Boronic Acid and Zn(II)-2,2’-Dipicolylamine Complex Based Chemical Receptors for the Selective Recognition and Binding of ATP, ADP, AMP and Phosphoinositides

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A thesis submitted in fulfilment of the requirements for the degree of Bachelor of Science in the

Dr. Krishna K. Damodaran’s Research Group
Department of Chemistry

June 2015
Declaration of Authorship

I, Jóhann D. Magnússon, declare that this thesis titled, 'Boronic Acid and Zn(II)–2,2’-Dipicolylamine Complex Based Chemical Receptors for the Selective Recognition and Binding of ATP, ADP, AMP and Phosphoinositides' and the work presented in it are my own. I confirm that:

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Date:
“If you want to live a happy life, tie it to a goal, not to people or objects.”

— A. Einstein
UNIVERSITY OF ICELAND

Abstract

Faculty of Inorganic Chemistry
Department of Chemistry
Bachelor of Science

Boronic Acid and Zn(II)–2,2′–Dipicolylamine Complex Based Chemical Receptors for the Selective Recognition and Binding of ATP, ADP, AMP and Phosphoinositides

by Jóhann D. Magnússon

Phosphate anions and their derivatives (e.g. phosphorylated proteins, DNA and phospholipids) are widespread in nature and thereof, cis-diol appended phosphate anion derivatives such as adenosine 5'-tri-, 5'-di- and 5'-monophosphate (ATP, ADP and AMP) and cell membrane lipids phosphoinositides (PIs) are ubiquitous. The selective recognition and binding of these biomolecules give insights into numerous biological processes. Therefore, these biomolecules should constitute an important class of targets in the research field of molecular recognition. In spite of this, there is a lack of general tools for the detection and separation of phosphoesters that combine selectivity and sensitivity. Small molecule based chemical receptors show excellent selectivity and sensitivity for phosphate anion derivatives but have limited activity under physiological conditions. Thus, development of biologically active small molecule based chemical receptors is a worthwhile task. This project aims to address this issue by designing and developing a series of novel small molecule based chemical receptors, targeted for the selective recognition and binding of phosphorylated biomolecules such as ATP, ADP, AMP and phosphoinositols. The reported are designed by combing two well-established recognition groups, namely, a boronic acid moiety; for the selective binding of cis-diol groups (adjacent hydroxyls); and a Zn$^{2+}$–2,2′–dipicolylamine complex moiety; for the selective binding of phosphate groups. Furthermore, fluorophore moiety is also introduced to monitor the binding events by standard spectroscopic methods.
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### Abbreviations

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<td>1,8-DCA</td>
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<td>9-AM-10-DPAMA</td>
<td>9-[N-amino(methyl)]-10-[N-dipicolylamino(methyl)]anthracene</td>
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Dedicated to my parents

Jósefína Kristbjörg Arnbjörnsdóttir

and

Magnús Torfason
Chapter 1

Introduction

Molecular recognition and sensing of phosphate derivatives of biological importance have been an area of considerable attention due to their significant roles in biological systems, including those associated with information transmission and storage of genetic information (DNA and RNA are polyanions) [1]. This is partly due to the significant roles that phosphate anions and phosphorylated species play in a myriad of biological systems and as industrially important components of medicinal drugs. Phosphorylation of proteins and metabolites is an essential mechanism for signal transduction and propagation in the cell, and plays a pivotal role in tuning and regulating a wide range of biological pathways [2]. Generally, biosensors (fluorescent protein luciferin–luciferase assay) and electrochemical sensors explored [2] so far suffer from disadvantages such as the requirement of cofactors, which sometimes lack in target areas, and bulkiness (large molecular size). An alternative approach is to develop sensors based on small molecule based artificial receptors which are designed to bind to a specific biological target and are desirable for their ease of synthesis, robustness, stability against changes in temperature and pH, long shelf life, and the ability to mimic the binding sites of biological molecules [3]. They can also be readily modified to enhance activity under physiological conditions and increase cell permeability [4].

1.1 Small Molecule Based Chemical Receptors

These versatile systems exhibit rapid response and are able to bind phosphorylated biomolecules with high selectivity, allowing real-time detection. Although, enormous efforts in the last 20 years have been dedicated to develop artificial receptors for phosphorylated molecules, these receptors have not been able to surpass the advantages of
their biosensor counterparts. Only a few limited number of receptors show excellent selectivity and sensitivity in aqueous biological conditions [3]. Interestingly, the bioanalytical applications of these artificial receptors are rarely explored. Receptors for anions can be separated into three broad classes: neutral, which depend mostly on hydrogen-bonding, dipole and $\pi - \pi$ interactions; charged, which bind via electrostatic interactions; and metal-based, which make use of metal-anion coordination. Initially, most anion-receptors were based on pure organic molecules containing hydrogen bonding donor groups to interact with the anion. More recently, there has been renewed interest in the design of anion receptors that combine organic ligands and metal centres [5]. Hamachi and co-workers [2] have shown that binuclear Zn(II) complex of 2,2’–dipicolylamine (see Figure 1.1) is an effective binding motif for phosphorylated protein/peptide recognition.

![2,2'-Dipicolylamine moiety and Boronic acid moiety](image)

**Figure 1.1**: Depiction of the molecular structures of a 2,2’–dipicolylamine moiety and a boronic acid moiety.

### 1.2 Cu(II)/Zn(II)–Dipicolylamine Complexes as Selective Receptors for Phosphate Groups

Zn(II)–dipicolylamine complex (Zn(II)–DPA) is an excellent metallo-receptor for phosphate anions, which utilizes coordination of metal to anion and binds anions in aqueous medium compared to the other non-bonding interactions (hydrogen bonding and electrostatic interactions). Thus, Zn(II)–DPA based receptors have been used as versatile binding motifs for phosphorylated biomolecules such as binding with a mono-phosphorylated protein, cooperative recognition of a multi-phosphorylated protein via a cross-linking interaction and selective detection of a phosphorylated protein using a hybrid-type sensor. Metal complexes of DPA continue to be popular for anion binding, and phosphate binding in particular. They have been used as chemosensors, as protein domain mimetics, for disruption of protein-protein interactions, and as fluorescent sensors to monitor the
progress of a biological reaction; and their use in these and other applications has been thoroughly reviewed [2], [6]. Another important recognition unit is boronic acid, that are excellent candidates for sensing and separation techniques for diol appended molecules such as saccharides and anions [7].

1.3 Boronic Acids as Selective Receptors for cis-Diol Groups

Boronic acids (see Figure 1.1) are also increasingly being used as anion-binding motifs, since they are Lewis acids and can therefore accept pairs of electrons from Lewis bases. The most common use of boronic acids as recognition motifs is in the recognition of 1,2– and 1,3–diols and as such have been used in the recognition of various biological materials and natural products including phosphoinositides, nucleic acids, metal ions and the neurotransmitter dopamine but most commonly saccharides and polysaccharides [4]. Boronic acids are extremely useful in this aspect as they form stable cyclic esters with diols in a reaction that is highly specific. The most successful example of a boronic acid-based enzyme inhibitor is the proteasome inhibitor PS-341, now approved by the FDA and marketed as the anti-tumour drug Velcade [8]. When boronic acids interact with 1,2–diols (aliphatic, aromatic or catechols) a 5-membered cyclic ester is formed. This strong, reversible interaction is favoured when the pH is above the pKa (9) of the boronic acid. The binding events in these systems can be easily monitored by introduction of a fluorophore moiety in the receptors.

1.4 Fluorophores in Small Molecule Based Chemical Receptors

Rigid, ring-fused and highly π–conjugated molecules such as naphthalene, anthracene, phenanthrene and their derivatives are well-known fluorophores [9]. By incorporation of fluorescent molecules in chemical receptors the selective recognition, binding and the binding affinities of the chemical receptors, to one of their target molecules, can be evaluated via fluorometric experimental techniques. Recently, Kubo et al have shown the fluorescent detection of multi-phosphates with an imine based Zn(II)-DPA appended phenylboronic acid receptor [10].

Thus, it will be interesting to develop new receptors based on multifunctional recognition groups based on the concept that Zn–DPA units and boronic acid will selectively recognise phosphorylated species and diols, respectively. The combinations of these two binding sites in a single receptor will enable us to detect phosphorylated molecules with
hydroxyl groups like phosphoinositides, \textit{ATP, ADP, AMP, etc.} and monitor their binding events spectroscopically.
Chapter 2

Objectives and Strategy

As mentioned above, the development of small molecule based chemical receptors for the selective recognition and binding of various phosphate anion derivatives is an area of current interest. In this project the focus was towards \textit{cis}-dial appended phosphate anion derivatives such as \textit{ATP}, \textit{ADP}, \textit{AMP} and phosphoinositides. (Figure 2.1).

\begin{figure}
\centering
\includegraphics[width=\textwidth]{mol structures.png}
\caption{Molecular structure diagrams of the targeted biomolecules. Shown are \textit{ATP}, \textit{ADP}, \textit{AMP} and a general example of a phosphoinositide (\textit{PI}).}
\end{figure}

The \textbf{objectives} of this project are as follows:
• To design small molecule based chemical receptors targeted for the selective recognition and binding of ATP, ADP, AMP and other phosphorylated biomolecules.

• To synthesize and characterize the small molecule based chemical receptors via simple multi-step synthesis.

• To investigate the binding properties of the small molecule based chemical receptors under physiological conditions.

One part of the design strategy was to utilize both a boronic acid moiety; for the selective binding of cis-diol groups; and a Zn$^{2+}$–2,2’-dipicolylamine complex moiety; for the selective binding of phosphate groups. In principle, by applying two different recognition groups selectivity and sensitivity is improved. The other important part of the design was the choice of a spacer, i.e. the molecular backbone that links the moieties together (Figure 2.2).

![An ATP molecule](image1)

![2,2'-Dipycolylamine Zn(II) complex](image2)

![The molecular backbone](image3)

![The spacer](image4)

**Figure 2.2:** A schematic representation of the idea behind the design of the chemical receptors.

The chemical role of the spacer is not solely as a backbone. The binding of the recognition groups, to one of their target species, should affect a change in the physical properties of the spacer, e.g. a spectral change. Logically, the change should be measurable. That way, the binding of the chemical receptors, to one of their target species, can be
observed and evaluated. With that in mind, the fluorophore anthracene was chosen as a spacer. We have chosen anthracene due to its unique properties. Anthracene is a cheap, commercially available chemical reagent and could selectively be substituted at the 9,10-positions. Anthracene can also be selectively substituted in the 1,8-positions via another cheap and commercially available chemical reagent, namely, 1,8-dichloro-9,10-anthraquinone. Also, given the projects time limits that was the most reasonable choice of fluorophore. Thereby, the binding of the fluorescent chemical receptors, to one of their target biomolecules, triggers a fluorescent spectral change measurable by fluorometric techniques. By methods of fluorescence titration experiments the binding affinities of the chemical receptors can also be evaluated. To conclude, the ultimate design of the chemical receptors is shown in Figure 2.3.

![Molecular structures of the chemical receptors designed.](image)

The general outline of the synthetic strategy can roughly be divided into six main steps but the details of the synthesis is discussed further in Chapter 4: Results and Discussion.

1. Synthesis of the symmetrically substituted dihaloanthracene derivative.
2. Phthalimide protection by displacement of one of the halogens.
3. Displacement of the other halogen by 2,2'-dipicolylamine.
4. Deprotection of the phthalimide to yield an amino group.
5. Coupling of the boronic acid via a Schiff base reaction.
6. Zn$^{2+}$-Complexation the 2,2'-dipicolylamine.
Chapter 3

Experimental Section

3.1 Materials and Methods

All starting materials and reagents were purchased from Sigma-Aldrich except anthracene (Merck), benzoyl peroxide (Merck), hydrobromic acid (Merck), paraformaldehyde (Merck), and were used as supplied. Tetrahydrofuran was freshly distilled from sodium wires and benzophenone, dichloromethane was freshly distilled from calcium hydride and isopropanol was freshly distilled from calcium chloride where the use of anhydrous solvents is stated. Other solvents were used without further purification. Deionized water was used for all the experiments. $^1$H–NMR spectra were recorded on Bruker Advance 400 spectrometer ($^1$H–NMR: 400 MHz).

3.2 Synthesis

3.2.1 9,10-Bis(bromomethyl)anthracene – A1a

As per the reported procedures [11], [12], anthracene (10.0 g, 56.1 mmol), paraformaldehyde (3.41 g, 114 mmol) and a phase-transfer catalyst hexadecyltrimethylammonium bromide (0.230 g, 0.631 mmol) were placed in a round-bottom flask under a source of N$_2$. Glacial acetic acid (20 mL) was added to the round-bottom flask to yield a heterogeneous mixture of white suspension. The reaction mixture was stirred thoroughly and effervescence took place. Then, hydrobromic acid (35 mL, 47%) was added dropwise to the reaction mixture over a period of 1 h. The reaction mixture turned gradually yellow in colour with formation of a yellow precipitate. The reaction mixture was set to reflux for 5 h and the reaction then discontinued as monitored by TLC (60%/40% DCM/pet. e.). The yellow precipitate was collected and washed with deionized water. The yellow
solid was left to dry overnight. The dry yellow solid was recrystallized from toluene. 
$^1$H–NMR in CDCl$_3$: 5.52 (s, 4H), 7.68 (dd, $J_1 = 6.9$ Hz, $J_2 = 3.2$ Hz, 4H) and 8.38 (dd, $J_1 = 6.9$ Hz, $J_2 = 3.2$ Hz, 4H)

### 3.2.2 9-Bromomethyl-10-[N-phthalimido(methyl)]anthracene – A1b

As per a similar reported procedure [13], phthalimide (27.9 mg, 0.190 mmol), potassium carbonate (79.1 mg, 0.572 mmol) and a phase-transfer catalyst tetrabutylammonium bromide (6.5 mg, 0.020 mmol) were placed in a round-bottom flask. Next, 99% aceton (25 mL) were added to the round-bottom flask and the resulting suspension stirred thoroughly for ca. 5 min. Then, 9,10-bis(bromomethyl)anthracene (201 mg, 0.552 mmol) was added to the cloudy reaction mixture which turned yellow in colour. The reaction flask was fitted with a drying tube (CaCl$_2$) and stirred at r.t. for 2 d. In two days, the cloudiness of the mixture had diminished. The reaction was then discontinued as monitored by TLC (60%/40% DCM/pet. e.). The solvent was removed via rotary evaporation to yield a bright yellow solid. The solid was then dissolved in a mixture of deionized water and DCM. The water layer was extracted further with DCM. The combined organic layers were then dried over Na$_2$SO$_4$. The solvent was removed via rotary evaporation to yield a bright yellow solid. Purification by column chromatography (silica, 60 – 200 µm), where it was started off with pet. e. and moved gradually on to 100% DCM, was tried. The spectroscopic data, of the compound recovered, did not match with that of the product expected.

### 3.2.3 9-Chloromethyl-10-[N-phthalimido(methyl)]anthracene – A1d

As per a similar reported procedure [13], 9,10-bis(chloromethyl)anthracene (502 mg, 1.82 mmol) was placed in a round-bottom flask. Next, DMF (30 mL) was added to the round-bottom flask to yield a slightly cloudy solution yellow in colour. Then, potassium phthalimide (271 mg, 1.45 mmol) was added to the reaction solution. The reaction solution was set to reflux (at 100 degrees centigrade) for 10 h. The solution became clear. In 10 h, a yellow-green precipitate had formed. The reaction was discontinued and the solution cooled down to r.t. The yellow-green solid was filtered away and the filtrate collected. Deionized water was added to the filtrate to yield a yellow solid. The yellow solid was collected and washed with deionized water. The solid was dried in the atmosphere. Purification by column chromatography (silica, 60 – 200 µm) using 30%/70% DCM/pet. e. (and in another try 60%/40% DCM/pet. e.) as the eluant was tried. The spectroscopic data, of the compound recovered, did not match with that of the product expected.
3.2.4 1,8-Dichloroanthracene – B1

As per the reported procedures [14], [15], 1,8-dichloro-9,10-anthraquinone (5.01 g, 17.94 mmol) and zinc dust (25.02 g, 382.6 mmol) were placed in a round-bottom flask. Then, a 25% ammonia solution (112 mL) was added to the thoroughly stirred reaction mixture to yield a heterogeneous mixture of dark-red colour and dark-gray zinc suspension. The reaction mixture was set to reflux and discontinued in 6 h as monitored by TLC (60%/40% DCM/pet. e.). The dark-gray solid was collected and washed with DCM. The aqueous filtrate was extracted with DCM. The organic layers were combined and dried over Na₂SO₄. The solvent was then removed via rotary evaporation to yield a coloured white solid. The coloured white solid was dissolved in 10 : 1 ratio solution (350 mL) of isopropanol to concentrated hydrochloric acid and set to reflux (90 degrees centrigrade) for ca. 3 h. The reaction solution turned gradually light green in colour. The reaction solution was then cooled down to r.t. and the solvent removed via rotary evaporation to yield a light green solid. The solid was extracted with DCM, the organic phase then washed with dilute K₂CO₃ solution (2x), deionized water (1x) and dried over Na₂SO₄. The solvent was removed via rotary evaporation to yield a light green solid. The solid was dried overnight. Finally, the solid was recrystallized from isopropanol to yield light green needles. (¹H–NMR in CDCl₃: 9.25 (s, 1H), 8.45 (s, 1H), 7.93 (d, J₁ = 8.5 Hz, 2H), 7.62 (d, J₁ = 7.2 Hz, 2H) and 7.41 (dd, J′₁ = 8.5 Hz, J″₁ = 7.2 Hz, 2H).)

and via different procedure:

As per the reported procedure [16], 1,8-dichloro-9,10-anthraquinone (2.77 g, 9.92 mmol) was placed in a round-bottom flask. Next, isopropanol (50 mL) was added to the round-bottom flask to yield a yellow solution. Upon thorough stirring, sodium borohydride (2.50 g, 66.1 mmol) was added directly to the reaction solution which turned red-brown in colour. The reaction mixture was set to reflux (90 degrees centigrade) for 3 d. The reflux condenser was fitted with a drying tube (CaCl₂). The reaction mixture turned progressively red in colour and a precipitate had formed. The reaction mixture was cooled down to r.t. and subsequently ice added to the mixture. Then, the mixture was hydrolysed using conc. hydrochloric acid in a dropwise manner. Vigorous foaming commenced and the mixture turned yellow in colour. A yellow precipitate was apparent. The yellow precipitate was collected and washed with deionized water, then suspended in deionized water and finally dried over P₄O₁₀. Purification by column chromatography (silica, 60 – 200 µm) using pet. e. as the eluant yielded a light green solid. Recrystallization from isopropanol furnished light green needles. (¹H–NMR in CDCl₃: 9.25 (s, 1H), 8.45 (s, 1H), 7.93 (d, J₁ = 8.5 Hz, 2H), 7.62 (d, J₁ = 7.2 Hz, 2H) and 7.41 (dd, J′₁ = 8.5 Hz, J″₁ = 7.2 Hz, 2H).)
3.2.5 1,8-Dimethylantracene – B2

As per the reported procedure [14], 1,8-Dichloroanthracene (470 mg, 1.90 mmol) and a catalyst [1,3-bis(diphenylphosphino)propane]dichloronickel(II) (15 mg, 0.028 mmol) were placed in a round-bottom flask under a source of N₂. Next, anhydrous THF was added to the round-bottom flask to yield a solution of red colour. The reaction solution was stirred thoroughly at r.t. for ca. 0.5 h. Then, a 3.4 M solution of the Grignard reagent methylmagnesium bromide in 2-methyltetrahydrofuran (3.0 mL, 10.2 mmol) was added to the reaction solution which in turn took a darker red colour – even brown. The reaction solution was set to reflux (70 degrees centigrade) overnight. The following day, the reaction was discontinued and the solution cooled down to r.t. The reaction solution was then quenched slowly with 3 M hydrochloric acid (15 mL). Afterwards, the solution was extracted with DCM, the organic layer washed with dilute K₂CO₃ (3x) and then dried over Na₂SO₄. The solvent was removed via rotary evaporation to yield a yellow-green solid. Purification by column chromatography (silica, 60 – 200 μm) using pet. e. as the eluant yielded a faintly green solid (340 mg, 81%). (¹H–NMR in CDCl₃: 8.65 (s, 1H), 8.14 (s, 1H), 7.91, (d, J₁ = 8.4 Hz, 2H), 7.42 (dd, J′₁ = 8.4 Hz, J′′₁ = 6.7 Hz, 2H), 7.36 and (d, J₁ = 6.7 Hz, 2H).)

3.2.6 1,8-Bis(bromomethyl)anthracene – B3

As per a similar reported procedure [17], 1,8-Dimethylantracene (200 mg, 0.970 mmol) and N-bromosuccinimide (345 mg, 1.94 mmol) were placed in a round-bottom flask under a source of N₂. Next, anhydrous tetrachloromethane (75 mL) was added to the reaction mixture. While stirring slowly, the reaction mixture turned pale yellow in colour as gentle reflux was commenced (at 80 degrees centigrade). In 0.75 h time, benzoyl peroxide (ca. 10 – 20 mg) was added to the reaction solution which in turn took a yellow-green colour. The reaction solution was refluxed for a further 3 h period of time. A white precipitate had formed against the walls of the round-bottom flask. The reaction solution was filtered while still hot. The filtrate was collected and the solvent removed via rotary evaporation to yield a bright yellow solid. The solid was dried further in vacuo. A TLC plate displayed three products (60%/40% DCM/pet. e.). Purification by column chromatography (silica, 60 – 200 μm) using 60%/40% DCM/pet. e. as the eluant yielded a bright yellow solid as the first product to elute. (¹H–NMR in CDCl₃: 9.01 (s, 1H), 8.53 (s, 1H), 8.03 (d, J₁ = 8.5 Hz, 2H), 7.62 (d, J₁ = 6.8 Hz, 2H) and 7.42 (dd, J₁ = 8.5 Hz, J′₁ = 6.8 Hz, 4H))
3.2.7 9-[N-Phthalimido(methyl)]anthracene – A1δ

As per the reported procedure [18], 9-Chloromethylanthracene (1.00 g, 4.41 mmol) and potassium phthalimide (1.02 g, 5.51 mmol) were placed in a round-bottom flask under a source of N₂. Next, DMF was added to the round-bottom flask to yield a yellow reaction mixture suspended with potassium phthalimide. The reaction mixture was stirred thoroughly and heated (at ca. 60 degrees centigrade) for approx. 18 h. Then, CFM was added to the reaction mixture and the reaction mixture washed with deionized water. The solvent was removed via rotary evaporation and further by use of a cold-trap (using liquid N₂) connected to a vacuum pump to yield a light yellow-green solid (1.33 g, 89.3%). The solid was further dried by heating (at ca. 60 degrees centigrade) in vacuo.

\(^{1}H\)–NMR in CDCl\(_{3}\): 8.66 (d, \(J = 9.0\) Hz, 2H), 8.48 (s, 1H), 8.01 (d, \(J = 8.4\) Hz, 2H), 7.75 (dd, \(J_1 = 5.5\) Hz, \(J_2 = 3.0\) Hz, 2H), 7.64 (dd, \(J_1 = 5.5\) Hz, \(J_2 = 3.0\) Hz, 2H), 7.60 (dd, \(J_1' = 7.7\) Hz, \(J_1'' = 7.7\) Hz, 2H), 7.48 (dd, \(J_1' = 7.4\) Hz, \(J_1'' = 7.4\) Hz, 2H) and 5.86 (s, 2H)

3.2.8 9-Bromomethyl-10-[N-phthalimido(methyl)]anthracene – A1d

As per the reported procedure [19], 9-[N-Phthalimido(methyl)]anthracene (500 mg, 1.48 mmol) was placed in a round-bottom flask under a source of Ar. Next, glacial acetic acid (50 mL) was added to the round-bottom flask to yield a light yellow-green cloudy reaction mixture. While stirring slowly, paraformaldehyde (400 mg, 13.3 mmol) was added to the reaction mixture. Then, a solution of 47% hydrobromic acid (10 mL) and glacial acetic acid (25 mL) was added dropwise to the reaction mixture over a time period of approx. 0.5 h. The reaction mixture became gradually more yellow in colour. The reaction mixture was set to reflux (at 60 – 80 degrees centigrade) for approx. 2.5 h. A yellow flaky precipitate had formed. The reaction mixture was cooled down to r.t. and subsequently, deionized water (150 mL) was added to the reaction mixture. The yellow solid was collected and washed with deionized water (ca. 1.5 – 2 L) until the wash was neutral in pH. The yellow solid was dried in the desiccator over P\(_4\)O\(_{10}\) overnight to yield the product (525 mg, 82.4%). \(^{1}H\)–NMR in CDCl\(_{3}\): 8.71 (dd, \(J_1 = 8.0\) Hz, \(J_2 = 1.8\) Hz, 2H), 8.35 (dd, \(J_1 = 7.8\) Hz, \(J_2 = 1.7\) Hz, 2H), 7.75 (dd, \(J_1 = 5.5\) Hz, \(J_2 = 3.0\) Hz, 2H), 7.68 – 7.60 (m, 6H), 5.86 (s, 2H) and 5.52 (s, 2H)
3.2.9 9-[N-Phthalimido(methyl)]-10-[N,N-dipicolylamino(methyl)]-anthracene – A2

As per the reported procedure [20], 9-bromomethyl-10-[N-phthalimido(methyl)]anthracene (452.8 mg, 1.05 mmol) was placed in a round-bottom Schlenk flask under a vacuum for ca. 1 h and then put under a source of Ar. Then, anhydrous DCM (ca. 70 mL) was added to the round-bottom flask. The solution went clear and orange in colour upon heating. Dipicolylamine (210 mg, 1.05 mmol) was placed in a separate round-bottom flask under a source of Ar. Then, anhydrous DCM (ca. 3 mL) was added to the flask. The solution of dipicolylamine was then transferred via syringe over to the main reaction flask. Next, diisopropylethylamine (300 mg, 2.3 mmol) was added dropwise via syringe to the reaction solution. The reaction solution was stirred overnight at r.t. The following day the reaction was discontinued as concluded by TLC (10%/90% - MeO-H/DCM). The solvent was removed via rotary evaporation to yield a red oily solid – even tar like. The product was dried under vacuum in a desiccator over weekend. Purification by column chromatography (silica, 60 – 200 µm) starting off with 50%/50% - DCM/pet. e. as the eluant up to (4%/96% - MeOH/DCM yielded the product as red oily solid. Once placed in a solution of DCM and the DCM evaporated overnight in the fume-hood yielded a light yellow-brown like dry solid (500 mg, 86.7%). (1H-NMR in CDCl₃: 8.63 (d, J = 8.9 Hz, 2H), 8.47 (two doublets, 4H), 7.72 (dd, J₁ = 5.5 Hz, J₂ = 3.0 Hz, 2H), 7.61 (dd, J₁ = 5.5 Hz, J₂ = 3.0 Hz, 2H), 7.58 – 7.53 (m, 4H), 7.47 (dd, J₁’ = 7.4 Hz, J₁'' = 7.4 Hz, 2H), 7.29 (d, J = 7.8 Hz, 2H), 7.09 (ddd, J₁’ = 7.4 Hz, J₁'' = 5.9 Hz, J₂ = 0.96 Hz, 2H), 5.81 (s, 2H), 4.73 (s, 2H) and 3.94 (s, 4H) )

3.2.10 9-[N-Amino(methyl)]-10-[N,N-dipicolylamino(methyl)]-anthracene – A3

As per the reported procedure [21], 9-[N-phthalimido(methyl)]-10-[N-dipicolylamino(methyl)]anthracene (400 mg, 0.730 mmol) and 64 – 65% hydrazine hydrate (0.39 mL, 8.04 mmol) were placed in a round-bottom flask under a source of N₂. Then, a 1:1.5 solvent mixture (ca. 25 mL) of CHCl₃:EtOH was added to the round-bottom flask to yield a clear solution yellow in colour. The reaction mixture was stirred thoroughly and refluxed overnight. The following day a white precipitate had appeared against the sides of the reaction flask. The reaction was discontinued and the reaction solution cooled down to r.t. as more and more white solid precipitated out. The solvent was removed via rotary evaporation to yield a white solid embedded with a yellow oily-like solid. The solid was extracted with CHCl₃ and the white solid filtered away. The white solid was washed further with CHCl₃. The organic layers, yellow in colour, were combined and
washed with deionized water, then 1 M NaOH and subsequently again with deionized water. Next, the organic layer was dried over Na₂SO₄ and the solvent thereafter removed via rotary evaporation to yield a oily yellow-brown solid. (¹H-NMR in CDCl₃: 8.46 (d, \( J = 4.8 \) Hz, 2H), 8.39 (dd, \( J_1 = 6.7 \) Hz, \( J_2 = 3.3 \) Hz, 2H), 8.28 (dd, \( J_1 = 7.0 \) Hz, \( J_2 = 3.0 \) Hz, 2H), 7.52 (ddd, \( J_1' = 9.2 \) Hz , \( J_1'' = 7.7 \) Hz, \( J_2 = 1.6 \) Hz, 2H), 7.45 (two doublet of doublets, 4H), 7.17 (d, \( J = 7.6 \) Hz), 7.08 (dd, \( J_1' = 6.6 \) Hz, \( J_1'' = 5.1 \) Hz, 2H), 4.82 (s, 2H), 4.59 (s, 2H) and 3.72 (s, 4H) )
Chapter 4

Results and Discussion

4.1 Synthesis of the Chemical Receptors

The synthesis of the chemical receptors is divided into two series depending on the substitution on the anthracene backbone – the A series which included 9,10-substitution and the B series which included 1,8-substitution (see Figure 2.3).

4.1.1 Synthesis of the A Series

The synthesis of the A series was further divided between three main routes as discussed below, namely, route 1, route 2 (Figure 4.2) and route 3 (Figure 4.3).

As mentioned above, anthracene can be selectively substituted in the 9,10-positions. The explanation for this preference of 9,10-substitution lies in aromaticity. The transition state of the electrophilic aromatic substitution proceeds via a carbocation intermediate. The 9,10-substitution leads to formation of the most stable carbocation intermediate, thus the lowest energy transition state and the kinetically favoured product (Figure 4.1).

In the synthesis of 9,10-bis(bromomethyl)anthracene, anthracene was first electrophilically substituted by formaldehyde to yield a hydroxymethyl substituted product. Subsequently, the hydroxyl group got protonated under acidic conditions and displaced by bromide via $S_N^2$ substitution reaction. Addition of hydrobromic acid to the reaction mixture resulted in a yellow solid, which was formed gradually, consistent with a bathochromic effect due to the bromomethyl substituents formed. However, problems were encountered with recrystallization and only a small portion of the crude product recrystallized. This is presumably due to incomplete washing of the crude product, which might have left
impurities, or too much heating while trying to dissolve the crude product for recrystallization. Anthracene and its derivatives are known to photodimerize via Diels-Alder like reaction, which the heating could have promoted. The heating or long exposures to light might even have led to polymerization of the product.

**Figure 4.1:** The diagram shows possible carbocation intermediates in the electrophilic aromatic substitution of anthracene. Substitution of the 9,10-positions proceeds through formation of the most stable carbocation which preserves the aromatic sextet of two rings.

The next step was to replace one of the bromine atoms by a phthalimide protection group to yield the monoprotected product. The problem with this synthesis was the low
solubility of 9,10-bis(bromomethyl)anthracene in acetone. The substitution of phthalimide for bromine proceeds via a mechanism similar to $S_N2$ mechanism. The reaction was successful only when the reactants were fully dissolved, preferably in polar aprotic solvent. Furthermore, there was possibility of bis-protected compound. The purification of this compound by column chromatography posed also problems. The elucidation of the crude product in two columns turned out to be unsuccessful. The spectroscopic data of these fractions recovered did not match with that of the expected product. The two well-recognizable doublet of doublets proton peaks of the phthalimide protection group were not visible in the $^1$H–NMR spectrum.

**Figure 4.2:** Reaction scheme showing route 1 and route 2 in the synthesis of the A series.
At this stage it was decided to run the reaction using 9,10-bis(chloromethyl)anthracene, which was readily available. In this case, DMF was used as a solvent in order to increase the solubility of the reactants. The solubility increased but the reaction was unsuccessful. The TLCs indicated at least two products, probably mono- and bis-protected, along with unreacted starting material as expected. The purification of these products by column chromatography was a failure. The spectroscopic data of any of the compounds recovered did not match with that of the expected product and there was no indication of the phthalimide peaks in the $^1$H–NMR spectrum.

Thus, we decided to tackle this problem by changing the reaction route. The problems with low solubility of the reactants, the chances of a mixture of mono- and bis-protected

Figure 4.3: Reaction scheme for the synthesis of the A series via route 3.
products and the difficulties associated with purification methods by column chromatography were time consuming. The low yield was not something to strive for either. Instead, using 9-chloromethylantracene as a starting material provided a more convenient route (see Figure 4.3). 9-chloromethylantracene dissolved completely in DMF and displacing the chlorine for a phthalimide protection group was successfully achieved. No further purification of the crude product was necessary as the reaction gave quantitative yield. The $^1$H–NMR reveals the well-recognizable two doublet of doublet peaks of phthalimide at 7.75 ppm (dd, $J_1 = 5.5$ Hz, $J_2 = 3.0$ Hz, 2H) and 7.64 ppm (dd, $J_1 = 5.5$ Hz, $J_2 = 3.0$ Hz, 2H).

The next step was bromomethylation of the phthalimide protected derivative. As discussed above the bromomethylation takes place in the 10-position. The 9-position already being phthalimide protected there was no chance of any mixed products. The reaction was successfully achieved. The $^1$H–NMR spectrum reveals the peak of the bromomethyl protons at 5.52 ppm (s, 2H). The $^1$H–NMR spectrum of the crude product obtained was pure enough to carry out the next step. No further purification of the crude product was carried out.

The following step was N-alkylation of 2,2’-dipicolylamine via $S_N2$ substitution reaction, which was successful. The only difficulty was associated with the purification of the crude product by column chromatography in order to separate it from unreacted starting material and the 2,2’-dipicolylamine. A silica column was performed two times to purify the crude product.

Deprotection of the phthalimide via reaction with hydrazine was done to yield 9-[N-amino(methyl)]-10-[N,N-dipicolylamino(methyl)]anthracene. Due to the polar nature of the product purification by column chromatography was not possible.

No further synthesis was achieved due to the project’s time limits. However, Figure 4.3 and Figure 4.4 show the target chemical receptors and the feasibility. Reaction of the amines with 2-, 3-, and 4-formylphenyl boronic acids, subjected to reduction with either sodium borohydride or sodium cyanoborohydride, followed by a complexation step with zinc nitrate would yield the A series chemical receptors.
4.1.2 Synthesis of the B Series

The synthesis of the B series substitution was done in the 1,8-position as discussed above (see Figure 4.5). By starting with 1,8-dichloro-9,10-anthraquinone and reducing, with zinc dust in an alkaline solution of ammonium hydroxide, 1,8-dichloro-9,10-bis(hydroxymethyl)anthracene could be obtained. Hydrolysis of this compound under acidic conditions will yield 1,8-dichloroanthracene. The problem with this synthesis was the purification of the crude product. The reaction was performed in several batches and a mixture of two products were obtained. Although, recrystallization of the crude
product gave the pure product but the other unwanted product recrystallized at some instances.

In order to avoid this problem, another synthesis was performed where the 1,8-dichloro-9,10-anthraquinone was reduced to 1,8-dichloroanthracene with sodium borohydride. Albeit, there was a mixture of two products we were able to isolate the pure product by column chromatography. This synthesis was more convenient because the crude product was flushed with petroleum ether (in silica) to get rid of the other undesired side product.

This was followed by a nickel-complex catalysed coupling reaction of 1,8-dichloroanthracene and methylmagnesium bromide to yield 1,8-dimethylanthracene. The 1,8-dimethylanthracene was then subjected to a NBS-bromination reaction to yield 1,8-bis(bromomethyl)anthracene. The NBS-bromination led to mono- and bis-brominated products and some other side products. Despite of this, the 1,8-bis(bromomethyl)anthracene was purified by column chromatography with much ease.

At this stage the 9,10-bis(halomethyl)anthracene derivatives had already been subjected to a substitution reaction with phthalimide but the results were unsuccessful. Thus, the
synthesis of the B series was discarded and we focused towards the synthesis of the A series via route 3.
Chapter 5

Conclusion

The specific aim of the project was to develop novel chemical receptors for the efficient recognition of phosphorylated molecules of biological interest. Phosphorylation (i.e. the attachment of a phosphate group) of proteins and metabolites is an essential mechanism for a wide range of biological processes and of utmost importance for cellular function. Therefore, it is highly desirable to develop tools for the detection, sensing and separation of this type of species. Although in recent years there have been important developments in this area, the current methods to recognize phosphorylated bio-molecules are far from ideal. They often lack sensitivity, selectivity and in some instances (e.g. biosensors) the shelf life of the systems are not ideal. Therefore, we set up to investigate novel approaches based on small molecules with desirable properties such as ease of synthesis, robustness, long shelf life, fast binding kinetics, fast clearance from blood circulation, and ease of formulation for widespread use.

The synthesis of the designed receptor molecules were partially achieved although, the final metal complexes were not acquired. All the compounds synthesized have been characterized by standard analytical techniques. As per the project’s time limits the synthesis of the chemical receptors was not complete and hence biological activity could not be investigated. This is due to the challenges involved in the mono-protection of the bis-halogen derivatives of anthracene, which was time consuming. Interestingly, the experimental results helped us to optimize the reaction conditions and opened a new pathway to deal with the synthesis of anthracene based compounds with two different substituents. These results indicated that synthesis via route 3 gave better results compared to other routes. Unfortunately, time was the major factor and by the time the synthesis of 9-[N-Amino(methyl)]-10-[N,N-dipicolylamino(methyl)]anthracene was complete the project’s time was practically out.
Chapter 6

Supplementary Information

6.1 Nuclear Magnetic Resonance Spectroscopy

6.1.1 General

The $^1$H–NMR spectra were recorded on a Bruker Advance 400 NMR spectrometer using CDCl$_3$ (s, 7.26 ppm) as the NMR solvent ($^1$H–NMR: 400 MHz).

6.1.2 9,10-Bis(bromomethyl)anthracene – A1a

![Figure 6.1: The $^1$H–NMR of 9,10-bis(bromomethyl)anthracene](image)

Figure 6.1: The $^1$H–NMR of 9,10-bis(bromomethyl)anthracene
6.1.3 1,8-Dichloroanthracene – B1

Figure 6.2: The $^1$H–NMR of 1,8-dichloroanthracene

6.1.4 1,8-Dimethylanthracene – B2

Figure 6.3: The $^1$H–NMR of 1,8-dimethylanthracene
6.1.5 1,8-Bis(bromomethyl)anthracene – B3

Figure 6.4: The $^1$H–NMR of 1,8-bis(bromomethyl)anthracene.

6.1.6 9-[N-Phthalimido(methyl)]anthracene – A1δ

Figure 6.5: The $^1$H–NMR of 9-[N-phthalimido(methyl)]anthracene.
6.1.7 9-Bromomethyl-10-[N-phthalimido(methyl)]anthracene – A1d

Figure 6.6: The $^1$H–NMR of 9-bromomethyl-10-[N-phthalimido(methyl)]anthracene.

6.1.8 9-[N-Phthalimido(methyl)]-10-[N-dipicolylamino(methyl)]-anthracene – A2

Figure 6.7: The $^1$H–NMR of 9-[N-Phthalimido(methyl)]-10-[N-DPA(methyl)]anthracene.
6.1.9 9-[N-Amino(methyl)]-10-[N-dipicolylamino(methyl)]-anthracene – A3

Figure 6.8: The $^1$H–NMR of 9-[N-amino(methyl)]-10-[N-DPA(methyl)]anthracene.
Bibliography


