can be achieved by dissolving tetrabutylammonium salts of dodecaborate clusters in methanol and precipitated by adding CsF in methanol. The different cations also influence the solution behaviour of the boron cluster. Alkali metal salts of $[B_{12}H_{12}]^{2-}$ ($Li^+$, $Na^+$, $K^+$) are very soluble in water and hygroscopic. Whereas the ammonium cations form the $B_{12}$ cluster enables the solubility in organic solvents. (**Scheme 4**) shows the various cation exchange reactions of the $[B_{12}H_{12}]^{2-}$ cluster.[43]
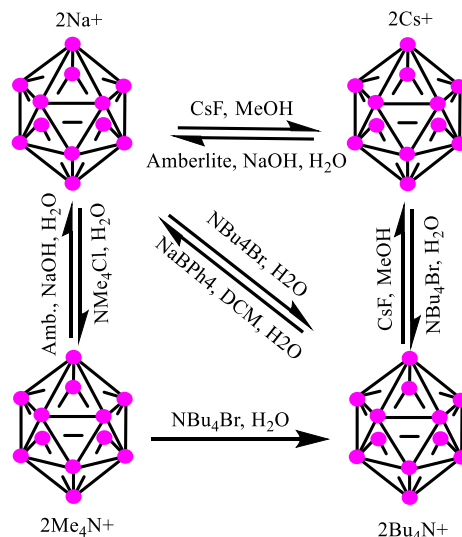
**Scheme 4.** Cation exchange of the closo-dodecaborate cluster.

## 1.5 Heteroatom Derivatives of *closo*-Dodecaborate [B$_{12}$H$_{12}$]$^{2-}$

Incorporation the [B$_{12}$H$_{12}$]$^{2-}$ cluster with organic molecules was intensively studied, general approach to link *closo*-dodecahydroborate with heteroatom-containing organic compounds is by substitution of a hydrogen atom on boron cluster. With the help of the heteroatom derivatives, then various reactions for linking with organic substances can be performed. The following sections will discuss the derivatives of [B$_{12}$H$_{12}$]$^{2-}$ in detail.

## 1.6 Halogenation of the *closo*-Dodecaborate Dianion

In the manner of its aromatic character, the chemistry of the [B$_{12}$H$_{12}$]$^{2-}$ anion undergo mainly aromatic substitution reactions. Several research groups have been studied and developed the halogenations of the *closo*-dodecaborate anion. The halogenation of the boron cluster dianion [B$_{12}$H$_{12}$]$^{2-}$ results in a significant change of the electronic properties of the molecule. The electron density is relocated from the boron atoms to the halogen substituents. This charge delocalization makes the halogenated *closo*-dodecaborate dianions interesting weakly coordinating anions (WCAs). Partly halogenated dodecaborate dianions are not easy to obtain as pure products and are often mixtures of molecules with different degree of halogenation [B$_{12}$H$_{12-n}$X$_n$]$^{2-}$ (X = Hal). Therefore, perhalogenated dodecaborates [B$_{12}$X$_{12}$]$^{2-}$ are preferred for application.

### 1.6.1 Fluorination of *closo*-Dodecaborate Dianion

Reaction of $[B_{12}H_{12}]^{2-}$ anion with hydrogen fluoride (HF) yielded the corresponding flourination derivatives with different substitution degrees $[B_{12}H_{12-x}F_x]^{2-}$, $x = 1–12$. [44] The substitution degree depends on the molar ratio of the reagents and the reaction temperature. The monofluoro derivative $[B_{12}H_{11}F]^{2-}$ was reported by heating $K_2[B_{12}H_{12}]$ in $KHF_2$ melt at 290 °C. Cesium salt was also prepared by reaction of $Cs_2[B_{12}H_{12}]$ with 1-(chloromethyl)-4-fluoro-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate) at room temperature in aqueous dimethylformamide. The structures of the prepared fluoro derivatives were determined by NMR spectroscopy and X-ray diffraction analysis and the substitution sequence was established. It was shown that a fluorine atom acts as deactivating *meta*-directing substituent. A nucleophilic aromatic substitution mechanism was proposed for the fluorination in liquid hydrogen fluoride [33], however some speculations reported that this reaction might proceed *via* electrophile-induced nucleophilic substitution mechanism.

### 1.6.2 Chlorination of *closo*-Dodecaborate Dianion

The monochloro derivative $[B_{12}H_{11}Cl]^{2-}$ has been reported for the first time by Knoth and co-workers in 1964, the reaction carried out by mixing of $(H_3O)_2[B_{12}H_{12}]·nH2O$ with anhydrous hydrogen chloride at 85 °C. [44a] Haeckel *et al*. reported in 1995 new approach to $[B_{12}H_{11}Cl]^{2-}$, the reaction of the tetrabutylammonium salt of $[B_{12}H_{12}]^{2-}$ with dichloromethane in the presence of trifluoroacetic acid[45] provided the desired monochloro dodecaborate in good yield. Chlorination of $[B_{12}H_{12}]^{2-}$ with chlorine in water gives a mixture of $[B_{12}H_{11}Cl]^{2-}$, $[1,2-B_{12}H_{10}Cl_2]^{2-}$, $[1,7-B_{12}H_{10}Cl_2]^{2-}$, $[1,2,3-B_{12}H_9Cl_3]^{2-}$, and $[1,7,9-B_{12}H_9Cl_3]^{2-}$ derivatives [46], which can be separated by chromatography. The perchloro derivative $[B_{12}Cl_{12}]^{2-}$ has been prepared by the treatment of $[B_{12}H_{12}]^{2-}$ with chlorine at 85 °C in acidic aqueous solution [47] or at 150 °C in water.[48]

### 1.6.3 Bromination of *closo*-Dodecaborate Dianion

Monobromo derivative $[B_{12}H_{11}Br]^{2-}$ was prepared by the reaction of $[B_{12}H_{12}]^{2-}$ with dibromomethane in the presence of trifluoroacetic acid. [47b] The treatment of $[B_{12}H_{12}]^{2-}$ with bromine in a water–tetrachloromethane mixture gave a mixture of mono, 1,2 and 1,7-dibromo derivatives, [46] which can be separated by chromatography. The bromine molecule reacts strongly with $[B_{12}H_{12}]^{2-}$in aqueous methanol at −10 °C, the reaction yielded a mixture of tetra-

and pentabromo derivatives [49], whereas the same reaction at 5 °C gives the hexabromo derivative $[B_{12}H_6Br_6]^{2-}$, decabromo derivative $[B_{12}H_2Br_{10}]^{2-}$ was achieved by heating $(H_3O)_2[B_{12}H_{12}] \cdot nH_2O$ with bromine in water at 80–90 °.[44a] The perbromo derivative $[B_{12}Br_{12}]^{2-}$ was prepared by the treatment of $[B_{12}H_6Br_6]^{2-}$ with bromine in the presence of chlorine in 50% aqueous methanol.

### 1.6.4 Iodination of *closo*-Dodecaborate Dianion

The synthesis of monoiodo derivative $[B_{12}H_{11}I]^{2-}$ was reported by the reaction of $[B_{12}H_{12}]^{2-}$ with one equivalent of iodine in aqueous methanol at 0 °C [44a] and by the reaction with diiodomethane in the presence of trifluoroacetic acid. [45] The use of two equivalents of iodine results in the disubstituted product which is mainly 1,7-isomer. The refluxing of $Na_2[B_{12}H_{12}]$ with iodine monochloride in 1,1,2,2-tetrachloroethane or nonyl  alcohol yielded the periodo derivative $[B_{12}I_{12}]^{2-}$ in good yield. [44a] [49] The crystal structures of monoiodo and periodo-*closo*-dodecaborate were determined as well.

### 1.7 Derivatives with Boron-Oxygen

### 1.7.1 Hydroxy Derivative of *closo*-Dodecaborate Dianion

Electrophilic substitutions of arenes have been extensively studied, and these studies established the effect of substituents on reactivity and regiospecificity of reactions at the aromatic ring. However, few reports have been published regarding the influence of substituents on subsequent reactions of the *closo*-$[B_{12}H_{12}]^{2-}$ cluster, which was first reported in 1960 and is the parent species of polyhedral boranes and carboranes.[50] The hydroxylated derivatives of  *closo*-$[B_{12}H_{12}]^{2-}$ cluster may have a more interesting chemistry because of their greater reactivity relative to the inert halogenated *closo*-$[B_{12}H_{12-n}X_n]^{2-}$ (X = F, Cl, Br, I) species.

  A variety of approaches for the synthesis of the hydroxy derivative of the *closo*-dodecaborate anion $[B_{12}H_{11}OH]^{2-}$ are reported in the literature. In 1964, Knoth *et al*. presented an efficient approach to monohydroxylation of *closo*-dodecaborate by heating the $[B_{12}H_{12}]^{2-}$ anion with 1-methylpyrrolidin-2-one at 180 °C in the presence of hydrochloric acid, the reaction provided the corresponding 1-methyl-1-pyrrolinio-2-yloxy derivative which can be easily hydrolysed with alkali to $[B_{12}H_{11}OH]^{2-}$.[51] The reaction of the $[B_{12}H_{12}]^{2-}$ anion with sulfones under acid conditions was another convenient approach to synthesis $B_{12}H_{11}OH$ in good yield. The reactions

with tetrahydrothiophene 1,1-dioxide or dipropyl sulfone gave the product of $[B_{12}H_{11}OS(O)C_4H_8]^{1-}$ which is can easily hydrolysed to afford the hydroxy derivative.[52] Further approaches to synthesize $B_{12}OH$ were reported, e.g., refluxing the $[B_{12}H_{12}]^{2-}$ anion in acidic propan-2-ol followed by hydrolysis of the formed isopropoxy derivative with concentrated hydrobromic acid. [51a] As well as, reaction of $[B_{12}H_{12}]^{2-}$ with acetyl chloride in acetone, and with sulfur dioxide at 60 °C under pressure, [51a] or by applying a mild oxidation of the *closo*-dodecaborate anion with oxalic acid. [53]

The syntheses of the hydroxy derivative by the reaction of the *closo*-dodecaborate with aqueous sulfuric acid at 90 °C have been described by Peymann *et al*. in 2000 in high yields (75−80%) [54]. Refluxing of $[B_{12}H_{12}]^{2-}$ in acetone-concentrated hydrochloric acid (HCl) yielded the dihydroxy isomers derivatives of $[B_{12}H_{10}(OH)_2]^{2-}$ as a mixture of the 1,2- and 1,7-isomers.[55] The pure $[1,2-B_{12}H_{10}(OH)_2]^{2-}$ isomer was obtained by alkaline hydrolysis of $[1,2-C_6H_5CH_2C(O)-OB_{12}H_{10}OH]$. [56] Ttrisubstituted hydroxy derivative was synthesized by heating of the $[B_{12}H_{12}]^{2-}$ anion in a mixture of acetone and concentrated hydrochloric acid at 100 °C. [54] The tetrasubstituted hydroxy derivative $[1,2,8,10-B_{12}H_8(OH)_4]^{2-}$ was obtained by the reaction of the $[B_{12}H_{12}]^{2-}$ anion with aqueous sulfuric acid at 175 °C.[54] The perhydroxy derivative $[B_{12}(OH)_{12}]^{2-}$ was prepared by heating the $[B_{12}H_{12}]^{2-}$ anion with 30% hydrogen peroxide under reflux.[57] [58] The perhydroxy derivative was also obtained from the reaction of the monocarboxylato derivative $[B_{12}H_{11}COO]^{2-}$ and hydrogen peroxide. [58]

Alkylation reaction of $[B_{12}H_{11}OH]^{2-}$ with alkyl halides in dimethyl sulfoxide and in the presence of potassium hydroxide [59] or in acetone with existence of potassium carbonate[31e] gives the alkoxy derivatives $[B_{12}H_{11}OR]^{2-}$ (**Scheme 5**). The synthesis of a boron-containing glucoside, ethoxy [60] and phenoxy derivative, [61] 1,7-dialkoxy derivatives, [62] and per-O-benzylated derivative [63] were also reported.



R = $C_2H_5$, $(CH_3)_2CH$, $CH_3(CH_2)_{15}$, $CH_2=CHCH_3$, $C_6H_5CH_2$, $4-NCC_6H_4CH_2$, $4-O_2NC_6H_4CH_2$, $4-O_2NC_6H_4CH_2CH_2$, $C_6H_4(CO)_2NCH_2CH_2$
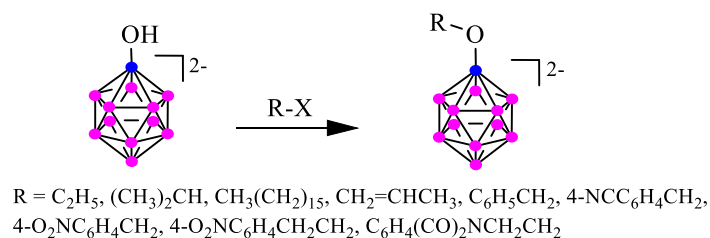
**Scheme 5.** Alkylation of $[B_{12}H_{11}OH]^{2-}$.

**1.7.2 Cyclic Oxonium Derivatives of *closo*-Dodecaborate Dianion.**

The discovery of oxonium derivatives offers a wide facile introduction of polyhedral boron hydrides into biological molecules. In the oxonium derivatives the boron cage is connected to a cyclic ether system via a covalent B-O+ bond. At present two different ways are known for the synthesis of oxonium derivatives. The first one is based on the reaction of boron hydrides with ethers in the presence of Lewis or Brønsted acids. This type of reaction is practically unknown in organic chemistry and is explained here in more detail (**Scheme 6**). The reaction involves the primary attack of an electrophilic agent on the borane hydride followed by a simultaneous elimination of hydride and electrophile resulting in a carbocation-like centre on the boron atom. Subsequently, a nucleophile attacks the positively charged boron atom to form a covalent bond. This mechanism is known as electrophile-induced nucleophilic substitution (EINS). In the absence of strong nucleophilic species even weak nucleophiles, such as ether solvent molecules, attack the boron atom giving the corresponding oxonium derivatives. [31d] In the presence of a Brønsted acid, a hydrogen molecule is eliminated whereas in the case of Lewis acid a simple abstraction of the hydride hydrogen atom by the acid results directly in a quasi-carbocation particle $[B_{12}H_{11}]^{1-}$. The intermediate $[B_{12}H_{11}]^{1-}$ is supported by quantum chemical calculations for protonation of the *closo*-dodecaborate anion $[B_{12}H_{12}]^{2-}$ [64]. The first oxonium derivatives were described by Young *et al.* in 1969. Here the 7,8-dicarba-*nido*-undecaborate anion reacts with $FeCl_3$ in tetrahydrofurane to two isomeric tetramethylene oxonium derivatives.[65] A tetrahydropyrane (THP) derivative of the dodecaborate cluster had first been published by Peymann *et al.* in 1996 and had prepared by alkylation of hydroxyundecahydro-*closo* dodecaborate with dibromopentane.[59] This potential synthesis strategy involves multiple reaction steps.
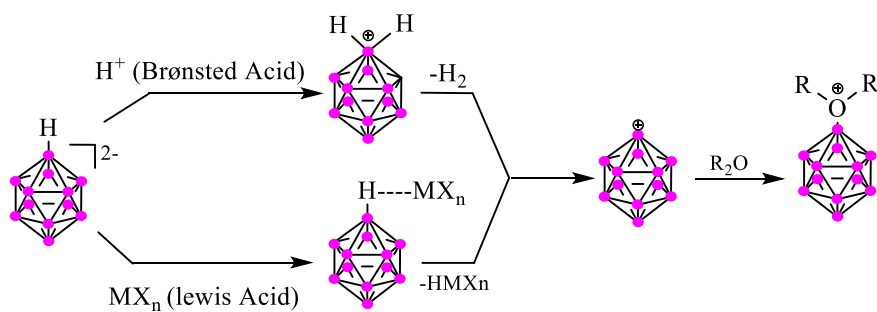


**Scheme 6.** Synthetic approaches of cyclic oxonium derivatives of $[B_{12}H_{12}]^{2-}$.

Between 2000 and 2008, Sivaev *et al*. developed a convenient method (Scheme 7).[66] [67] For both synthesis ways the EINS was used. At first, the synthesis of the THF and dioxane derivative of the cluster respectively was obtained via the reaction of $[B_{12}H_{12}]^{2-}$ with $BF_3 \cdot OEt_2$ in the corresponding cyclic ethers. Recently a new synthesis way was proposed by same research group in 2008 in which $[B_{12}H_{12}]^{2-}$ reacts with hydrogen chloride in 1,4-dioxane in the presence of $NaBF_4$.
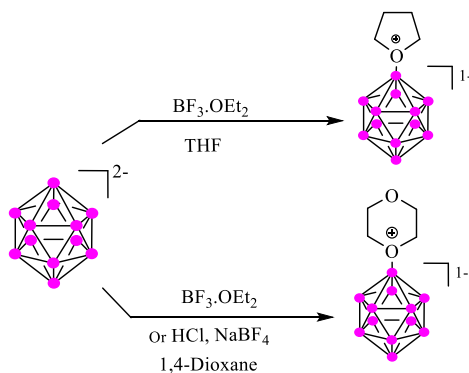


**Scheme 7.** Synthesis of oxonium derivatives of THF and 1,4-dioxane.

Oxonium derivatives of boron hydrides are known with higher stability; however, they could act as alkylating agents. Especially attractive for the cyclic oxonium derivatives, breaking of acarbon–oxygen bond resulting in formation of a moiety having a boron cluster separated from a carbocationic centre by chain of 4–5 atoms. In such a way, molecules with a reasonable length spacer between the boron cage and the property-determining part of the molecule could be prepared. In this regard, it should be noted that active use of this type of compounds in synthesis started only 30 years after they had been prepared for the first time.

The ring opening reactions have a wide range of application, mainly in the preparation of boron compounds for BNCT. In 1997 the first nucleophilic ring opening reactions (RORs) are published by Peymann *et al.,* they have reported the opening of the tetrahydropyrane ring with hydroxide and fluoride as nucleophile. [68] In 2000 Sivaev *et al*. described also nucleophilic RORs with the cyclic oxonium derivatives (THF and dioxane) and thereby established a facile method for functionalization of a wide range of compounds [66]. Ring opening reactions are possible with oxygen, sulfur, phosphorus, halogens, or carbon as nucleophiles. In 2007, Semioshkin *et al*. published the reaction of oxonium derivatives with various amines and the preparation of novel $B_{12}$- containing piperazines and amino acids. [69]

## 1.8 Derivatives with Boron–Sulfur Bond

$[B_{12}H_{11}SH]^{2-}$ derivative is gained great interest in medicinal chemistry field because its sodium salt $Na_2[B_{12}H_{11}SH]^{2-}$ has been used as an agent for boron neutron capture therapy for cancer. The mercapto derivative was obtained for the first time by the reaction of $(H_3O)_2[B_{12}H_{12}]\cdot nH_2O$ with hydrogen sulfide in the early 1960's. [51a] The reaction provided a mixture of the mono- and dimercapto derivatives as well as the hydroxy derivatives, yield of the monomercapto derivative $[B_{12}H_{11}SH]^{2-}$ after work-up did not exceed 25%. The interaction of the $[B_{12}H_{12}]^{2-}$ anion with thiocarbonyl compounds in acid media followed by alkaline hydrolysis of the formed intermediates. Alkylation of the mercapto derivative with alkyl halides is reported, the general approach provided a mixture of the corresponding sulfide and sulfonium derivatives. [31a] [70] [71] The cyclic sulfonium derivative was also obtained by reacting with .1,4-dibromobutane. [31a]
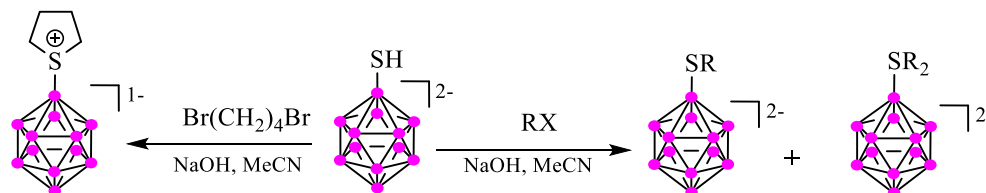


**Scheme 8.** Alkylation approaches of $[B_{12}H_{11}SH]^{2-}$.

## 1.9 Derivatives with Boron–Phosphorous Bond

Pyrolysis of trimethylphosphine–borane complex provided the trimethylphosphonium derivatives of the dodecaborate anion $[B_{12}H_{11}PMe_3]^{1-}$ with 60% yield. [36] Recently, Bernard *et al*. reported that the reaction of a molar excess of $[N(n\text{-}C_4H_9)_4]_2$-closo-$[B_{12}H_{11}I]$ with tetrakis(triphenylphosphine)palladium (0), Pd(0)L$_4$, yields to the formation of the title monoanionic compound, *closo*-$[1\text{-}B_{12}H_{11}P(C_6H_5)_3][N(n\text{-}C_4H_9)_4]$ in 54% yield (**Scheme 9**).[72]
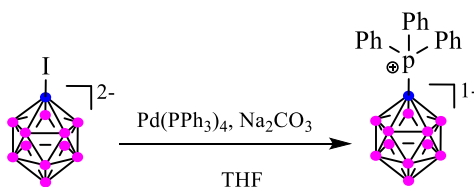


**Scheme 9.** B-P bond formation via palladium-catalyzed coupling of $[B_{12}H_{11}I]^{2-}$ .

## 1.10 Derivatives with Boron–Carbon Bond

Synthesizing $B_{12}$-cluster bearing carbonyl moiety has facilized preparation of various monosubstituted derivatives of the *closo*-dodecaborate anion. Monocarbonyl derivative $[B_{12}H_{11}CO]^{1-}$ has been reported by reaction of $[B_{12}H_{12}]^{2-}$ with oxalyl chloride in dichloromethane or acetonitrile at room temperature, the desired mono-product was obtained in quantitative yield. [73] [74] Due to high reactivity of carbonyl derivatives of the *closo*-dodecaborate anion toward nucleophiles, this compound served as starting material for synthesis of various derivatives of the $[B_{12}H_{12}]^{2-}$ anion. [75] [76] [77] The ketone derivatives $[B_{12}H_{11}COR]^{2-}$ (R = $CH_2C_6H_5$, $CH_2CH_2C_6H_5$, $C_6H_5$, $1\text{-}C_{10}H_7$, $4\text{-}O_2NC_6H_4$, $4\text{-}ClC_6H_4$, $4\text{-}BrC_6H_4$) were obtained from the reaction of sodium salt of $[B_{12}H_{12}]$ with the corresponding acyl chlorides in acetone as solvent. [78]

  Palladium-catalysed cross coupling methodology was also used for incorporation carbon moieties to boron cluster. The phenyl and alkyl derivatives were synthesized by the coupling of $(Bu_4N)_2[B_{12}H_{11}I]$ with PhMgBr and Alkyl-MgBr in 1,4-dioxane [79] or with PhZnCl in tetrahydrofuran. [80] Corresponding derivatives of $[B_{12}H_{11}R]^{2-}$ (R = Ph, $CH_3$, $C_{18}H_{37}$) have been obtained in good yield 54-76%.[79] The monomethyl derivative was obtained also by the reaction of $[B_{12}H_{11}I]^{2-}$ with refluxing trimethylaluminium(**Scheme 10**). [81]
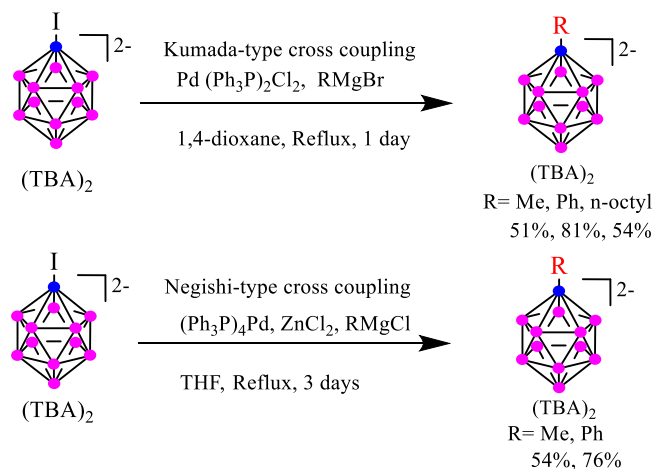


**Scheme 10.** Palladium-catalyzed coupling reaction of $(Bu_4N)_2[B_{12}H_{11}I]$.

  Himmelspach *et al*. reported the synthesis of salts of the ethynyl-*closo*-dodecaborate dianion $[HC{\equiv}C\text{-}closo\text{-}B_{12}H_{11}]^{2-}$by a microwave-assisted palladium-catalysed cross-coupling reaction starting from the iodinated cluster $[closo\text{-}B_{12}H_{11}I]^{2-}$and $Me_3SiC{\equiv}CMgBr$. The preparation of

salts by a microwave-assisted synthesis demonstrates the value of this technique for cross-coupling reactions of iodinated closo-$B_{12}$ clusters. [82] The desired product was achieved in high yield (77%) compared to the previous reported examples (**Scheme 11**).
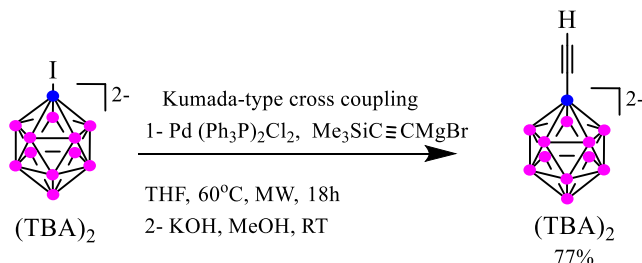


**Scheme 11.** Microwave-assisted palladium-catalyzed cross-coupling of $B_{12}I$.

Recently, the percyanated dodecaborate anion $[B_{12}(CN)_{12}]^{2-}$ was prepared by Kamin and co-workers, the reported method involves heating of $[B_{12}I_{12}]^{2-}$ with CuCN in the presence of a palladium catalyst at elevated pressure and temperature in a microwave reactor. The fully cyanided cluster was isolated as a tetraethylammonium salt in yields of up to 39%. [83]

## 1.11 Derivatives with Boron–Nitrogen Bond

In 1964, Hertler *et al.* described the reaction of hydroxylamine-O-sulfonic acid with $[B_{12}H_{12}]^{2-}$, hydroxylamine-O-sulfonic acid reacts smoothly with $[B_{12}H_{12}]^{2-}$ in aqueous solution to give the corresponding mono or diamino derivatives. [84] The amino derivative of *closo*-$B_{12}$ was a promising starting material for incorporate N-containing organic compounds to the $B_{12}$-cage (**Scheme 12**). Trimethylamine derivatives were prepared by treatment of the amino derivatives with dimethyl sulfate in alkaline solution. [84] A variety of alkylamine derivatives were also prepared by deprotonation of $[B_{12}H_{11}NH_3]^-$ with strong bases (NaH or KOH) followed by the reaction with alkyl halides (**Scheme 12**). [85] [86]

Sivaev *et al*. reported the reaction of $[B_{12}H_{11}NH_3]^-$ with aromatic aldehydes in methanol and in the presence of catalytic amounts of alkali gives N-protonated Schiff bases $[B_{12}H_{11}NH=CHR]^-$ (R = $C_6H_5$, 2-$C_6H_4OMe$, 4-$C_6H_4OMe$, 4-$C_6H_4SMe$, 4-$C_6H_4NMe_2$, 4-$C_6H_4NHCOMe$, 4-$C_6H_4CN$, 4-$C_6H_4Br$, 4-$C_6H_4Cl$, 3,4-$C_6H_3O_2CH_2$, 1-$C_{10}H_7$, 2-$C_{10}H_7$, CH=CHMe, CH=CHPh). The Schiff bases reduced with $NaBH_4$ in aqueous 2-propanol to provide almost quantitatively the corresponding monoalkylamino derivatives $[B_{12}H_{11}NH_2CH_2R]^-$ (R = 2-$C_6H_4OMe$, 3,4-$C_6H_3O_2CH_2$, 4-$C_6H_4NHCOMe$, 4-$C_6H_4CN$). *closo*-dodecaborate derivatives containing amino, carboxy, and

isothiocyanate functions on aromatic rings, $[B_{12}H_{11}NH_2CH_2C_6H_4\text{-}4\text{-}NH_2]^-$, $[B_{12}H_{11}NH_2CH_2C_6H_4\text{-}4\text{-}COOH]^-$, and $[B_{12}H_{11}NH_2CH_2C_6H_4\text{-}4\text{-}NCS]^-$, were also prepared[31f], in general the reaction was limited for reaction of aromatic aldehydes while aliphatic aldehydes didn't show good reactivity (**Scheme 12**). Reactions to guanidinium derivatives and arylamides starting from $[B_{12}H_{11}NH_3]^{1-}$ were described [87]. Alkylation of the N atom to di- and tri-substituted ammonium salts is possible, but selective monoalkylation could not be achieved.[88] Nucleophilic aromatic substitution of *closo*-$[B_{12}H_{11}NH_3]^{1-}$ with 1-chloro-2,4-dinitrobenzene has been described,[89] resulting in a mixture of two compounds with very poor yield. $(Bu_4N)_2[B_{12}H_{12}]^{2-}$ reacts with the amino group p-aminopyridine at 190°C to a B-N bond.[90] Under similar conditions, the cluster reacts with the amino group of aniline, but undergoes further hydroamination reactions to a mixture of compounds in poor yield. [89] The alkylamide and aromatic amide derivatives can be prepared by reaction of the amino derivative with acyl chlorides (**Scheme 12**). [91] [92]



**Scheme 12.** Current methodologies for B-N bond formation.

## 1.12 Scope of the Thesis

The lack of effective, non-hygroscopic and easy-handling, economic, and diversified ways of functionalizing *closo*-dodecaborate $[B_{12}H_{12}]^{2-}$ is the main reason why these moieties did not find a wide application in the synthesis of boron-containing biomolecules until recently. This thesis presents the generation and attractive development of novel methodologies to functionalize *closo*-dodecaborate dianions. The thesis focuses on development of three main derivatives, first, preparation of cyclic oxonium derivatives of the *closo*-dodecaborate anion using cheap commercial available aqueous (37%) HCl, the modified procedure provided the desired products in good yield, as well as, using non-hygroscopic and easy-handling material simplifying achieving highly pure cyclic oxonium derivatives in short time which are important substrate for nucleophilic ring-opening methodology that lead to the preparation of a wide spectrum of its functional derivatives. Second, obtaining mono-halo tetrabutylammonium salt of *closo*-dodecaborate dianion by reaction of $(TBA)_2[B_{12}H_{12}]$ with N-halosuccinimid in acetonitrile, the new approch provided highly pure monhalo *closo*-dodecaborate in quantitative yield. Third, we describe a productive approach of B-N bond formation on the *closo*-dodecaborate anion via palladium-catalyzed cross coupling assisting with microwave irradiation. Palladium-catalyzed cross coupling amination of Iodo-undecahydro-*closo*-dodecaborate $[B_{12}H_{11}I]^{2-}$ provides a general avenue for the formation of boron-cluster containing organic molecules.

At the outset of this project, we investigated the reactivity of the $[B_{12}H_{11}I]^{2-}$ anion in Pd-catalyzed amination reactions. Although the derivatization of such anions and their use in transition-metal-catalyzed reactions have been studied in the past, however, there were no reports on their use in Pd-catalyzed B-N bond formation. The method developed serves as a milder, cheaper, and more reproducible route for the construction of the B-N bond. The new approach shows a wide scope of applications and allows the incorporation of aromatic amines, N-containing heterocycles, and amides in excellent yield.

**1.13 A Novel Synthesis of Cyclic Oxonium Derivatives of *closo*- Dodecaborate Anion with Aqueous HCl**

Historically, THF and dioxane derivatives of *closo*-dodecaborate cluster respectively were obtained via the reaction of $[B_{12}H_{12}]^{2-}$ with $BF_3 \cdot OEt_2$ in the corresponding cyclic ethers. Recently a new synthesis way was proposed in 2008 by Sivaev *et al.* in which $[B_{12}H_{12}]^{2-}$ reacts with hydrogen chloride in 1,4-dioxane in the presence of $NaBF_4$. While tetrahydropyrane (THP) derivative of the dodecaborate cluster had first been published by Peymann *et al.* in 1996 and had prepared by alkylation of hydroxyundecahydro-*closo*dodecaborate with dibromopentane (see section **1.6.2**).

  Recently, we developed in our lab a new simple, cheap, and effective approach to synthesize THF, 1,4-dioxane, and tetrahydropyrane oxonium derivatives by simple modification on Sivaev approach. We noticed that using 4M of commercially available aqueous 37% HCL in appropriate water miscible cyclic ether would drive the reaction to complete conversion and provides almost same yield as the one has been reported. The cyclic oxonium derivatives of THF, 1,4-dioxane, and tetrahydropyrane have been obtained in 70-75% yield in two hours reaction time. The obtained products have been characterized with ^{11}B-NMR, ^{1}H-NMR, and mass spectroscopy and the obtained results completly match the reported results (**Scheme 13**).  Main advantages of this method over the previously published is summarized in very good yield of the goal product, high reproducibility specially with large scale reaction, use of non-hygroscopic and easy-handling tetrabutylammonium *closo*-dodecaborate as the starting material, as well as its diversified way for synthesis of cyclic oxonium derivatives of $[B_{12}H_{12}]^{2-}$.

  Our new general methodology was tested for introducing other substituted cyclic ethers like morpholine, 4,4-dimethylmorpholine, and tetrahydro-2H-pyran-4-carbaldehyde, due to immiscibility of these compounds in water, they don't show good reactivity, therefore we don't manage to get desired product.
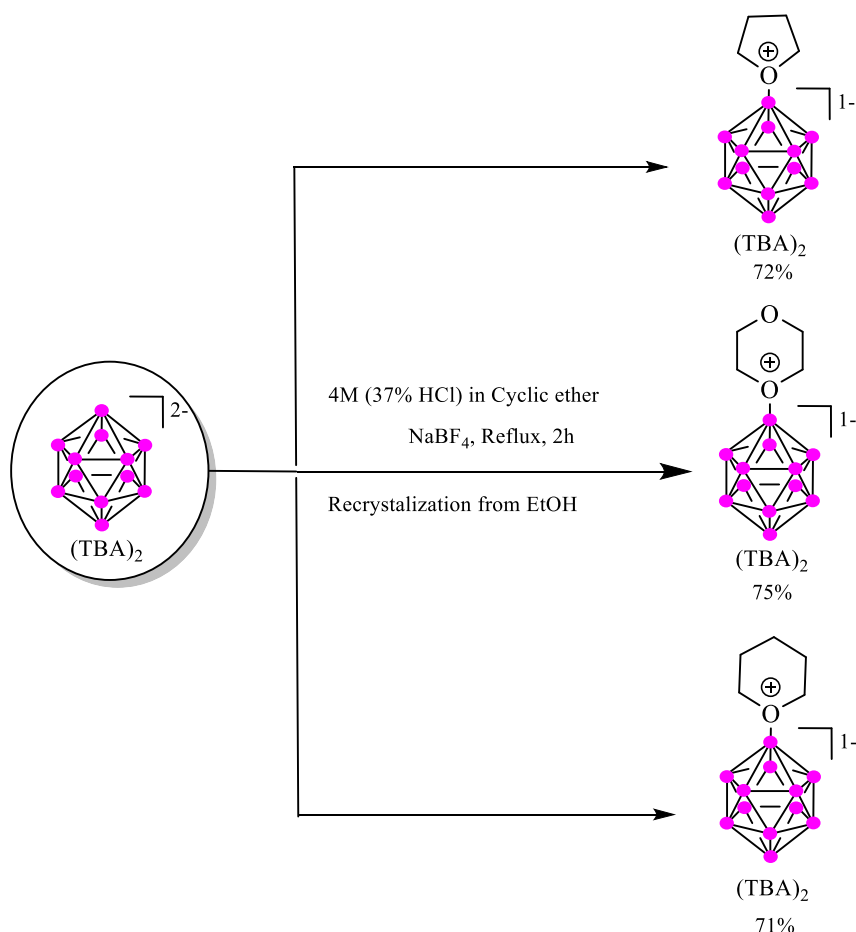
**Scheme 13.** Aqueous HCl mediated synthesis of cyclic oxonium derivatives of (TBA) $[B_{12}H_{12}]^{2-}$.

General procedure: To suspension of (1.0 mmol) $(Bu_4N)_2[B_{12}H_{12}]$ and (5.0 mmol) $NaBF_4$ in 70 ml of THF, 1,4-dioxane, or tetrahydropyrane, 1.0 ml of 4 M solution HCl in THF, 1,4-dioxane, or tetrahydropyrane was added. The reaction mixture was heated at reflux for 2 h, allowed to cool to room temperature, filtered and concentrated near to dryness under reduced pressure. The residue was recrystallized from ethanol. The Precipitate washed with a small amount of ethanol and dried in air to give the desired product.

### 1.14 A Novel Halogenation of *closo*-Dodecaborate

A new approach of synthesis highly pure mono-halo of tetrabutylammonium *closo*-dodecaborate has been developed in our lab. The new method represents mild condition, fast, and effective approach to obtain a highly pure mono-halo derivative (TBA)_2 $[B_{12}H_{11}X]^{2-}$(X=Cl, Br, I). The desired products were obtained by reaction of one equivalent of (TBA)_2 $[B_{12}H_{12}]^{2-}$ with one

equivalent of N-halosuccinimid in acetonitrile. The addition of N-halosuccinimide carried out portion-wise at -10-0 $^o$C in case of iodination and bromination process and the reaction mixture stirred for one hour at room temperature providing quantitative yield of desired product and succinimide as a side product which can be easily removed by extraction the crude mixture with DCM and water. While chlorination of (TBA)$_2$ [B$_{12}$H$_{12}$]$^{2-}$ need reflux the mixture for one hour for complete conversion, the desired mono-chloro of (TBA)$_2$ [B$_{12}$H$_{12}$]$^{2-}$ was collected in 85% yield by column chromatography.



**Scheme 14.** Synthetic approach of (TBA)$_2$ [B$_{12}$H$_{11}$X]$^{2-}$ where (X= I, Br, Cl).
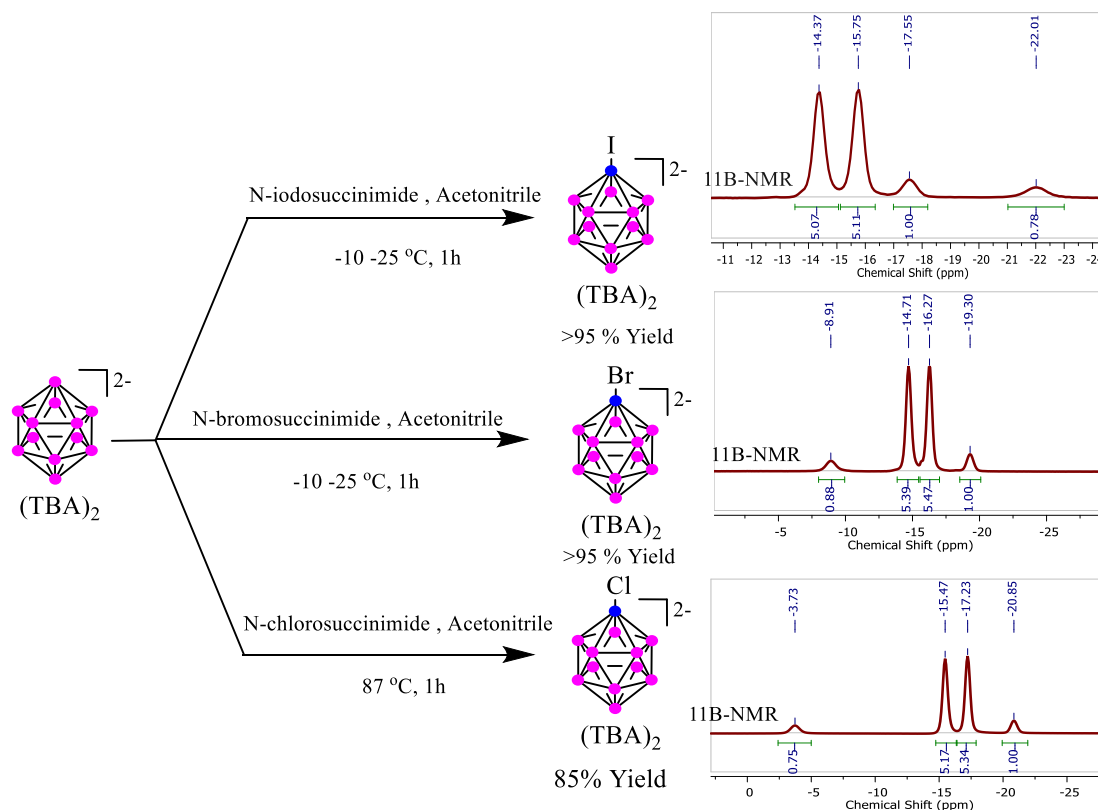
The procedure and the work-up yields much purer material than any of the literature procedures. Yields are very high, and work-up is very straight-forward. In our experience, this method is the only method which yields the mono-iodinated cluster B$_{12}$H$_{11}$I$^{2-}$ without any di- or tri-iodinated product or any starting B$_{12}$H$_{12}$$^{2-}$. The methods described before all yield mixtures, which were difficult to impossible to separate.

## 1.15 Microwave-Assisted Palladium Cross-Coupling Amination of Iodo-Undecahydro-*closo*-Dodecaborate with Large Versatility

As discussed in detail in section 1.10, the synthesis of the B-N derivative of $[B_{12}H_{12}]^{2-}$ still limited and challenging. Collectively, for many years a major limitation for expanding the application of the *closo*-dodecaborate has been attributed to its poor reactivity and lack of derivatization methods. On the other hand, palladium- catalyzed cross-coupling reactions have emerged as an extremely powerful, versatile, and tolerant tool for many functional groups. This new catalytic system demonstrated an effective approach to cross-couple aryliodides, arylbromides, and arylchlorides, thereby facilitating transformations of synthetically challenging substrates. Among existing ligand platforms, biaryl phosphine ligands significantly increased the efficacy of Pd-catalyzed C–C, C–N, and C–O bond formation. Despite of wide application of this methodology for aromatic substrates, effective methodologies for metal-catalysed B–N cross-coupling for ionic boron clusters are lacking. In fact, B-iodo-dodecaborates have been used in Pd-catalysed cross-coupling, so we decided to start our investigations using B-iodo dodecaborate as starting electrophile. Successfully, the iodo-dodecaborate shows high reactivity for coupling multiple N-containing organic compounds nucleophiles onto the boron-rich clusters. Selection of optimal ligand and other reaction parameters (such as Pd source, base, solvent, and temperature) can vary for different substrate combinations, the reason is because of the effect of the electronic and steric properties of the nitrogen-based nucleophiles when compared to other cross-coupling processes such as the Kumada-type cross coupling reaction. The amine and amides can differ in nucleophilicity and p$K_a$ which means that the rate determining step of the catalytic cycle can vary with substrate, resulting in the difficulty in selecting the optimal conditions. We postulated that employing a combination of commercially available Pd (0) precursor (Pd$_2$dba$_3$, dba = dibenzylideneacetone) and the biaryl phosphine ligand DavePhos, which has been previously shown to dramatically improve catalytic activity across a large pool of aryl-based substrates, will efficiently forming the catalytically active species [Ligand-Pd$^0$][93]. Importantly, this ligand-catalyst system greatly improved the catalytic conversion of iodo-dodecaborate producing B-N bond in nearly quantitative conversion in most cases within 15 minutes using microwave irradiation.
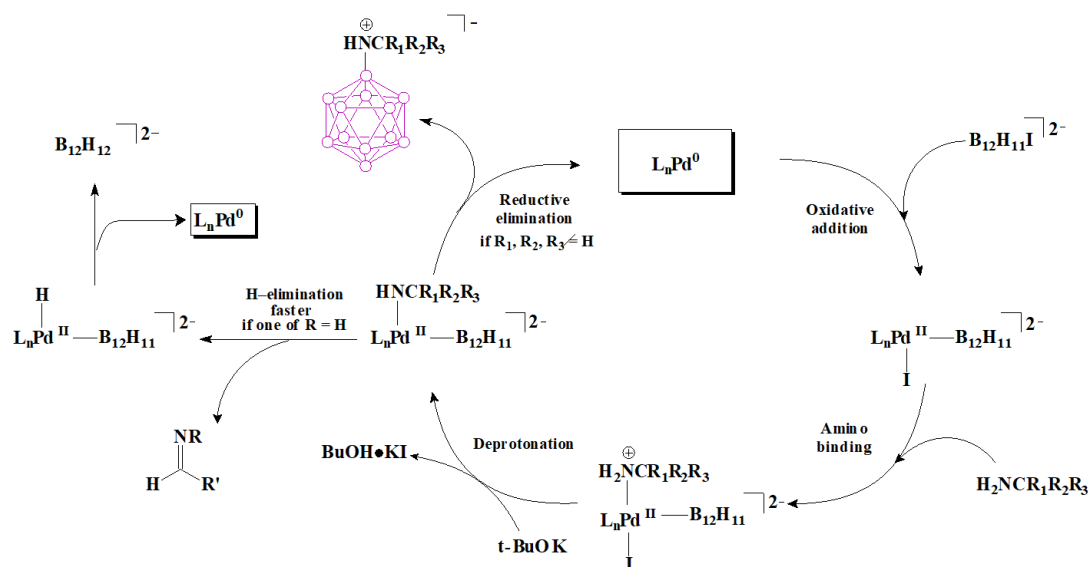
**Figure 4.** Proposed catalytic cross-coupling cycle for Pd-catalyzed amination of $[B_{12}H_{11}I]^{2-}$.

Pd-catalyzed cross-coupling generally attributes three elementary steps: oxidative addition of an electrophile, transmetallation of a nucleophile, and coupling of the electrophile and nucleophile by reductive elimination. (**Figure 4**) represent our proposed mechanism for developed Pd-catalyzed B-N cross coupling methodology.

In cross-coupling reaction of polyhedral borane, the published reports exhibited that the most reactive boron-halogen bond is a B-I bond because it's classified as the weakness bond compared to B-Cl and B-Br, therefore, other B-X (X = F, Cl, or Br) bonds are too unreactive. The lack of reactivity in B-bromo and B-chloro polyhedral boranes in cross-coupling reactions was attributed to the stronger B-X bonds, compared to aryl C-X bonds, which prevented oxidative addition of B-bromo and chlor-carboranes as well as dodecaborate. This difference in B-X bond strength allowed radio labelling of carboranes with $^{76}$Br and $^{18}$F because the resulting B-bromo- and B-fluoro-carboranes do not undergo oxidative addition in the presence of commonly used Pd catalysts.[94]

Herein, we developed an unprecedented and efficient method for the formation of the B−N bond via microwave-assisted palladium-catalyzed amination of the Iodo-undecahydro-*closo*-dodecaborate $[B_{12}H_{11}I]^{2-}$. The development of Pd-catalyzed B−N coupling will significantly contribute to the streamlining of the synthesis of small molecule pharmaceutical agents, allowing more efficient syntheses and facilitating a modular approach to analogue synthesis. The

significance of this methodology in this regard stems from the prevalence of boron containing organic amines in biologically active molecules.[95]

As model for our initial experiments, we chose the reaction of **1** with aniline (**2a**). We varied catalytic systems, bases, and reaction conditions. Selected examples are presented in **Table 1**. We observed dramatic effects of the ligand, base, solvent, and temperature in these initial experiments. To best facilitate the $[B_{12}H_{11}I]^{2-}$ amination, we assessed several Pd/ligand system combinations. The dba ligand is known to coordinate to palladium phosphine complexes and improve the rate of oxidative addition to aryl halides and halocarboranes.[95] According to the literature, notably, that electron-rich ligands such as dialkyl biaryl phosphines, PCy3 and P$_t$Bu$_3$ show high reactivity towards B-N and B-O bond formation in carboranes.[96] For $[B_{12}H_{11}I]^{2-}$ amination, our investigation show that only DavePhos gave the desired aniline derivatives. JohnPhos, XPhos, and t-BuDavePhos gave no conversion, and SPhos and BrettPhos led to conversion of the starting material, but not to a cross-coupling product. [95] The previous finding leads us for general speculation about the possible mechanistic pathway of the amination of $[B_{12}H_{11}I]^{2-}$ using Davephos as ligand. The first stage of this type of reaction is the oxidative addition, in our case, the mono-iodo derivative $B_{12}$-I to the $Pd^0$ complex. We speculated that using Davephos as electron donor phosphines ligands would stabilize the metal center as well as facilitate the insertion of Pd. We conclude that due to the large volume of the 12-vertex of dodecaborate compared to aryl compounds, which is weakening the B-I bond and therefore make oxidative addition step possible. The presence of N(Me)$_2$ group on the body of Davephos would also stabilize the complex in this stage and facilitate the transmetallation process.
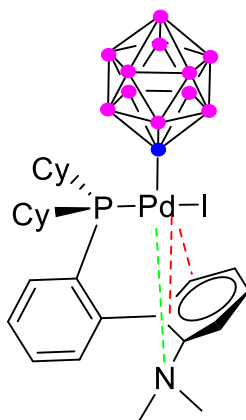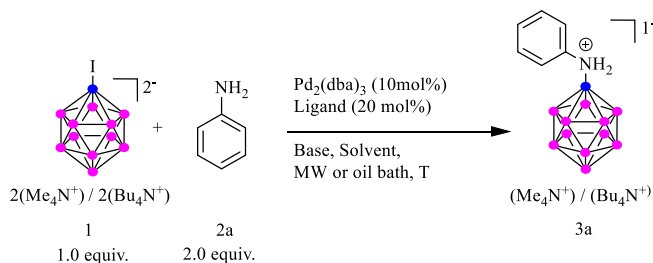


**Figure 5.** Proposed intermediate formation in oxidative addition step.

We should be noted that the electron donating properties of the *closo*-dodecabrate anion are more obvious than those of *closo*-carboranes and aromatic compounds; therefore, the binding of *closo*-dodecaborate anion will increase the electron density in the metal center than the carborane analogous. It can be assumed that the formation of the Pd-N bond will be energetically more favorable for nitrogen depleted in electron density, which should shift the equilibrium towards the formation of an ammonium complex. Deprotonation of this complex with bases should also proceed faster for the same reason. The last stage in this reaction concludes two main competing reaction pathways. The first pathway is reductive elimination and the formation of the desired product of the cross-coupling amination. The second way is the process of the so-called *beta*-elimination which is possible in the presence of protons at the carbon atom bound to nitrogen, transferring of proton instead of amine group providing $[B_{12}H_{12}]^{2-}$ as major product and desired B-N product as minor product.

As important as the choice of ligand was the choice of the heating method. Microwave irradiation proved to be far more effective than conventional heating (compare, e.g., entries **5** and **6** of **Table 1**). Using conventional heating, higher temperatures resulted in higher conversion of **1**, but not to **3a**; rather $[B_{12}H_{12}]^{2-}$ was formed, with the amination product in less than 10% yield. We therefore replaced conventional heating by microwave irradiation. We had shown before that microwave irradiation greatly increased yield and reduced reaction time in a Sonogashira-type cross coupling of **1.** (See section 1.9). Also, under microwave irradiation conditions the reaction underwent complete conversion within 15 minutes with very little side product formation, emphasizing the advantage of using microwave in these types of reactions. Kumada-type cross-coupling with **1** has been reported to proceed by conventional heating but required longer reaction times (see section 1.9).

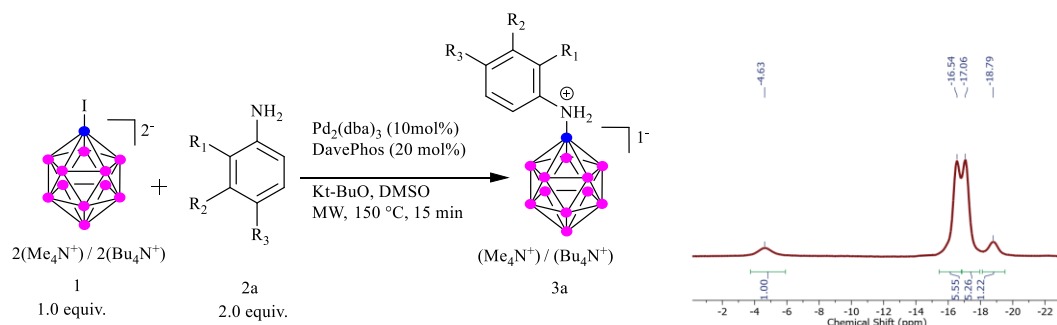**Table 1.** Screening of bases and reaction conditions.



| Entry | Base | Solvent | Ligand | $T$ (°C) | Time (h) | Consumption of 1 (%) | Yield[c] 3a (%) |
|---|---|---|---|---|---|---|---|
| 1 | $Cs_2CO_3$ | 1,4-dioxane | DP | 100[b] | 0.5 | 20 | <15 |
| 2 | KO$t$-Bu | 1,4-dioxane | DP | 100[b] | 0.5 | 50 | 30 |
| 3 | KO$t$-Bu | DMF | DP | 150[b] | 0.5 | 90 | 45 |
| 4 | KO$t$-Bu | DMF | DP | 150[b] | 1 | >95 | 45 |
| 5 | KO$t$-Bu | DMSO | DP | 150[a] | 48 | 95 | <5 |
| 6 | KO$t$-Bu | DMSO | DP | 150[b] | 0.25 | 100 | 65 |
| 7 | KO$t$-Bu | DMSO | BP | 150[b] | 0.25 | 90 | <5 |
| 8 | KO$t$-Bu | DMSO | JP | 150[b] | 0.25 | 0 | 0 |
| 9 | KO$t$-Bu | DMSO | tBP | 150[b] | 0.25 | 0 | 0 |
| 10 | KO$t$-Bu | DMSO | SP | 150[b] | 0.25 | 95 | <5 |
| 11 | KO$t$-Bu | DMSO | XP | 150[b] | 0.25 | 0 | 0 |

Reaction conditions: 1 (1.0 equiv.), aniline (2.0 equiv.), base (2.5 equiv.), $Pd_2(dba)_3$ (10 mol%), ligand (20 mol%), solvent (concentration of 1 = 0.1 M). [a] Oil bath, [b] Microwave irradiation. [c] Determined from 11B-NMR. Ligands: DP = DavePhos, JP = JohnPh, SP = SPhos, tBP = t-BuDavePhos, BP = BrettPhos, XP = XPhos.

Solvents like THF, 1,4-dioxane, toluene, and DME gave low conversion, most likely due to the low solubility of **1**, catalyst and/or base, and perhaps due to their lower boiling points (compare entry **2** of **Table 1** with entry **6**). Solvents with high polarity and high boiling point, such as DMF and DMSO, gave the desired aniline derivatives in better yields. Notably, only little of the undesired $[B_{12}H_{12}]^{2-}$ as side product was obtained using DMSO and DMF. DMSO proved to be the most effective solvent, as it could be removed more easily, and was used for further optimization of the B–N coupling reaction conditions. Also, the choice of base was important. $Cs_2CO_3$ (entry **1**) was less effective than KO$t$-Bu (entry **2**). KO$t$-Bu was therefore used for all subsequent reactions. Catalyst loading was important for the yield. Ten mol% of $Pd_2(dba)_3$

yielded >65% of the desired product, 5 mol% only between 20-30%. In most cases, we obtained a mixture of the B-N product and $[B_{12}H_{12}]^{2-}$ in a ratio that varied depending on the reaction conditions. In terms of yield, the optimum was obtained with a ratio of **1** to aniline of 1:2.

**Table 2.** Pd-catalyzed cross-coupling of 1 with substituted anilines.



| Entry | Product 3 | R₁, R₂, R₃ | Conversion[a] [%] | Yield[b] [%] |
|-------|-----------|-----------|-------------------|--------------|
| 1 | 3a | H, H, H | 85 | 65 |
| 2 | 3b | H, H, NO₂ | 100 | 84 |
| 3 | 3c | H, H, CN | 100 | 84 |
| 4 | 3d | H, H, CO₂Me | 100 | 81 |
| 5 | 3e | H, H, Me | >95 | 70 |
| 6 | 3f | H, H, OPh | >90 | 52 |
| 7 | 3g | H, H, OEt | >90 | <10 |
| 8 | 3h | H,H, N(Me)₂ | >90 | <10 |
| 7 | 3i | H, H, F | > 90 | 52 |
| 8 | 3j | H, H, Cl | < 5 | Trace[c] |
| 9 | 3k | H, H, Br | < 2 | Trace[c] |
| 10 | 3l | H, H, I | 0 | 0 |
| 11 | 3m | NO₂, H, H | 100 | 83 |
| 12 | 3n | H, NO₂, H | 100 | 83 |
| 13 | 3o | NO₂, H, NO₂ | >95 | 78 |

Reaction conditions: **1** (1.0 equiv.), Substituted aniline (2.0 equiv.), K$_t$BuO (2.5 equiv.), Pd₂(dba)₃ (10 mol%), DavePhos (20 mol%), DMSO (0.1M). [a] Conversion determined by $^{11}$B-NMR and referred to the consumption of **1**. [b] Isolated yield. [c] Based on $^{11}$B-NMR of reaction mixture.

Using Pd$_2$(dba)$_3$ (10 mol%), DavePhos as ligand (20 mol%), and KO$t$-Bu (2.5 equiv.) in DMSO as optimized protocol, we assessed the scope of substituted anilines as coupling partners. For electron-deficient anilines, yields were high (**Table 2**, **3b**, **3c**, **3d**, **3m**, **3n, 3o**), except for $p$-fluoroaniline (**3i**). $p$-Phenoxyaniline provided the B-N product in 52% yield (**3f**), while for the few examples of electron-rich anilines, amination yields were poor <10% and hydrogen transfer was dominant, resulting in [B$_{12}$H$_{12}$]$^{2-}$ as major product (**3g**, **3h**).

No conversion was observed with chloro-, bromo- and iodo-aniline under the standard reaction conditions, and the starting material **1** remained untouched. This is possibly due to the faster rate of oxidative addition of palladium with the haloanilines in comparison to that with **1**. Collectively, the reaction proceeds well with electron-withdrawing groups in aniline; electron-donating groups react, albeit with lower yield.

Given the promising results achieved, we envisioned that our methodology might also allow us to prepare other types of aromatic primary amines. Coupling of 1-naphthylamine was possible, but only with low yield (**Scheme 15**). This might be due to steric reasons or to the larger electron density on the N atom.
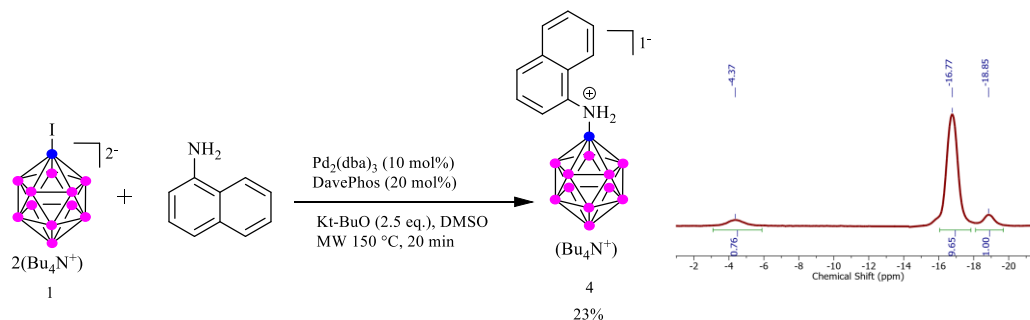


**Scheme 15.** Cross-coupling reaction of 1-naphthylamine with 1.

On our next milestone, we have decided to examine the use of a variety N-containing heterocycles as nucleophiles in the coupling with **1**, the ability to combine aromatic heterocyclic fragments with boron cluster is an important and challenging subset of this field with implications both in academic and industrial settings. Heterocyclic structures are often key fragments of biologically active molecules and many drugs contain nitrogen-rich heterocyclic moieties such as pyrrol, imidazole, and indole. Notably, our new methodology allows for the direct formation of a wide range of N-containing heterocycles. Adiverse set of N-containing heterocyclic structures and sensitive functional groups were tested, the desired B-N products

were obtained in excellent yield (**Scheme 16**, **5a-e**). According to ^{1}H-NMR and mass spectra of the obtained B-N product of Pyrrol, indole, and carbazole, the final products were obtained as dianion, while pyrazole and imidazole derivatives were collected as (1-) form and in excellent yield. As expected, pyridine did not react (**5f**).
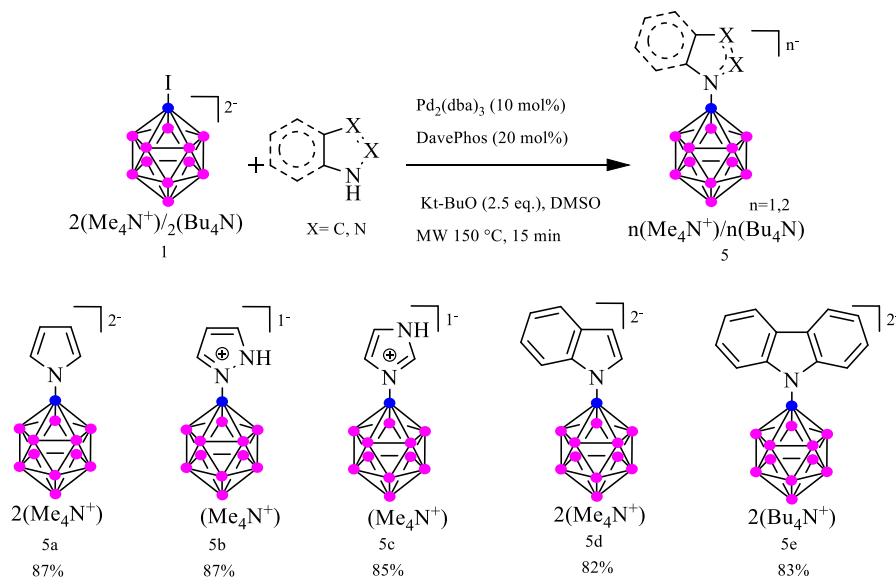


**Scheme 16.** Cross-coupling of 1 with N-containing heteroaromatics.

As we have shown in this part, rapid conversion, high yields, use of economic catalysts, and reactions with a number of different nucleophiles which can be coupled to the dodecaborate cage are particular advantages of this methodology. To expand the applicability of our approach, we investigated the coupling of amides with the dodecaborate cage. We could carry out amidation of **1** under our standard conditions, and the reaction proceeds with aromatic and aliphatic amides, and sulfonamides (**Scheme 17**). 2-nicotinamide and propanamide were successfully coupled in excellent yield. Sulfamide and *p*-toluene sulfonamide were coupled, however, with poor isolated yield. The amide coupling products were isolated as protonated species, as described before for the benzoyl derivative, where the H atom could be located on the carbonyl oxygen [87]. We successfully coupled also secondary amides; acetanilide and N- methylacetamide reacted with **1** in excellent yields (**Scheme 17**, **6e** and **6f,** respectively).
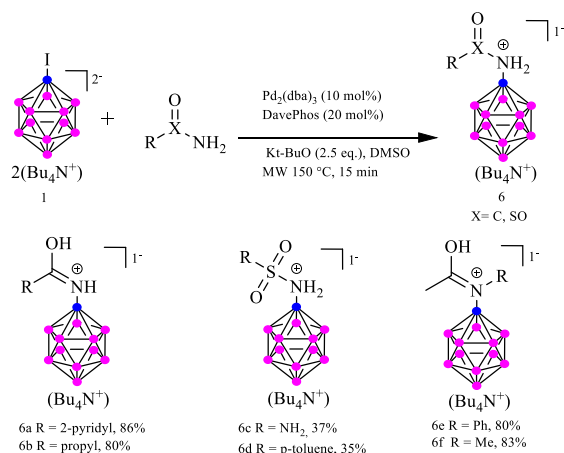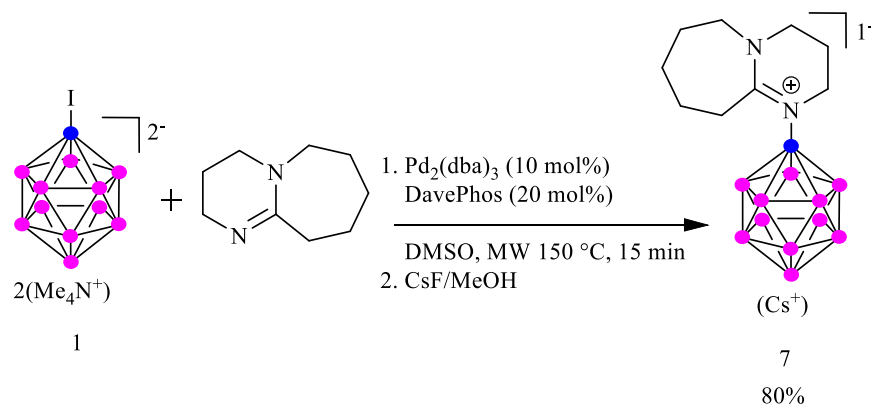
**Scheme 17.** Cross-coupling of 1 with amides.

  The reaction that we describe here is possible with aromatic amines, NH-containing heteroaromatics, and amides. For secondary primary aliphatic amines, our current methodology did not prove effective; rather, we obtained $[B_{12}H_{12}]^{2-}$ as a major product, and the B-N product in single-digit percent yields. We have not yet identified the source of the H atom in any of the reactions, but we suspect that the Pd (II) amine complex undergoes hydride elimination.

  It should be pointed out that because there were some limitations when this reaction was carried out to secondary amines. Actually, this is linked to the amine possessing hydrogen atoms α to the nitrogen atom which enables the Pd (II) amine complex to undergo β-hydride elimination to find suitable conditions for secondary and primary alkyl amines in this coupling process, we undertook an intensive screening of a variety of ligands and reaction variables. According to literature support of Buchwald-type cross coupling reactions, we have speculated that employing bulky ligands e.g., P(o-tolyl)$_3$, BrettPhos, or ligands that are not as electron-rich at the phosphorus atom e.g., JohnPhos, and t-BuDavePhos, as well as using chelating agents like BINAP, could be helpful for the formation of the desired B-N product [93]; however, despite all the previous trials, formation of the B-N product was observed in a very low yield around 5-10% in comparison to the reductive elimination product, $[B_{12}H_{12}]^{2-}$, which was observed in high amounts. An extensive search of alternative bases (milder, stronger, or less nucleophilic) was carried out; however, KO*t*-Bu and NaO*t*-Bu were the only bases which led to the formation of the desired product to a certain extent.    Regarding the solvent, DMSO was the only solvent to afford any little desired product formation while no product was observed with other solvents.

Additionally, decreasing the reaction temperature and /or reaction time does not show any progress in product formation.

In the search for alternative bases, an unexpected B-N bond formation product occurred with DBU. We obtained a product where one of the N atoms of DBU was bonded to a boron atom, a reaction that could be driven to complete conversion and excellent yield by using two equivalents of DBU (**Scheme 18**).



**Scheme 18.** Cross-coupling of 1 with DBU.

In summary, we have developed a new Pd-catalysed cross-coupling of iodo-*closo*-dodecaborate with aromatic amines, with amides, with NH heteroaromatics, and with sulfonamides. Rapid conversion, high yields, use of readily available catalysts, and reactions with several different nucleophiles that can be coupled to the dodecaborate cage are advantages of this methodology. DavePhos as ligand, DMSO as solvent, KO-*t*Bu as base, and microwave irradiation were essential. Further computational studies on reaction mechanism, and experimental studies to extend the scope of these reactions to include secondary and aliphatic primary amines, are currently underway in our laboratory.

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# Chapter 2

## Synthesis and Application of Heptamethine Indocyanine Dye

This chapter is derived from the content of the following manuscript.

# Chapter 2

# **Introduction**

## 2.1 Cyanine Dye

The name cyanine dye was derived from the ancient Greek word "κύανος" (kyanos) which means dark blue referring to colour of very first cyanine, which was discovered in 1856 by G. Williams.[1] Originally, cyanine dyes were used as textile dyes, but their application was negligible. Due to their ability to impart light sensitivity, they were applied in silver halide photography.[2] Nowadays, cyanine dyes are used in semiconducting materials, laser materials, optical recording media, and more recently they have a broad scope of biological applications and in biomedical imaging.[3] [4]

## 2.2 Structure and Resonance Forms

Cyanine dyes, a class of polymethine dyes, they are classified as fluorescent molecules that contain two nitrogen heterocyclic centers, behaving as both electron acceptors and donors that are connected by a mono- or polymethine chain (**Figure 1**).[5] The polymethine bridge consists of a conjugated chain with $n$ generally ranging from 0 to 10, as shown in (**Figure 1**).[5] The conjugation of π-electrons are distributed between the electron donor and electron acceptor groups results in a delocalized cationic charge across the polymethine bridge. Cyanine dyes are named according to the number of methine groups ($n = 0, 1, 2, 3$) in their polymethine chain. As example, cyanine dye with a seven carbons linker (n = 3) is known as heptamethine cyanine (Cy7). If n = 0, 1, or 2, then they named as monomethine Cy1, trimethine Cy3, and pentamethine Cy5 cyanine, respectively.
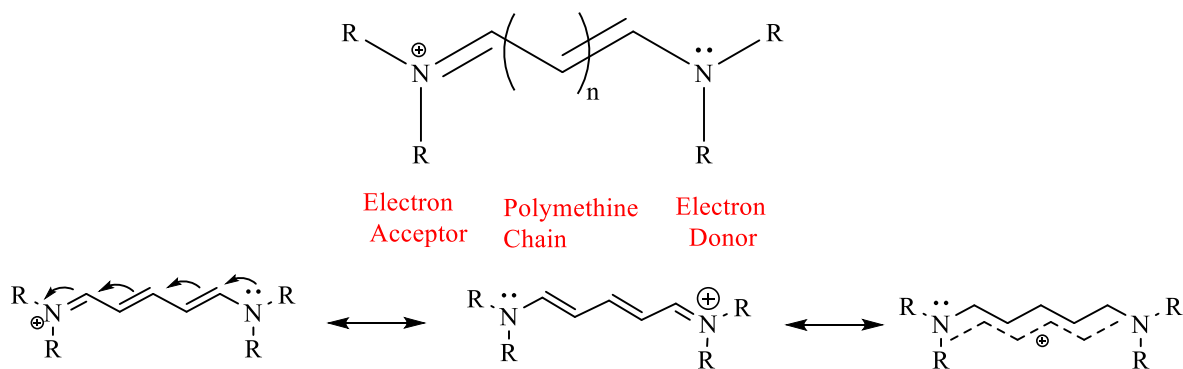
**Figure 1.** Basic skeleton of a polymethine cyanine dye.

Moreover, the substitution of nitrogen atoms of the dyes results in open chain cyanines (streptocyanines) and closed chain cyanines exhibiting two equal or different heterocyclic end groups. Hemicyanines contain one terminal heterocyclic structure while the other nitrogen atoms do not carry any cyclic group, which is shown in (**Figure 2**).[6]
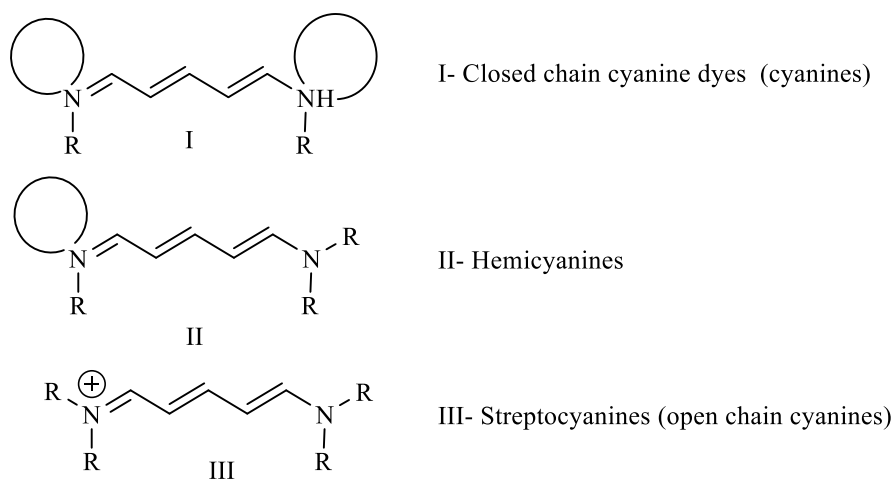


I- Closed chain cyanine dyes (cyanines)

II- Hemicyanines

III- Streptocyanines (open chain cyanines)

**Figure 2.** Chemical structures of cyanine dyes with different N-substitutions.

A variety of heterocyclic groups is illustrated in (**Figure 3**) including quinoline which was the base of the first cyanine dye more than 100 years ago. Nowadays, commonly used heterocycles in cyanine dyes for optical imaging are the following: 3*H*-indole, 3*H*-benzindole, benzoxazole, benzothiazole, or benzindoles.[7] [8]
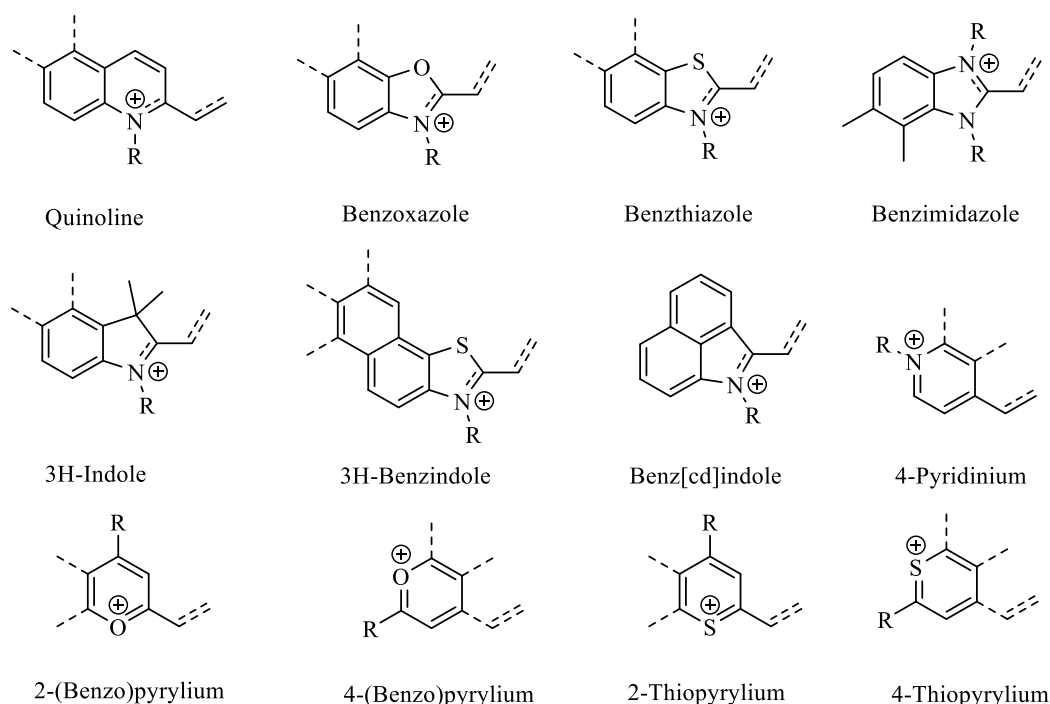
| Quinoline | Benzoxazole | Benzthiazole | Benzimidazole |
| --- | --- | --- | --- |
| 3H-Indole | 3H-Benzindole | Benz[cd]indole | 4-Pyridinium |
| 2-(Benzo)pyrylium | 4-(Benzo)pyrylium | 2-Thiopyrylium | 4-Thiopyrylium |

**Figure 3.** Heterocyclic end groups of cyanine dyes.

Cyanine dyes are classified based on its structure to symmetrical and unsymmetrical, symmetric dyes are characterized by the same terminal groups, while asymmetric dyes contain two differing moieties, (**Figure 4**). These two classes of cyanine dyes show wide variety of differences with respect to spectral characteristics and binding behavior with different type of cells. [2]
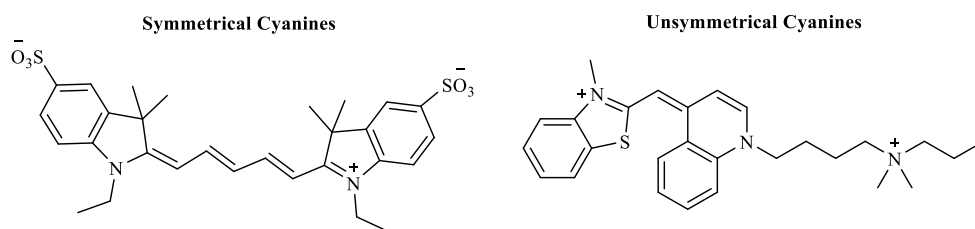


**Figure 4.** Classification of cyanine dyes.

The stability of cyanine dyes depends on the number of methine group and particularly heptamethine dyes are less stable. However, the stability of heptamethine dyes is increased when incorporating a cyclic group into the conjugated system, cyclohexene rings are often introduced into a methine bridge to increase rigidity and make the dyes more stable by introducing more

rigidity to the system. Furthermore, indocarbocyanine dyes enable many possibilities for substitutions which will be discussed in the following sections.[9] In addition, heptamethine cyanines dyes (Cy7, **Figure 5**), containing seven carbon atoms in the linker, are particularly valued for their absorption and emission maxima located in the center of the near-infrared (NIR) window (~800 nm). Among large number of symmetric and asymmetrical heptamethine cyanine dye, indocyanine green (ICG) illustrated in (**Figure 5**) is the most widely used in biomedical application, because ICG dyes typically absorb and fluoresce in the near-infrared region (750 – 1100 nm) of the electronic spectrum.[10] ICG was originally designed as a blood-pooling agent and is nowadays applied as diagnostic imaging probe including fluorescence angiography in ophthalmology,[11] imaging of lymphoid disorders,[12] or fluorescence-guided surgery. [13]
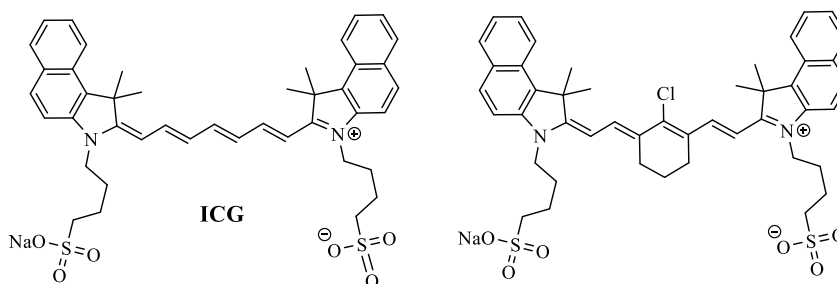


**Figure 5.** Indocyanine green (ICG) and heptamethine cyanine dye containing a cyclohexenyl ring on the polymethine chain.

Despite of the expanding medical uses of ICG, the dye suffers from several disadvantages, high aggregation in aqueous solution and low fluorescence quantum yields as well as the limited approaches for synthesizing more derivatives the things can make it compatible in biomedical field.

Despite these limitations, ICG has shown biocompatibility, which has attracted researchers to offer several methods to developed derivatives with functional groups for further reactivity. Furthermore, various modifications of indocyanine green have been developed to enhance the chemical and photostability. [14] [15] For instance, Strekowski *et al*. reported a novel approach of introducing a cyclohexenyl ring into the polymethine chain, the new form of cyanine dye enhances the rigidity of the polymethine bridge, which increases the solubility, fluorescence emission efficiency (quantum yield) and photostability. Additionally, the new design provides an active site for further functionalization at the central ring, see (**Figure 6**). [16]
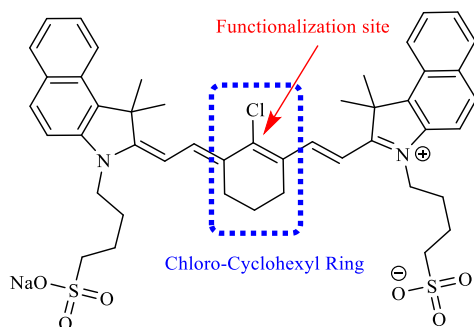
**Figure 6.** Heptamethine cyanine dye containing a cyclohexenyl ring on the polymethine chain.
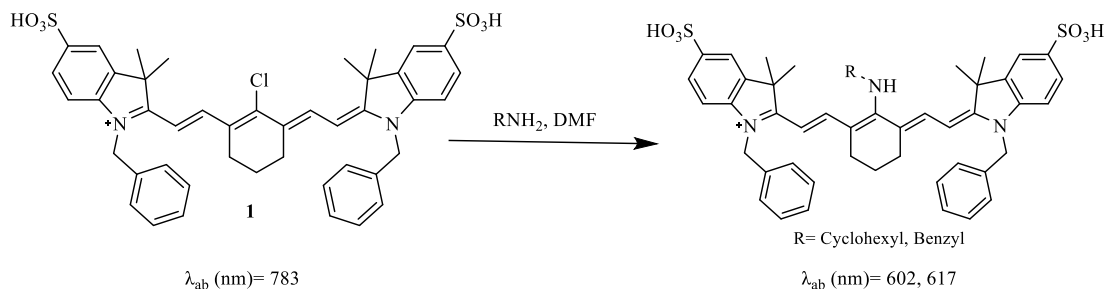
   The substitution of heptamethines at the central position has been widely studied as well as their spectral properties; (**Scheme 1**) represents a numbering system for the heptamethine cyanine dyes. Despite the widespread applications of cyanine dyes and its substitution, the strategies used to modify the heptamethine chain remain limited. A typical methods start from the C4′-chloro substituted cyanine and rely on an electron-transfer mediated $S_{RN}1$ reaction with N, O, or S nucleophiles [17],[18],[19] or palladium-catalyzed Suzuki [20] or Sonogashira[21] coupling reactions (**Scheme 1, 1a**). The second approach involves the preparation of a Schiff base intermediate from a substituted cyclohexene in the Vilsmeier-Haack reaction (**Scheme 1, 1b**). However, these methodologies provide moderate yields for specific substituents cyclohexenes because of harsh conditions employed in the synthesis.
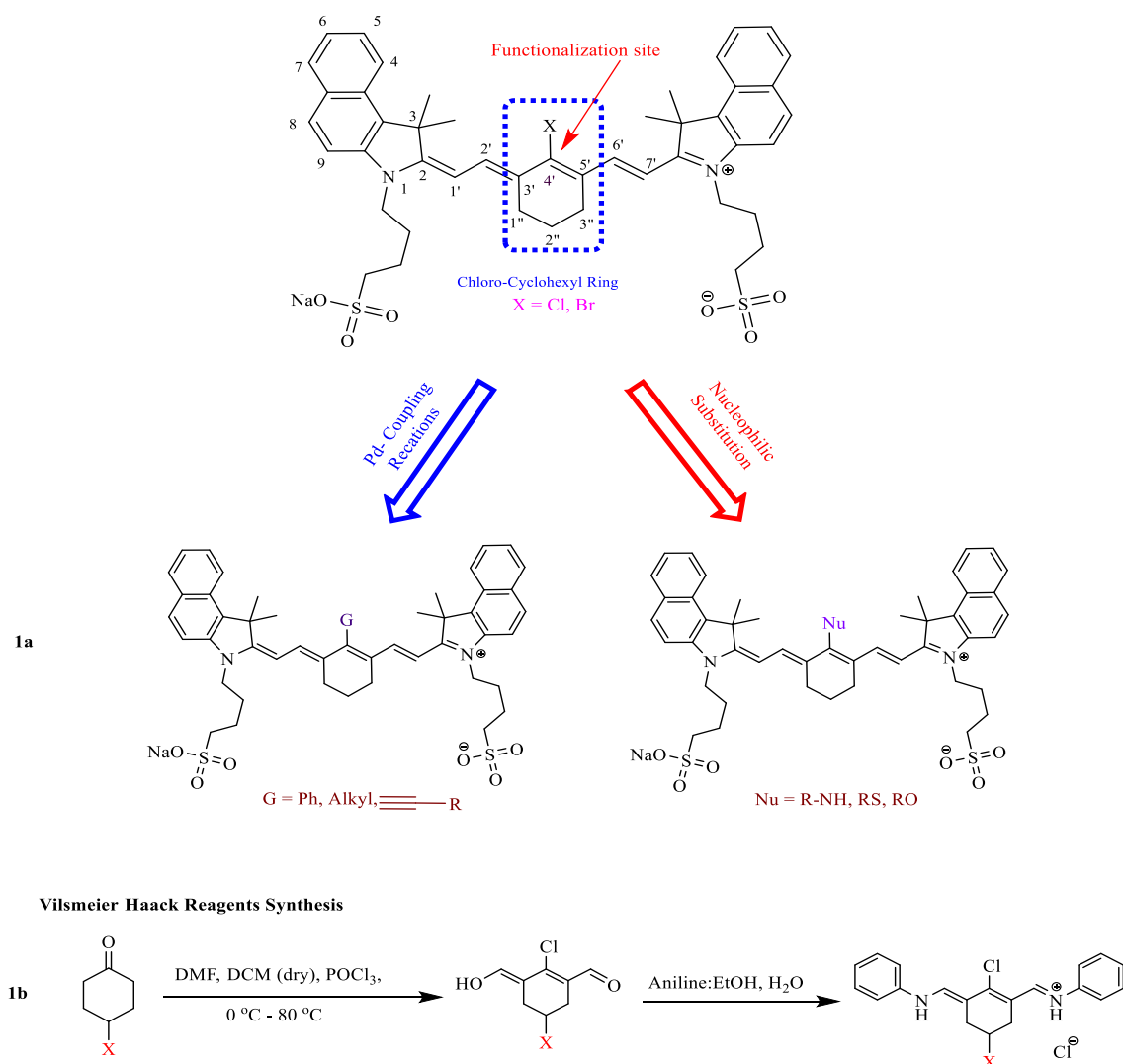
**Scheme 1.** A numbering system for the heptamethine cyanine dyes. 1a) Strategies used to modify the heptamethine chain, 1b) Schiff base intermediate synthesis strategy *via* Vilsmeier-Haack reaction**.**

The amine derivatives of indocyanine dye were intensively studied. In 2005, Peng *et al*. reported the synthesis of new heptamethine cyanine dyes containing robust C−N bonds, the dyes were synthesized from dye **1** by an apparent $S_{NR}1$ reaction. Generally, Peng *et al*. studied the trend of spectral properties of new amino derivatives of indocyanine dyes and demonstrated that the lower the electron density on the amine, the longer the wavelength of the absorption maximum, with little change in the emission maximum. This study indicated a characteristic absorption band at 600-700 nm, and a hypsochromic shift from that of the *meso*-chloro dye, (**Figure 8**). The emission wavelength varied from 700 to 800 nm,[22] as well as significantly large

Stokes shifts (≥ 140 nm) comparing its precursor chloro-substituted dye. These characteristics were useful as well as expected of the chromophoric systems that are good NIR fluorophore suitable for fluorescence imaging.



**Scheme 2.** Synthetic and photophysical properties for the amino derivatives of heptamethine cyanine dyes. [22]

According to reported studies which explain the influence of the central substitution, the results proposed that the increasing of the electron-donating ability of the central substituent **Y** results in localization of the cationic charge on the central carbon atom of the polymethine chain. The photophysical properties of the cyanine dye will be affected if substituent enable to delocalization of the charge on the $\pi$-conjugated skeleton.[23]
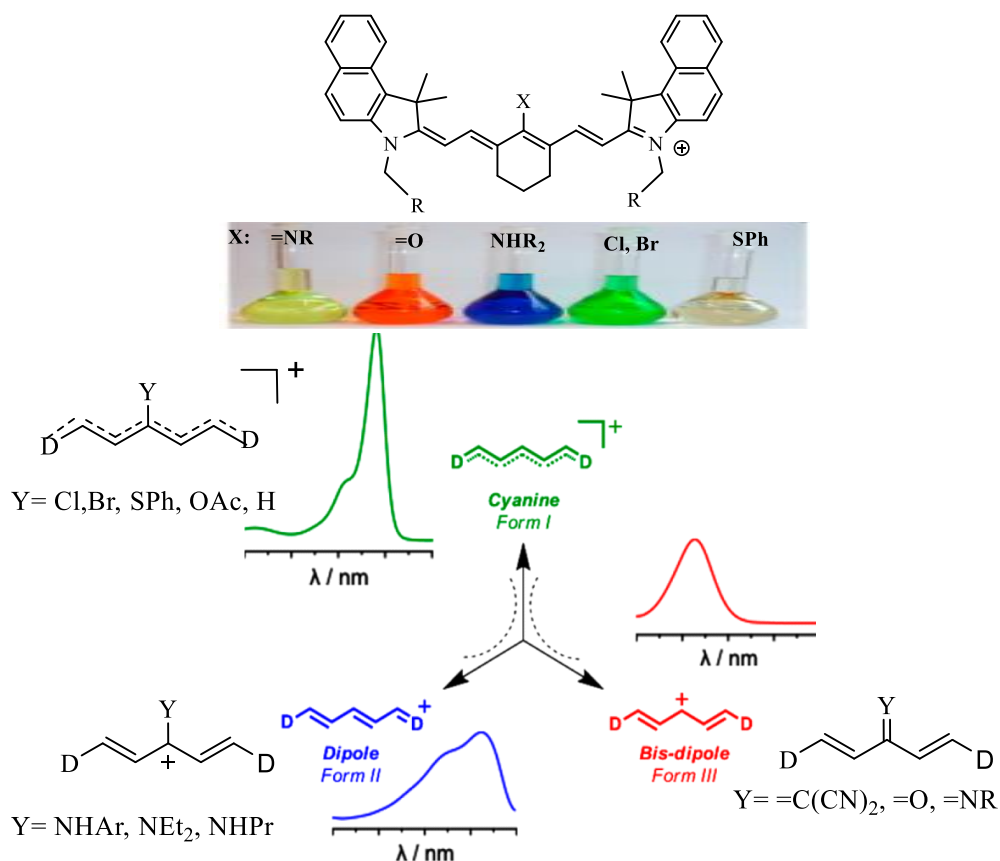
**Figure 7.** Graphical representation of the effects of *meso*-substitution on electronic spectra properties. Adapted from ref [23].

Among different type of substituents have been reported on C4′ position of heptamethine dye, the compounds are often substituted at the C4′ position with phenols (**Figure 7**). Cyanines modified at the C4′ position with an O-alkyl substituent is desirable because these are likely to be quite stable, while maintaining the excellent optical and physical properties associated with C4′-O substitution. However, such molecules have only three reports been published so far, in 2006, Zhang *et al.* reported a successful and privilege designed for synthesizing a novel NIR carbocyanine fluorescent probe containing galactose. The researchers provided an approach to incorporate the monosaccharide in the central hydrophobic core of the chromophore system and anchored by ether linkage to the conformationally constrained cyclohexenyl group, (**Figure 8**).[24]
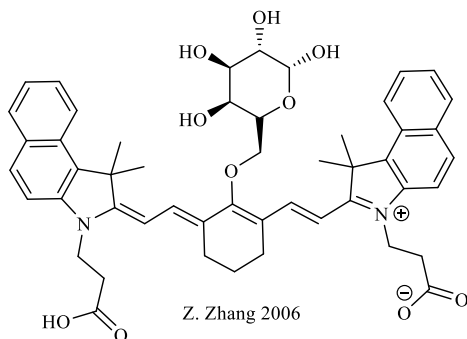
**Figure 8.** Synthesis of indocarbocyanine dye containig galactose.

Strekowski *et al*. reported in 1990 a novel approach to derivatization of cyanine dyes in reactions of compounds containing MeO- group at the central position of cyclohexyl in good yield and the product showed high stability.[25]

Classical Smiles rearrangement is reported that enables the efficient synthesis of previously inaccessible C4′-O-alkyl heptamethine cyanines. The key reaction involves N- to O-transposition with selective electrophile incorporation on nitrogen, (**Figure 9**).[26]
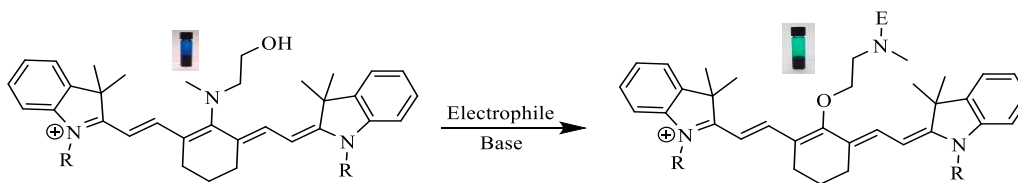


**Figure 9.** Synthesis of C4′-O-alkyl heptamethine cyanines, adapted from ref [26].

Recently, L. Štacková *et al*. reported a high yield novel of incorporation of a substituted pyridine residue into the heptamethine scaffold. The direct transformation of Zincke salts to cyanine dyes carried out under mild conditions. The new method provided an access to new generation of cyanine derivatives and incorporating a diverse substituents and different substitution patterns at the chain in high yield, see (**Scheme 3**).[27]
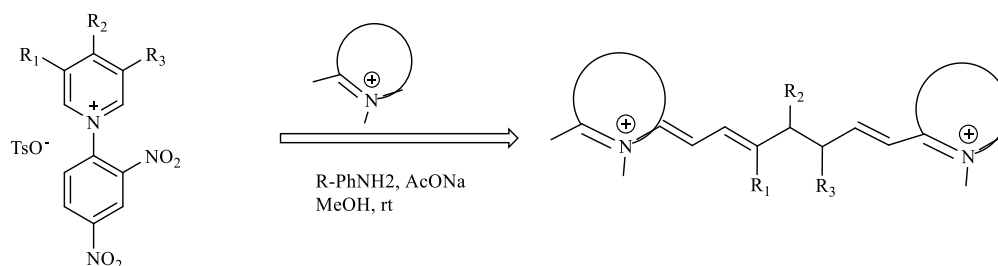


**Scheme 3.** Heptamethine cyanine chain by the ring opening of Zincke salts.

## 2.3 ICG in Ophthalmology

The inner limiting membrane ILM is thin, transparent, and cannot always be removed in a continuous curvilinear manner in cataract surgery, therefore, it may be difficult for most vitreoretinal surgeons to remove the ILM completely without damaging other parts of the retina. The dyes used in ophthalmology facilitate the removal of inner limiting membrane (ILM) and epiretinal membrane (ERM) by staining the tissue of interest. Staining the membrane makes it easier to be located, ensures its complete removal, and decreases the risk of damaging the retina. A few cyanine dyes were suggested for staining of the ILM during vitreoretinal surgery i.e. indocyanine green is known as the dye has an affinity to ILM and stains well the membrane in patients is controversial when it comes to its toxicity experiment results, it is also not stable in aqueous solution and needs to be prepared freshly each time in a clinic. Histologic examinations have shown that ICG stains the ILM, ICG has been postulated to bind to protein structure of the collagens (collagen IV alpha chain and laminin) of a cellular ILM, and thereby staining the ILM.[28]

In 2000, surgeons started using ICG as a stain agent in macular hole surgery. Due to that the macular holes are more likely to close if there have been deeper dissections, and peeling the ILM results in such dissections, ICG has been utilized to the ILM peeling by increasing visualization, as result, ICG make ILM stiffness and a very good contrast to the retina, therefore the peeling process is facilitated. (See **Figure 10**).
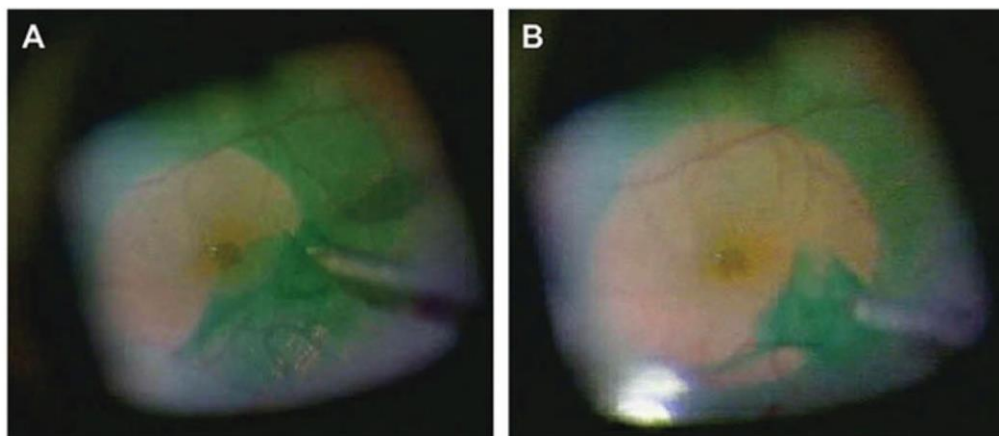


**Figure 10.** ILM membrane peeling with ICG in macular hole surgery. A) The green-stained ILM is easier to view and peel, B) Removal of the ILM. Adapted from ref. [29]

## 2.4 Synthetic Strategies of Indocarbocyanines

Indocarbocyanines have been classically synthesized by condensation under basic conditions (sodium acetate) using two equivalents of a nucleophilic quaternary salt and one equivalent of an electrophilic reagent. The quaternary salt is usually an active methylene base, e.g., commercially available 2,3,3-trimethylindolenine, also known as Fisher's base, see (**Scheme 4**).
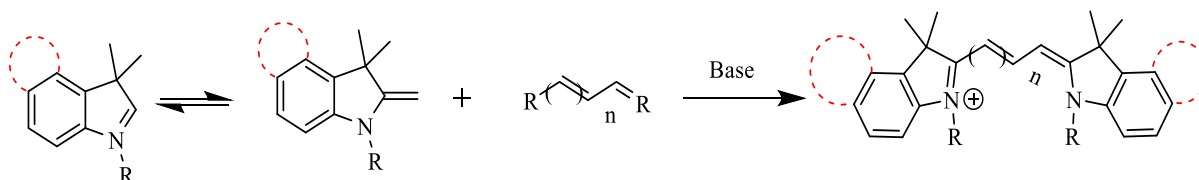


**Scheme 4.** General synthesis of cyanine dye**.**

Waggoner *et al.* presented sufficient synthetic strategies to yield benzindocyanines with shifted absorption maxima due to the additional benzene substitution of the indolenine.[30] Heptamethine dyes are typically prepared with a rigid cyclohexenyl ring in the polymethine chain to increase stability. The synthesis was carried out by condensing a Vilsmeier-Haack reagent (glutaconic aldehyde cyclohexene-bridged) with quaternized indolenines to yield chloro-substituted heptamethines thereby providing a reactive *meso*-position in the polymethine chain for further derivatization.[31] Furthermore, Patonay *et al.* presented that using butanol/benzene mixtures without the requirement of sodium acetate as catalyst, would provide the symmetric and asymmetric heptamethine dyes in (72-95%) yields by.[5b]
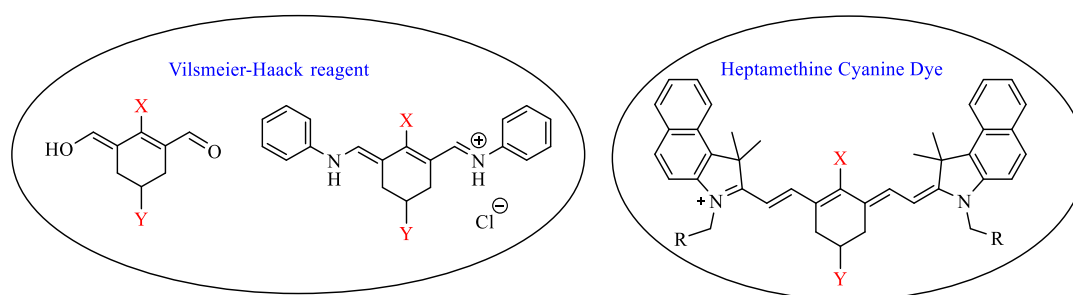


**Figure 11.** General components for the synthesis of polymethine dyes.

## 2.5 Derivatizations of Indocarbocyanines

Since synthetic approaches could provide a variation of polymethine chain length, structural diversity of cyanine dyes is mainly achieved by modification of the heterocycle system. Existence of the aromatic system and the indole-nitrogen substituents on the heterocyclic system provide two positions for determination of dye properties. In addition, the carbon atom in the centre of polymethine chain would undergo many modifications. (**Figure 12**) illustrates the three main positions including (a) *meso*-position, (b) the aromatic system, and (c) the indole-nitrogen substituents.
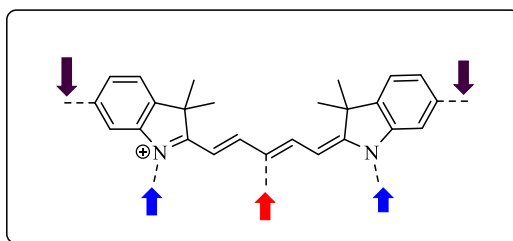


**Figure 12.** Modification positions on cyanine dye skeleton.

## 2.5.1 Substituents at *meso*-Position

The cyanine dyes with *meso*-substitute might contain either alkyl, phenyl, or halogen atoms. The known reactions on *meso*-substituted dyes are undergo by nucleophilic substitution ($S_N$) or palladium-catalyzed cross coupling thereby replacing the *meso*-halogen. [28] In contrast, introducing different groups of aromatic moieties to *meso*-substituted indocyanines are well developed via Stille, Heck, and Sonogashira cross coupling, (see **Scheme 5**).[32]
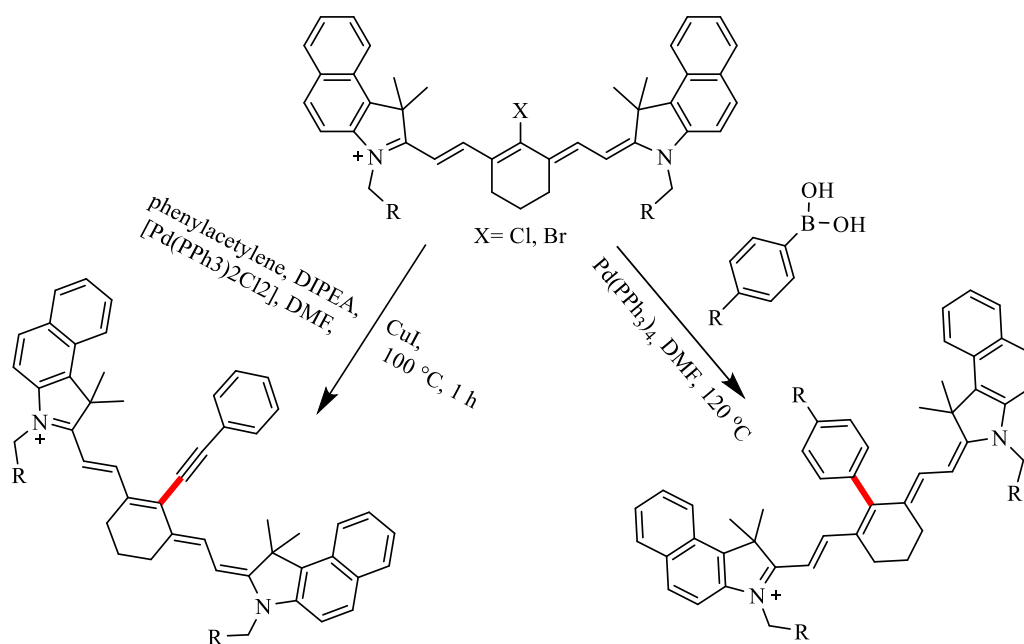
**Scheme 5.** Palladium-catalyzed cross coupling of *meso*-substituted heptamethine cyanine dye.

Introducing a cyclohexene group to heptamethine dyes can be easily modified by nucleophilic substitution via a radical-nucleophilic aromatic ($S_{RN}1$) mechanism.[31] A variety of nucleophiles such as thiols, phenolates and amines have been replaced the chlorine atom in the centre of heptamethine cyanine dye (see **Scheme 6**). [33] [34] Additionally, functional groups such as NHS ester or isothiocyanates derivatives have been reported, as well as their application as active biomolecules. [35]
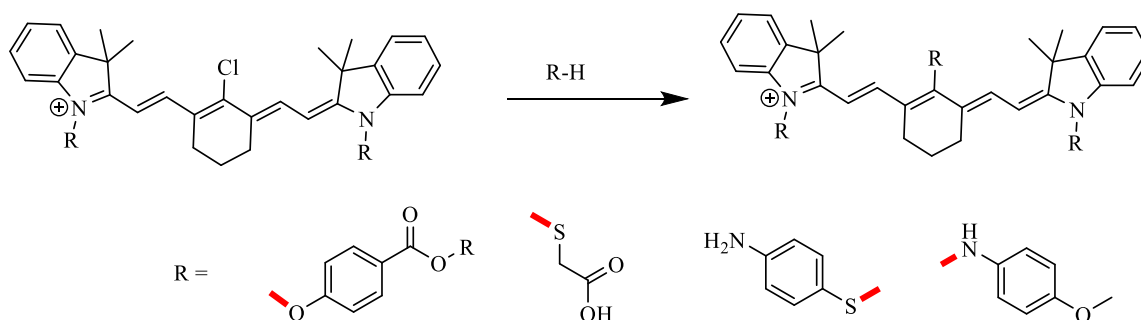


**Scheme 6.** Nucleophilic substitution at *meso*-position.

### 2.5.2 Substitution at Aromatic System

Cyanine dyes undergo an aromatic substitution can be easily modified. Introducing reactive groups should be inserted before the indolenine formation. Aromatic group of indolenines are usually carry sulfo-group, since they are inert during dye synthesis and they enhance the water

solubility due to electrostatic repulsion, which is necessary for biomedical applications. Common sulfo-substituted indolenines are illustrated in (**Scheme 7**). Waggoner *et al*. reported a general approach to sulfonate indolenines by using well known Fischer indole synthesis. [30, 36] Indolenines were prepared from sulfo-substituted hydrazinobenzene and methyl isopropyl ketone 73% yields (see **Scheme 7**). Sulfonated benzindolenines were prepared from sulfonated naphthylamines and applied in further dye synthesis bearing up to four sulfonic acid groups was also published.
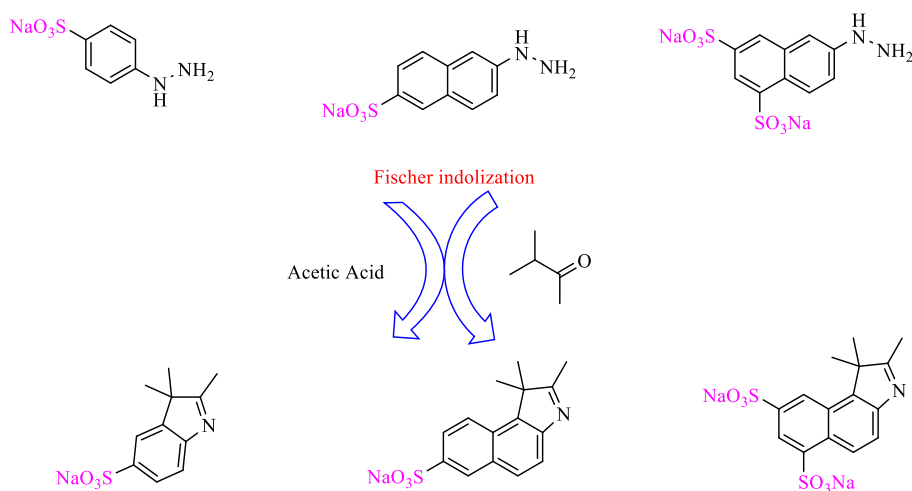


**Scheme 7.** Synthesis of sulfonated trimethylindolenine and trimethylbenzindolenine derivatives.

Further derivatization of trimethylindolenines have been yielded the functionalized indoles illustrated in (**Figure 13**) including carboxylic acids, nitro and amino groups directly attached to the aromatic system.[37] Furthermore, carboxyl and amino benzyl indolenine derivatives have been reported. [38]
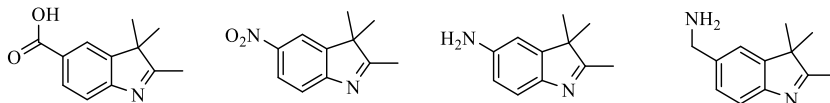


**Figure 13.** Further aromatic substitutions of trimethylindolenines.

### 2.5.3 Substituents at Indole Nitrogen

Indole-nitrogen atom in the heterocyclic system has been alkylated by nucleophilic substitutions of halo-alkanes. Generally, when the indolenines alkylated with short, halogenated alkanes such as methyl and ethyl iodide, the resulting plymethine dyes are usually non-polar. Ernst *et al*. provided new approach to increase water solubility of the dye by the incorporation of alkyl-

sulfonates in dye structures. Reaction of 1,4-butanesultone with indolenines yielded the alkylated indolenines with sulfobutyl chains.[39] Nitrogen alkylation are illustrated in (**Scheme 8**) represents a new synthetic route to amino terminated indolenines. Romieu *et al.* demonstrated the reaction of benzindolenines with (4-bromobutyl) phthalimide affording quaternary salts in high yields. Moreover, condensation with a carboxylated benzindolenine and subsequent deprotection with hydrazine yielded a cyanine-based amino acid is also reported. [16]
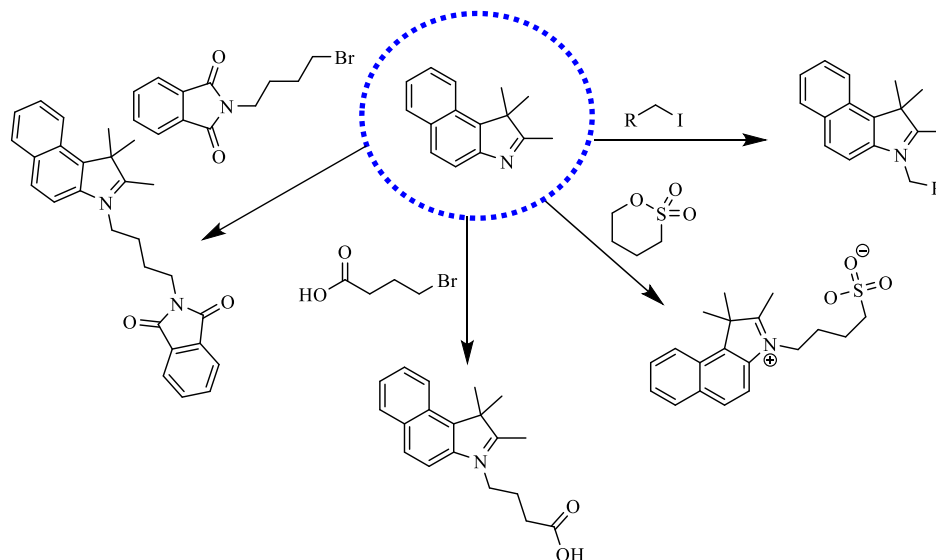


**Scheme 8.** Nitrogen alkylation of sulfonated trimethylindoleine with variant functional groups.

## 2.5.4 Aggregation Behaviour of Cyanine Dyes

Because their wavelength extends from the visible (VIS) and NIR region, heptamethine cyanine dyes are applied in biomedical imaging. Generally, the shift the absorption bands can be affected by two factors: (1) elongation of the $\pi$-conjugated polymethine chain and (2) terminal substituents that have their own $\pi$-electron systems. As the length of the polymethine bridge is increased by single vinylene unit, the wavelength of absorption and emission maxima shifts to a longer wavelength, usually by 100 nm.[40] As a result the long chain cyanine dye has tendency to absorb light greater than 700 nm, the thing lead to increase photodegradation. Moreover, trimethines which has smaller fluorescence quantum yields than penta- and heptamethines, and higher molar absorption coefficients are also achieved for red-shifted dyes (**Figure 14**). .[30]
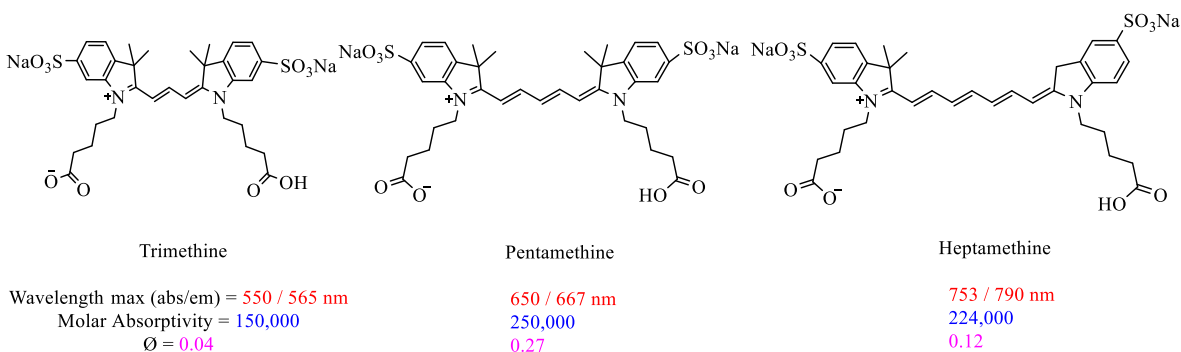
Trimethine

Wavelength max (abs/em) = 550 / 565 nm
Molar Absorptivity = 150,000
Ø = 0.04

Pentamethine

650 / 667 nm
250,000
0.27

Heptamethine

753 / 790 nm
224,000
0.12

**Figure 14.** Optical properties of varying polymethine lengths measured in PBS solution.

Cyanine dyes form self-aggregation because they possess strong intermolecular van der Waals-like attractive forces. Aggregation led to strongly changes in the UV-visible to near-infrared absorption and fluorescence band in solution, as well as decreasing fluorescence quantum yields comparison to the monomeric species. Generally, aggregates are classified in H-aggregates and J-aggregates. H-aggregates (H for hypochromic) exhibiting a hypsochromic shift (towards the blue) compared to the monomer band forming sandwich-type aggregates in a plane-to-plane stacking. Conversely, J-aggregates (J is named after Jelly, who was among the first to investigate these shifts) aggregates showing dye orientation in head-to-tail arrangement (end-to-end stacking) resulting in red-shifted (bathochromic) peaks (**Figure 15**). [41] While H-aggregates reveal broad peaks and negligible fluorescence, J-aggregates are distinguished by a narrow band and show enhanced fluorescence.[42]
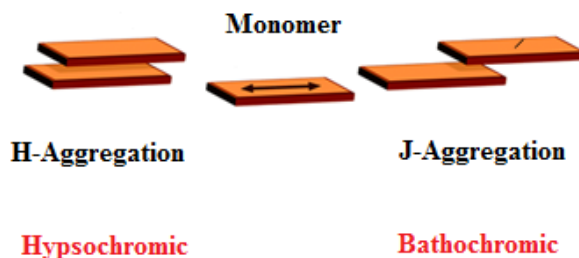


**Figure 15.** Schematic presentation of the relationship between spectroscopic shift and the arrangement of molecules in H- and J-aggregates.

The formation of aggregates on the cyanine dye are based on its structure and its environment such as solvent polarity, concentration, pH, and temperature parameters.[43] Many studies have

been reported to synthesizing new cyanine dyes with decreased aggregation in solution. For instance, aggregation can be reduced by incorporating sterically bulky groups, such as cyclodextrin [44], and attaching sulfonate or sulfonato alkyl moieties [45] to the heterocyclic rings. Generally, the aggregation can be inhibited in case of existence a highly steric interactions between the dyes because their molecular orbitals poorly overlap. Cyanine dyes that contain two alkyl sulfonate substituents, one attached to each nitrogen atom on the end-heterocyclic rings, prevent intermolecular interactions with other dye molecules. Additional sulfonate moieties on the terminal aromatic rings further stabilize the dye and results in aggregation inhibition. The negatively charged sulfonic group provides a sphere of solvation, which increases its solubility in polar solvents and thus achieve less aggregation.

## 2.6 Scope of the Chapter 2

Despite a variety of investigation studies available for the applicability of the cyanine dyes towards near-infrared in *vivo* imaging studies; stable green dye in aqueous solutions with affinity for the ILM staining still be highly desirable. Here we aim to expand the approaching of designing and synthesizing various near-infrared heptamethine indocyanine green dye possessing selectivity as staining agent for ILM and ERM. Taking into our account that the new dyes would stain the tissue of interest specifically and sufficiently, would be non-toxic to surrounding cells, and would be stable in aqueous solution.

## 2.7 Novel Synthesis of Heptamethine Cyanine Green Dyes

Indocyanine green (ICG) meets the requirements of an NIR dye, it absorbs and emits light at 785 nm and 815 nm, respectively, in aqueous solution and at 805 nm and 835 nm in blood, ICG has high molar absorptivity (250,000 $cm^{-1}$ $M^{-1}$) as well as it has low toxicity. However, there are limitations to the use of ICG, most importantly their high aggregation in aqueous solution as well as the general challenge to synthesize more derivatives employing multifunctionality and capability to work as biomedical agent.

According to the reported findings, ICG derivatives that possess a conformationally constrained heptamethine moiety would be enhanced its chemical and photochemical stability in aqueous solution. Introducing a cyclohexene or cyclopentene rings into a methine bridge increase rigidity and make the dyes more stable, but there is a lack of literature dealing with modification at the meso position of this ring. Nucleophilic substitution through an $S_{RN}1$ reaction of the chlorine or

bromine atom at the meso-position of the methine bridge allows for modification of the dye structure with little effect on the dyes optical profile because this position is not conjugated into the dye scaffold. [35a]

Recently, carbon–carbon coupling at the meso-position has been explored utilizing an adapted Suzuki-Miyaura method, but this method known as an expensive because using of expensive palladium catalyst, and it's generally applies to water soluble cyanine dyes, and requires difficult purification of the product and unreacted halo version by column chromatography as well as Suzuki coupling often requires strong base which is not suitable for use with sensitive heptamethine cyanines because they undergo hydrolysis or decomposition in strong base.[46]

As a result, utilizing this new strategy chemically modified water-soluble heptamethine cyanine dyes with aryl- or alkyl-ether, thioether, or amine, linkages have been prepared.[47] In this section we present the synthesis and characterization of a novel set of several heptamethine cyanine derivatives potentially would be applicable for biomedical applications in the field of opthalmology and imaging. We speculated that introducing a bulky group on methine bridge would be useful method to enhance the solubility of dyes in aqueous solution by reducing the chance of aggregation.

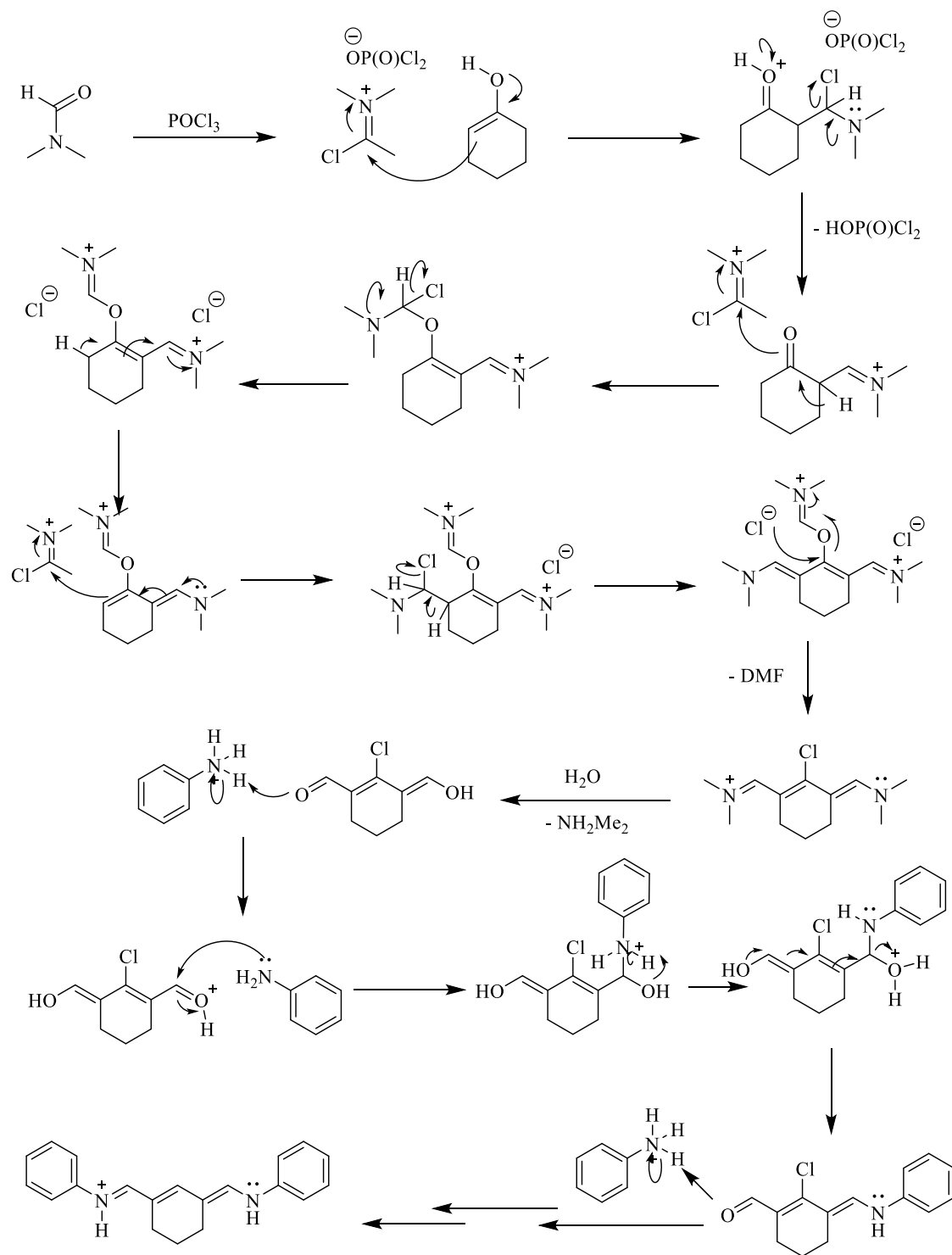### 2.7.1 Synthesis of Heptamethine Cyanine Derivatives

The synthesis of our cyanine dyes began with the preparation of the indolinium salts $S_1$ and $S_2$, 1,1,2-Trimethylbenz[*e*]indole and 2,3,3-trimethyl-3H-indole were alkylated with 1,4-sultone under reflux for two hours to afford compounds $S_1$ and $S_2$ in excellent yield [30]. As described in (**Scheme 9**), a series of *meso*-substituted heptamethine cyanines dyes **4a**-**f** has been synthesized by the classic stepwise condensation reaction between two equivalents of indolinium salts $S_1$ and a dianil linker. The first step in synthesizing heptamethine cyanine dye is the construction of the linker. The synthesis of the linker involves the Vilsmeier-Haack reagent, *N*-chloromethylene-*N,N*-dimethylammonium dichlorophosphonate, which is formed from the reaction of *N,N*-dimethylformamide (DMF) or *N*-methylformamide (NMF) and POCl₃. Hydrolysis of iminium functionalities in the intermediate provides a bisaldehyde which is transformed later into the pentamethinium salt by treatment with anilinium hydrochloride. Subsequently, the condensation reaction takes place, after which the dyes are then purified by dissolving in a minimal amount of methanol followed by precipitation in diethyl ether followed by using column chromatography

(10–50% methanol/DCM). In addition to the substituted cyclohexanone and cyclohexene, cyclopentene has also been tested. An acceptable yield of the product was obtained when 2.5-3.0 equivalents of the indolinium salts $S_1$ was used. Sodium acetate was used in the reaction as a base to remove the acidic proton of the methyl group of the heterocyclic salt. The mechanistic pathway to the pentamethinium salt is postulated in the reaction (**Scheme 10**).
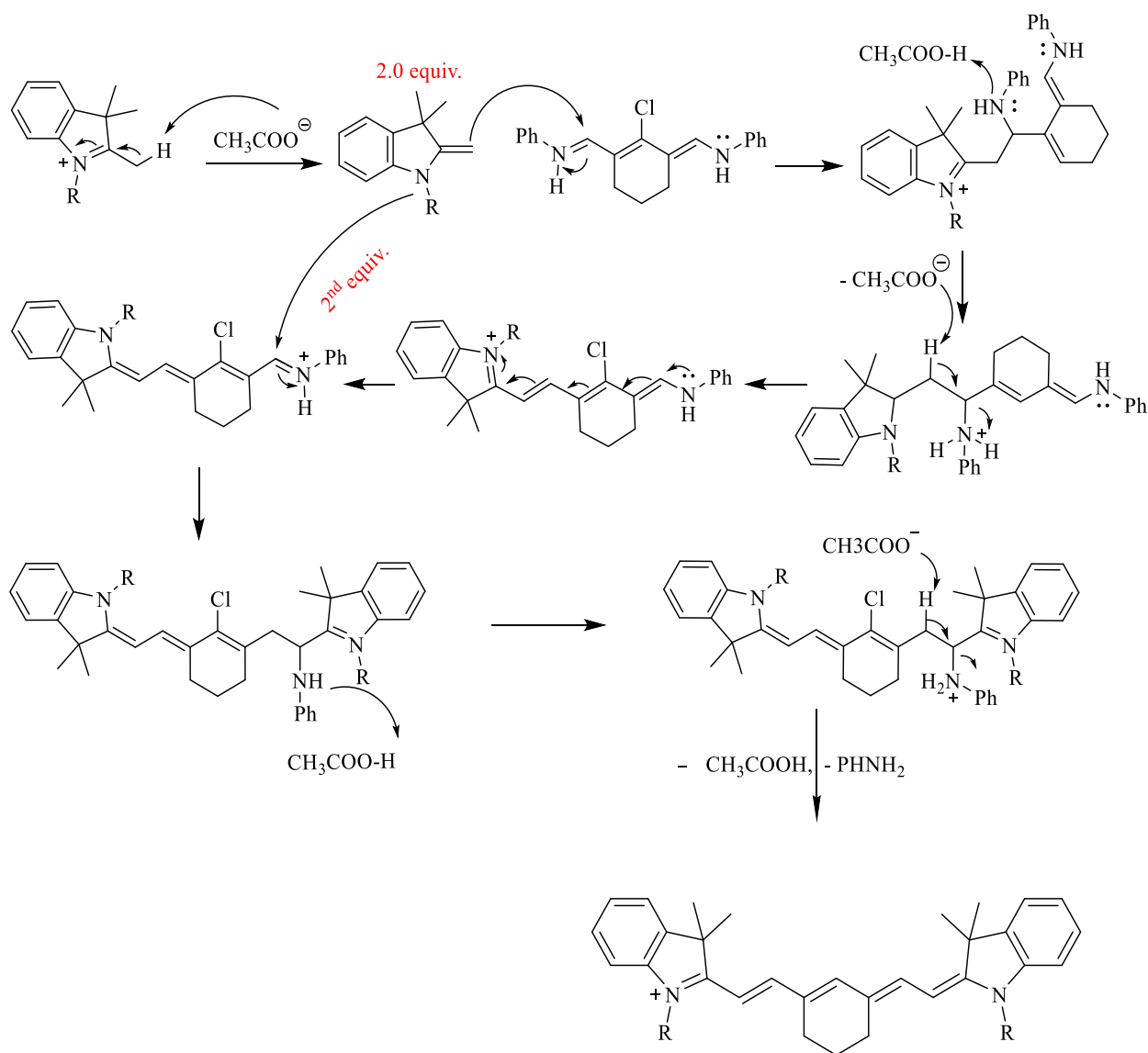
The mechanism for the condensation reaction between the indolenine base and the Vilsmeier regaent is shown in the (**Scheme 11**). An acceptable yield of the product was obtained when 3.0 equivalent of the heterocyclic base was used. Sodium acetate was used in the reaction as a base to remove the acidic proton of the methyl group of the heterocyclic salt, (**Scheme 9**) represents complete synthetic route.
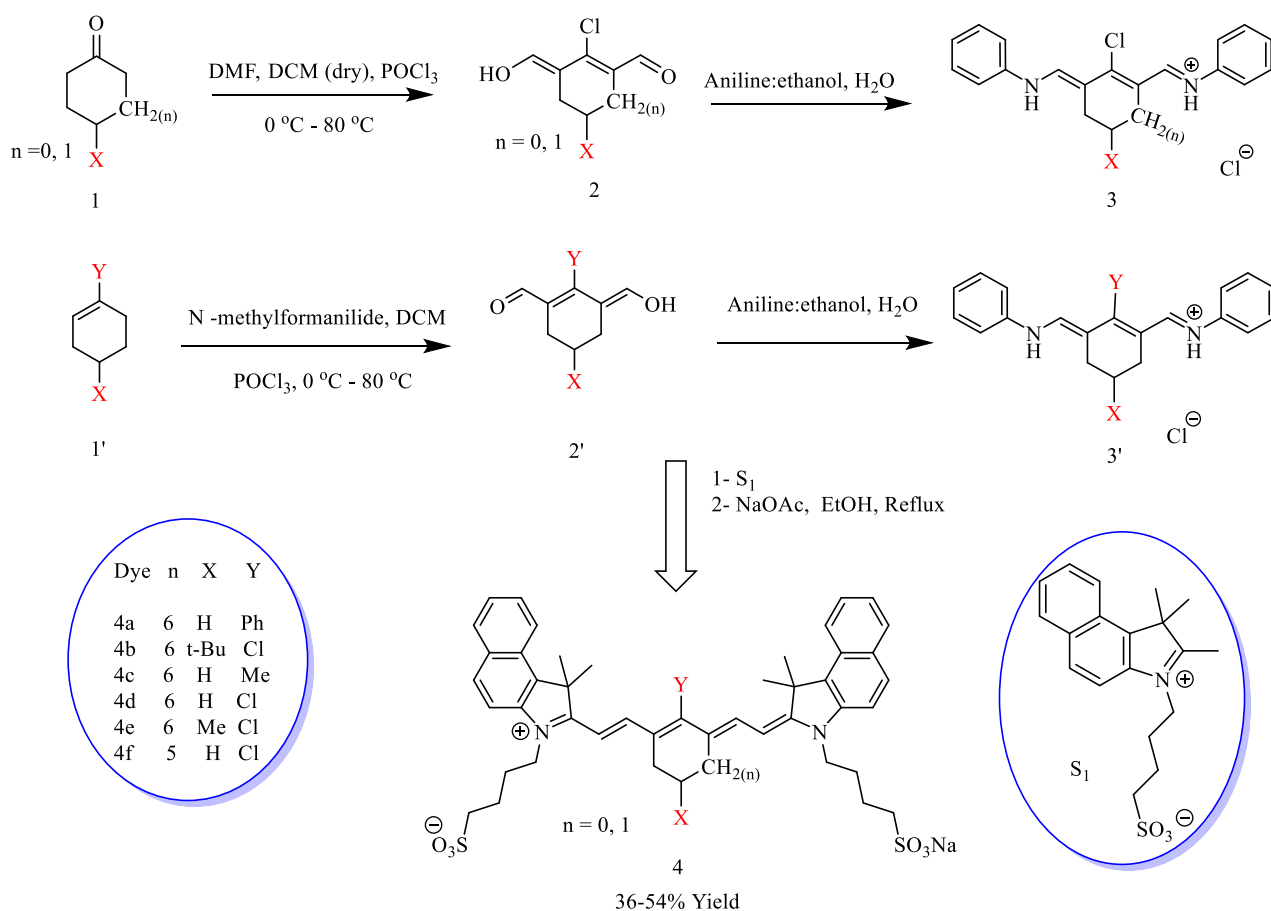


**Scheme 9.** Synthetic route of substituted heptamethine indocyanine dye.

**Scheme 10.** Proposed mechanism of Vilsmeier reagent synthesis.

**Scheme 11.** Proposed mechanism of condensation reaction between the indolenine base and the Vilsmeier reagent.

A derivative of the heptamethine cyanine dye with a substitution of a methylene group with dimethyl quaternary ammonium nitrogen, dye **5**, known as quaternary cyanine or QuatCy, was also synthesized according to the literature.[48] QuatCy has been made starting from synthesizing Vilsmeier-Haack reagent **B**, applying standard conditions furnished **B** in good yield, subsequently, treated one equivalent of **B** with one equivalent of MeI in methanol provided C in good yield, (**Scheme 12**). The previously prepared trimethyl benzindolonium salt **S₁** is the other

component of the QuatCy synthesis. Condensation of $S_1$ and $C$ in refluxing ethanol afforded dye 5.
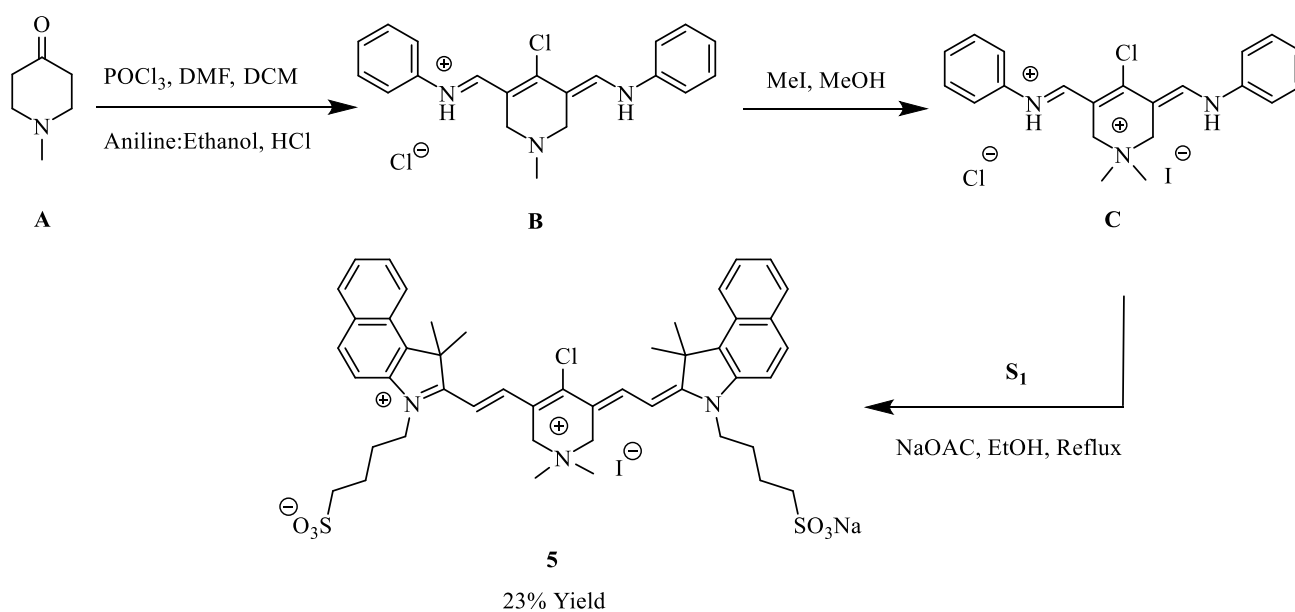


**Scheme 12.** Synthesis of QuatCy (5) converging from two key fragments, the Vilsmeier-Haack product C and the trimethyl benzindolozinidinium salt $S_1$.

A cyanine dye, **6**, with a methoxy group at the *meso* position was synthesized by preparing the methoxy-dienamine linker according to (**Scheme 13**); the key step is the synthesis of the *keto-dienaminone via* organocatalyzed condensation of cyclopentanone and N,N-dimethyl acetal. Martins *et al.* reported that the removal of methanol produced during the reaction enhance the reaction yield and reaction rate.[49] Treating the dienaminone with methyl triflate and stirring the mixture for 1 hour at room temperature provides the methoxy dienamine which can be mixed with two equivalents of the indolinium salt $S_1$ and sodium acetate to yield the corresponding dye in good yield.
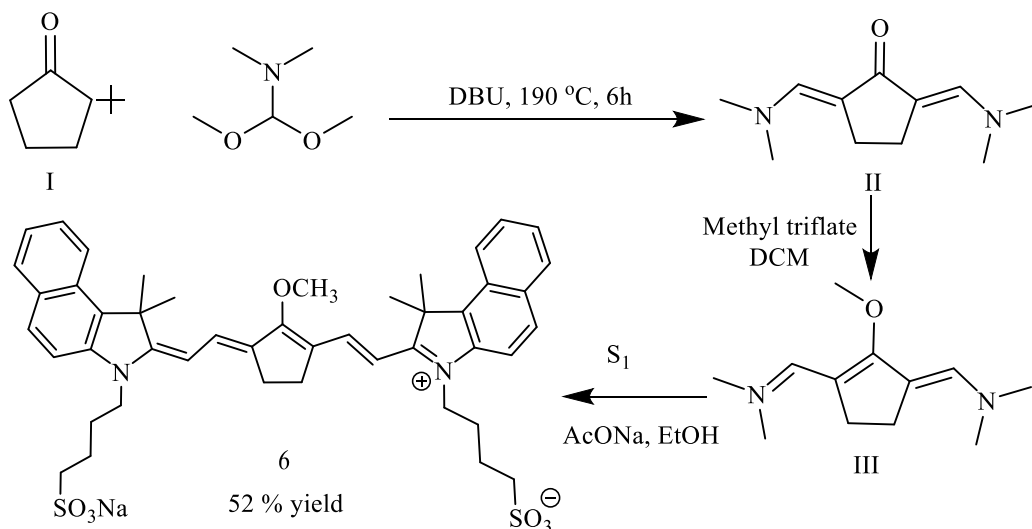
**Scheme 13.** Synthesis of 6 by condensation of compound III and S$_1$.

The synthetic pathway for the ICG-analogue unsymmetrical cyanine dye **7** is outlined in (**Scheme 14**), as the first step 2,3,3-trimethyl-3H-indole was alkylated with 1,4-sultone under reflux for two hours to afford compound **S$_2$** in excellent yield. one equivalent of **S$_1$** was treated with one equivalent of glutaconaldehyde dianil hydrochloride in acetic anhydride at 100 $^{o}$C for 1 h to yield compound B in a quantitative yield. The intermediate B was refluxed for half an hour with one equivalent of **S$_2$** in ethanol and triethylamine to provide the asymmetric ICG-analogue dye **7** in good yield.
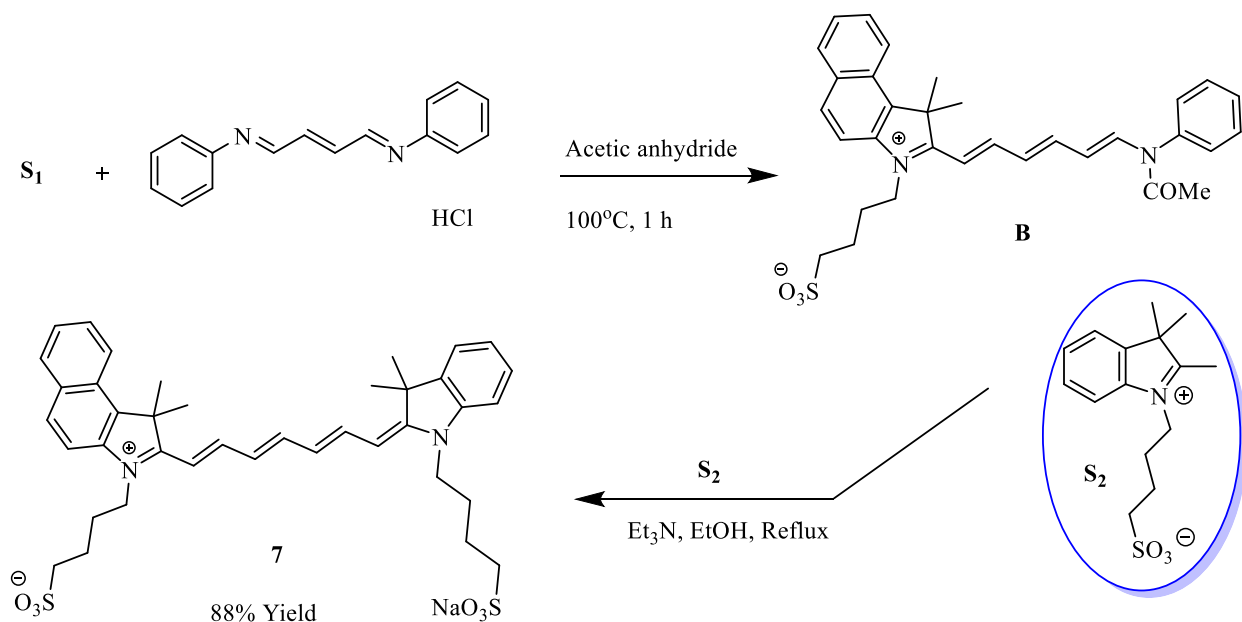


**Scheme 14.** Synthetic pathway for asymmetric ICG-analogue **7**.

## 2.7.2 Photophysical Properties of Heptamethine Cyanine Dyes

Optical properties for NIR heptamethine cyanine dyes were studied by collecting the spectral data of UV-vis/NIR absorption and fluorescence emission (**Figure 18** and **Table 1**). The UV-vis/NIR absorption ($\lambda_{max}$) and fluorescence emission ($\lambda_{em}$) of the dyes were measured with uniform concentrations in methanol. Additionally, the absorption spectra of NIR dyes were obtained with the specific concentration in phosphate buffer solution at pH of 7.4 (PBS) in order to investigate aggregation formation and solvent effect. The data relating to the spectral properties of the NIR dyes are presented in **Table 1**. Interestingly, despite the structural similarities, the dyes exhibit notable different absorption spectra, whose maxima are spread over the 780−850 nm range. This feature highlights the dramatic influence of the central substitution on the electronic structure of these molecules. The $\lambda_{max}$ of the prepared dyes in methanol varies from 796 nm and 820 nm for those containing different substituents at *meso*-position, following the order: **4a** (Y = Ph, 796 nm), **4c** (Y = Me, 805 nm), and **4d** (Y = Cl, 850 nm). The $\lambda_{max}$ of heptamethine cyanines with the *meso*-chlorine substitution is generally about 806-820 nm. In general, changing the X-substituents and keeping the Y = Cl did not show a significant change in $\lambda_{max}$ (~818-820 nm). Absorption shifts in cyanines are generally seen when the methine chain substitutions are changed, the most notable absorption shifts come from the alternate conjugation pathway in the *meso*-phenyl dye **4a** and the strain caused by the cyclopentenyl ring on the methine chain in dye **4f**.

For comparison we investigated the parent cyanine dye absorption band at 805 nm (dye **4d**), with other substituents (X and Y). The absorption band of dye **4a** with substituents X = H and Y = experienced a bathochromic shift to 820 nm in the presence of methanol. In contrast, the absorption band of dye **4b** with substituent (X = t-butyl, Y = Cl) showed a hypsochromic shift to 797 nm comparative to parent dye. Further, dye **4c** substituent such as X = H, Y = Me showed a similar behavior to the dye **4a** with substituent of X = H, Y = Ph. Additionally, the dye **4e** with substituent (X = Me, Y = Cl) caused a bathochromic shift to 821 nm comparative to the parent dye **4d**. However, the addition of five-membered ring dye **4f** instead of six-membered ring to the core of parent cyanine dye, endows dramatically bathochromic shifted to 846 nm. Moreover, in aqueous solution (PBS buffer) the cyanine derivatives **4e, 4f** exhibited a hypsochromic shift compared to the parent dye **4d**. Further in the aqueous solution, the $\lambda_{max}$ of cyanine derivatives showed a large wavelength shift compared to that in methanol. This indicated the dye molecules

are most likely aggregated in the aqueous solution even at low concentrations. In addition, the dye **6**, with cyclopentenyl ring and *meso*-methoxy substituent showed a high hypsochromic shift around 65 nm compared to its parent dye **4f** with *meso*-chlorine substituent. However, the dye **5** with *meso*-chlorine substituent and quaternary ammonium salt at cyclohexyl ring showed a high similarity with the comparable dyes (*i.e.*, **4b**, **4d** and **4e**). In addition, a blue-shifted shoulder appeared for the dyes **4a, 4b, 4c, 4d,** and **4e** in the absorbance spectra as shown in (**Figure 18a**). Shoulders of this kind are indicative of H-aggregates. Concentration dependences of dye **6** and dye **7** compared to other dyes was determined to explore H-aggregation further, but very less compared to other dyes in methanol.
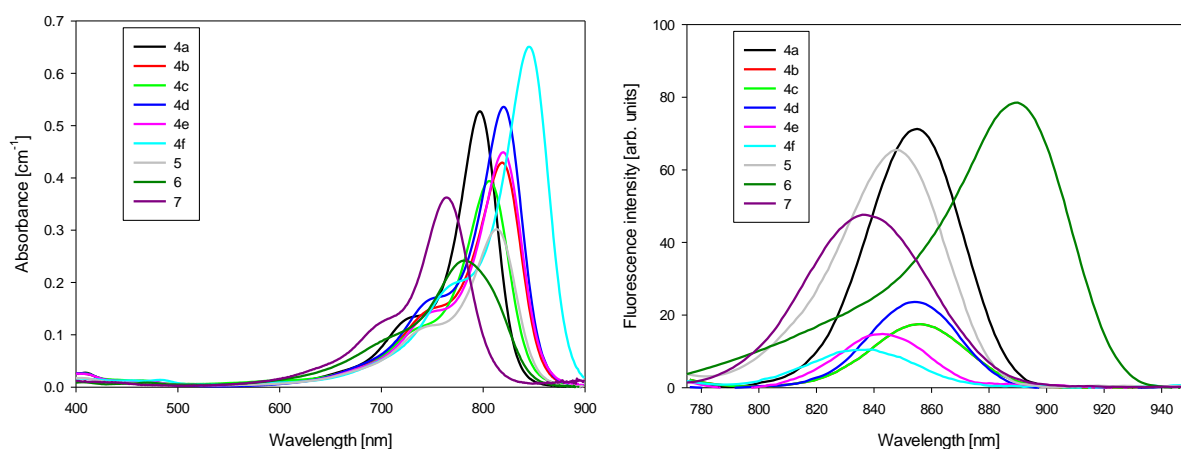


**Figure 16.** (a) VIS spectrum of all dyes at 6.0 µM in MeOH. (b) Emission spectra of the dyes in MeOH. For excitation wavelength see Table **1**.

**Table 1.** Optical properties of substituted heptamethine cyanine dyes in MeOH.

| Dye | $n$ | X | Y | $\lambda_{max}$ | $\varepsilon_{MeOH}$ $(\times 10^3)$ | $\lambda_{em}$ | $\lambda_{ex}$ | $\phi_{MeOH}$ |
|---|---|---|---|---|---|---|---|---|
| ICG[a] | - | --- | --- | 785 | 101 | 790 | 717 | 0.016 |
| 4a | 6 | H | Ph | 796 | 180 | 816 | 725 | 0.030 |
| 4b | 6 | t-Bu | Cl | 818 | 17 | 829 | 745 | 0.007 |
| 4c | 6 | H | Me | 805 | 130 | 824 | 732 | 0.010 |
| 4d | 6 | H | Cl | 820 | 130 | 832 | 746 | 0.004 |
| 4e | 6 | Me | Cl | 819 | 170 | 837 | 746 | 0.008 |
| 4f | 5 | --- | Cl | 845 | 214 | 832 | 767 | 0.003 |
| 5 | 6 | Q. Cy | Cl | 814 | 164 | 740 | 744 | 0.006 |
| 6 | 5 | --- | OMe | 782 | 141 | 696 | 708 | 0.014 |
| 7 | - | --- | --- | 765 | 203 | 695 | 705 | 0.060 |

Optical properties of the dyes are listed in **Table 1**. The molar absorptivity of these dyes is relatively large, and the values range from 130,000–214,000 L mol$^{-1}$ cm$^{-1}$. Interestingly, the two dyes 4f and **7** showed the highest molar absorptivity values with 214,000 and 203,000 L mol$^{-1}$ cm$^{-1}$, respectively. These two dyes differ in existence of the ring at the methine chain (five membered ring for **4f**) while dye **7** contains a linear bridge. The *meso*-phenyl group in **4a** and the strain put on the methine chain by the cyclopentenyl group in **4f** can prevent *cis–trans* isomerization increasing the molar absorptivity.[50] Subsequently, the higher molar absorptivity was observed for dye **4f** (214,000 L mol$^{-1}$ cm$^{-1}$) while the lowest molar absorptivity was observed for dye **4c and 4d** with the *meso*-methyl and chloro substituent with a value of 130,000 L mol$^{-1}$ cm$^{-1}$.

Quantum yields were determined according to the literature:

$$\Phi_{dye} = \Phi_{Fluo} \times \frac{I_{dye} * A_{Fluo}}{I_{Fluo} * A_{dye}} \times \frac{\eta_{MeOH}^2}{\eta_{H2O}^2}$$

where $\Phi_{Fluo}$ is the quantum yield of fluorescein (in 0.1 M NaOH) and is equal to 0.89, $\Phi_{dye}$ is the quantum yield of dye, $I$ is the integrated area under the curve, A is the absorbance at excitation wavelength, and η is the refractive index of the solvents ($\eta_{methanol}$= 1.331, $\eta_{H2O}$ = 1.333).[51]

Fluorescence quantum yields of the synthesized dyes range from 0.3% to 6.0 %. The dyes **4b, d, e, f,** and dye **5** showed similar quantum yields ranging from 0.3-0.8%. The t-butyl and methyl substitutions at the 2" position of the cyclohexene showed only a small effect on quantum yield as they are not conjugated within the system. A trend is observed with decreasing quantum yield as the rigidity of the cyclic system increases with the addition of carbons, e.g., the dye **4f** with a *meso*-chlorine and cyclopentenyl ring had the lowest quantum yield at 0.3 % while the dye with the *meso*-phenyl **4a** had the higher quantum yield of 3%. Dye **7** represented the highest quantum yield with value 6%. In contrast, substituted cyanine dyes are indicating extremely weak fluorescence in PBS buffer solution. Furthermore, near infrared dyes suffered from low quantum yield, as a result of long wavelength photon requires low band gap molecules, in which non radiative process tend to dominate over the fluorescence.[52]

### 2.7.3 Filter-Membrane Staining and Solubility Characterization

The aim of this experiment is to determine the staining power of the dyes, as well as assessing their solubility in water and PBS for a concentration of 0.1%. To achieve this, a filter-membrane experiment was designed. A Millipore Multiscreen BV 96-well filtration plate was used, whose polyvinylidene fluoride filter mimics the ILM with respect to staining properties, as well as a collagen cell carrier (CCC) membrane, which is similar to the ERM with respect to staining. Solubility was assessed by preparing a 0.1% (w/v) solution of the dyes in water and in PBS. The size of the pellet and the colour intensity of the supernatant account are considered as a qualitative evaluation of the solubility.

The results are summarized in (**Figure 17**). Dye **6** was completely insoluble in water as well as in PBS. The filter wells show a consistent staining of different tones of green, across all the used dyes including ICG. On the other hand, the membranes appear to have no consistent green staining at all, with the exception of **7** and ICG. BIP stained the membrane in blue, and the green staining of the filter indicates a considerable solvatochromic shift.

| Dye | 4a | 4b | 4c | 4d | 4e | 4f | 5 | 7 | BIP | ICG |
|---|---|---|---|---|---|---|---|---|---|---|
| Filter | | | | | | | | | | |
| Membrane | | | | | | | | | | |
| Solution | | | | | | | | | | |
| Precipitate H$_2$O/PBS | +++ / +++ | ++ / +++ | - / +++ | + / +++ | - / +++ | +++ / +++ | +++ / +++ | - / - | - / - | + / +++ |

**Figure 17.** Filter-membrane staining and solubility test.

## 2.7.4 Toxicity Measurements

Dyes used for staining of ILM must be non-toxic. We therefore assessed the toxicity of the dyes synthesized. Solutions/suspensions of 0.1% in water were tested. Cells were incubated at 37 °C for 15 minutes, and then the dye was washed off with PBS. After washing, MTT was used to determine the viability. As control, PBS was used. Results are shown in (**Figure 18**).
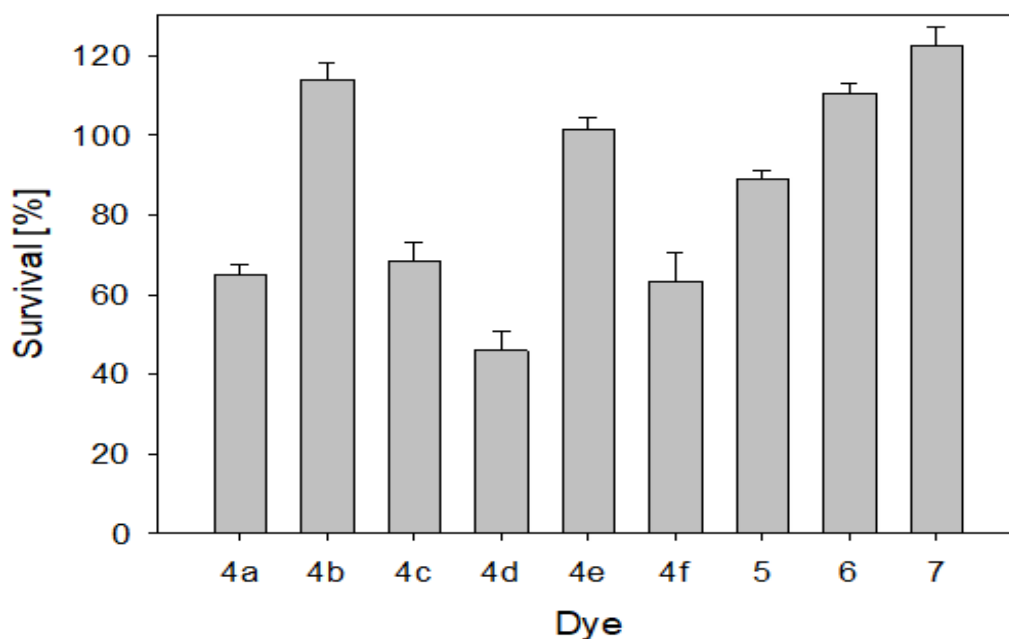


**Figure 18.** Toxicity of the dyes measured as cell survival [%] with their corresponding error.

Of the water-soluble dyes, **4b**, **4e**, **6** and **7** were the only dyes which showed no toxicity. Dye **5** resulted in a survival rate of 80%. Dyes **4a**, **4c** and **4f** showed a survival rate around 60%. The dye **4d** had a higher toxicity compared to the rest, with a survival rate below 50%, and therefore could not be considered as a dye suitable for surgical procedures.

In conclusion, ssolubility in water of most bis-benzoindole dyes was limited as well as their membrane staining; however, they all showed a consistent filter staining. The dyes **4b**, **4e,** and **7** showed the highest survival rate with no toxicity. Considering the overall properties, the asymmetric dye **7** had appropriate solubility and staining properties and lack of toxicity, thus it could be evaluated further for use in ophthalmologic surgery.

A series of substituted heptamethine cyanine dyes has been synthesized using substituted dianil and dienaminone linkers, carrying a benzoindole unit at both or at least one end of the heptamethine chain. The effects of the substitutions on the dyes properties like, optical properties, solubility and stability in aqueous solutions as well as membrane staining and toxicity have been studied. The spectral data of an UV-vis/NIR absorption and fluorescence emission of the NIR heptamethine cyanine dyes showed that in spite of the structural similarities, the dyes exhibit notable different absorption spectra, highlighting the influence of the central substitution on the electronic structure of these molecules. Solubility in water of most bis-benzoindole dyes was limited, and only **4e** also showed low toxicity in cell culture. The asymmetric dye **7** had appropriate solubility and staining properties and lack of toxicity, these two dyes could be evaluated further for use in ophthalmologic surgery.

## 2.8 References

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# Chapter 3

## Outlook

# Chapter 3

# Chapter 3

# **Outlook**

We would like to expand our investigations by exploring potential methodologies for the synthesis and characterization of first generation of Boron-Boron bond of dodecahydro-*closo*-dodecaborate anion $[B_{12}H_{12}]^{2-}$ via either palladium or cupper-catalysed cross-coupling borylation principle. The great finding will enrich the field by overcoming the drawbacks of derivatization methods of $[B_{12}H_{12}]^{2-}$. $B_{12}$-boronic acids and esters are versatile reagents that can utilize in the preparation of various boron–oxygen, boron–nitrogen, and boron–carbon bonds. In addition, the use of organoboranes for cross-coupling processes is particularly attractive owing to their high stability and low toxicity and it's known as an inexpensive and economical source.

Homo cross-coupling of an electron-rich dodecahydro-*closo*-dodecaborate anion will yield first symmetrical bicluster product, as well as cross coupling of dodecahydro-*closo*-dodecaborate anion with another ionic and neutral boron cluster. We aim to utilize our new methodology to synthesize such interesting compounds, and explore their electronic, chemical, and physical properties.

Th initial results were promising, and we managed to see the 1- and 2- peaks of the desired product by mass spectroscopy.
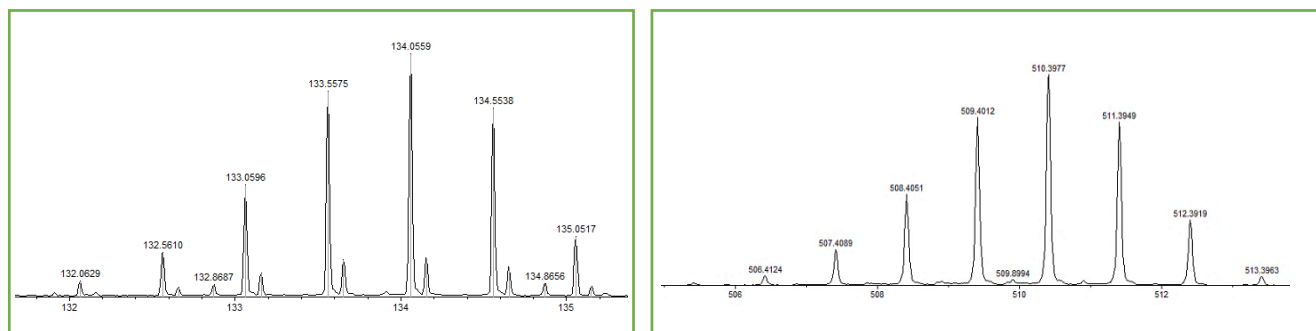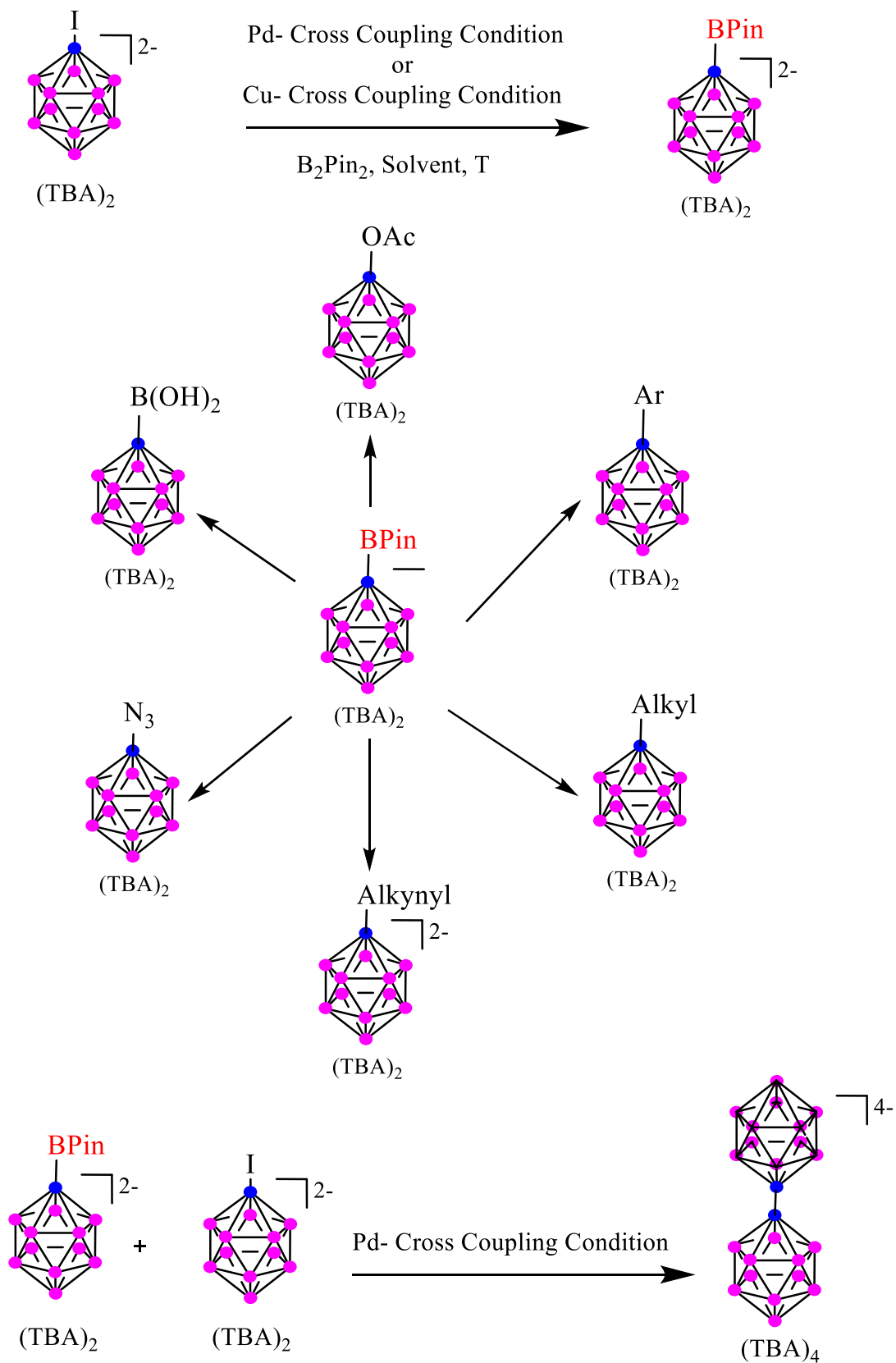


**Figure 1.** The Ms spectra of $B_{12}H_{11}B(CH_3)_4C_2O_2$.

**Scheme 1:** Outlook of the thesis.

# Chapter 4

# Appendices

# Chapter 4

## 4.1 Appendix 1: Manuscripts for Chapter 1

# Microwave-Assisted Palladium-Catalyzed Cross-Coupling Amination of Iodo-undecahydro-*closo*-dodecaborate with Large Versatility

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Supporting information for this article is given via a link at the end of the document

Abstract: For many years, a major limitation for expanding the application of the closo-dodecaborate has been its poor reactivity and lack of derivatization methods. On the other hand, palladium-catalyzed cross-coupling reactions have emerged as an extremely powerful, versatile tool, tolerant for a large number of functional groups. Herein, we describe an unprecedented and efficient method for the formation of B−N bonds via microwave-assisted palladium-catalyzed amination of iodo-undecahydro-closo-dodecaborate $[B_{12}H_{12}]^{2-}$. The method allows mild and reproducible formation of B-N bonds with aromatic amines, HN-containing heterocycles, and amides.

Boron clusters have gained considerable interest in drug design [1] and material science [2]. Icosahedral dicarba-*closo*-dodecaboranes (carboranes, of which three isomers exist) belong to the most hydrophobic building blocks known. In contrast, dodecaborates with two negative charges possess good water solubility with alkali counter ions. Nevertheless, they can interact very strongly also with hydrophobic surfaces such as the interior of cyclodextrins [3] or the exterior of cucurbiturils [4]. This unexpected behavior stems in part from the weak coordination of water around the boron clusters [5]. This effect has been named superchaotropic [3b, 6]. For making full use of it, dodecaborates should ideally be linked to organic residues exhibiting suitable and complementary characteristics, both in drugs and in materials. While the chemistry for organo-carboranes is well-established, methods for linking the *closo*-dodecaborates to organic residues are less developed [7]. Heteroatoms such as N, [8] S, [9] and O [10] can be introduced and functionalized further by reactions with nucleophiles [11]. Few reports have been published on methods to synthesize B-N derivatives (see Scheme 1). The first B-N derivative of $[B_{12}H_{12}]^{2-}$ was reported by Hertler *et al.* in 1964 [8]. Much later, Sivaev *et al.* prepared derivatives of $[B_{12}H_{11}NH_3]^{1-}$ with aldehydes followed by reduction of the resulting imine [12]. However, this approach is limited to aromatic aldehydes. Later, reactions to guanidinium derivatives and aroyl chlorides with $[B_{12}H_{11}NH_3]^{1-}$ were described [13]. Alkylation of the N-atom to di- and tri-substituted ammonium salts is possible, but selective monoalkylation could not be achieved [11b, 11c]. Nucleophilic aromatic substitution of *closo*-$[B_{12}H_{11}NH_3]^{1-}$ with 1-chloro-2,4-dinitrobenzene results in a mixture of two compounds with very poor yield [11e]. Direct functionalization of $[B_{12}H_{12}]^{2-}$ with organic nitrogen compounds has been described, but the methods suffer from harsh conditions and/or poor selectivity [11e, 14]. For more general use in the design of drugs or new materials, milder, more general, and more selective methods for linking the dodecaborate cluster to amines are therefore needed. Herein, we describe a general approach to B-N bond formation on the *closo*-dodecaborate anion via palladium-catalyzed cross-coupling

(Buchwald-Hartwig reaction), assisted by microwave irradiation. Palladium-catalyzed cross-coupling amination of iodo-undecahydro-*closo*-dodecaborate $[B_{12}H_{11}I]^{2-}$ (**1**) provides a general avenue for linking the boron cluster to organic residues. Although **1** has been studied in transition-metal-catalyzed reactions [15], Pd-catalyzed B-N bond formation in **1** has not been reported.
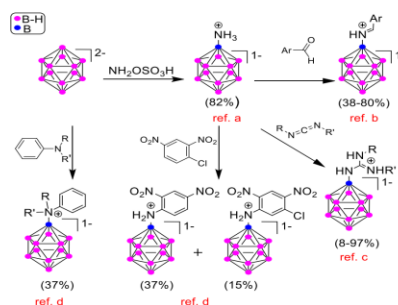


Scheme 1. Current methodologies for preparation of B-N derivatives of $[B_{12}H_{12}]^{2-}$. Ref. a [8], ref. b [12], ref. c [13], ref. d [11e].

As model for our initial experiments, we chose the reaction of **1** with aniline (**2a**). We varied catalytic systems, bases, and reaction conditions. Selected examples are presented in Table 1. We observed dramatic effects of the ligand, base, solvent, and temperature in these initial experiments. To best facilitate the $[B_{12}H_{11}I]^{2-}$ amination, we assessed several Pd/Ligand system combinations. Only DavePhos gave the desired aniline derivatives. JohnPhos, XPhos, and t-BuDavePhos gave no conversion, and SPhos and BrettPhos led to conversion of the starting material, but not to a cross-coupling product.
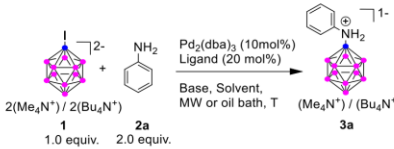
As important as the choice of ligand was the choice of the heating method. Microwave irradiation proved to be far more effective than conventional heating (compare, e.g., entries 5 and 6 of Table 1). Using conventional heating, higher temperatures resulted in higher conversion of **1**, but not to **3a**; rather $[B_{12}H_{12}]^{2-}$ was formed, with the amination product in less than 10% yield. We therefore replaced conventional heating by microwave irradiation. We had shown before that microwave irradiation greatly increased yield and reduced reaction time in a Sonogashira coupling of **1** [16]. Also here, under microwave irradiation conditions the reaction underwent complete conversion within 15 min with very little side product formation, emphasizing the advantage of using microwave in these types of reactions. Kumada-type cross-

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coupling with **1** has been reported to proceed by conventional heating, but required longer reaction times [15b].Solvents like THF, 1,4-dioxane, toluene, and DME gave low conversion, most likely due to the low solubility of **1**, catalyst and/or base, and perhaps due to their lower boiling points (compare entry 2 of Table 1 with entry 6). Solvents with high polarity and high boiling point, such as DMF and DMSO, gave the desired aniline derivatives in better yields. Notably, only little of the undesired $[B_{12}H_{12}]^{2-}$ as side product was obtained using DMSO and DMF. DMSO proved to be the most effective solvent, as it could be removed more easily,

and was used for further optimization of the B–N coupling reaction conditions.
Also, the choice of base was important. $Cs_2CO_3$ (entry 1) was less effective than KO$t$-Bu (entry 2). KO$t$-Bu was therefore used for all subsequent reactions. Catalyst loading was important for the yield. Ten mol% of $Pd_2(dba)_3$ yielded >65% of the desired product, 5 mol% only between 20-30%. In most cases, we obtained a mixture of the B-N product and $[B_{12}H_{12}]^{2-}$ in a ratio that varied depending on the reaction conditions. In terms of yield, the optimum was obtained with a ratio of **1** to aniline of 1:2
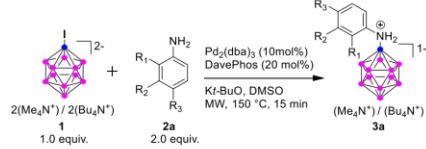
**Table 1**: Screening of bases and reaction conditions.



| Entry | Base | Solvent | Ligand | T (°C) | Time (h) | Consumption of 1 (%) | Yield$^c$ 3a (%) |
|---|---|---|---|---|---|---|---|
| 1 | $Cs_2CO_3$ | 1,4-dioxane | DP | 100$^b$ | 0.5 | 20 | <15 |
| 2 | KO$t$-Bu | 1,4-dioxane | DP | 100$^b$ | 0.5 | 50 | 30 |
| 3 | KO$t$-Bu | DMF | DP | 150$^b$ | 0.5 | 90 | 45 |
| 4 | KO$t$-Bu | DMF | DP | 150$^b$ | 1 | >95 | 45 |
| 5 | KO$t$-Bu | DMSO | DP | 150$^a$ | 48 | 95 | <5 |
| 6 | KO$t$-Bu | DMSO | DP | 150$^b$ | 0.25 | 100 | 65 |
| 7 | KO$t$-Bu | DMSO | BP | 150$^b$ | 0.25 | 90 | <5 |
| 8 | KO$t$-Bu | DMSO | JP | 150$^b$ | 0.25 | 0 | 0 |
| 9 | KO$t$-Bu | DMSO | tBP | 150$^b$ | 0.25 | 0 | 0 |
| 10 | KO$t$-Bu | DMSO | SP | 150$^b$ | 0.25 | 95 | <5 |
| 11 | KO$t$-Bu | DMSO | XP | 150$^b$ | 0.25 | 0 | 0 |

Reaction conditions: 1 (1.0 equiv.), aniline (2.0 equiv.), base (2.5 equiv.), Pd$_2$(dba)$_3$ (10 mol%), ligand (20 mol%), solvent (concentration of 1 = 0.1 M). $^a$ Oil bath, $^b$ Microwave irradiation. $^c$ Determined from 11B-NMR. Ligands: DP = DavePhos, JP = JohnPh, SP = SPhos, tBP = t-BuDavePhos, BP = BrettPhos, XP = XPhos.

**Table 2.** Pd-catalyzed cross-coupling of 1 and substituted anilines



| Entry | Product 3 | R$_1$, R$_2$, R$_3$ | Conversion$^a$ [%] | Yield$^b$ [%] |
|---|---|---|---|---|
| 1 | **3a** | H, H, H | 85 | 65 |
| 2 | **3b** | H, H, NO$_2$ | 100 | 84 |
| 3 | **3c** | H, H, CN | 100 | 84 |
| 4 | **3d** | H, H, CO$_2$Me | 100 | 81 |
| 5 | **3e** | H, H, Me | >95 | 70 |
| 6 | **3f** | H, H, OPh | >90 | 52 |
| 7 | **3g** | H, H, OEt | >90 | <10 |
| 8 | **3h** | H,H, N(Me)$_2$ | >90 | <10 |
| 7 | **3i** | H, H, F | > 90 | 52 |
| 8 | **3j** | H, H, Cl | < 5 | Trace$^c$ |
| 9 | **3k** | H, H, Br | < 2 | Trace$^c$ |
| 10 | **3l** | H, H, I | 0 | 0 |
| 11 | **3m** | NO$_2$, H, H | 100 | 83 |
| 12 | **3n** | H, NO$_2$, H | 100 | 83 |
| 13 | **3o** | NO$_2$, H, NO$_2$ | >95 | 78 |

Reaction conditions: **1** (1.0 equiv.), Substituted aniline (2.0 equiv.), KO-$t$Bu (2.5 equiv.), Pd$_2$(dba)$_3$ (10 mol%), DavePhos (20 mol%), DMSO (0.1M). $^a$ Conversion determined by $^{11}$B-NMR and referred to the consumption of **1**. $^b$ Isolated yield. $^c$ Based on $^{11}$B-NMR of reaction mixture.

Using $Pd_2(dba)_3$ (10 mol%), DavePhos as ligand (20 mol%), and KO$t$-Bu (2.5 equiv.) in DMSO as optimized protocol, we assessed the scope of substituted anilines as coupling partners. For electron-deficient anilines, yields were high (Table 2, **3b**, **3c**, **3d**, **3m**, **3n**, **3o**), except for $p$-fluoroaniline (**3i**). p-Phenoxyaniline provided the B-N product in 52% yield (**3f**), while for the few examples of electron-rich anilines, amination yields were poor <10% and hydrogen transfer was dominant, resulting in $[B_{12}H_{12}]^{2-}$ as major product (**3g**, **3h**).
No conversion was observed with chloro-, bromo- and iodo-aniline under the standard reaction conditions, and the starting material **1** remained untouched. This is possibly due to the faster rate of oxidative addition of palladium with the haloanilines in comparison to that with **1**. Collectively, the reaction proceeds well with electron-withdrawing groups in aniline; electron-donating groups react, albeit with lower yield.
Given the promising results achieved, we envisioned that our methodology might also allow us to prepare other types of aromatic primary amines. Coupling of 1-naphthylamine was possible, but only with low yield (Scheme 2). This might be due to steric reasons or to the larger electron density on the N atom.
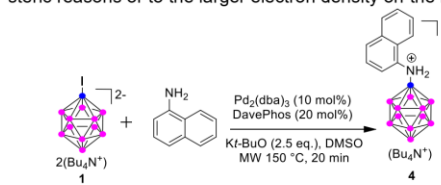


**Scheme 2.** Cross-coupling reaction of 1-naphthylamine and **1**.

We examined the coupling of various N-containing heterocycles with **1**. Such heterocycles react in Buchwald-Hartwig reactions [17]. The ability to combine aromatic heterocyclic fragments with a boron cluster is an important and challenging task. Heterocyclic structures are often key fragments of biologically active molecules

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and many drugs contain N-heterocycles such as pyrrole, imidazole, and indole. A set of N-containing heterocyclic structures was tested; the desired B-N products were obtained in excellent yield (Scheme 3, **5a-e**). As expected, pyridine did not react (**5f**).
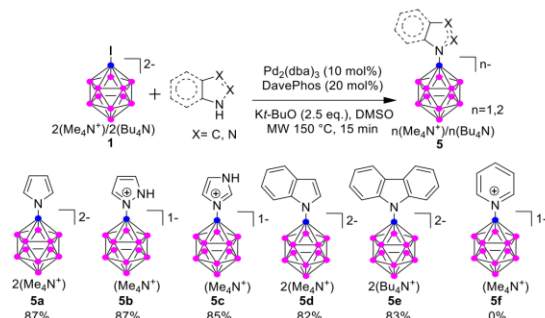


**Scheme 3.** Coupling of **1** to N-containing heterocycles

To expand the applicability of our approach, we investigated the coupling of amides with the dodecaborate cage. We could carry out amidation of **1** under our standard conditions, and the reaction proceeds with aromatic and aliphatic amides, and sulfonamides (Scheme 4). 2-Nicotinamide and propanamide were successfully coupled in excellent yield. Sulfamide and *p*-toluene sulfonamide were coupled, however, with poor isolated yield. The amide coupling products were isolated as protonated species, as described before for the benzoyl derivative, where the H atom could be located on the carbonyl oxygen [13]. We successfully coupled also secondary amides; acetanilide and N-methylacetamide reacted with **1** in excellent yields (**6e** and **6f**, respectively).

The reaction that we describe here is possible with aromatic amines, NH-containing heterocycles, and amides. For secondary primary aliphatic amines, our current methodology did not prove effective; rather, we obtained $[B_{12}H_{12}]^{2-}$ as a major product, and the B-N product in single-digit percent yields. We have not yet identified the source of the H atom in any of the reactions, but we suspect that the Pd(II) amine complex undergoes hydride elimination [18].
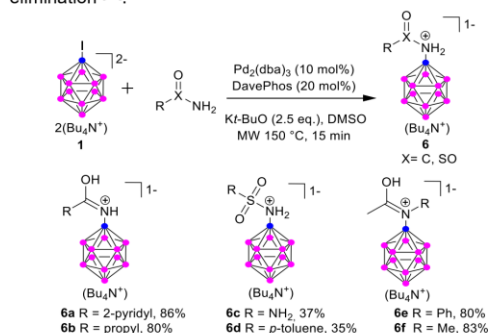


**Scheme 4.** Cross-coupling of **1** with amides.

In the search for alternative bases, an unexpected B-N bond formation product occurred with DBU. We obtained a product where one of the N atoms of DBU was bonded to a boron atom, a reaction that could be driven to complete conversion and excellent yield by using two equivalents of DBU (Scheme 5)
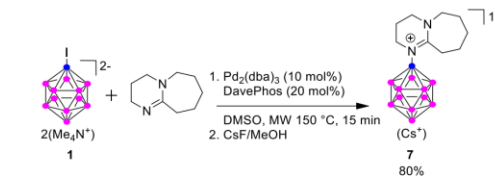


**Scheme 5.** Cross-coupling of **1** with DBU.

In summary, we have developed a new Pd-catalyzed cross-coupling of iodo-*closo*-dodecaborate with aromatic amines, with amides, with NH heterocycles, and with sulfonamides. Rapid conversion, high yields, use of readily available catalysts, and reactions with several different nucleophiles that can be coupled to the dodecaborate cage are advantages of this methodology. DavePhos as ligand, DMSO as solvent, KO-*t*Bu as base, and microwave irradiation were essential. Further computational studies on reaction mechanism, and experimental studies to extend the scope of these reactions to include secondary and aliphatic primary amines, are currently underway in our laboratory.

**Keywords:** catalysis • cross-coupling • cluster • boron

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# Chapter 4

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# Chapter 4
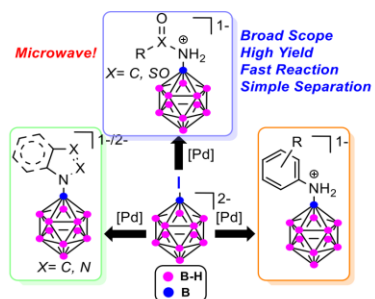
Improved synthesis of halo- and oxonium derivatives of *closo*-dodecaborate(2-)

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## Highlights

- A simple method, avoiding non-aqueous acid, to produce cyclic oxonium derivatives of dodecaborate $B_{12}H_{12}^{2-}$ has been designed.

- Using N-halosuccinimide, pure mono-halogenated dodecaborate $B_{12}H_{11}Hal^{2-}$ with Hal = Cl, Br, I can be obtained

- These derivatives are known as useful synthons for incorporating dodecaborate into organic moieties

## Abstract

Convenient methods have been developed to synthesize cyclic oxonium derivatives and pure mono-halo derivatives of dodecaborate.

## Graphical Abstract
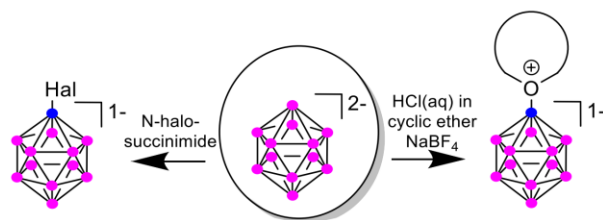


## Keywords

dodecaborate, cyclic oxonium dodecaborate, monohalogenated dodecaborate, N-halosuccinimde

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## 1 Introduction

After the prediction of the structure of the $[B_{12}H_{12}]^{2-}$ anion in 1955 by Longuet-Higgins and Roberts,[1] and its isolation by Pitochelli and Hawthorne in 1960,[2] various researchers developed approaches to the preparation of this anion with high yields and more accessible starting materials.[3, 4] Effective methods for the functionalization of the $[B_{12}H_{12}]^{2-}$ anion were developed and a large number of its derivatives were obtained,[5] which found their application in many fields. Thus, the presence of a charge in such compounds allows them to be used in extraction and in catalysis as weakly coordinating anions.[6] The use of $Na_2B_{12}H_{11}SH$ as an agent for boron neutron capture therapy (BNCT) is of great interest.[7]

In recent years, new properties of $[B_{12}H_{12}]^{2-}$ not found in organic compounds have been discovered. The boron cluster and its derivatives are well water-soluble with the proper counter ion, but nevertheless they are able to bind to the hydrophobic surfaces in the interior of cyclodextrins.[8-10]. These properties resemble those of chaotropic anions, but are much more intense in their nature; the anions have therefore been called „super-chaotropic". This can lead to a number of interesting applications in material science, analytics, and medicinal chemistry.[11-13]

Based on the above, the search for new methods and modification of old methods of functionalization of this anion remains an urgent task. Since many methods of synthesis are more than 50 years old, in practice it turns out that not all the results described are well reproducible.

This article describes the modification of already known derivatives of $[B_{12}H_{12}]^{2-}$ and is aimed at facilitating and/or improving the synthesis of oxonium and monohalogen derivatives of the dodecahydro-closo-dodecaborate anion. These intermediates are commonly used to link the dodecaborate cluster to organic moieties (Scheme 1).
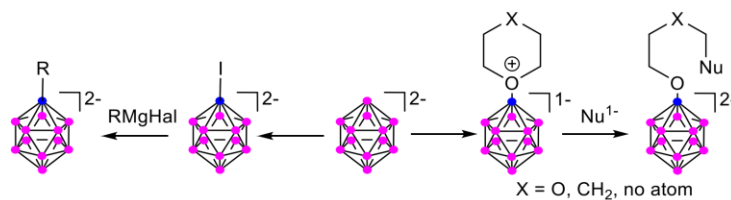


**Scheme 1.** Cross-coupling and ring-opening reactions to attach $B_{12}H_{12}^{2-}$ to organic residues.

Dodecaborate clusters containing a cyclic oxonium group (tetrahydrofuranium, hexahydropyrylium, 1,4-dioxanium) are presently the most widely used derivatives to attach the boron cluster to nucleophiles. These derivatives all present a long alkyl or alkoxy linker between the cluster and the second part of the molecule.

Different methods for the preparation of the oxonium derivatives are being used, depending on the desired cyclic structure. Usually, a strong acid such as *p*-toluenesulfonic acid or $HBF_4$ (prepared in situ from $NaBF_4$ and HCl under anhydrous couditions) or $BF_3$ etherate is used. Work-up is not straight-forward in the present methods, and differs according to the ethers used. [5, 14].

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The reaction of mono-halogenated dodecaborates in cross-coupling reactions is gaining increasing importance for the preparation of dodecaborate derivatives. [15, 16] As substrate for these reactions, mono-halododecaborate (preferentially iodododecaborate) has been used. Its preparation by the methods described so far has led to mixtures of mono-, di-, and tri-halogenated compounds, with several positional isomers in the clusters containing two and three halogen atoms. Often, unreacted cluster is also present when trying to limit the amount of halogen source to the stoichiometric minimum. The presence of the non-, di-, and tri-halogenated clusters makes isolation, purification, and characterization of the cross-coupling compounds difficult.

The monochloro derivative $[B_{12}H_{11}Cl]^{2-}$ has been reported for the first time by Knoth and co-workers in 1964.[17] The reaction was carried out by mixing of $(H_3O)_2[B_{12}H_{12}] \cdot nH_2O$ with anhydrous hydrogen chloride at 85 °C. Haeckel et al. reported in 1995 the reaction of the tetrabutylammonium salt with dichloromethane in the presence of trifluoroacetic acid. [18] The reaction provided the desired monochloro dodecaborate in good yield. Chlorination of $[B_{12}H_{12}]^{2-}$ with chlorine in water gives a mixture of $[B_{12}H_{11}Cl]^{2-}$, $[1,2-B_{12}H_{10}Cl_2]^{2-}$, $[1,7-B_{12}H_{10}Cl_2]^{2-}$, $[1,2,3-B_{12}H_9Cl_3]^{2-}$, and $[1,7,9-B_{12}H_9Cl_3]^{2-}$ derivatives,[19] which can be separated by chromatography.

The monobromo derivative $[B_{12}H_{11}Br]^{2-}$ was achieved before by the reaction of $[B_{12}H_{12}]^{2-}$ with dibromomethane in the presence of trifluoroacetic acid.[20] Treatment of $[B_{12}H_{12}]^{2-}$ with bromine in a water–tetrachloromethane mixture gave a mixture of mono-, 1,2- and 1,7-dibromo derivatives,[19] which can be separated by chromatography. The reaction of $[B_{12}H_{12}]^{2-}$ with bromine in aqueous methanol at –10 °C was shown to give a mixture of tetra- and pentabromo derivatives,[21] whereas the same reaction at 5 °C gives the hexabromo derivative $[B_{12}H_6Br_6]^{2-}$. The decabromo derivative $[B_{12}H_2Br_{10}]^{2-}$ was achieved by heating $(H_3O)_2[B_{12}H_{12}] \cdot nH_2O$ with bromine in water at 80–90 °C [22]. The perbromo derivative $[B_{12}Br_{12}]^{2-}$ was prepared by the treatment of $[B_{12}H_6Br_6]^{2-}$ with bromine in the presence of chlorine in 50% aqueous methanol.

The synthesis of the monoiodo derivative $[B_{12}H_{11}I]^{2-}$ was reported by the reaction of $[B_{12}H_{12}]^{2-}$ with one equivalent of iodine in aqueous methanol at 0 °C [22] and by the reaction with diiodomethane in the presence of trifluoroacetic acid.[18] The use of two equivalents of iodine (dissolved in $CCl_4$) with $Na_2B_{12}H_{12}$ in water results in the disubstituted product which is mainly the 1,7-isomer.[23]

We found that one general method, not employing any other acid that aqueous hydrochloric acid, allows to attach any water-soluble cyclic ether to the cluster, thereby allowing one single and simple procedure for obtaining these valuable intermediates.

Likewise, we found a method to produce highly pure monohalo-dodecaborates (with the halogen being chlorine, bromine, or iodine) without risk for di- and tri-halogenation, and without non-halogenated material.

These procedures might be of value for obtaining pure starting materials for further reactions.

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## 2 Materials and Methods

*closo*-Dodecaborate was purchased as Cs salt from BASF. It was converted to the tetrabutylammonium salt by precipitation from an aqueous solution with $Bu_4NCl$ dissolved in water. All other chemicals and solvents were from Sigma Aldrich or Carl Roth.

Abbreviations used: N-chlorosuccinimide (NCS); N-bromosuccinimide (NBS); N-iodosuccinimide (NIS)

NMR spectra were taken on a JEOL 400 spectrometer.

Mass spectra were recorded on a Bruker micrOTOF spectrometer in negative mode.

### 2.1 Synthesis of oxonium derivatives

General procedure: To a suspension of (1.0 mmol) $(Bu_4N^+)_2[B_{12}H_{12}]^{2-}$ and (5.0 mmol) $NaBF_4$ in 70 ml of THF, 1,4-dioxane, or tetrahydropyrane, 1.0 ml of a 4 M solution HCl (prepared by mixing 1 volume of 37% HCl in water with 3 volumes of cyclic ether) in THF, 1,4-dioxane, or tetrahydropyrane was added. The reaction mixture was heated to reflux for 2 h, allowed to cool to room temperature, filtered and concentrated near to dryness under reduced pressure. The residue was recrystallized from ethanol. The precipitate was washed with a small amount of ethanol and dried in air to give the desired product. $^{11}B$ and $^1H$ NMR of $(Bu_4N^+)$ $[C_5H_{10}O\text{-}closo\text{-}B_{12}H_{11}]^{1-}$ agrees with the literature.[24] The spectral data of $(Bu_4N^+)$ $[C_4H_8O_2\text{-}closo\text{-}B_{12}H_{11}]^{1-}$ and $(Bu_4N^+)$ $[C_4H_8O\text{-}closo\text{-}B_{12}H_{11}]^{1-}$ agree with the literature.[25]

### 2.2 Reaction with N-halo-succinimide

$(Bu_4N^+)_2$ $[B_{12}H_{12}]^{2-}$ was reacted with one equivalent of N-halosuccinimide in acetonitrile. The addition of N-halosuccinimide, dissolved in acetonitril was carried out in a slow dropwise addition over the course of 30 mins at -10-0 °C in case of iodination and bromination. The reaction mixture was then stirred for one hour at room temperature providing quantitative yield of desired product and succinimide as a side product which could be easily removed by distributing the crude mixture with DCM (containing the halogenated cluster) and water (containing the other reaction products and acids). Chlorination of $(Bu_4N)_2$ $[B_{12}H_{12}]^{2-}$ needed reflux of the mixture for one hour for complete conversion, the desired mono-chloro derivative $(Bu_4N^+)_2$ $[B_{12}H_{11}Cl]^{2-}$ was collected in 85% yield by flash column chromatography on silica gel with 1:10 acetonitrile to DCM as an eluent.

**$(Bu_4N)_2B_{12}H_{11}I$:** $^{11}B$-NMR (129 MHz, acetone-$d_6$) δ = -21.47 (S, IB, B-I), -17.12 (d, $J$= 135,1B, B-H) -15.26 (d, $J$= 137, 5B, B-H), -13.73 (d, $J$= 171, 5B, B-H). HRMS (ESI/TOF) m/z calcd for $B_{12}H_{11}I$ $(M+C_{16}H_{36}N)^{-1}$: 510.3958; Found: 510.4064.

**$(Bu_4N)_2B_{12}H_{11}Br$:** $^{11}B$-NMR (129 MHz, acetone-$d_6$) δ = -19.30(d, $J$= 128,1B, B-H), -16.27(d, $J$= 134, 5B, B-H), -14.71(d, $J$= 136, 5B, B-H), -8.91(S, IB, B-Br). HRMS (ESI/TOF) m/z calcd for $B_{12}H_{11}Br$ $(M+C_{16}H_{36}N)^{-1}$: 463.4089; Found: 463.4095.

**$(Bu_4N)_2B_{12}H_{11}Cl$:** $^{11}B$-NMR (129 MHz, acetone-$d_6$) δ = -20.8 (d, $J$= 129,1B, B-H), -17.2 (d, $J$= 129, 5B, B-H), -15.5 (d, $J$= 131, 5B, B-H), -3.7 (S, IB, B-Br)). HRMS (ESI/TOF) m/z calcd for $B_{12}H_{11}Cl$ $(M+C_{16}H_{36}N)^{-1}$: 418.4595; Found: 418.4802.

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## 3    Results and Discussion

### 3.1    Reaction to oxonium derivatives
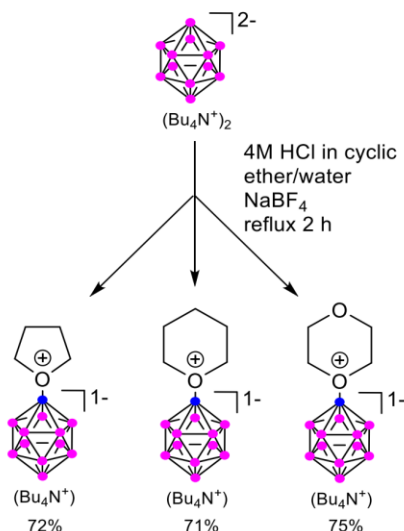


**Scheme 2**. Aqueous HCl mediated synthesis of cyclic oxonium derivatives of $(Bu_4N^+)_2 [B_{12}H_{12}]^{2-}$.

Using 4 M of commerially available aqueous 37% HCl in the desired water-miscible cyclic ether, the reaction goes to complete conversion and provides almost the same isolated yields as the ones reported before. The cyclic oxonium derivatives of THF, 1,4-dioxane, and tetrahydropyrane were obtained in 70-75% yield in two hours reaction time. The obtained products were characterized with $^{11}$B-NMR, $^1$H-NMR, and mass spectroscopy and the obtained results completely matched the reported results (Scheme 2).   The main advantages of this method over the previously published ones is in very good yield of the goal product, high reproducibility specially with large scale reaction, use of non-hygroscopic and easy-handling tetrabutylammonium *closo*-dodecaborate as the starting material, and identical conditions for synthesis of water-soluble ethers.

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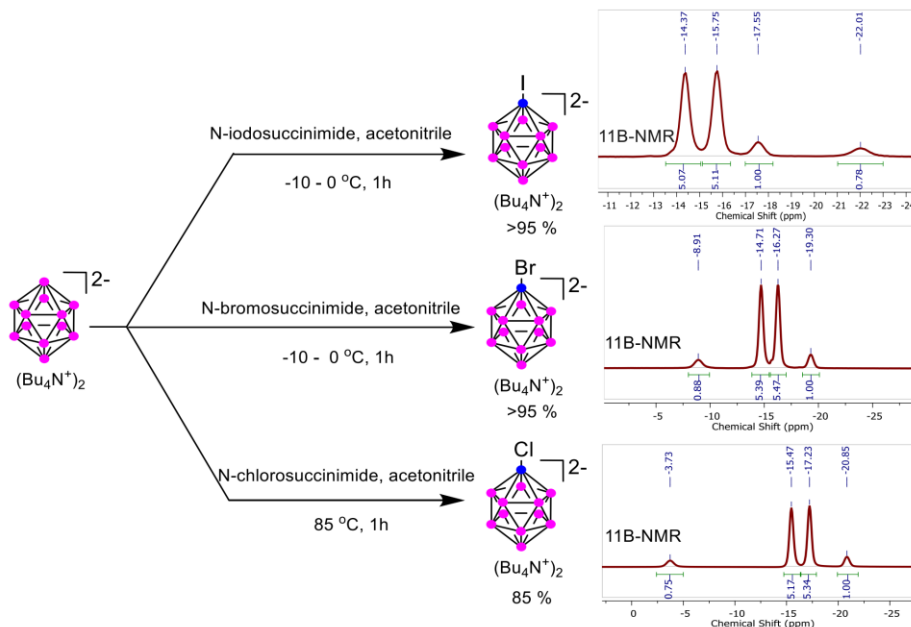
## 3.2 Halogenation



**Scheme 3**. Synthetic approach of $(Bu_4N)_2$ $[B_{12}H_{11}X]^{2-}$ (X= I, Br, Cl).

Halogenation proceeds very smoothly with NBS and NIS, even at low temperatures, and is complete within one hour. For chlorination, the temperature needs to be increased to reflux, and results in slightly lower yields than the bromination and iodination reactions.

For iodination, a substoichiometric amount of NIS leads to a mixture of mono- and non-iodinated material, with no di-iodinated material detectable by NMR spectroscopy. An excess of NIS leads to a mixture of mono- and di-iodinated material, with no unreacted or tri-iodinated products detectable. We therefore assume that iodination with NIS proceeds fast for the first substitution, and then subsequently slower for the second and third substitution. This is in contrast to iodination with molecular iodine or iodine monochloride.

The procedure yields therefore much purer material than any of the literature procedures. Yields are very high, and work-up is very straight-forward.

In our experience, this method is the only method which yields the mono-halogenated cluster $B_{12}H_{11}Hal^{2-}$ without any di- or tri-halogenated product or any starting $B_{12}H_{12}^{2-}$. The methods described before all yield mixtures, which were difficult to impossible to separate.

## 4 Conclusion

A simple method for synthesizing oxonium derivatives of cyclic ethers with dodecaborate has been designed. A novel method to obtain $[B_{12}H_{11}Hal]^{2-}$ with

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excellent purity has been developed. These methods will have impact on the further development of dodecaborate functionalization.

One or both of these reactions might be transferable to other boron clusters such as $[B_{10}H_{10}]^{2-}$, $[CB_{11}H_{12}]^{1-}$ or cobalta-bis-carbollide.

## 5 Acknowledgments

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# Chapter 4

## 4.1.1 Supporting Information for Chapter 1

**Martials and Methods**

Commercially available reagents were used without further purification, unless otherwise stated. B12H11I was prepared according to the literature procedure with some modification.1 4-Nitroaniline (>99%), 2,4-dinitroaniline, 2-nitroaniline, 3-nitroaniline, 4-cyanoaniline, N-methyl 4-aminobenzoate (98%), imidazole, pyrazole, pyrrol, indole carbazole (98%), iodine (>99%), Davephos, Ru-Phos, Brettphos, Johnphos, Sphos, and tertbutyl Davephos (99%), Pd2(dba)3 (99%), anhydrous K3PO4 (>98%), KtBuO (>98%), K2CO3 (>99.0%), toluene (99.8%) and 1,4-dioxane (99.8%) were from Sigma-Aldrich and were used as received. Dichloromethane, acetonitrile, and silica gel (Grade 60, 230-400 Mesh) were from Carl Roth. Celite (545 filter aid, not acid washed, powder) was from Fisher. Deuterated solvents were from SigmaAldrich and Deutero.

All cross-coupling reactions were performed in an oven dried 10 mL reaction round bottom flask. Thin-layer chromatography (TLC) plates (Macherey-Nagel). TLC samples for borane-containing compounds were stained with 1 wt. % PdCl2 in 6 M HCl and were developed using a heat gun.

1H NMR, 11B NMR, and 13C NMR spectra were recorded on a JOEL 400 MHz spectrometer at 25 °C. Chemical shifts were referenced relative to residual solvent. The following abbreviations are used to describe peak patterns where appropriate: br = broad, s =singlet, d = doublet, t = triplet, q = quartet, m = multiplet. MestReNova V10.0.2-15465 S3 software was used to visualize the spectra. Coupling constants (J) are reported in Hertz (Hz). Mass spectra in the negative-ion mode were recorded with a Bruker Ultraflex TOF spectrometer. The reactions have been performed in an open vessel microwave oven (Discover, CEM).

**Synthesis of oxonium derivatives**

To a suspension of (1.0 mmol) $(Bu_4N^+)_2[B_{12}H_{12}]^{2-}$ and (5.0 mmol) $NaBF_4$ in 70 ml of THF, 1,4-dioxane, or tetrahydropyrane, 1.0 ml of a 4 M solution HCl (prepared by mixing 1 volume of 37% HCl in water with 3 volumes of cyclic ether) in THF, 1,4-dioxane, or tetrahydropyrane was added. The reaction mixture was heated to reflux for 2 h, allowed to cool to room temperature, filtered and concentrated near to dryness under reduced pressure. The residue was recrystallized from ethanol. The precipitate was washed with a small amount of ethanol and dried in air to give the desired product. 11B and 1H NMR of the $(BU_4N^+)$ $[C_5H_{10}O\text{-closo-}B_{12}H_{11}(1-)]$ is matched with the literature. [59] The spectral data of $(BU_4N^+)$ $[C_4H_8O_2\text{-closo-}B_{12}H_{11}(1-)]$ and $(BU_4N^+)$ $[C_4H_8O\text{-closo-}B_{12}H_{11}(1-)]$ is matched with the literature. [66]

**Reaction with N-halo-succinimide**

$(BU_4N+)_2$ $[B_{12}H_{12}]^{2-}$ was reacted with one equivalent of N-halosuccinimide in acetonitrile. The addition of N-halosuccinimide was carried out in a slow dropwise addition over the course of 30 mins at -10-0 oC in case of iodination and bromination. The reaction mixture was then stirred for one hour at room temperature providing quantitative yield of desired product and succinimide as a side product which can be easily removed by distributing the crude mixture with DCM (containing the halogenated cluster) and water (containing the other reaction products and acids). Chlorination of $(Bu_4N)_2$ $[B_{12}H_{12}]^{2-}$ needs reflux of the mixture for one hour for complete conversion, the desired mono-chloro derivative $(BU_4N^+)_2$ $[B_{12}H_{11}Cl]^{2-}$ was collected in 90% yield by flush column chromatography with 1:10 acetonitrile to DCM as an eluent.

**$(Bu_4N)_2B_{12}H_{11}I$**: $^{11}$B-NMR (129 MHz, acetone-d6) δ = -21.47 (S, IB, B-I), -17.12 (d, J= 135,1B, B-H) -15.26 (d, J= 137, 5B, B-H), -13.73 (d, J= 171 , 5B, B-H). HRMS (ESI/TOF) m/z calcd for B12H11I (M-I)-1: 141.2056; Found: 141.2065, HRMS (ESI/TOF) m/z calcd for $B_{12}H_{11}I$ (M+C16H36N)-1: 510.3958; Found: 510.4064.

**$(Bu_4N)_2B_{12}H_{11}Br$**: $^{11}$B NMR (129 MHz, acetone-d6) δ = -19.30(d, J= 128,1B, B-H), -16.27(d, J= 134, 5B, B-H), -14.71(d, J= 136, 5B, B-H), -8.91(S, IB, B-Br). HRMS (ESI/TOF) m/z calcd for B12H11Br (M-Br)-1: 141.2056; Found: 141.2065, HRMS (ESI/TOF) m/z calcd for $B_{12}H_{11}Br$ (M+C16H36N)-1: 463.4089; Found: 463.4095.

**$(Bu_4N)_2B_{12}H_{11}Cl$**: $^{11}$B NMR (129 MHz, acetone-d6) δ = -20.8 (d, J= 129,1B, B-H), -17.2 (d, J= 129, 5B, B-H), -15.5 (d, J= 131, 5B, B-H), -3.7 (S, IB, B-Br)). HRMS (ESI/TOF) m/z calcd for B12H11Cl (M-Cl)-1: 141.2056; Found:141.2059, HRMS (ESI/TOF) m/z calcd for $B_{12}H_{11}Cl$ (M+C16H36N)-1: 418.4595; Found: 418.4802.

**Experimental Procedures**

**General procedure for the synthesis of (Bu₄N)₂B₁₂H₁₁I (1)**



(Bu₄N)₂
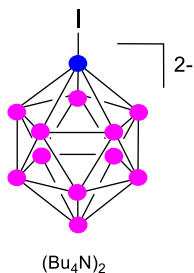
(1.0    g, 2.5 mmol, 1.0 equiv) $Cs_2B_{12}H_{12}$ was added to an 250 round bottom flask. The flask was charged with 30 ml of phosphate buffer solution (0.1 M, pH=7.2), the resulting mixture was heated up to 40 °C for 3-5 mins until all $Cs_2B_{12}H_{12}$ was completely dissolved and the solution became clear. Subsequently, $I_2$ (592 mg, 2.4 mmol, 1.0 equiv) dissolved in 100 ml MeOH was added dropwise to the reaction vessel over 30-40 mins. After completion of the addition of $I_2$, the reaction mixture was stirred for 3-5 mins until the reaction solution became colorless. Completion of the reaction was determined by ^{11}B-NMR. The contents of the flask were subsequently concentrated under reduced pressure yielding a white powder. The powder was washed several time with MeOH and  then filtered through a filter paper on a fritted funnel to yield a white solid, which was dissolved in water and treated with 2.4 equivalent of tetrabutylammonium bromide resulting in a white precipitate. The precipitate was dried *in vacuo* to produce the title compound, (1.77 g, 96 % yield). ^{11}B-NMR (129 MHz, acetone-$d_6$) δ = -21.47 (S, IB, B-I), -17.12 (d, J= 135,1B, B-H) -15.26 (d, J= 137 , 5B, B-H) , -13.73 (d, J= 171 , 5B, B-H). HRMS (ESI/TOF) m/z calcd for $B_{12}H_{11}I$ (M-I)[-1]: 141.2056; Found: 141.2065, HRMS (ESI/TOF) m/z calcd for $B_{12}H_{11}I$ (M+$C_{16}H_{36}N$)[-1]: 510.3958; Found: 510.4064.

**General procedure for the synthesis of (Me₄N)₂B₁₂H₁₁I (1)**



(Me₄N)₂
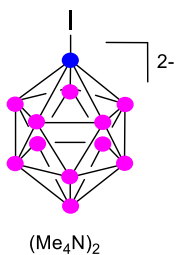
The above procedure was used. The water solution was treated with 2.4 equivalent of tetramethylammonium bromide resulting in a white precipitate, the precipitate was dried *in vacuo* to produce the title compound, (1.9 g, 90 % yield).

# Chapter 4

## General procedure for Pd-catalyzed cross-coupling amination

An oven-dried 10 mL round bottom flask was charged with 1.0 equiv of $(Me_4N)_2$ or $(Bu_4N)_2B_{12}H_{11}I)$, $Pd_2(dba)_3$ (10-15 mol%), Davephos ligand (20 mol%), amine (2.0 equiv), and K$t$BuO (2.5 equiv), subsequently 2.0 ml of anhydrous DMSO or DMF was added. The reaction flask was filled with $N_2$ and connected to a condenser. The round bottom flask was submitted to microwave irradiation at 150 $^o$C, power 300 W, for 15 mins with stirring (high) until the starting $(Bu_4N)_2/(Me4N)_2B_{12}H_{11}I$ had been completely consumed as judged by ^{11}B-NMR and TLC. The mixture was cooled to room temperature and then filtrated through a funnel filled with cotton, celite and filter paper. The resulting solution was concentrated under reduced pressure. The crude material was checked by TLC (usually by using acetonitrile: DCM (1:4 or 1:2) after that the mixture was purified by column chromatography on silica gel (eluting with acetonitrile-DCM mixtures).

## Experimental details and characterization data

### Remarks

1- We did not notice an effect of using $Bu_4N$ or $Me_4N$ as counter cation.
2- All compounds can be obtained as solid by precipitating with acetonitrile: $Et_2O$ (1:10) or acetone-$Et_2O$ (1:10).
3- In case of using $(Me_4N)_2[B_{12}H_{11}I]^{2-}$, we noticed in some compounds that methyl peak in ^{1}H-NMR and ^{13}C -NMR disappeared, most probably due to exchange of the cation with potassium from $Bu_tOK$.

## $(Bu_4N)[B_{12}H_{11}NH_2C_6H_5]$ (3a)
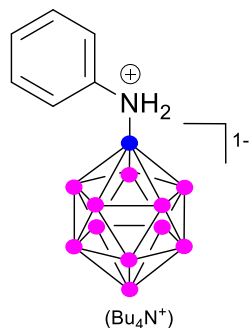


Following the general procedure using $(Bu_4N)_2$ $B_{12}H_{11}I$ (100 mg, 0.133 mmol, 1.0 equiv), $Pd_2(dba)_3$ (18.0 mg, 0.02 mmol, 0.15 equiv), Davephos ligand (16.0 mg, 0.04 mmol, 0.30 equiv.), K$t$BuO (37.0 mg, 0.333 mmol, 2.5 equiv) aniline (25 $\mu$L, 0.266 mmol, 2.0 equiv), DMSO 2ml. The crude product was purified by flash column chromatography on silica gel (Acetonitrile/DCM = 1:5) to afford the product as a yellow oil (41 mg, 65 % yield). ^{1}H NMR (400 MHz, acetone-$d_6$) $\delta$ = 0.94 – 1.06 (t, $J$=7.4, 12H), 1.37 – 1.51 (dt, $J$=14.9, 7.4, 8H), 1.76 – 1.89 (m, 8H), 3.38 – 3.50 (m, 8H), 7.22 – 7.29 (t, $J$=7.2, 1H), 7.31 – 7.44 (m, 4H), 7.81 – 7.93 (s, 1H). ^{13}C NMR (400 MHz, Acetonitrile-$d_3$) $\delta$ = 13.78, 20.29, 24.26, 59.24, 124.13, 127.97, 129.62, 139.14. ^{11}B NMR (129

MHz, acetone-$d_6$) δ = -18.79(1B, $J$=130, d), -17.06 (5B, $J$=103, d), -16.54 (5B, $J$=103, d), -4.63(1B, s). HRMS (ESI/TOF) m/z calcd for $C_6H_{18}NB_{12}$ (M+H)$^{-1}$: 234.2638; Found: 234.2689.

**(Bu$_4$N)[B$_{12}$H$_{11}$NH$_2$C$_6$H$_5$-4-NO$_2$](3b)**
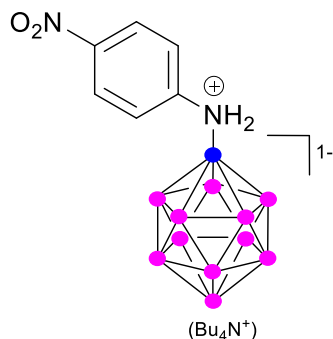


Following the general procedure using (Bu$_4$N)$_2$ B$_{12}$H$_{11}$I (100 mg, 0.133 mmol, 1.0 equiv), Pd$_2$(dba)$_3$ (12.2 mg, 0.0133 mmol, 0.10 equiv), Davephos ligand (10.5 mg, 0.0266 mmol, 0.20 equiv.), K$^t$BuO (37.0 mg, 0.333 mmol, 2.5 equiv), 4-nitroaniline (36.7mg, 0.266 mmol, 2.0 equiv), DMSO 2ml. The crude product was purified by flash column chromatography on silica gel (acetonitrile/DCM = 1:5 to 1:2) to afford the product as a orange oil in (60 mg, 84 % yield). $^1$H NMR (401 MHz, Acetonitrile-$d_3$) δ = 0.89 – 0.98 (t, $J$=7.3, 24H), 1.26 – 1.41 (h, $J$=7.3, 16H), 1.51 – 1.65 (p, $J$=8.6, 8.2, 16H), 3.01 – 3.13 (m, 16H), 5.97 – 6.08 (s, 1H), 6.96 – 7.06 (d, $J$=8.4, 2H), 7.83 – 7.92 (d, $J$=9.5, 2H). $^{13}$C NMR (101 MHz, Acetonitrile-$d_3$) δ = 1.32, 13.79, 20.26, 24.28, 59.24, 113.70, 120.80, 125.89, 127.09. $^{11}$B NMR (129 MHz, Acetonitrile-$d_3$) δ = -18.82(1B, d, $J$=130), -16.81(10B, d, $J$=103), -4.66 (1B, s). HRMS (TOF) m/z calcd for $C_6H_{17}N_2O_2B_{12}$ (M+H)$^{-1}$: 279.2489; Found: 279.2554. $C_{22}H_{52}N_3O_2B_{12}$ (M+Bu4N)$^{-1}$: 520.5268; Found: 520.5412. $C_6H_{16}N_2O_2B_{12}$ (M)$^{-2}$: 139.1208; Found: 139.1227.

**(Bu$_4$N)[B$_{12}$H$_{11}$NH$_2$C$_6$H$_5$-4-CN] (3c)**
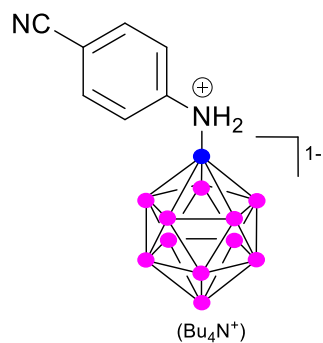


Following the general procedure using (Bu$_4$N)$_2$ B$_{12}$H$_{11}$I (100 mg, 0.133 mmol, 1.0 equiv), Pd$_2$(dba)$_3$ (12.2 mg, 0.0133 mmol, 0.10 equiv), Davephos ligand (10.5 mg, 0.0266 mmol, 0.20 equiv.), K$t$BuO (37.0 mg, 0.333 mmol, 2.5 equiv) 2-nitroaniline (32mg, 0.266 mmol, 2.0 equiv), (DMSO 2ml). The crude product was purified by flash column chromatography on silica gel (acetonitrile/DCM = 1:5 to 1:2) to afford the product as yellow oil in (55 mg, 84 % yield). $^1$H NMR (401 MHz, Acetonitrile-$d_3$) δ = 0.93 – 0.99 (t, $J$=12.0, 12H), 1.29 – 1.40 (h, $J$=14.5, 8H), 1.54 – 1.64 (p, $J$=10.0, 11H), 3.04 – 3.10 (m, $J$=5.3, 9H), 7.16 – 7.29 (s, 1H), 7.34 – 7.38 (d, $J$=9, 2H), 7.70 – 7.75 (d, $J$=9, 2H). $^{13}$C NMR (101 MHz, Acetonitrile-$D_3$) δ = 13.78, 20.28, 24.26, 41.29, 59.23, 119.23, 124.94, 133.92, 143.55. $^{11}$B NMR (129 MHz, Acetonitrile-$d_3$) δ = -18.89 (1B, d, $J$=130 ), -17.21( 5B, d, $J$=100 ), -16.73( 5B, d, $J$=100 ), -

4.82( 1B, s). HRMS (TOF) m/z calcd for $C_7H_{16}N_2B_{12}$ (M)$^{-2}$: 129.1259; Found: 129.1276. HRMS (TOF) m/z calcd for $C_6H_{18}N_2O_2B_{12}$ (M+H)$^{-1}$ 259.2591; Found: 259.2652.
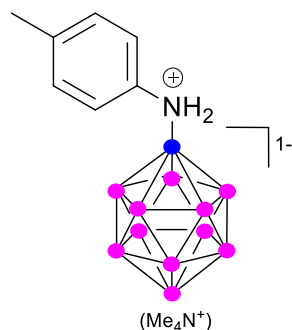
### (Me₄N)[B₁₂H₁₁NH₂C₆H₅-4-CO₂Me] (3d)
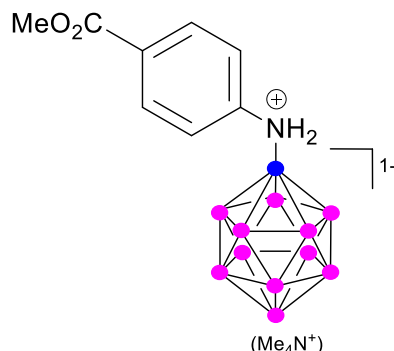


Following the general procedure using $(Me_4N)_2$ $B_{12}H_{11}I$ (150 mg, 0.361 mmol, 1.0 equiv), $Pd_2(dba)_3$ (33 mg, 0.0361 mmol, 0.10 equiv), Davephos ligand (28.5 mg, 0.0722 mmol, 0.20 equiv.), KtBuO (101 mg, 0.903 mmol, 2.5 equiv), 4-aminobenzoate (109 mg, 0.722 mmol, 2.0 equiv), (DMF 3.6ml). The crude product was purified by flash column chromatography on silica gel (acetonitrile/DCM = 1:5 to 1:2) to afford the product as yellow oil in (102 mg, 81 % yield).$^1$H NMR (401 MHz, Acetonitrile-$d_3$) δ = 0.11 − 1.79 (11H), 3.86 (s, 12H), 7.27 − 7.36 (m, 2H), 7.41 − 7.57 (d, $J$=5.6, 2H), 7.94 − 8.04 (s, 1H).$^{11}$B NMR (129 MHz, Acetonitrile-$d_3$) δ = -18.98(1B, d, $J$=113 ), -16.94 (10B,d, $J$=121 ) -4.71(1B, s).HRMS (TOF) m/z calcd for $C_8H_{20}NO_2B_{12}$ (M)$^{-2}$: 145.6311; Found: 145.6315. HRMS (TOF) m/z calcd for $C_8H_{21}NO_2B_{12}$ (M+H)$^{-1}$ 292.2694; Found: 292.2735.
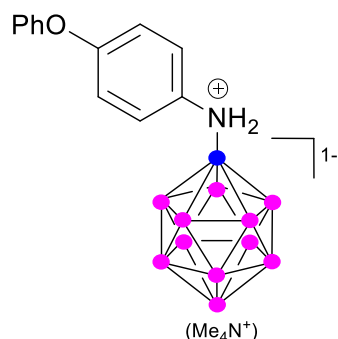
### (Me₄N)[B₁₂H₁₁NH₂C₆H₅-4-Me] (3e)



Following the general procedure using $(Me_4N)_2$ $B_{12}H_{11}I$ (150 mg, 0.361 mmol, 1.0 equiv), $Pd_2(dba)_3$ (33 mg, 0.0361 mmol, 0.10 equiv), Davephos ligand (28.5 mg, 0.0722 mmol, 0.20 equiv.), K$^t$BuO (101 mg, 0.903 mmol, 2.5 equiv), 4-aminobenzoate (109 mg, 0.722 mmol, 2.0 equiv), (DMSO 3.6ml). The crude product was purified by flash column chromatography on silica gel (acetonitrile/DCM = 1:5 to 1:2) to afford the product as yellow oil in (55 mg, 70 % yield). $^1$H NMR (401 MHz, Acetonitrile-$d_3$) δ = 2.32 (s, 3H), 2.36 (s, 12H), 7.01(s, 1H), 7.08-7.10( d, 2H, $J$= 8), 7.17-7.19 (d, $J$=8, 2H). $^{13}$C NMR (101 MHz, Acetonitrile-$d_3$) δ = 1.32, 20.72, 123.67, 129.82, 136.46, 137.62. $^{11}$B NMR (129 MHz, Acetonitrile-$d_3$) δ = -19.20 (1B, d, $J$=132 ), -17.05(10B,d, $J$=126 ), -4.58 (1B, s). HRMS (TOF) m/z calcd for $C_7H_{20}NB_{12}$ (M+H)$^{-1}$: 248.2784;          Found:          248.2852.
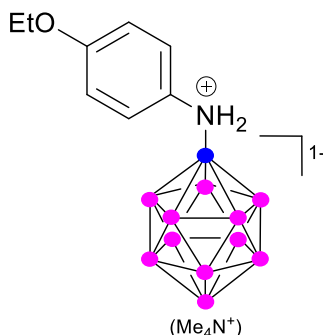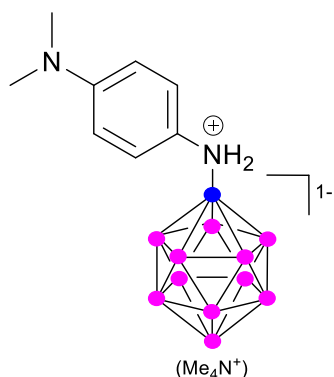
**(Me₄N)[B₁₂H₁₁NH₂C₆H₅-4-phenoxy] (3f)**

Following the general procedure using (Me₄N)₂ B₁₂H₁₁I (100 mg, 0.204 mmol, 1.0 equiv), Pd₂(dba)₃ (18.5 mg, 0.0204 mmol, 0.10 equiv), Davephos ligand (16 mg, 0.041 mmol, 0.20 equiv.), K*t*BuO (57 mg, 0.51 mmol, 2.5 equiv), 4-phenoxyaniline (186 mg, 0.408 mmol, 2.0 equiv), (DMSO 2.4ml). The crude product was purified by flash column chromatography on silica gel (acetonitrile/DCM = 1:5 to 1:2) to afford the product as yellow oil in (35 mg, 52 % yield). $^1$H NMR (401 MHz, Acetonitrile-$d_3$) δ = 0.46-1.49 (11H, BH), 2.27(s, 12H), 6.96-6.99(m, 2H), 7.01-7.03(m, 2H), 7.14-7.16(1H), 7.18-7.42 (4H). $^{13}$C NMR (101 MHz, Acetonitrile-$d_3$) δ = 1.32, 56.23, 119.50, 119.88, 124.69, 125.61, 131.02, 134.61, 157.04, 157.91. $^{11}$B NMR (129 MHz, Acetonitile-$D_3$) δ = -19.08( d, 1B, $J$=137 ), -17.00 (d, 10B, $J$=121 ), -4.56 (s,1B). HRMS (TOF) m/z calcd for C₁₂H₂₁NOB₁₂ (M)$^{-2}$: 162.6415; Found: 162.6427, m/z calcd for C₁₂H₂₂NOB₁₂ (M+H)$^{-1}$: 326.2904, Found: 326.2961.

**(Me₄N)[B₁₂H₁₁NH₂C₆H₅-4-ethoxy] (3g)**

Following the general procedure using (Me₄N)₂ B₁₂H₁₁I (100 mg, 0.204 mmol, 1.0 equiv), Pd₂(dba)₃ (18.5 mg, 0.0204 mmol, 0.10 equiv), Davephos ligand (16 mg, 0.041 mmol, 0.20 equiv.), K*t*BuO (57 mg, 0.51 mmol, 2.5 equiv), 4-ethoxyaniline (137.2 mg, 0.408 mmol, 2.0 equiv), (DMSO 2.4ml). The crude was tested by $^{11}$B-NMR and the spectrum showed that conversion of (Me4N)₂ B₁₂H₁₁I was >90%, the reaction provided B₁₂H₁₂ as major product and less than 10% of B-N product.
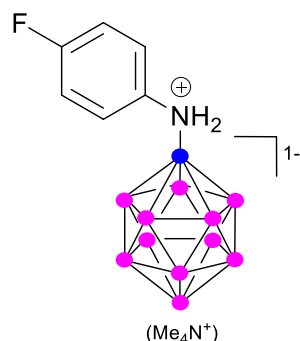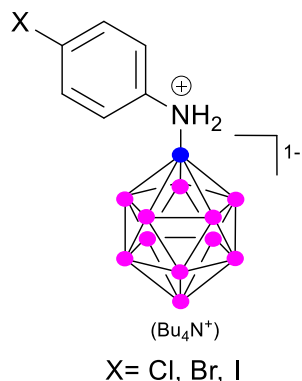
**(Me₄N)[B₁₂H₁₁NH₂C₆H₅-4-N(Me)₂] (3h)**

Following the general procedure using (Bu4N)₂ B₁₂H₁₁I (100 mg, 0.133 mmol, 1.0 equiv), Pd₂(dba)₃ (12.2 mg, 0.0133 mmol, 0.10 equiv), Davephos ligand (10.5 mg, 0.0266 mmol, 0.20 equiv.), K$t$BuO (37.0 mg, 0.333 mmol, 2.5 equiv), N,N-dimethy-p-phenylenediamine (40mg, 0.266 mmol, 2.0 equiv), (DMSO 2ml).The crude was tested by ^{11}B-NMR and the spectrum showed that conversion of (Me₄N)₂ B₁₂H₁₁I was >90%, the reaction provided B₁₂H₁₂ as major product and less than 10% of B-N product.

**(Me₄N)[B₁₂H₁₁NH₂C₆H₅-4-F] (3i)**

Following the general procedure using (Me₄N)₂ B₁₂H₁₁I (100 mg, 0.204 mmol, 1.0 equiv), Pd₂(dba)₃ (18.5 mg, 0.0204 mmol, 0.10 equiv), Davephos ligand (16 mg, 0.041 mmol, 0.20 equiv.), K$t$BuO (57 mg, 0.51 mmol, 2.5 equiv), 4-phenoxyaniline (186 mg, 0.408 mmol, 2.0 equiv), (DMSO 2.4ml). The crude product was purified by flash column chromatography on silica gel (acetonitrile/DCM = 1:5 to 1:2) to afford the product as yellow oil in (41 mg, 52 % yield). ^{1}H NMR (401 MHz, Acetonitrile-$d_3$) δ = 0.4-1.4(11H, BH, 2.25 (s, 12H), 7.09-7.12(d,2H), 7.23- 7.24 (d,2H). ^{11}B NMR (129 MHz, Acetonitrile-$d_3$) δ = -19.03 (1B, d, $J$=141 ), -16.87 (10B, d, $J$=117 ), -4.63 (s, 1B). ^{19}F NMR (377 MHz, Acetonitrile-$d_3$) δ = -117.04. ^{13}C NMR (101 MHz, Acetonitrile-$d_3$) δ = 115.95, 116.18, 125.73, 125.81, 135.25. HRMS (TOF) m/z calcd for C₆H₁₇NFB₁₂ (M+H)$^{-1}$: 252.2533, Found: 252.2599.

**(Bu$_4$N) [B$_{12}$H$_{11}$NH$_2$C$_6$H$_5$-4-Cl (3j) , (Bu$_4$N)[B$_{12}$H$_{11}$NH$_2$C$_6$H$_5$-4-Br (3k) (Bu$_4$N)[B$_{12}$H$_{11}$NH$_2$C$_6$H$_5$-4-I (3l)**



X= Cl, Br, I

Following the general procedure using (Bu$_4$N)$_2$ B$_{12}$H$_{11}$I (100 mg, 0.133 mmol, 1.0 equiv), Pd$_2$(dba)$_3$ (12.2 mg, 0.0133 mmol, 0.10 equiv), Davephos ligand (10.5 mg, 0.0266 mmol, 0.20 equiv.), K$t$BuO (37.0 mg, 0.333 mmol, 2.5 equiv), 4-haloaniline (....mg, 0.266 mmol, 2.0 equiv), (DMSO 2ml). The crude was tested by ^{11}B-NMR and the spectrum showed that conversion of (Bu$_4$N)$_2$ B$_{12}$H$_{11}$I was <5%, We expected that the reaction provided C-N product of haloaniline as major product and less than 2-5 % of B-N product.

**(Bu$_4$N)[B$_{12}$H$_{11}$NH$_2$C$_6$H$_5$-2-NO$_2$] (3m)**
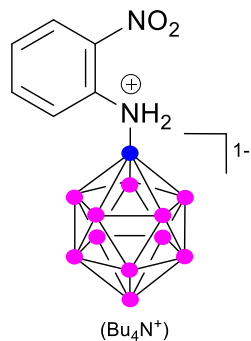


Following the general procedure using (Bu$_4$N)$_2$ B$_{12}$H$_{11}$I (100 mg, 0.133 mmol, 1.0 equiv), Pd$_2$(dba)$_3$ (12.2 mg, 0.0133 mmol, 0.10 equiv), Davephos ligand (10.5 mg, 0.0266 mmol, 0.20 equiv.), K$t$BuO (37.0 mg, 0.333 mmol, 2.5 equiv) 2-nitroaniline (37mg, 0.266 mmol, 2.0 equiv), (DMSO 2ml). The crude product was purified by flash column chromatography on silica gel (acetonitrile/DCM = 1:5 to 1:2) to afford the product as orange oil in (60 mg, 83 % yield). ^{1}H NMR (401 MHz, Acetonitrile-$d_3$) δ = 0.29 − 1.59 (BH,12H), 6.20 − 6.32 (d, $J$=12.4, 1H), 7.04 − 7.17 (d, $J$=13.8, 1H), 7.78 − 7.92 (s, 1H), 8.12 − 8.25 (s, 1H), 8.54 − 8.74 (s, 1H).. ^{13}C NMR (101 MHz, Acetonitrile-$d_3$) δ = 100.00, 112.37, 120.78, 125.62, 133.27, 133.91. ^{11}B NMR (129 MHz, Acetonitrile-$d_3$) δ = -18.82, -16.81(10B, ^{11}B NMR (129 MHz, acetone-$D_6$) δ = -19.12(1B, d, $J$=130), -16.99 (5B, d, $J$=103), -16.12(5B,d, $J$=103), -5.19( s, 1B) HRMS (TOF) m/z calcd for C$_6$H$_{17}$N$_2$O$_2$B$_{12}$ (M+H)$^{-1}$: 279.2489; Found: 279.2553. C$_6$H$_{16}$N$_2$O$_2$B$_{12}$ (M)$^{-2}$: 139.6247; Found: 139.1230.

**(Me$_4$N)[B$_{12}$H$_{11}$NH$_2$C$_6$H$_5$-2-NO$_2$] (3m)**
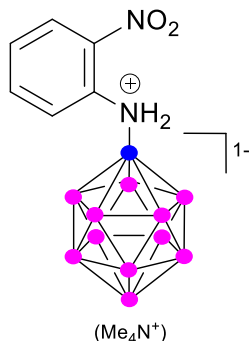


Following the general procedure using (Me$_4$N)$_2$ B$_{12}$H$_{11}$I (150 mg, 0.361 mmol, 1.0 equiv), Pd$_2$(dba)$_3$ (33 mg, 0.0361 mmol, 0.10 equiv), Davephos ligand (28.5 mg, 0.0722 mmol, 0.20 equiv.), K$t$BuO (101 mg, 0.903 mmol, 2.5 equiv) 3-nitroaniline (100mg, 0.722 mmol, 2.0 equiv), (DMF 3.6ml). The crude product was purified by flash column chromatography on silica gel (acetonitrile/DCM = 1:5 to 1:2) to afford the product as orange oil in (60 mg, 83 % yield).^{1}H NMR (401 MHz, acetone-$d_6$) δ = 0.48 – 1.81 (m, 12H), 3.23 – 3.39 (s, 12H), 6.25 – 6.54 (m, 1H), 7.02 – 7.27 (d, 1H), 7.78 – 7.90 (d, $J$=8.7, 1H), 8.13 – 8.29 (d, $J$=9.0, 1H), 8.57 – 9.20 (s, 1H). ^{13}C NMR (101 MHz, acetone-$D_6$) δ = 29.84, 55.96, 117.38, 123.40, 125.97, 134.19, 163.24, 167.20. HRMS (TOF) m/z calcd for C$_6$H$_{16}$N$_2$O$_2$B$_{12}$ (M)$^{-2}$: 139.6247; Found: 139.1232.

**(Me$_4$N)[B$_{12}$H$_{11}$NH$_2$C$_6$H$_5$-3-NO$_2$] (3n)**
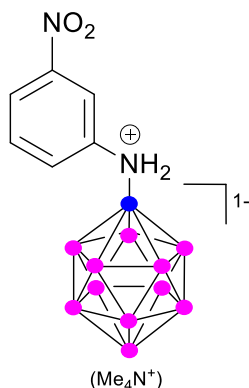


Following the general procedure using (Me$_4$N)$_2$ B$_{12}$H$_{11}$I (150 mg, 0.361 mmol, 1.0 equiv), Pd$_2$(dba)$_3$ (33 mg, 0.0361 mmol, 0.10 equiv), Davephos ligand (28.5 mg, 0.0722 mmol, 0.20 equiv.), K$t$BuO (101 mg, 0.903 mmol, 2.5 equiv) 3-nitroaniline (100mg, 0.722 mmol, 2.0 equiv), (DMF 3.6ml). The crude product was purified by flash column chromatography on silica gel (acetonitrile/DCM = 1:5 to 1:2) to afford the product as orange oil (60 mg, 83 % yield). ^{1}H NMR (401 MHz, Acetonitrile-$d_3$) δ 0.28 – 1.78 (s, 12H), 2.81 – 3.24 (s, 12H), 7.50 – 7.62 (m, 1H), 7.65 – 7.67 (m, 1H),8.07 – 8.12 (m, 1H), 8.37 – 8.42 (m, 1H). ^{13}C NMR (101 MHz, Acetonitrile-$d_3$) δ = 1.32, 49.92, 119.31, 122.96, 130.59, 131.00, 140.26, 148.93. ^{11}B NMR (129 MHz, Acetonitrile-$d_3$) δ = -18.70 (1B, d, $J$=130), -17.32 (5B, d, $J$= 103), -16.48 (5B, d, $J$=103), -4.83(1B, s). HRMS (TOF) m/z calcd for C$_6$H$_{16}$N$_2$O$_2$B$_{12}$ (M)$^{-2}$: 139.6247; Found: 139.1211.
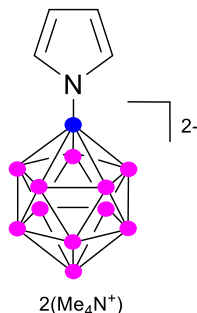
**(Me₄N)[B₁₂H₁₁NH₂C₆H₅-2,4-dinitro] (3o)**
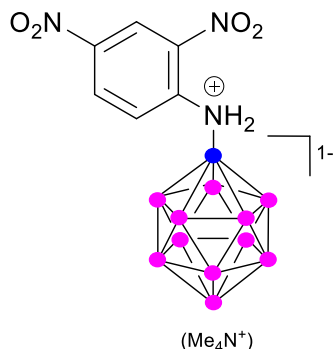
zsFollowing the general procedure using (Me₄N)₂ B₁₂H₁₁I (150 mg, 0.361 mmol, 1.0 equiv), Pd₂(dba)₃ (33 mg, 0.0361 mmol, 0.10 equiv), Davephos ligand (28.5 mg, 0.0722 mmol, 0.20 equiv.), K*t*BuO (101 mg, 0.903 mmol, 2.5 equiv), 2,4-dinitroaniline (132.2 mg, 0.722 mmol, 2.0 equiv), (DMF 3.6ml). The crude product was purified by flash column chromatography on silica gel (acetonitrile/DCM = 1:5 to 1:2) to afford the product as yellow oil in (130 mg, 78 % yield). $^1$H NMR (401 MHz, acetone-$d_6$) δ = 0.8-1.6 (BH, 11H)3.38 (s, 12H), 7.82-7.88(d, J=10, 1H), 7.86, 8.42- 8.46(d, J=10, 1H), 8.84- 8.88(d, *J*=3, 1H), 9.14(s, 1H).$^{13}$C NMR (101 MHz, acetone-$d_6$) δ = 29.84, 55.93, 117.66, 121.93, 124.82, 127.38, 133.29, 153.15.$^{11}$B NMR (129 MHz, acetone-$d_6$) δ = -18.43( d, 1B, *J*=128 ), -16.76( d, 5B, *J*=114 ), -16.00( d, 5B, *J*=114), -5.90 (s, 1B). ). HRMS (TOF) m/z calcd for C₆H₁₅N₃O₄B₁₂ (M)$^{-2}$: 161.6134; Found: 161.6146.
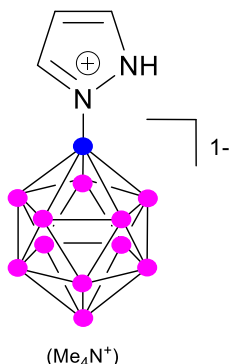
**(Bu₄N)[B₁₂H₁₁NH₂-naphthyl] (4)**
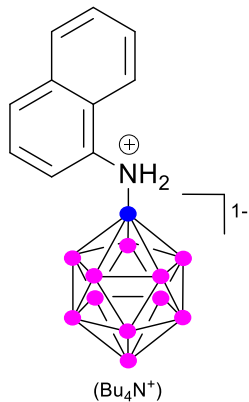
Following the general procedure using (Bu₄N)₂ B₁₂H₁₁I (100 mg, 0.133 mmol, 1.0 equiv), Pd₂(dba)₃ (12.2 mg, 0.0133 mmol, 0.10 equiv), Davephos ligand (10.5 mg, 0.0266 mmol, 0.20 equiv.), K*t*BuO (37.0 mg, 0.333 mmol, 2.5 equiv), 1-naphthyl amine (36 mg, 0.266 mmol, 2.0 equiv), (DMSO 2ml). The crude product was purified by flash column chromatography on silica gel (acetonitrile/DCM = 1:5 to 1:2) to afford the product as yellow oil in (16 mg, 23 % yield). $^1$H NMR (401 MHz, Acetonitrile-$d_3$) δ = 0.96- 0.99(s, 12H), 1.31-1.40(m, 8H), 1.56-1.64 (m, 8H),3.06-3.10 (m, 8H), 7.38-7.40(d, 1H), 7.49- 7.53(t, 1H), 7.57-7.64(m, 2H), 7.86-7.88(d, 1H), 7.94-7.96(d, 1H), 8.13- 8.15(d, 1H). $^{13}$C NMR (101 MHz, Acetonitrile-$d_3$) δ = 12.86, 19.37, 23.35, 58.39, 121.36, 123.02, 124.99, 126.08, 126.54, 127.58, 128.15, 134.05. $^{11}$B NMR (129 MHz, acetone-$d_6$) δ = -18.85(d, 1B, *J*=135 ), -16.77(d, 10B, *J*=126 ), -4.37(s, 1B). HRMS (TOF) m/z calcd for C₁₀H₂₀NB₁₂ (M+H) $^{-1}$: 284.2786, Found: 284.2866.
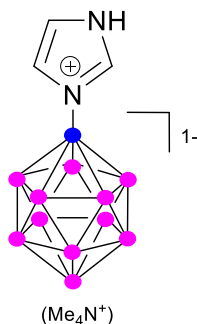
## (Me₄N)[B₁₂H₁₁.pyrrole] (5a)

Following the general procedure using (Me₄N)₂ $B_{12}H_{11}I$ (100 mg, 0.204 mmol, 1.0 equiv), $Pd_2(dba)_3$ (18.5 mg, 0.0204 mmol, 0.10 equiv), Davephos ligand (16 mg, 0.041 mmol, 0.20 equiv.), K$t$BuO (57 mg, 0.51 mmol, 2.5 equiv), pyrrol (33 mg, 0.408 mmol, 2.0 equiv), (DMSO 2.4ml). The crude product was purified by flash column chromatography on silica gel (acetonitrile/DCM = 1:5 to 1:2) to afford the product as yellow oil in (74mg, 87 % yield). $^1$H NMR (401 MHz, acetone-$d_6$) δ = 5.12-5.13(t, J= 2.0, 2H), 6.27 (b, 2H). $^{13}$C NMR (101 MHz, acetone-$d_6$) δ = 29.84, 105.39, 125.23. $^{11}$B NMR (129 MHz, acetone-$d_6$) δ = -20.46 (d, $J$= 127, 1B), -17.50 (d, $J$= 146, 5B), -16.23 (d, $J$= 146, 5B), -2.96 (s,1B). HRMS (TOF) m/z calcd for $C_4H_{15}NB_{12}$ (M)$^{-2}$: 103.6204, Found: 103.6217.

## (Me₄N)[B₁₂H₁₁.pyrazole] (5b)

Following the general procedure using (Me₄N)₂ $B_{12}H_{11}I$ (100 mg, 0.204 mmol, 1.0 equiv), $Pd_2(dba)_3$ (18.5 mg, 0.0204 mmol, 0.10 equiv), Davephos ligand (16 mg, 0.041 mmol, 0.20 equiv.), K$t$BuO (57 mg, 0.51 mmol, 2.5 equiv), pyrazole (33 mg, 0.408 mmol, 2.0 equiv), (DMSO 2.4ml). The crude product was purified by flash column chromatography on silica gel (acetonitrile/DCM = 1:5 to 1:2) to afford the product as yellow oil in (60 mg, 87% yield). $^1$H NMR (401 MHz, Acetonitrile-$d_3$) δ = 6.50- 6.51( t, $J$=3, 1H), 7.80- 7.81(d, $J$=2, 1H), 7.90- 7.91(d, $J$=2, 1H),. $^{13}$C NMR (101 MHz, Acetonitrile-$d_3$) δ = 108.41, 133.35, 138.35. $^{11}$B NMR (129 MHz, Acetonitrile-$d_3$) δ = -18.40 (d, $J$=126, 1B), -16.61(d, $J$=130, 10B), -5.86 (s, 1B). HRMS (TOF) m/z calcd for $C_3H_{15}N_2B_{12}$ (M+H)$^{-1}$: 209.2432, Found: 209.2474.
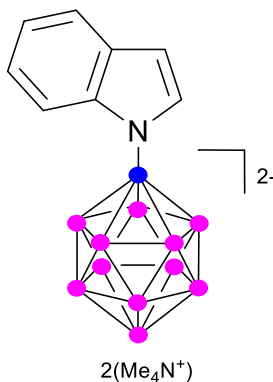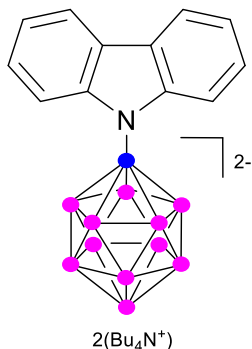
## (Me₄N)[B₁₂H₁₁-imidazole] (5c)



(Me₄N⁺)

Following the general procedure using (Me₄N)₂ B₁₂H₁₁I (100 mg, 0.204 mmol, 1.0 equiv), Pd₂(dba)₃ (18.5 mg, 0.0204 mmol, 0.10 equiv), Davephos ligand (16 mg, 0.041 mmol, 0.20 equiv.), K$t$BuO (57 mg, 0.51 mmol, 2.5 equiv), imidazole(56 mg, 0.408 mmol, 2.0 equiv), (DMSO 2.4ml). The crude product was purified by flash column chromatography on silica gel (acetonitrile/DCM = 1:5 to 1:2) to afford the product as yellow oil in (36mg, 85 % yield). ^{1}H NMR (401 MHz, acetone-$d_6$) δ = 7.32-7.33(d, J=4.0, 2H), 8.32(s, 1H). ^{13}C NMR (101 MHz, acetone-$d_6$) δ =118.32, 125.93, 135.91. ^{11}B NMR (129 MHz, acetone-d6) δ = -18.23(d, $J$= 131, 1B), -15.79(d, J= 127, 10B), -4.93(s, 1B). HRMS (TOF) m/z calcd for $C_3H_{15}N_2B_{12}$ (M+H)[-1]: 209.2421, Found: 209.2476.
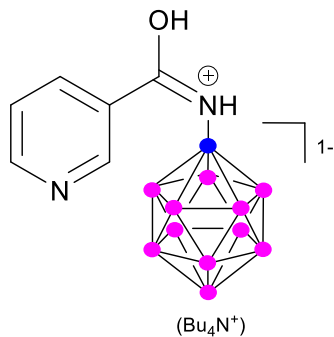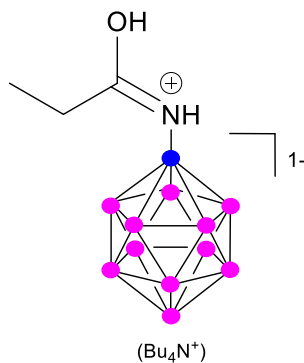
## (Me₄N)[B₁₂H₁₁·indole] (5d)



2(Me₄N⁺)

Following the general procedure using (Me₄N)₂ B₁₂H₁₁I (100 mg, 0.204 mmol, 1.0 equiv), Pd₂(dba)₃ (18.5 mg, 0.0204 mmol, 0.10 equiv), Davephos ligand (16 mg, 0.041 mmol, 0.20 equiv.), K$t$BuO (57 mg, 0.51 mmol, 2.5 equiv), indole (92 mg, 0.408 mmol, 2.0 equiv), (DMSO 2.4ml). The crude product was purified by flash column chromatography on silica gel (acetonitrile/DCM = 1:5 to 1:2) to afford the product as a yellow oil in (55mg, 82 % yield).^{1}H NMR (401 MHz, acetone-$D_6$) δ = 3.00 (s, 12H), 6.27-6.31(dt, 1H), 6.49-6.66(dt,1H) , 7.13- 7.73(d, 1H), 7.73(s, 1H), 8.21- 8.24(d, 1H). ^{13}C NMR (101 MHz, acetoneE-$D_6$) δ = 41.04, 115.22, 116.29, 117.48, 120.55, 122.16, 129.89, 131.92, 143.15.^{11}B NMR (129 MHz, acetone-$D_6$) δ = -19.57 (1B, d, $J$=-- ), -17.58 (5B, d, $J$=153 ), -16.38 (5B, d, $J$=129 ), -3.32(1B, s). HRMS (TOF) m/z calcd for $C_8H_{17}NB_{12}$ (M)[-2]: 128.6283, Found: 128.6305.

## (Bu$_4$N)[B$_{12}$H$_{11}$.carbazole] (5e)

Following the general procedure using (Bu$_4$N)$_2$ B$_{12}$H$_{11}$I (100 mg, 0.133 mmol, 1.0 equiv), Pd$_2$(dba)$_3$ (12.2 mg, 0.0133 mmol, 0.10 equiv), Davephos ligand (10.5 mg, 0.0266 mmol, 0.20 equiv.), K$t$BuO (37.0 mg, 0.333 mmol, 2.5 equiv), Carbazole (88 mg, 0.266 mmol, 2.0 equiv), (DMSO 2ml). The crude product was purified by flash column chromatography on silica gel (acetonitrile/DCM = 1:5 to 1:2) to afford the product as a yellow oil in (61 mg, 83 % yield). $^1$H NMR (401 MHz, acetone-$d_6$) δ = 0.84 – 0.99 (t, J=6.0, 24H), 1.22 – 1.41 (m, 16H), 1.58 – 1.77 (m, 16H), 3.16 – 3.30 (m, 16H), 6.82 – 6.93 (m, 2H), 7.04 – 7.18 (m, 2H), 7.79 – 7.90 (d, J=6.7, 2H), 9.07 – 9.16 (d, J=8.9, 2H). $^{11}$B NMR (129 MHz, acetone-$d_6$) δ = -17.84 (d,1B), -17.15 (d, J= 129, 5B), -16.05 (d, J= 129, 5B), -4.09 (s, 1B). $^{13}$C NMR (101 MHz, acetone-$d_6$) δ = 13.91, 20.29, 24.38, 59.16, 116.51, 118.18, 118.70, 123.43, 124.93, 148.20. HRMS (TOF) m/z calcd for C$_{12}$H$_{20}$NB$_{12}$(M)$^{-2}$: 153.6363, Found: 153.6396.

## (Bu$_4$N)[B$_{12}$H$_{11}$-3-nicotinamide] (6a)

Following the general procedure using (Bu$_4$N)$_2$ B$_{12}$H$_{11}$I (100 mg, 0.133 mmol, 1.0 equiv), Pd$_2$(dba)$_3$ (12.2 mg, 0.0133 mmol, 0.10 equiv), Davephos ligand (10.5 mg, 0.0266 mmol, 0.20 equiv.), K$t$BuO (37.0 mg, 0.333 mmol, 2.5 equiv), 3-nicotinamide (33mg, 0.266 mmol, 2.0 equiv), (DMSO 2ml). The crude product was purified by flash column chromatography on silica gel (acetonitrile/DCM = 1:5) to afford the product as yellow oil in (58mg, 86% yield). $^1$H NMR (401 MHz, acetone-$D_6$) δ = 0.94-0.98 (t, 12H), 1.38- 1.47 (m, 8H), 1.75-1.83 (m, 8H), 3.38-3.42 (m,8H), 6.28 (s, 1H), 7.38-7.41(m, 1H), 8.06- 8.09 (1H), 8.60-8.62 (1H), 8.97 (1H). $^{13}$C NMR (101 MHz, acetone-$d_6$) δ = 13.87, 20.26, 24.40, 29.84, 59.26, 123.81, 134.00, 134.87, 149.29, 151.55, 167.38. $^{11}$B NMR (129 MHz, acetone-$d_6$) δ = -18.96 (d, J=137, 1B), -16.80 (d, J= 121,10B), -6.57 (s, 1B). HRMS (TOF) m/z calcd for C$_6$H$_{15}$N$_2$OB$_{12}$ (M)$^{-2}$: 130.1155, Found: 130.1255.

## (Bu₄N)[B₁₂H₁₁-propanamide] (6b)

Following the general procedure using $(Bu_4N)_2$ $B_{12}H_{11}I$ (100 mg, 0.133 mmol, 1.0 equiv), $Pd_2(dba)_3$ (12.2 mg, 0.0133 mmol, 0.10 equiv), Davephos ligand (10.5 mg, 0.0266 mmol, 0.20 equiv.), K$t$BuO (37.0 mg, 0.333 mmol, 2.5 equiv), propanamide (26mg, 0.266 mmol, 2.0 equiv), (DMSO 2ml). The crude product was purified by flash column chromatography on silica gel (acetonitrile/DCM = 1:5) to afford the product as yellow oil in ( 49mg, 80 % yield). $^1$H NMR (401 MHz, Acetonitrile-$d_3$) δ = 0.94-0.99 (Me, 15H), 1.32-1.37 (3, 7H), 1.57-1.65 (m, 8H), 2.5 (m, 2H), 3.06- 3.12 (m, 8H). $^{13}$C NMR (101 MHz, Acetonitrile-$d_3$) δ = 10.82, 13.78, 20.30, 24.31, 30.88, 59.29, 176.07, 207.56. $^{11}$B NMR (129 MHz, Acetonitrile-$d_3$) δ = -20.3 (d, $J$=123, 1B), -17.3 (d, $J$=148, 5B), -16.0 (d, $J$=150 , 5B),, -5.2 (s, 1B). HRMS (TOF) m/z calcd for $C_3H_{17}NOB_{12}$ (M)$^{2-}$: 106.6256, Found:106.6189, HRMS (TOF) m/z calcd for $C_3H_{17}NOB_{12}$ (M+H)$^{1-}$: 214.2585, Found: 214.2448.

## (Bu₄N)[B₁₂H₁₁-sulfamide] (6c)
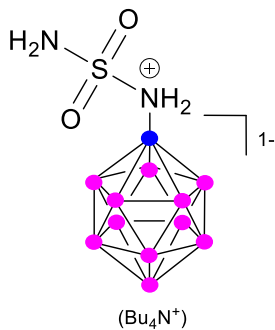
Following the general procedure using $(Bu_4N)_2$ $B_{12}H_{11}I$ (100 mg, 0.133 mmol, 1.0 equiv), $Pd_2(dba)_3$ (12.2 mg, 0.0133 mmol, 0.10 equiv), Davephos ligand (10.5 mg, 0.0266 mmol, 0.20 equiv.), K$t$BuO (37.0 mg, 0.333 mmol, 2.5 equiv), sulfamide (26mg, 0.266 mmol, 2.0 equiv), (DMSO 2ml). The crude product was purified by flash column chromatography on silica gel (acetonitrile/DCM = 1:5) to afford the product as yellow oil in (18 mg, 28 % yield). $^1$H NMR (401 MHz, acetone-$d_6$) δ = 0.84 – 0.99 (t, $J$=6.0, 12H), 1.22 – 1.41 (m, 8H), 1.58 – 1.77 (m, 8H), 3.16 – 3.30 (m, 8H), 5.8(broad s). $^{11}$B NMR (129 MHz, acetone-$D_6$) δ = -20.14 (d, $J$=132 , 1B), -17.16(d, $J$= 184, 5B), -16.45(d, $J$= 184, 5B), -6.04(s, 1B). HRMS (TOF) m/z calcd for $N_2H_{14}O_2SB_{12}$ (M)$^{-2}$: 118.0989, Found:118.1006.

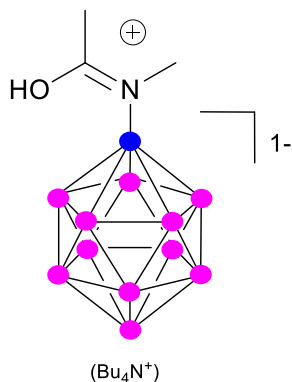## (Bu₄N)[B₁₂H₁₁-p-toluenesulfonamide] (6d)
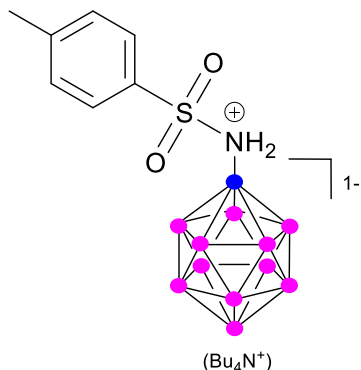
Following the general procedure using (Bu₄N)₂ B₁₂H₁₁I (100 mg, 0.133 mmol, 1.0 equiv), Pd₂(dba)₃ (12.2 mg, 0.0133 mmol, 0.10 equiv), Davephos ligand (10.5 mg, 0.0266 mmol, 0.20 equiv.), K$t$BuO (37.0 mg, 0.333 mmol, 2.5 equiv), p-toluenesulfonamide (46mg, 0.266 mmol, 2.0 equiv), (DMSO 2ml). The crude product was purified by flash column chromatography on silica gel (acetonitrile/DCM = 1:5) to afford the product as yellow oil in (16mg, 35 % yield). $^{1}$H NMR (401 MHz, acetone-$d_6$) δ = 0.84 – 0.99 (t, $J$=6.0, 12H), 1.22 – 1.41 (m, 8H), 1.58 – 1.77 (m, 8H), 2.15 (s, 3H), 3.16 – 3.30 (m, 8H), 7.37-7.39(d, $J$=8,2H), 7. 72-7.74(d, $J$=8,2H). $^{13}$C NMR (101 MHz, acetone-$d_6$) δ = 13.9, 20.3, 21.3, 24.5,  59.3, 126.9, 122, 128.7, 130.1.$^{11}$B NMR (129 MHz, acetone-$D_6$) δ = -20.21 (d, $J$=132 , 1B), -17.37 (d, $J$= 184, 5B), -16.46(d, $J$= 184, 5B), -5.84(s, 1B). HRMS (TOF) m/z calcd for C₇H₁₉NO₂SB₁₂ (M)$^{-2}$: 155.6171, Found:155.6033, HRMS (TOF) m/z calcd for C₇H₁₉NO₂SB₁₂ (M+C₁₆H₃₆N)$^{-1:}$ 553.5193, Found: 553.4841. HRMS (TOF) m/z calcd for C₇H₁₉NO₂SB₁₂ (M+H)$^{-1:}$ 312.2415, Found: 312.2200.
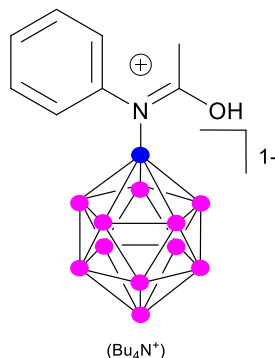
## (Bu₄N)[B₁₂H₁₁-N-methylacetamide] (6e)

Following the general procedure using (Bu₄N)₂ B₁₂H₁₁I (100 mg, 0.133 mmol, 1.0 equiv), Pd₂(dba)₃ (12.2 mg, 0.0133 mmol, 0.10 equiv), Davephos ligand (10.5 mg, 0.0266 mmol, 0.20 equiv.), K$t$BuO (37.0 mg, 0.333 mmol, 2.5 equiv), N-methylacetamide (20 mg, 0.266 mmol, 2.0 equiv), (DMSO 2ml). The crude product was purified by flash column chromatography on silica gel (acetonitrile/DCM = 1:5 to 1:3) to afford the product as yellow oil in (51mg, 83% yield). $^{1}$H NMR (401 MHz, acetone-$d_6$) δ = 0.94-0.97 (t, 12H), 1.40-1.42 (m,8H), 1.80-1.82(m,8H),  , 2.37(s, 3H), 2.60(s,3H), 3.31-3.43(m,8H), 7.91(s), 11.54(s).$^{13}$C NMR (101 MHz, acetone-$d_6$) δ = 13.13, 18.31, 19.55, 23.66, 25.46, 53.19, 58.56, 173.67. $^{11}$B NMR (129 MHz, acetone-$d_6$) δ = -19.89 (d, $J$=117,

1B), -18.02 (d, $J$=156, 5B), -16.76 (d, $J$=156, 5B), -3.05(s, 1B). HRMS (TOF) m/z calcd for $C_3H_{18}NOB_{12}$ $(M)^{2-}$: 106.6256, Found:106.6236, HRMS (TOF) m/z calcd for $C_3H_{18}NOB_{12}$ $(M+C_{16}H_{36}N)^{1-:}$ 455.5364, Found: 455.5306. HRMS (TOF) m/z calcd for $C_3H_{19}NOB_{12}$ $(M+H)^{1-:}$ 214.2585, Found: 214.2543.

## (Bu₄N)[B₁₂H₁₁-acetanilide] (6f)

Following the general procedure using $(Bu_4N)_2$ $B_{12}H_{11}I$ (100 mg, 0.133 mmol, 1.0 equiv), $Pd_2(dba)_3$ (12.2 mg, 0.0133 mmol, 0.10 equiv), Davephos ligand (10.5 mg, 0.0266 mmol, 0.20 equiv.), K$t$BuO (37.0 mg, 0.333 mmol, 2.5 equiv), acetanilide (35 mg, 0.266 mmol, 2.0 equiv), (DMSO 2ml). The crude product was purified by flash column chromatography on silica gel (acetonitrile/DCM = 1:5 to 1:3) to afford the product as yellow oil in (55mg, 80% yield). $^1$H NMR (401 MHz, acetone-$d_6$) δ = 0.96-0.99(t, 12H), 1.38, 1.40- 1.47 (m, 8H), 1.76, - 1.84 (m, 8H), 2.09 (s, 3H), 7.18-7.20 d, 2H), 7.34-7.40 (m, 3H), 11.75 (b, 1H). $^{13}$C NMR (101 MHz, acetone-$d_6$) δ = 13.88, 18.03, 20.34, 24.40, 59.29, 125.60, 127.15, 128.40, 129.53, 130.13, 175.63. $^{11}$B NMR (129 MHz, acetone-$d_6$) δ = -19.0 (d, $J$=115, 1B), -17.4 (d, $J$=130, 5B), - 15.8 (d, $J$=130, 5B), -2.8 (s, 1B). HRMS (TOF) m/z calcd for $C_8H_{19}NOB_{12}$ $(M)^{2-}$: 137.6336, Found:137.6318, HRMS (TOF) m/z calcd for $C_8H_{19}NOB_{12}$ $(M+C_{16}H_{36}N)^{1-:}$ 517.5523, Found: 517.5437. HRMS (TOF) m/z calcd for $C_8H_{19}NOB_{12}$ $(M+H)^{1-:}$ 276.2745, Found: 276.2683.

## Cs [B₁₂H₁₁.DBU] (7)
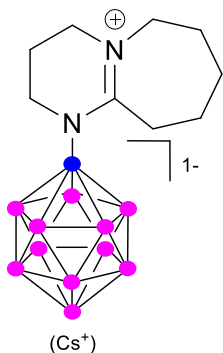
Following the general procedure using $(Me_4N)_2$ $B_{12}H_{11}I$ (100 mg, 0.204 mmol, 1.0 equiv), $Pd_2(dba)_3$ (18.5 mg, 0.0204 mmol, 0.10 equiv), Davephos ligand (16 mg, 0.041 mmol, 0.20 equiv.), DBU (62 mg, 0.408 mmol, 2.0 equiv), (DMSO 2.4ml). The crude product was purified by flash column chromatography on silica gel (acetonitrile/DCM = 1:5 to 1:2) to afford the product as Me₃N-salt as a light-yellow oil. The purified

compound was dissolved in MeOH and precipitated as Cs-salt (white solide in (70 mg, 80 % yield) by adding 1.5 equiv. Of CsF in MeOH.^{1}H NMR (401 MHz, Acetonitrile-$d_3$) δ = 1.57(m, 4H), 1.66- 1.68(m, 2H), 1.80-1.84(m, 2H), 1.94-2.21(m, 2H), 3.28- 3.46(m, 4H), 3.69-3.70(m, 2H). ^{13}C NMR (101 MHz, Acetonitrile-$d_3$) δ = 22.51, 25.86, 27.23, 29.41, 30.74, 50.09, 50.55, 54.78. ^{11}B NMR (129 MHz, Acetonitrile-$d_3$) δ = -18.28(d,1B), -17.07(d, $J$= 121, 5B), -16.29(d, $J$= 113, 5B), -2.70(s, 1B). HRMS (TOF) m/z calcd for $C_9H_{27}N_2B_{12}$ $(M+H)^{-1:}$ 293.3375, Found: 293.3425.

[1]  W. H. Knoth, H. C. Miller, J. C. Sauer, J. H. Balthis, Y. T. Chia, E. L. Muetterties, *Inorg. Chem.* **1964**, *3*, 159-167.

## 4.2 Appendix 2: Manuscript of Chapter 2

# Synthesis and Characterization of NIR Heptamethine Cyanine Dyes with Intrinsic Ophthalmology Targeting

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Herein, a novel series of substituted heptamethine cyanine dyes have been synthesized using substituted dianil and dienaminone linkers. Substitutions include methyl, phenyl, chloro, and methoxy groups at the meso-position, as well as methyl and t-butyl groups at the 2"-position of the commonly used cyclohexene residue in heptamethine cyanines. Moreover, ICG analouge (unsymmetric) cyanine dye and another two dyes have been prepared by introducing a cyclopentenyl ring into the polymethine chain, or by replacing the cyclohexyl methylene with a dialkyl ammonium moiety. The effect of substitutions on the methine bridge on the dyes properties like, optical properties, solubility and stability in aqueous solutions have been studied. As an application, the synthesized dyes were tested for their potential utilization in ophthalmology, including filter-membrane staining experiments, where the ILM and ERM compositions are mimicked by the filter and the collagen cell carrier, respectively.

## Introduction

Due to large Stokes' shifts, and high molar extinction coefficients, as well as the ability to generate strong fluorescence emission at the range of 700 to 1000 nm, near infrared (NIR) heptamethine cyanine dyes have become an increasingly important contributor in imaging science. Clinically, Indocyanine Green (ICG) (Chart 1a) is being used in NIR imaging of blood vessels for angiography *in vivo*, in applications such as angiography in colorectal surgery[1] and in ophthalmology.[2] Another ophthalmic use is the staining of the inner limiting membrane (LM) during vitreoretinal surgery.[3] Staining the ILM makes it easier to be located, ensures its complete removal, and decreases the risk of damaging the retina.[4] ICG is the only green dye available presently for this purpose. We found recently that isotonic solutions of ICG show no toxicity when tested in cell culture.[5] ICG has, however, a number of disadvantages in daily clinical routine. The major disadvantage is that it is not soluble in salt solutions, and that its solutions in either water or isotonic solutions with glucose or other similar agents are not stable, and precipitates are formed within one day. It is therefore necessary to prepare fresh solutions every day in the hospital, with its known problems such as quality control and sterility of the injection solution. ICG can form stable solutions when suitable higher-molecular-weight excipients are added.[6] In preliminary studies we found, however, that a solution of ICG stabilized by excipients is no longer capable of ILM staining. Alternative cyanine dyes would therefore have great clinical potential.

Like ICG, many of the known cyanine dyes suffer from several drawbacks, for example, cyanine dyes are well-known to form self-aggregates as they possess strong intermolecular van der Waals-like attractive forces.[7] [8] [9] [10]. Dye aggregation causes significant changes in the UV-visible to near-infrared absorption and fluorescence emission in aqueous solution, as well as decreases the fluorescence quantum yield relative to the monomeric form. In addition, they have low photostability, which arises due to the *cis-trans* disruption of the extended conjugation.[10]

Apart from ICG, several other cyanine dyes have been suggested for application in ophthalmologic surgery. A family of pentamethine dyes has been synthesized and tested in vitro by Langhals et al.[11] A heptamethylene dye BIP, differing from ICG by the lack of the two condensed benzene rings on the indole units, has been suggested by us for staining of the anterior lens capsule.[12]
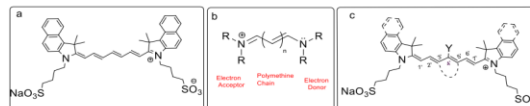


**Chart 1. a)** Indocyanine green. **b)** Basic skeleton of a polymethine cyanine dye. **c)** Sites of modification of heptamethine dyes.

The basic structure of an indolene cyanine dye offers several sites for modification (Chart **1**c). One or both of fused benzene rings of ICG could be replaced by H atoms. An additional ring in the polyene chain can introduce rigidity. Finally, the replacement of the H atom on the central polyene atom by other groups is possible.

Introducing a cyclohexenyl ring into the polymethine chain as reported by Patonay and co-workers was found to enhance the rigidity of the polymethine bridge, which increases the solubility, fluorescence quantum yield, and photostability, as well as provides a site for further functionalization at the central

ring, as shown in Chart **1**c, which is known as a "convertible cyanine dye".[13] The substitution of heptamethines at the central position has been widely studied including their effect on chemical and photophysical properties.[14] [15] [16] [17]

Aqueous solubility and charge modification, photostability and lack of toxicity are sometimes needed to derive superior biomedical applications.[18] For instant, in 2006, Zhang *et al.* reported a novel NIR carbocyanine dye containing galactose. They provided an approach to incorporate the monosaccharide in *meso*-position and anchored by ether linkage to the conformationally constrained cyclohexenyl group, the suggested design with existence of a nonionic polyhydroxyl moiety on the dye skeleton enhances the dye solubility and minimizes aggregation in aqueous solutions.[19]

In this work, several representative heptamethine cyanine dyes have been prepared and extensively studied for their common chemical structures, photophysical properties, stability in aqueous solutions, as well as their potential application to stain the ILM membrane for ophthalmology purposes. The synthesis of series of substituted heptamethine cyanines has been described using substituted dianil linkers. Substituents include methyl, phenyl, chloro, and methoxy groups at the meso-position, as well as methyl and t-butyl groups at the 2"-position of the commonly used cyclohexene in heptamethine cyanines. Introducing a cyclopentenyl ring to the dye skeleton and synthesis of ICG analogue (unsymmetric) cyanine dye have also been explored. The effect of methine bridge substitutions on the dyes' optical properties, stability in aqouse solutions, and potential utilization in ophthalmology have been reported.

## Results and discussion

### Synthesis

The synthesis of heptamethine cyanine began with the preparation of the indolinium salts $S_1$ and $S_2$,( 1,1,2-Trimethylbenz[*e*]indole, 2,3,3-trimethyl-3H-indole were alkylated with 1,4-sultone under reflux for two hours to afford compounds $S_1$ and $S_2$ in excellent yield[20]. As described in Scheme 1, a series of *meso*-substituted heptamethine cyanines dyes **4a-f** has been synthesized by the classic stepwise condensation reaction between two equivalents of indolinium salts $S_1$ and a dianil linker. The first step in synthesizing heptamethine cyanine dye involves the construction of the linker. The synthesis of the linker involves the Vilsmeier-Haack reagent, *N*-chloromethylene-*N,N*-dimethylammonium dichlorophosphonate, which is formed from the reaction of *N,N*-dimethylformamide (DMF) or *N*-methylformamide (NMF) and POCl₃. Hydrolysis of iminium functionalities in the intermediate provides a bisaldehyde which is transformed later into the pentamethinium salt by treatment with anilinium hydrochloride. Subsequently, the condensation reaction takes place, after which the dyes are then purified by using column chromatography (10–50% methanol/DCM). In addition to the substituted cyclohexanone and cyclohexene, cyclopentene has also been tested. An acceptable yield of the product was obtained when 2.5-3.0 equivalents of the indolinium salts $S_1$ was used. Sodium acetate was used in the reaction as a base to remove the acidic proton of the methyl group of the heterocyclic salt.
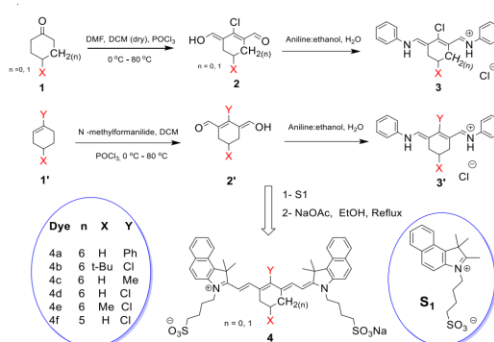


**Scheme 1.** Synthesis of *meso*-substituted heptamethine cyanines.

A derivative of the heptamethine cyanine dye with a substitution of a methylene group with dimethyl quaternary ammonium nitrogen, dye **5**, known as quaternary cyanine or *QuatCy*, was also synthesized according to the literature.[21] *QuatCy* has been made starting from synthesizing Vilsmeier-Haack reagent **B**, applying standard conditions furnished **B** in good yield, subsequently, treated one equivalent of **B** with one equivalent of MeI in methanol provided **C** in good yield,(Scheme 2). Condensation of $S_1$ and **C** in refluxing ethanol afforded dye **5**.
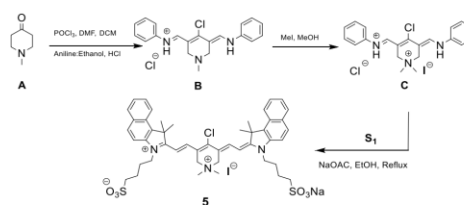


**Scheme 2.** Synthesis of *QuatCy* (**5**) converging from two key fragments, the Vilsmeier-Haack product **C** and the trimethyl benzindolozinidinium salt $S_1$.

A cyanine dye, **6**, with a methoxy group at the *meso* position was synthesized by preparing the methoxy-dienamine linker according to Scheme 3; the key step is the synthesis of the *keto*-dienaminone *via* organocatalyzed condensation of cyclopentanone and N,N-dimethyl acetal. Martins *et al.* reported that the removal of methanol produced during the reaction enhance the reaction yield and reaction rate.[22] Treating the dienaminone with methyl triflate and stirring the mixture for 1 hour at room temperature provides the methoxy dienamine which can be mixed with two equivalents of the indolinium salt $S_1$ and sodium acetate to yield the corresponding dye in good yield.
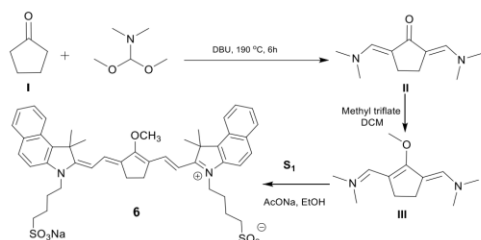
**Scheme 3.** Synthesis of **6** by condensation of compound **III** and **S₁**.

The synthetic pathway for the ICG-analogue unsymmetrical cyanine dye **7** is outlined in Scheme 4, as the first step 2,3,3-trimethyl-3H-indole was alkylated with 1,4-sultone under reflux for two hours to afford compound **S₂** in excellent yield. one equivalent of **S₁** was treated with one equivalent of glutaconaldehyde dianil hydrochloride in acetic anhydride at 100 ºC for 1 h to yield compound **B** in a quantitative yield. The intermediate **B** was refluxed for half an hour with one equivalent of **S₂** in ethanol and triethylamine to provide the asymmetric ICG-analaoue dye **7** in good yield.
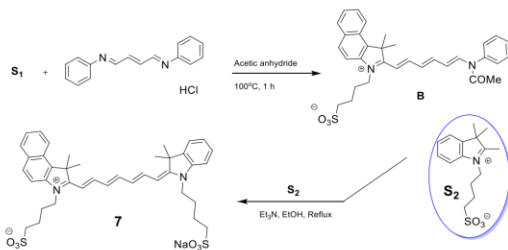


**Scheme 4:** Synthetic pathway for asymmetric ICG-analogue **7**.

**Photophysical Properties**

Optical properties for NIR heptamethine cyanine dyes were studied by collecting the spectral data of UV-vis/NIR absorption and fluorescence emission (Figure 1 and Table 1). The UV-vis/NIR absorption ($\lambda_{max}$) and fluorescence emission ($\lambda_{em}$) of the dyes were measured with uniform concentrations in methanol. Additionally, the absorption spectra of NIR dyes were obtained with the specific concentration in phosphate buffer solution at pH of 7.4 (PBS) in order to investigate aggregation formation and solvent effect. The data relating to the spectral properties of the NIR dyes are presented in Table 1. Interestingly, despite the structural similarities, the dyes exhibit notable different absorption spectra, whose maxima are spread over the 780–850 nm range. This feature highlights the dramatic influence of the central substitution on the electronic structure of these molecules. The $\lambda_{max}$ of the prepared dyes in methanol varies from 796 nm and 820 nm for those containing different substituents at *meso*-position, following the order: **4a** (Y = Ph, 796 nm), **4c** (Y = Me, 805 nm), and **4d** (Y = Cl, 850 nm). The $\lambda_{max}$ of heptamethine cyanines with the *meso*-chlorine substitution is generally about 806-820 nm. In general, changing the X-substituents and keeping the Y = Cl did not show a significant

change in $\lambda_{max}$ (~818-820 nm). Absorption shifts in cyanines are generally seen when the methine chain substitutions are changed, the most notable absorption shifts come from the alternate conjugation pathway in the *meso*-phenyl dye **4a** and the strain caused by the cyclopentenyl ring on the methine chain in dye **4f**.

For comparison we investigated the parent cyanine dye absorption band at 805 nm (dye **4d**), with other substituents (X and Y). The absorption band of dye **4a** with substituents X = H and Y =ph experienced a bathochromic shift to 820 nm in the presence of methanol. In contrast, the absorption band of dye **4b** with substituent (X = t-butyl, Y = Cl) showed a hypsochromic shift to 797 nm comparative to parent dye. Further, dye **4c** substituent such as X = H, Y = Me showed a similar behavior to the dye **4a** with substituent of X = H, Y = Ph. Additionally, the dye **4e** with substituent (X = Me, Y = Cl) caused a bathochromic shift to 821 nm comparative to the parent dye **4d**. However, the addition of five-membered ring dye **4f** instead of six-membered ring to the core of parent cyanine dye, endows dramatically bathochromic shifted to 846 nm. Moreover, in aqueous solution (PBS buffer) the cyanine derivatives **4e, 4f** exhibited a hypsochromic shift compared to the parent dye **4d**. Further in the aqueous solution, the $\lambda_{max}$ of cyanine derivatives showed a large wavelength shift compared to that in methanol (see ESI). This indicated the dye molecules are most likely aggregated in the aqueous solution even at low concentrations. In addition, the dye **6**, with cyclopentenyl ring and *meso*-methoxy substituent showed a high hypsochromic shift around 65 nm compared to its parent dye **4f** with *meso*-chlorine substituent. However, the dye **5** with *meso*-chlorine substituent and quaternary ammonium salt at cyclohexyl ring showed a high similarity with the comparable dyes (*i.e.*, **4b**, **4d** and **4e**). In addition, a blue-shifted shoulder appeared for the dyes **4a, 4b, 4c, 4d,** and **4e** in the absorbance spectra as shown in (Figure 2a). Shoulders of this kind are indicative of H-aggregates. Morever, hypsochromic shift of dye **6** has been observed compared to its analogue ICG.

Further, in aqueous solution, cyanine derivatives showed large wavelength shifting comparatively to that in methanol solution, indicating that the dyes show aggregation in aqueous solution already at low concentrations.
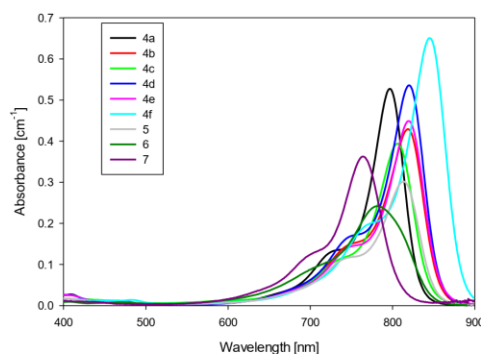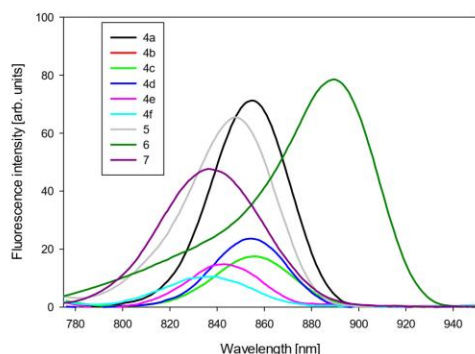
Figure 1. (a) VIS spectrum of all dyes at 6.0 μM in MeOH. (b) Emission spectra of the dyes in MeOH. For excitation wavelength see Table 1.

Optical properties of the dyes are listed in Table 1. The molar absorptivity of these dyes is relatively large and the values range from 130,000–214,000 L mol$^{-1}$ cm$^{-1}$. Interestingly, the two dyes **4d** and **4f** showed the highest molar absorptivity values with 210,000 and 214,000 L mol$^{-1}$ cm$^{-1}$, respectively. The *meso*-phenyl group in **4a** and the strain put on the methine chain by the cyclopentenyl group in **4f** can prevent *cis–trans* isomerization increasing the molar absorptivity.[23] Subsequently, the higher molar absorptivity was observed for dye **4f** (214,000 L mol$^{-1}$ cm$^{-1}$) while the lowest molar absorptivity was observed for dye **4c** with the *meso*-methyl substituent with a value of 130,000 L mol$^{-1}$ cm$^{-1}$. Interestingly, dye **7** showed the higher molar absorptivity value 203,000 L mol$^{-1}$ cm$^{-1}$ than its analogue ICG which is recorded 101,000 L mol$^{-1}$ cm$^{-1}$ as molar absorptivity value.

Table 1. Optical properties of substituted heptamethine cyanine dyes in MeOH.

| Dye | n | X | Y | λmax | εMeOH (×10³) | λem | λex | ØMeOH |
|-----|---|---|---|------|--------------|-----|-----|-------|
| ICGª | - | --- | --- | 785 | 101 | 790 | 717 | 0.016 |
| 4a | 6 | H | Ph | 796 | 180 | 816 | 725 | 0.030 |
| 4b | 6 | t-Bu | Cl | 818 | 17 | 829 | 745 | 0.007 |
| 4c | 6 | H | Me | 805 | 130 | 824 | 732 | 0.010 |
| 4d | 6 | H | Cl | 820 | 130 | 832 | 746 | 0.004 |
| 4e | 6 | Me | Cl | 819 | 170 | 837 | 746 | 0.008 |
| 4f | 5 | --- | Cl | 845 | 214 | 832 | 767 | 0.003 |
| 5 | 6 | Q. Cy | Cl | 814 | 164 | 740 | 744 | 0.006 |
| 6 | 5 | --- | OMe | 782 | 141 | 696 | 708 | 0.014 |
| 7 | - | --- | --- | 765 | 203 | 695 | 705 | 0.060 |

Quantum yields were determined according to the literature

$$\Phi_{dye} = \Phi_{Fluo} \times \frac{I_{dye} * A_{Fluo}}{I_{Fluo} * A_{dye}} \times \frac{\eta_{MeOH}^2}{\eta_{H2O}^2}$$

where $\Phi_{Fluo}$ is the quantum yield of fluorescein (in 0.1 M NaOH) and is equal to 0.89, $\Phi_{dye}$ is the quantum yield of dye, $I$ is the integrated area under the curve, $A$ is the absorbance at excitation wavelength, and $\eta$ is the refractive index of the solvents ($\eta_{methanol}$= 1.331, $\eta_{H2O}$ = 1.333).[24]

Fluorescence quantum yields of the synthesized dyes range from 0.3% to 6.0 %. Dyes **4b, d, e, f,** and dye **5** showed almost similar quantum yields ranging from 0.4-0.8%. While the dyes **4b** and **4e** showed only a small effect on quantum yield as they are not conjugated within the system. A trend is observed with decreasing quantum yield as the rigidity of the cyclic system increases with the addition of carbons, e.g., the dye **4f** with a *meso*-chlorine and cyclopentenyl ring had the lowest quantum yield at 0.3 % while the dye with the *meso*-phenyl **4a** had higher quantum yield of 3%. For ICG, dye **4c**, and dye **6** , the quantum yields ranging from 1.0-1.6%. Interstingly, the best results reported for quantum yield was for dye **7** which is showe higher quantum yield with 6% Generally, the long wavelengths exhibited by cyanine dyes are caused by the delocalization of electrons throughout the molecule. Cyclic structures within the dyes lead to rigidity enhancing the stability of the molecule in the excited state, ultimately increasing the quantum yield. In contrast, substituted cyanine dyes are indicating extremely weak fluorescence in PBS buffer solution. Furthermore, near infrared dyes suffered from low quantum yield, as a result of long wavelength photon requires low band gap molecules, in which non radiative process tend to dominate over the fluorescence.[25]

**Filter-Membrane Staining and Solubility Characterization**

The aim of this experiment is investigating the staining ability of new potential dyes for ILM and ERM, as well as assessing their solubility in water and PBS for a concentration of 0.1%. To achieve this, a filter-membrane experiment was designed. A Millipore MultiScreen BV 96-well filtration plate was used, whose polyvinylidene fluoride filter mimics the ILM with respect to staining properties, as well as a collagen cell carrier (CCC) membrane, which is similar to the ERM with respect to staining. Solubility was assessed by preparing a 0.1% (w/v) solution of the dyes in water and in PBS. The size of the pellet and the color intensity of the supernatant account are considered as a qualitative evaluation of the solubility. The results are summarized in Figure 2. Dye 6 was completely insoluble in water as well as in PBS.

The filter wells show a consistent staining of different tones of green, across all the used dyes including ICG. On the other hand, the membranes appear to have no consistent green staining at all, with the exception of **7** and ICG. BIP stained the membrane in blue, and the green staining of the filter indicates a considerable solvatochromic shift.
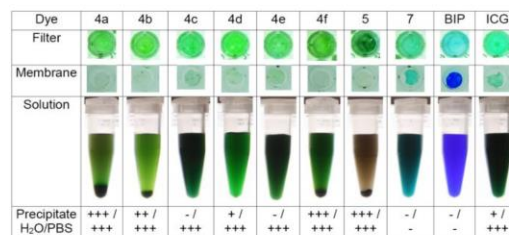


Figure 2. The pictures of the individual wells of the stained filters, membranes for all prepared heptamethine cyanine dyes dissolved in H₂O/PBS.

The filter wells show a consistent staining of different tones of green, across all the used dyes, except for dye 6, which showed a blueish color. That of dye **4b**, however, is very faint and practically unperceivable when dissolved in water. Moreover, dye **4a** did not dissolve properly. On the other hand, the supernatant on these dissolved wells was read under spectrophotometry from 400 nm to 850 nm. The results were plotted into graph shown in (Figures 00).

**Toxicity Measurements**

Dyes used for staining of ILM must be non-toxic. We therefore assessed the toxicity of the dyes synthesized. Solutions/suspensions of 0.1% in water were tested. Cells were incubated at 37 °C for 15 minutes, and then the dye was washed off with PBS. After washing, MTT was used to determine the viability. As control, PBS was used. [5] Results are shown in Fig. 3.

In order to test the toxicity of the dyes **4a**, **4b**, **4c**, **4d**, **4e**, **4f**, **5** and **6**, these were prepared as a 0.1% (w/v) solution in 5% glucose and distilled water.

Of the dyes soluble in water, **4e** and **7** are the only dyes which show no toxicity. All other dyes are either too toxic or not soluble even in water, and would therefore not be suitable as dyes for surgical procedures.
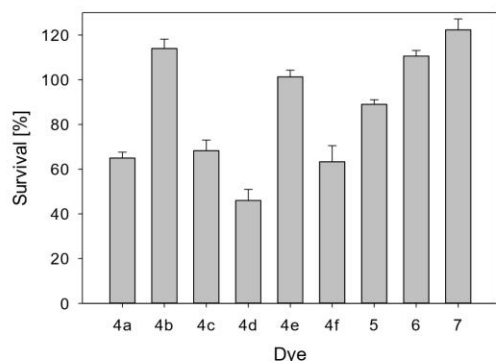


**Figure 3**. Toxicity of the dyes measured as cell survival [%] with their corresponding error.

## Conclusions

A series of substituted heptamethine cyanine dyes has been synthesized using substituted dianil and dienaminone linkers, carrying a benzoindole unit at both or at least one end of the heptamethine chain. The effects of the substitutions on the dyes properties like, optical properties, solubility and stability in aqueous solutions as well as membrane staining and toxicity have been studied. The spectral data of an UV-vis/NIR absorption and fluorescence emission of the the NIR heptamethine cyanine dyes showed that in spite of the structural similarities, the dyes exhibit notable different absorption spectra, highlighting the influence of the central substitution on the electronic structure of these molecules. Solubility in water of most bis-benzoindole dyes was limited, and only **4e** also showed low toxicity in cell culture. The asymmetric dye **7** had appropriate solubility and staining properties and lack of toxicity toxicity, these two dyes could be evaluated further for use in ophthalmologic surgery.

## Author contributions

MAJ designed the syntheses, carried out most of the syntheses of the symmetric dyes, did the physcial-chemical measurements, and wrote the first draft of the manuscript. MA and EA carried out the synthesis of the asymmetric dye. JW developed the staining model. AG synthesized some of the symmetric dyes. PC carried out and evaluated the cell toxicity experiments. EM carried out and evaluated the staining experiment. AM consulted for all ophthalmologic questions. DG designed the study. All authors contributed to the writing of the manuscript.

Conflicts of interest

There are no conflicts to declare.

## Acknowledgments

## Notes and references

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## 4.2.1 Supporting Information for Chapter 2

**Experimental procedures and characterization data**

**S₁: 4-(1,1,2-trimethyl-1H-benzo[e]indol-3-ium-3-yl)butane-1-sulfonate**
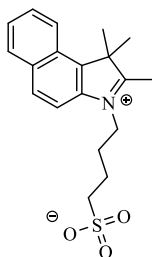


Trimethyl-4,5-benzo-3*H*-indole (3.1 g, 15 mmol) and 1,4-butane sultone (2.1 g, 15 mmol) were mixed in a round-bottomed flask (25 mL) under a nitrogen atmosphere, and the reaction solution was stirred at 80 °C for 4 h and then cooled at room temperature. By addition of acetone to the reaction mixture, the residue was dissolved, and the product was crystallized. The reaction product, 2,3,3-trimethyl-1-(sulfobutyl)-4,5-benzoindolium inner salt, was separated by filtration and rinsed with acetone. The product was obtained as a gray crystal (1.17 g, 23%). δ H (400MHz, DMSO-d6) d 1.75 (s, 6H), 1.78 (m, 2H, J= 7.2), 2.03 (m, 2H J= 7.6), 2.52 (t, 2H, J= 7.2), 2.95 (s, 3H), 4.61 (t, 2H, J= 7.6), 7.69–7.80 (m, 2H, J= 8.3), 8.20 (d, 2H, J= 8.9), 8.27 (d, 1H, J= 8.9), 8.36 (d, 1H, J= 8.3).

**S₂: N-((E)-(6-((E)-(phenylimino)methyl)-4,5-dihydro-[1,1'-biphenyl]-2(3H)-ylidene)methyl)aniline.**
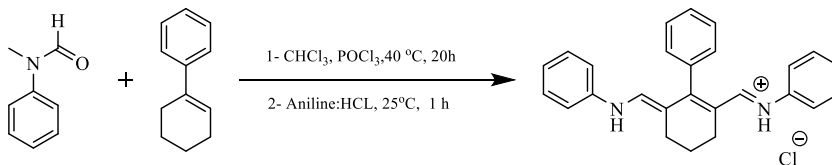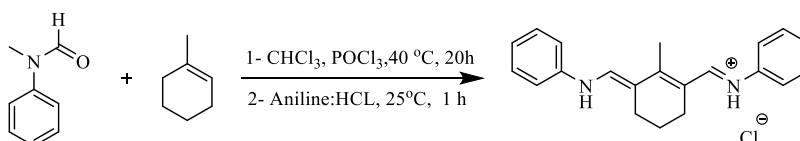


Chloroform (2 mL) was cooled down to 0 °C and *N*-methyl-*N*-phenylformide (NMF) (2.6 mL, 18.96 mmol, 3.0 equiv.) was added to it. POCl₃ (1.8 mL, 18.96 mmol, 3.0 equiv.) was added dropwise over 30 mins and stirred for 1 h at 0 °C. Afterwards, 1-phenyl-1-cyclohexene (1.0 mL, 6.32 mmol, 1.0 equiv.) was added and heated at 45 °C for 20 h. The reaction was cooled down at ice to 0 °C, distilled water (2.0 mL) was added gently to the reaction mixture, followed by 1.5 g of K₂CO₃ and stirred for 10 mins at room temperature. This was followed by addition of aniline: conc. HCl

(5:1) (2.0 ml) in water 1.5 mL and reaction mixture was stirred for additional 30 mins. After cooling the reaction mixture $K_2CO_3$ was added dropwise over 15 mins till the evolving $CO_2$ stopped. The reaction was left at 0 °C for 15 mins for precipitates to form which were filtered and washed with cold water. To get pure compounds, the products were precipitated from acetonitrile (100 ml) with vigorously stirring and leave it for 10 min in ice bath to get dark purple solid before filtration (2.021g, 88%).

δ H (400MHz, Methanol-d4) δ2.0-2.25(m, 2H), 2.64 (t, J = 6.1 Hz, 4H), 2.59 (t, J = 6.0 Hz, 2H),), 7.21-7.25 (m, 2H), 7.25-7.45 (m, 4H), 7.5-7.65 (m, 4H), 7.4-7.5(m, 4H), 7.5-7.57 (s, 2H). HRMS calculated for $C_{26}H_{24}N_2$, calculated (M+H): 365.49.42; found 365.159.

**S₃: N-((E)-(2-methyl-3-((E)-(phenylimino)methyl)cyclohex-2-en-1-ylidene)methyl)aniline.**



Chloroform (5.0 mL) was cooled down to 0 °C and *N*-methyl-*N*-phenylformide (NMF) (5.3 mL, 37.91 mmol, 3.0 equiv.) was added to it. $POCl_3$ (3.1 mL, 37.91 mmol, 3.0 equiv.) was added dropwise over 30 mins and stirred for 1 h at 0 °C. Afterwards, 1-methyl-1-cyclohexene (1.5 mL, 12.634 mmol, 1.0 equiv.) was added and heated at 45 °C for 20 h. The reaction was cooled down at ice to 0 °C, distilled water (4.0 mL) was added gently to the reaction mixture, followed by 3.0 g of $K_2CO_3$ and stirred for 10 mins at room temperature. This was followed by addition of aniline:conc HCl (5:1) ( 5.0 ml) in water 4.0 mL and reaction mixture was stirred for additional 30 mins. After cooling the reaction mixture $K_2CO_3$ was added dropwise over 15 mins till the evolving $CO_2$ stopped. The reaction was left at 0 °C for 15 mins for precipitates to form which were filtered and washed with cold water. To get pure compounds, the products were precipitated from acetonitrile (100 ml) and washed it by aceton (20 mlX2) with vigorously stirring and leave it for 10 min in ice bath to get dark purple solid before filtration (11.6 g,42%). δ H (400MHz, Methanol-d4) 1.94 (t, J = 6.3 Hz, 2H) 2.52 (m, 7H), 7.31 − 7.25 (m, 2H), 7.51 − 7.39 (m, 8H), δ 8.48 (s, 2H). Calculated for $C_{21}H_{22}N_2^+$ calculated (M+H) $^+$: 303.42; found 303.17.

**S₄:N-((E)-(5-(tert-butyl)-2-chloro-3-((E)-(phenylimino)methyl)cyclohex-2-en-1- yli-dene)methyl)aniline.**
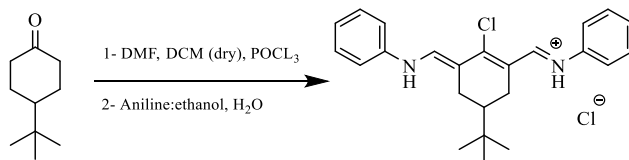


Anhydrous DMF (6.5 ml) was dissolved in anhydrous dichloromethane (6.0 mL), and POCl₃ (4 eq., 52 mol, 7.0 g) was added dropwise (ice-water bath) under nitrogen atmosphere. To this thick reaction mixture, a solution of 4-(*t*-butyl) cyclohexanone (13.0 mmol, 2.0 g) in dichloromethane (6.5 mL) was added, and yellow mixture was refluxed for ~3 h. Bright orange-red solution was cooled to room temperature, poured into ice, and organic phase was removed. The aqueous phase was extracted with dichloromethane several times, the combined organic phases were dried over anhydrous magnesium sulfate, and the solution was treated with aniline (3.8 g), and the solvents were removed by rotary evaporation. The residue was dissolved in methanol, and concentrated HCl (2 mL) was added (ice-water bath). After stirring for ~10 min precipitate formed (dark red), water was added, and dark red solid was separated by vacuum filtration, and then dried under vacuum (4.54g, 98 %). δ H (400MHz, chloroform-d): 1.05 (s, 9H), 1.49 (tt, 2 J = 12.5 Hz, 3 J = 3.5 Hz, 1H),) 2.34 (d, 3 J = 14, 2H), 3.28 (d, 3 J = 14 Hz, 2H), 7.18 (t, 3 J = 7.5 Hz, 2H7.30 (t, 3 J = 7.5 Hz, 4H),), 7.61 (d, 3 J = 7.5 Hz, 4H), 8.39 (s, 2H). HRMS calculated for $C_{26}H_{24}N_2$, calculated (M+H)⁺: 378.94; found 379.14.

**S₅: (1*E*,1'*E*)-1,1'-(2-chlorocyclohex-1-ene-1,3-diyl)bis(*N*-phenylmethanimine).**
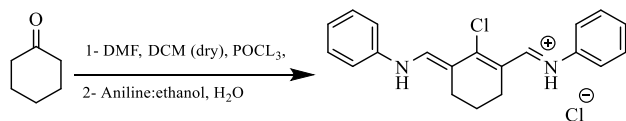


Anhydrous DMF (15 ml) was dissolved in anhydrous dichloromethane (15 mL), and POCl₃ (4.2 eq., 128.4 mmol, 17.4 g, 10.6ml) was added dropwise (ice-water bath) under nitrogen atmosphere. To this thick reaction mixture (colorless) a solution of cyclohexanone (30.569 mmol, 3.0 g) was added dropwise at 0°C, and yellow mixture was refluxed for ~3 h at 65°C. After three hours bright orange-red solution was cooled to room temperature, poured into ice, and organic phase was removed. The aqueous phase was extracted with dichloromethane several times (3 X

75 ml), the combined organic phases were dried over anhydrous magnesium sulfate, and the solution was treated with aniline: EtOH ( 1:1) (2.5ml:2.5ml), color changed to dark red directly, the reaction mixture allowed to stirring for 10 mins, then the solvent were removed by rotary evaporation. The residue was dissolved in methanol (3.0 ml), and concentrated HCl (2 mL) was added (ice-water bath). After stirring for ~10 min precipitate formed (dark red), water was added, and dark red solid was separated by vacuum filtration, and then washed many times with cold water ( 300-400 ml), after that precipitated again by Et$_2$O. Recrystallizing the product by tert-butyl methyl ether: n-Hexane (1:1) (50 ml) then column chromatography 5:95 MeOH: DCM (200mlx 2) to get rid of polar spot and get pure solid. (1.767 g, 18 %). $^1$H NMR (400 MHz, MeOD) δ 2.00 (s, 2H), 2.74 (t, J = 6.2 Hz, 4H), 7.31 (ddd, J = 8.4, 5.6, 3.0 Hz, 2H), 7.49 (d, J = 2.0 Hz, 3H), 8.66 (s, 2H), 7.50 (s, 3H). HRMS calculated for C$_{20}$H$_{19}$N$_2$Cl, calculated (M+H) $^+$: 323.84; found 323.12.

**S$_6$: (Z)-2-chloro-3-(hydroxymethylene) cyclopent-1-ene-1-carbaldehyde.**
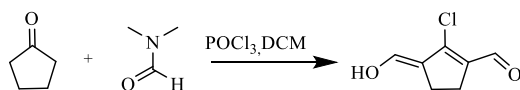


25 mL of anhydrous DMF and 25 mL of dichloromethane are added under argon to a 500 mL flask fitted with a mechanical stirrer. The mixture is cooled with a water/NaCl bath to 4-5°C. A solution of 22.5 ml of POCl$_3$ in 22.5 mL of dichloromethane previously cooled to 4°C in the refrigerator is slowly added drop by drop. The reaction mixture is cooled to 0°C with a NaCl/ice bath under continuous stirring. The addition requires 45 minutes. The reaction mixture becomes milky and it is allowed to stand for 30 minutes at 4-5°C. A solution of 5ml mL of cyclopentanone is added dropwise. The color of the reaction mixture turns to yellow. At the end of the addition, the mixture is stirred for a further 15 minutes and then heated at reflux for 5 hours. Its color turns to orange and then to dark red. It is then cooled to room temperature. The dichloromethane solvent is evaporated in vacuo and the residue is thrown into 200 g of ice and is stirred for 2 hours. The pH of the solution is brought to 5 with 50% aqueous NaOH. A dark precipitate form. The mixture was stirred overnight, and the violet crystals are collected onto a fritted glass filter. (5.5 g, 42.0 %). δ H (400 MHz, Methanol-d4) 2.59 – 2.70 (2 H, m), 2.98 – 3.05 (2 H, m), 6.98 –

7.01 (1 H, s), 9.53 – 9.60 (1 H, s). HRMS calculated for $C_{20}H_{19}N_2Cl$, calculated $(M+H)^+$: 323.84; found 323.12.

**S$_7$: (E)-2-chloro-3-(hydroxymethylene)-5-methylcyclohex-1-ene-1-carbaldehyde.**



15 mL of anhydrous DMF and 15 mL of dichloromethane are added under argon to a 500 mL flask fitted with a mechanical stirrer. The mixture is cooled with a water/NaCl bath to 4-5°C. A solution of 13.0 ml of POCl3 in 15 mL of dichloromethane previously cooled to 4°C in the re-frigerator is slowly added drop by drop. The reaction mixture is cooled to 0°C with a NaCl/ice bath under continuous stirring. The addition requires 45 minutes. The reaction mixture becomes milky and it is allowed to stand for 30 minutes at 4-5°C. A solution of 5.0 ml mL of 4-methyl cyclohexanone is added dropwise. The color of the reaction mixture turns to yellow. At the end of the addition, the mixture is stirred for a further 15 minutes and then heated at reflux for 5 hours. Its color turns to orange and then to dark red. It is then cooled to room temperature. The dichloromethane solvent is evaporated in vacuo and the residue is thrown into 200 g of ice and is stirred for 2 hours. The pH of the solution is brought to 5.0 with 50% aqueous NaOH. A dark precipitate form. The mixture is stirred overnight, and the violet crystals are collected onto a frit-ted glass filter. (5.5 g, 42 %). $^1$H-NMR (400 MHz, MeOD-d3): 1.04 (d, 3H), 1.92-1.60 (m, *3H*), 2.75-7.50 (m, 2H), 7.30 (s, 1H), δ 10.15 (s, 1H). HRMS calculated for $C_{20}H_{19}N_2Cl$, calculated (M+H): 323.84; found 323.12.

**S₈:(E)-4-chloro-1,1-dimethyl-3-((phenylamino)methylene)-5-((E)-(phenyliminio)methyl)-1,2,3,6-tetrahydropyridin-1-ium chloride iodide.**
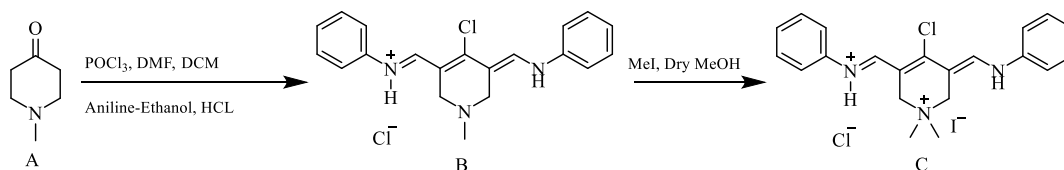


A solution of DMF (1.8 ml, 23.39 mmol) and dichloromethane (1.8 ml) was cooled in ice with stirring under nitrogen. Phosphorous oxychloride (2.68 g, 17.50 mmol) in dichloromethane (1.8 ml) was added dropwise over 10 min. I-Methylpiperidin-4-one **A** (0.5g, 4.41 mmol) was added dropwise over 10 min. The solution turned yellow. The mixture was heated at 70 0C for 3h and become orange. The reaction mixture was cooled down and poured over cold water (10 ml). Solution was concentrated under vacuum and the yellow residue was washed twice with ether and air dried. After that an ethanol solution (2.8 ml) of aniline (0.56 ml, 6.11 mmol) was slowly added into a solution of dried dianal (0.5 g, 2 .66 mmol) in DMF (1.84 ml) and HCl (0.69 ml). The reaction temperature was maintained around 10-15° C. with an ice-water bath during the addition of aniline. After the reaction mixture was stirred for an additional 20 minutes, it was poured, with stirring, into 75 ml of diethyl ether. The deep-purple solid was collected by filtration and washed twice with cold water, washed twice with ether and then dried under vacuum at ambient temperature overnight to obtain the Pproduct **B** as purple solid (0.79 g, 75%). Compound B (0.075 g, 0.20 mmOl) and methyl iodide (0.433 g, 3.05 mmol) were placed into a seal tube in dry methanol (6 ml) under N₂. The reaction mixture was heated at 40 ⁰C for 28 h. The solvent was removed under vacuum and the product was washed twice with ether and precipitated from methanol/ether. Product **C** was obtained as a red burgundy solid in (0.086g, 84%). The obtained crude product was used directly to synthesize the corresponding dye.

**S₉:  bis((dimethylamino)methylene)cyclohexan-1-one**.



Cyclopentanone (1.0 equiv, 1.500 g, 15.283 mmole ), was added to the mixture of N,N-dimethylformamide dimethyl acetal (4 equiv., 7.65 g, 64.19 mmole) and DBU (10 mol%, 233 mg, 1.53 mmole) to a 50 mL round-bottom flask equipped with a stir bar. Connect a condenser at

60 °C to the flask and allow the reaction mixture to stir at 190 °C. After completion (16 h), allow the reaction mixture to cool down to room temperature and observe the crystallization of product.Isolation of 2,5-Bis((dimethylamino)methylene)cyclopentanone (Time for Completion: 30 min) Wash the crystals of product with MTBE (3 × 5 mL). Dry the product under vacuum. OPTIONAL STEP: Recrystallize from hot acetone. δ H (400 MHz, Chloroform-d) 2.72 – 2.89 (4H, s), 2.93 – 3.45 (12 H, s), 7.04 – 7.07 (2 H, s).

**S₁₀: (E)-N-((3-((dimethylamino)methylene)-2-methoxycyclopent-1-en-1-yl)methylene)-N-methylmethanaminium trifluoromethanesulfonate.**
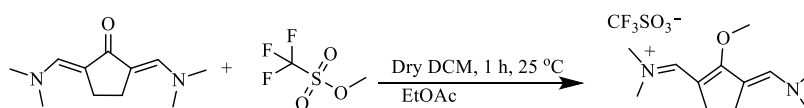


Compound S9 (210 mg, 1.08 mmole, 1.0 equiv.) was suspended in 11 ml of DCM ( dry), followed by the dropwise addition of ( 147 Ml, 1.2 equiv., 1.297 mmole ) of methyl triflate during 10 mins. After stirring the mixture for 1.0 hour at room temperature, the solvent was removed under reduced pressure and the precipitate washed two times with EtOAc, then the product dried under air. δ H (400 MHz, Chloroform-d) 2.69 – 2.93 (4 H, s), 3.04 – 3.37 (12 H, d, *J* 17.3), 3.82 – 4.07 (3 H, s), 7.27 – 7.30 (2 H, s).

**Synthesis and Characterization of Dye**

A solution of Vilsmeier-Haack reagent (1.0 equiv.), benzindolium inner salt (2.5 equiv.), and anhydrous sodium acetate (2.5 equiv.) in absolute ethanol (20 mL) was refluxed for 2-20 h under N₂. Reaction was mentoring by TLC, upon completion of the reaction the mixtures were allowed to cool to room temperature, then ethanol was removed under reduced pressure before the dye was dissolved in 3 ml MeOH and precipitated in diethyl ether (30 mL X 2). The precipitate was purified by column chromatography and the purity tested by HPLC.

**Dye-4a**: **4-(2-((E)-2-((E)-6-((E)-2-(1,1-dimethyl-3-(4-sulfobutyl)-1,3-dihydro-2H-benzo[e]indol-2-ylidene)ethylidene)-3,4,5,6-tetrahydro-[1,1'-biphenyl]-2-yl)vinyl)-1H-benzo[e]indol-3-ium-3-yl)butane-1-sulfonate**
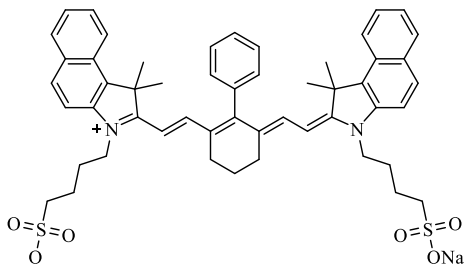


$^1$H NMR (400 MHz, Methanol-d4): δ 1.25 (s, 12H), 1.96 (bs, 8H), 2.10 (bs,2H), 2.80 (bs, 4H), 2.93-2.90 (m, 4H), 4.18 (bs, 4H), 6.36 (d, $J$ = 13.9 Hz, 2H),8.08 (d, $J$ = 8.6 Hz, 2H), .28 (d, $J$ = 6.6Hz, 2H), 7.41 (t, $J$ = 7Hz, 2H), 7.55 (d, $J$ = 8Hz 4H), 7.68-7.64 (m, 3H), 7.92 (t, 4H). Yield 36 %. HRMS (ESI-TOF): [M + H] $^+$ Calculated for $C_{52}H_{56}N_2O_6S_2$ $m/z$ 867.15; found $m/z$ 867.37.

**Dye-4b**: **4-(2-((E)-2-((E)-5-(tert-butyl)-2-chloro-3-((E)-2-(1,1-dimethyl-3-(4-sulfobutyl)-1,3-dihydro-2H-benzo[e]indol-2-ylidene)ethylidene)cyclohex-1-en-1-yl)vinyl)-1,1-dimethyl-1H-benzo[e]indol-3-ium-3-yl)butane-1-sulfonate.**
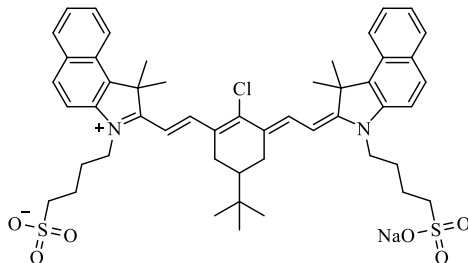


$^1$H NMR (400 MHz, Methanol-d4): δ 1.13 ( s, 12H), 1.96-2.00 (m, 4H), 2.03 (s, 12H), 2.08-2.12 (m, 4H), 2.67-2.76 (m, 3H), ), 2.90 (t, 4H, J = 7.6 Hz), 3.15-3.18 (m, 2H), 4.37 (t, 4H, J = 7.2 Hz), 6.41 (d, 2H, J = 14 Hz), 7.48 (dt, 2H, J = 7.6, 0.8 Hz), 7.63 (dt, 2H, J = 8.4, 1.2 Hz), 7.79 (d, 2H, J = 9.2 Hz), 7.98 (d, 2H, J = 8.4 Hz), 8.02 (d, 2H, J = 10.8 Hz), 8.26 (d, 2H, J = 8.4 Hz), 8.55 (d, 2H, J = 14 Hz), Yield = 54%. HRMS (ESI-TOF): [M-H]$^-$ calculated for $C_{50}H_{59}N_2ClO_6S_2$, $m/z$ 882.60; found $m/z$ 881.37.

**Dye-4c:4-(2-((E)-2-((E)-3-((E)-2-(1,1-dimethyl-3-(4-sulfobutyl)-1,3-dihydro-2H-benzo[e]indol-2-ylidene)ethylidene)-2-methylcyclohex-1-en-1-yl)vinyl)-1,1-dimethyl-1H-benzo[e]indol-3-ium-3-yl)butane-1-sulfonate.**
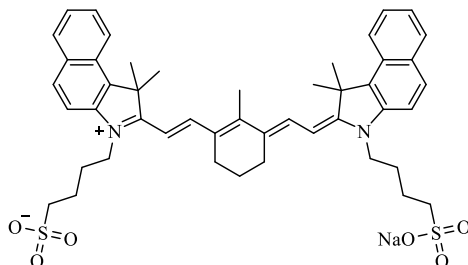


δ H (400 MHz, Methanol-d4) 1.21 – 1.38 (1 H, d, J 12.1), 1.79 – 2.09 (8 H, d, J 16.3), 2.46 – 2.60 (1 H, s), 2.59 – 2.73 (5 H, d, J 16.0), 2.82 – 2.96 (3 H, m), 3.18 – 3.40 (4 H, m), 4.13 – 4.38 (2 H, s), 6.15 – 6.40 (1 H, d, J 13.1), 7.36 – 7.50 (1 H, t, J 11.5), 7.54 – 7.72 (2 H, d, J 8.8), 7.86 – 8.11 (2 H, t, J 9.2), 8.16 – 8.40 (3 H, t, J 11.9). Yield = 47 %, HRMS (ESI-TOF): [M - H]⁻ calculated for $C_{47}H_{54}N_2O_6S_2$, 806.08; found 805.3423.

**Dye-4d:    4-(2-((E)-2-((E)-2-chloro-3-((E)-2-(1,1-dimethyl-3-(4-sulfobutyl)-1,3-dihydro-2H-benzo[e]indol-2-ylidene)ethylidene)cyclohex-1-en-1-yl)vinyl)-1,1-dimethyl-1H-benzo[e]indol-3-ium-3-yl)butane-1-sulfonate**.
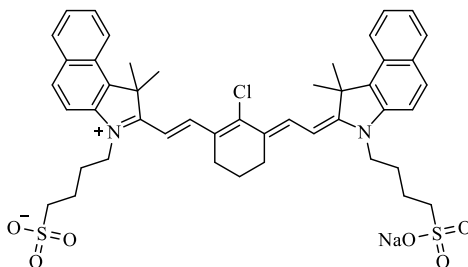


δ $_H$ (400 MHz, Chloroform-*d*) 1.17 – 1.34 (2 H, m), 1.47 – 1.58 (5 H, m), 1.63 – 1.70 (1 H, m), 1.90 – 2.00 (6 H, m), 2.42 – 2.56 (2 H, m), 7.42 – 7.48 (2 H, d, *J* 7.6), 7.50 – 7.60 (2 H, q, *J* 8.2, 7.6), 7.70 – 7.80 (1 H, dd, *J* 13.9, 8.5), 7.80 – 7.89 (2 H, dd, *J* 8.5, 5.0), 7.91 – 8.02 (3 H, dd, *J* 12.5, 8.4), 8.12 – 8.19 (1 H, d, *J* 8.5). . Yield = 55%. HRMS calculated for $C_{46}H_{51}ClN_2O_6S_2$, calculated (M-H)⁻: 826.27 found 825.281.

**Dye-4e:** **4-(2-((E)-2-((E)-2-chloro-3-((E)-2-(1,1-dimethyl-3-(4-sulfobutyl)-1,3-dihydro-2H-benzo[e]indol-2-ylidene)ethylidene)-5-methylcyclohex-1-en-1-yl)vinyl)-1,1-dimethyl-1H-benzo[e]indol-3-ium-3-yl)butane-1-sulfonate.**
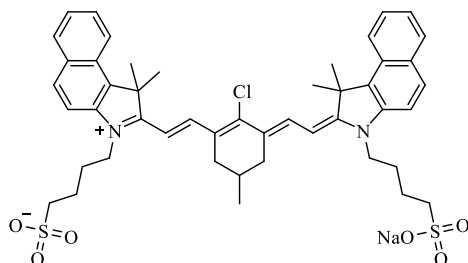


δ H (401 MHz, Methanol-d4) 1.05 − 1.20 (2 H, d, J 7.0), 1.18 − 1.40 (3 H, m), 1.92 − 2.08 (3 H, m), 2.15 − 2.34 (1 H, m), 2.80 − 3.07 (5 H, m), 3.23 − 3.41 (3 H, m), 3.40 − 3.55 (0 H, m), 4.23 − 4.46 (2 H, t, J 7.4), 6.24 − 6.47 (2 H, d, J 14.2), 7.35 − 7.58 (3 H, m), 7.56 − 7.75 (2 H, d, J 4.2), 7.91 − 8.08 (6 H, m), 8.15 − 8.31 (10 H, d, J 8.6), 8.45 − 8.63 (2 H, d, J 14.0)$^{).}$. Yield = 54%. HRMS calculated for $C_{47}H_{52}ClNaN_2O_6S_2$, calculated (M+Na): 862.29; found 885.12.

**Dye-4f: 4-(2-((E)-2-((E)-2-chloro-3-((E)-2-(1,1-dimethyl-3-(4-sulfobutyl)-1,3-dihydro-2H-benzo[e]indol-2-ylidene)ethylidene)cyclopent-1-en-1-yl)vinyl)-1,1-dimethyl-1H-benzo[e]indol-3-ium-3-yl)butane-1-sulfonate.**
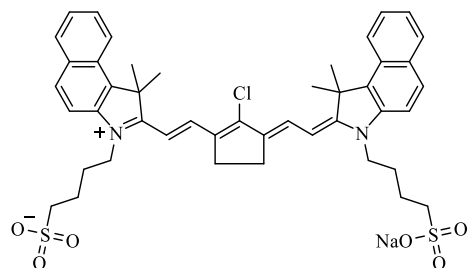


^{1}H NMR (400 MHz, CD3OD): δ 1.96-2.00 (m, 4H). 2.03 (s, 12H), 2.08-2.12 (m, 4H), 2.90 (t, 4H, J = 7.6 Hz), 8.67-2.76 (m, 3H), 3.15-3.18 (m, 2H), 4.37 (t, 4H, J = 7.2 Hz), 6.41 (d, 2H, J = 14 Hz), 7.48 (dt, 2H, J = 7.6, 0.8 Hz), 7.63 (dt, 2H, J = 8.4, 1.2 Hz), 7.98 (d, 2H, J = 8.4 Hz), 7.79 (d, 2H, J = 9.2 Hz), 8.02 (d, 2H, J = 10.8 Hz), 8.26 (d, 2H, J = 8.4 Hz), 8.55 (d, 2H, J = 14 Hz), Yield = 52 %. HRMS calculated for $C_{45}H_{49}ClN_2O_6S_2$, calculated (M-H)$^-$: 811.27; found 811.17.

**Dye-05:** **4-(2-((E)-2-((E)-4-chloro-5-((E)-2-(1,1-dimethyl-3-(4-sulfobutyl)-1,3-dihydro-2H-benzo[e]indol-2-ylidene)ethylidene)-1,1-dimethyl-1,2,5,6-tetrahydropyridin-1-ium-3-yl)vinyl)-1,1-dimethyl-1H-benzo[e]indol-3-ium-3-yl)butane-1-sulfonate iodide**.
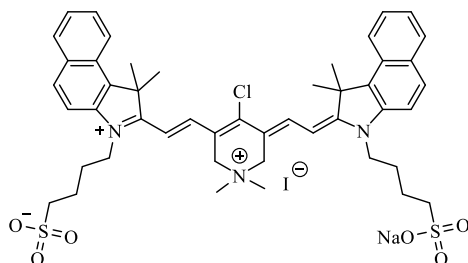


The overall yield of 1 in three steps was 23%.

δ $_H$ (400 MHz, Methanol-$d_4$) 0.77 − 0.99 (0 H, d, *J* 27.3), 1.15 − 1.42 (2 H, d, *J* 46.1), 1.46 − 1.70 (0 H, d, *J* 26.6), 1.98 − 2.03 (18 H, s), 2.68 − 2.86 (0 H, s), 2.80 − 3.07 (6 H, s), 3.71 − 3.90 (8 H, m), 4.31 − 4.48 (1 H, s), 6.24 − 6.42 (1 H, d, *J* 20.9), 7.44 − 7.59 (1 H, m), 7.60 − 7.76 (5 H, m), 7.95 − 8.16 (2 H, d, *J* 8.5), 8.21 − 8.37 (2 H, d, *J* 8.6), 8.36 − 8.52 (2 H, d, *J* 14.4). HRMS calculated for Chemical Formula: $C_{46}H_{50}ClN_3O_6S_2$, calculated (M-CH$_4$)$^-$: 840.492; found 840.284.

**Dye-06: 4-(2-((E)-2-((E)-3-((E)-2-(1,1-dimethyl-3-(4-sulfobutyl)-1,3-dihydro-2H-benzo[e]indol-2-ylidene)ethylidene)-2-methoxycyclopent-1-en-1-yl)vinyl)-1,1-dimethyl-1H-benzo[e]indol-3-ium-3-yl)butane-1-sulfonate**
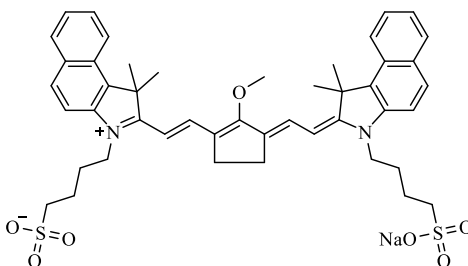


δ $_H$ (400 MHz, Methanol-$d_4$) 1.11 − 1.23 (0 H, s), 1.53 − 1.67 (1 H, s), 1.87 − 2.01 (22 H, s), 2.75 − 2.97 (16 H, d, *J* 11.7), 3.21 − 3.39 (6 H, d, *J* 16.0), 3.62 − 3.74 (1 H, s), 4.14 − 4.32 (6 H, s), 4.34 − 4.50 (2 H, s), 4.54 − 4.71 (2 H, m), 5.85 − 6.05 (2 H, m), 7.32 − 7.39 (0 H, d, *J* 7.5), 7.40 − 7.50 (11 H, m), 7.51 − 7.73 (4 H, s), 7.86 − 8.02 (5 H, d, *J* 8.5), 8.00 − 8.15 (3 H, dd, *J* 20.9, 14.0), 8.15 − 8.24 (3 H, d, *J* 8.0). Yield = 52 %. HRMS calculated for Chemical Formula: $C_{46}H_{52}N_2O_7S_2$, calculated (M-H)$^-$: 809.05; found 807.345.

**Dye-07: 4-(2-((1E,3E,5E)-7-((E)-3,3-dimethyl-1-(4-sulfobutyl)indolin-2-ylidene)hepta-1,3,5-trien-1-yl)-1,1-dimethyl-1H-benzo[e]indol-3-ium-3-yl)butane-1-sulfonate.**
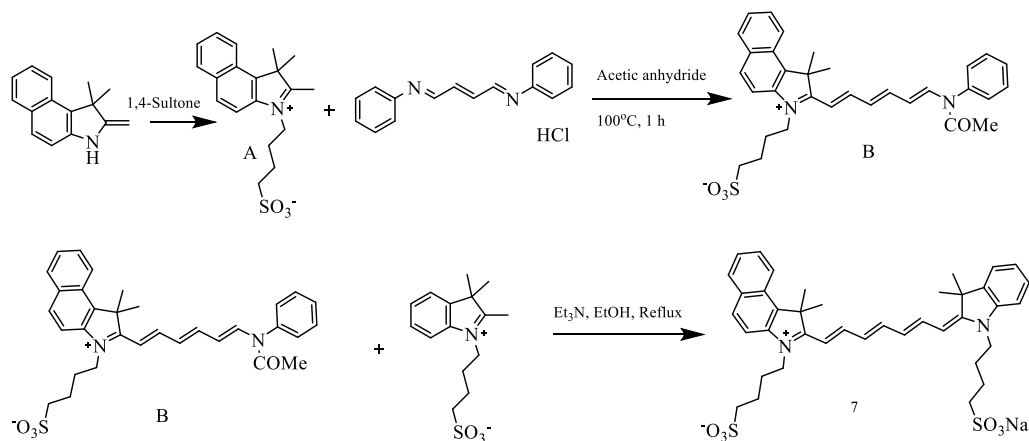


A suspension of compound (A) (3.0 g, 8.66 mmol) and glutaconaldehyde dianil hydrochloride (2.5 g, 8.66 mmol) in acetic anhydride (38 mL) was heated at 120°C for 1 h. After cooling, the reaction mixture was concentrated under reduced pressure to dryness and dissolved in DCM, then the gummy product was precipitaed after addition of diethyl ethr, the resulting precipitate (B) was dried and used directly in next step without further purification. To a solution of **B** (0.500 g, 0.90 mmol) in pyridine (6.0 mL) was added 4-(2,3,3-trimethyl-3H-indol-1-ium-1-yl)butane-1-sulfonate, (0.273 g, 0.90 mmol) at room temperature. After stirring at 40 °C for 2 h, the solvent was removed under reduced pressure. The crude product was subjected to column chromatography on SiO$_2$ with DCM/MeOH (v:v = 10:1) to afford the dye **7** (yield = 88%) as a green ssolid. δ $_H$ (400 MHz, Methanol-$d_4$) 1.22–1.35 (6 H, s), 1.62 – 1.71 (4 H, s), 1.88 – 2.00 (17 H, s), 2.83 – 2.96 (7 H, d, $J$ 6.7), 3.13 – 3.25 (2 H, s), 4.02 – 4.15 (0 H, s), 4.19 – 4.31 (0 H, s), 6.22 – 6.33 (0 H, d, $J$ 20.3), 6.37 – 6.48 (0 H, d, $J$ 13.0), 6.51 – 6.66 (0 H, m), 7.13 – 7.22 (1 H, m), 7.22 – 7.29 (0 H, s), 7.32 – 7.39 (1 H, s), 7.39 – 7.50 (1 H, m), 7.57 – 7.69 (4 H, dd, $J$ 9.1, 3.7), 7.82 – 7.93 (0 H, m), 7.93 – 8.02 (3 H, m), 8.18 – 8.27 (1 H, d, $J$ 8.2). HRMS calculated for Chemical Formula: C$_{39}$H$_{46}$N$_2$O$_6$S$_2$, calculated (M-H)$^-$: 701=93; found MS (ESI) m/z: 701.29.

**4.3 Appendix 3: NMR spectra of Chapter 1**

**Link:**

https://drive.google.com/file/d/16S62_oZj1p8yWdaufL4r0Y4Ca8MYTUyv/view?usp=sharing

**4.4 Appendix 3: NMR spectra of Chapter 2**

**Link:**

https://drive.google.com/file/d/1Ms33brNzCOTci87YA196upfDJGNYyFMC/view?usp=sharing