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Synthesis, Spectroscopic Evaluation and Preliminary UV-Vis Titration Study of 1,2-bis[*N,N'*-6-(3-pyridylmethylamido)pyridyl-2-carboxyamido]butane as a Potential Anion Receptor

Nurul Shafiqah Shohimi[1], Nafisah Mansor[1], Nur Shuhaila Haryani Haris[1] and Maisara Abdul Kadir[1,2]*
[1]Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia
[2]Advanced Nano Materials (ANoMa) Research Group, Faculty of Science and Marine Environment, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia
*Corresponding author (e-mail: maisara@umt.edu.my)

In an effort to produce a new receptor that can bind with a wide range of anions, a butyl group was combined as a spacer with two pre-organized amide moieties as the anion binding sites. This study presents the synthesis of a new compound, namely 1,2-bis[*N,N'*-6-(3-pyridylmethylamido)pyridyl-2-carboxyamido]butane, from the reaction between *N*-6-[(3-*N*-pyridylmethylamino) carbonyl]-pyridine-2-carboxylic acid methyl ester and butane-1,4-diamine in toluene. Characterization of this compound was accomplished using Fourier Transform Infrared (FTIR), ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR), UV-Vis spectroscopy and mass spectrometry. FTIR analysis showed the main absorption peaks of ν_{str}(N-H), ν_{str}(C=C), ν(C=O) amide and ν_{bend}(N-H) at 3267 cm^{-1}, 1234 cm^{-1},1667 cm^{-1} and 1521 cm^{-1}, respectively. ^{1}H NMR analysis indicated the presence of methyl groups (δ_H 1.68-4.71 ppm), aromatic protons at 7.38-8.65 ppm and amides N-H resonance at 9.45-10.00 ppm. In the ^{13}C NMR spectrum, methyl groups (27.72 ppm and 28.99 ppm), aromatic pyridine (124.09-149.32 ppm) and carbonyl groups (164.12 ppm and 163.52 ppm) were present. The UV-Vis spectrum showed absorbance peaks for carbonyl and pyridine at λ_{max} 274 nm ($n \rightarrow \pi^*$) and 224 nm ($\pi \rightarrow \pi^*$), respectively. Titration of chloride and nitrate anions with 1,2-bis[*N,N'*-6-(3-pyridyl methylamido)pyridyl-2-carboxyamido]butane gave inconsistent changes in the absorbance of pyridine moieties which reflected the decrease of anions to the receptor contacts. In contrast, the absorbance of 1,2-bis[*N,N'*-6-(3-pyridylmethylamido)pyridyl-2-carboxyamido]butane consistently decreased when titrated with chromate due to the presence of hydrogen bonding interactions between the host and analyte. This finding shows the promising potential of 1,2-bis[*N,N'*-6-(3-pyridylmethylamido)pyridyl-2-carboxyamido]butane to act as an anion receptor for chromate.

Keywords: Butane; diamide; chromate; nitrate; spacer; analyte

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Anthropogenic activities leading to household, agricultural, and industrial waste have increased anionic pollutants in the environment. For example, chloride, nitrate, chromate and phosphate ions may be considered pollutants when present in water supplies beyond certain levels, which in turn affects human health and the environment [1-4]. Tetrahedral anions exhibit large radii, low charge densities and high hydrophobicity. Due to these properties, tetrahedral-like anions are difficult to identify in water [5]. Measures have been taken to remove these pollutants from water sources such as rivers, lakes and groundwater using several methods such as ion exchange, filtration, adsorption, reverse osmosis, solvent extraction, chemical precipitation, evaporation and concentration, electro-dialysis and biomethods [6]. However, these methods require high cost and high tech equipment. Thus, tremendous efforts have been made to produce adsorbents from synthetic anion receptors, as this approach is the simplest, cheapest and most effective method for water remediation. The main concept in anion receptor chemistry is the supramolecular interaction between the host and analyte *via* non-covalent interactions such as hydrogen bonding, phi-stacking and electro-static attraction (Figure 1).

Amide-functionalized compounds have been commonly utilized as anion receptors due to the presence of directed hydrogen bonding sites at the N-H amide moieties [7, 8]. This can be attributed to the inherent stability and neutrality of the amide bond (CO-N), which enables the formation of hydrogen bonds [9]. This unique structural arrangement contributes to its impressive binding capabilities and favourable characteristics that enable the formation of hydrogen bonding interactions with anionic species within the cavity, giving a stable anion-receptor complex. Much attention has been paid to amide-based receptors because amide groups are water-soluble and can form strong hydrogen-bonding interactions with anion [10].

To enhance binding performance, many receptors with flexible structures have been designed to capture a wide range of anions [11, 12]. This includes incorporating aliphatic chains and acyclic structures with a higher degree of freedom. Incorporating an aliphatic group at the spacer enhances the ligand's basicity. This leads to the formation of coordination compounds that exhibit a slightly higher degree of stability in solution. Moreover, the increased length of the spacer provides the ligand with enhanced flexibility, enabling it to undergo bending and rotation during coordination with anions [13].

Thus, this study comprises the synthesis, characterization and anion titration studies of a new compound, namely 1,2-bis[*N,N'*-6-(3-pyridylmethyl amido)pyridyl-2-carboxyamido]butane, which was derived from 2,6-pyridinecarboxamide. This compound was synthesised by combining two pre-organized amides with a butyl spacer (-C_4H_8-). The molecule was incorporated with 3-aminomethyl pyridine groups as the pendant arms to support the formation of a molecular cleft structure when the anions are encapsulated within the amide moieties, as well as to enhance the acidity of the compound for efficient anion binding [14]. In theory, the oxoanions should be accommodated inside the anion pocket through weak hydrogen bonding interactions (NH···O). The butyl spacer was employed to ensure a flexible anion receptor structure that could fold on itself, allowing the molecular host to encapsulate the anions, as shown in Figure 2.

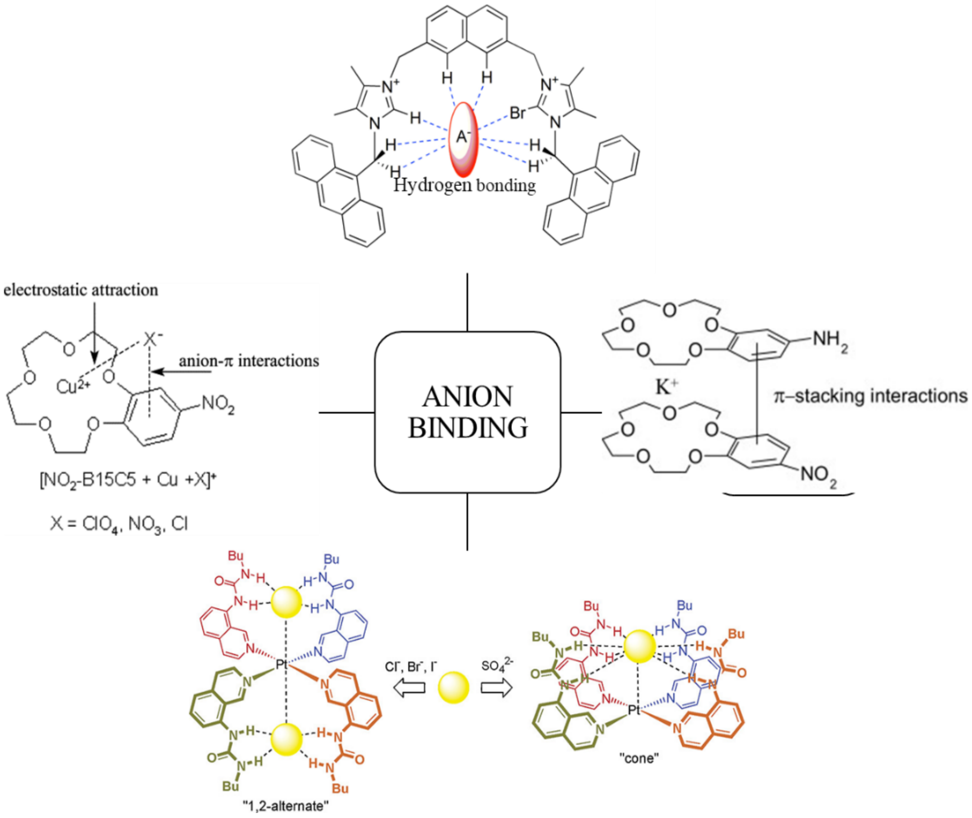


Figure 1. Types of anion binding.

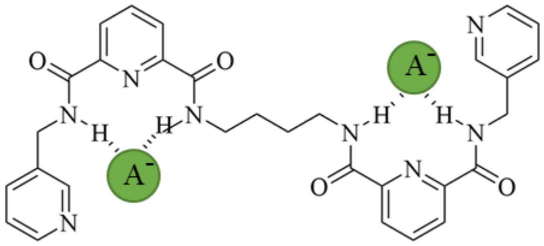


Figure 2. Encapsulation of anions by NH moieties through hydrogen bonding interactions in the studied molecule.

EXPERIMENTAL

Dimethyl pyridine-2,6-dicarboxylate, 3-aminomethyl pyridine, butane-1,4-diamine, toluene, dichloromethane, sodium bicarbonate, sodium sulphate, dilute hydrochloric acid and methanol were used without further purification.

Synthesis of Precursor

Dimethyl pyridine-2,6-dicarboxylate (1.09 g, 5.1 mmol) and 3-aminomethyl pyridine (0.52 mL, 5.1 mmol) were suspended in toluene (40 mL) and heated under reflux in an inert atmosphere. The reaction was monitored by thin-layer chromatography (TLC) until completion. Next, the toluene was removed using a rotary evaporator. The residue was dissolved in dichloromethane (100 mL), washed with dilute hydrochloric acid (0.2 M, 2 x 100 mL) and then the dichloromethane layer was discarded. The aqueous extract was neutralized with sodium bicarbonate, extracted with dichloromethane (3 x 100 mL), filtered, and dried *in vacuo*. The residue was purified by flash column chromatography on silica gel by eluting with methanol : dichloromethane (0.4:9.6).

Synthesis of 1,2-bis [*N,N’*-6-(3- pyridylmethylamido) pyridyl-2-carboxyamido]butane

A mixture of *N*-6-[(3-*N*-pyridylmethylamino) carbonyl]-pyridine-2-carboxylic acid methyl ester (0.5 g, 1.8 mmol) and butane-1,4-diamine (0.09 mL, 0.92 mmol) was suspended in toluene (40 mL), refluxed under an inert atmosphere and monitored by thin-layer chromatography (TLC) until the reaction was completed. After 24 h, the product was precipitated from the reaction as a light-yellow solid.

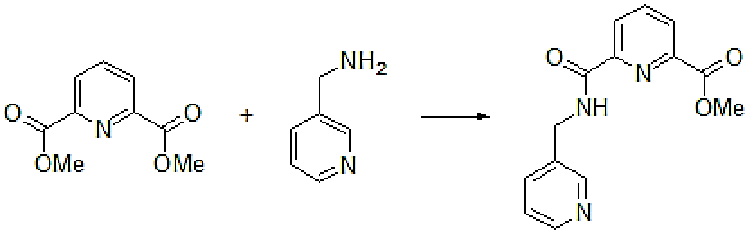
Anion Titration Methods

A stock solution of 1,2-bis[*N,N*’-6-(3-pyridylmethyl amido)pyridyl-2-carboxyamido]butane (10 mL, 3.33 x 10^{-5} M) with potassium chloride, potassium nitrate and potassium chromate (10 mL, 1.0 x 10^{-2} M) was prepared in acetonitrile. A series of working solutions were prepared by adding 3 mL of the stock solution into five separate 10 mL volumetric flasks. To each of the 10 mL volumetric flasks was added 10 μL, 20 μL, 30 μL, 40 μL, and 50 μL of the anion solution. These working solutions were diluted with acetonitrile before being analysed by UV.

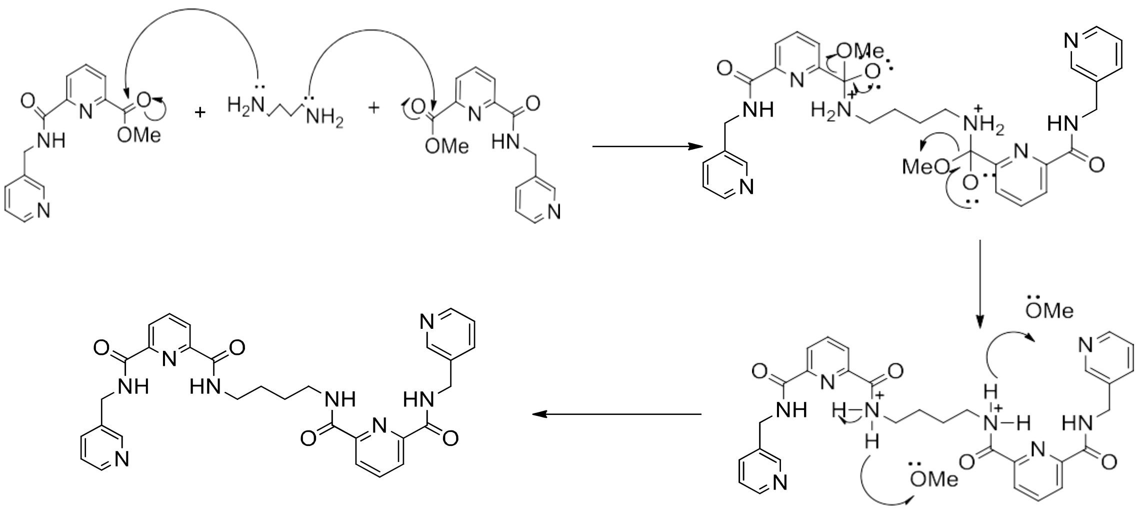
RESULTS AND DISCUSSION

The main precursor, *N*-6-[(3-pyridylmethylamino) carbonyl]-pyridine-2-carboxylic acid methyl ester was prepared from a reaction between dimethyl pyridine-2,6-dicarboxylate and 3-aminomethyl pyridine in toluene (Scheme 1). The mixture was heated under reflux in an inert atmosphere until the reaction was completed. The synthesis of this precursor was facile and straightforward, with the product being obtained directly as a white solid with a good yield (approximately 70 %). The precursor was further reacted with butane-1,4-diamine in toluene for 55 hours, where a sticky precipitate was formed. The toluene was removed using a rotary evaporator to give 1,2-bis[*N,N’*-6-(3-pyridylmethylamido)pyridyl-2-carboxyamido]butane as a light yellow solid with a yield of 80 %.

Scheme 2 shows the mechanism for the formation of 1,2-bis[*N,N’*-6-(3-pyridylmethylamido) pyridyl-2-carboxyamido]butane. A similar mechanism has been proposed in a previous report using propane-1,3-diamine as the diamine group [15]. The reaction starts with a nucleophilic attack by an aminopyridine nitrogen atom on a carbonyl carbon in dimethyl pyridine-2,6-dicarboxylate. This attack forms a sp^3 hybridized intermediate of an electronegative methoxy group (O-CH_3). The lone pair at the oxygen atom forms a pi bond, which causes the methoxy group to leave the compound. To stabilize the compound, a hydride shift occurs which shifts the positive charge to the carbon. Then, a nucleophilic attack by the amine nitrogen atom on the carbonyl group attached to *N*-6-[(3-pyridylmethylamino)carbonyl]-pyridine-2-carboxylic acid methyl ester occurs. This nucleophilic reaction produces a sp^3 hybridized intermediate with a negatively charged oxygen atom, and as a result, the methoxy group leaves, leading to the formation of a new symmetrical diamide compound.



Scheme 1. Equation for the synthesis of *N*-6-[(3-pyridylmethylamino)carbonyl]-pyridine-2-carboxylic acid methyl ester.



Scheme 2. Mechanism for the formation of 1,2-bis[*N,N'*-6-(3-pyridylmethylamido)pyridyl-2-carboxyamido]butane.

The CHN elemental analysis data for this compound is listed in Table 1. Theoretical and experimental values are in excellent agreement, indicating that the product was of good purity.

In the FTIR spectrum, an (N-H) stretching peak was present at 3267 cm^{-1}. The low frequency (N-H) stretching indicated a steric effect with methyl near the amino group. Meanwhile, the strong frequency band at 2929 cm^{-1} was due to (C-H) alkyl groups consistent with the formation of sp^3 hybridization. The carbonyl group had shifted to a lower frequency at 1667 cm^{-1}, suggesting the formation of a hydrogen bond between the amide moieties and the pendant pyridyl group [16].

Table 1. CHN data of 1,2-bis[*N,N'*-6-(3- pyridylmethylamido)pyridyl-2-carboxyamido]butane.

	C%	H%	N%
Experimental	63.59	5.34	19.78
Theoretical	62.89	5.483	19.59

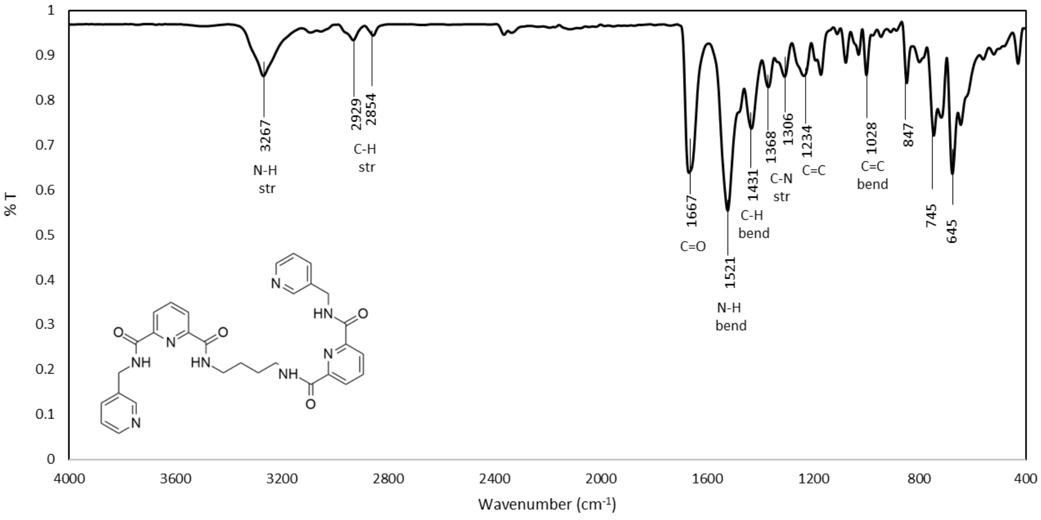


Figure 3. FTIR spectrum of 1,2-bis[*N,N'*-6-(3-pyridylmethylamido)pyridyl-2-carboxyamido]butane.

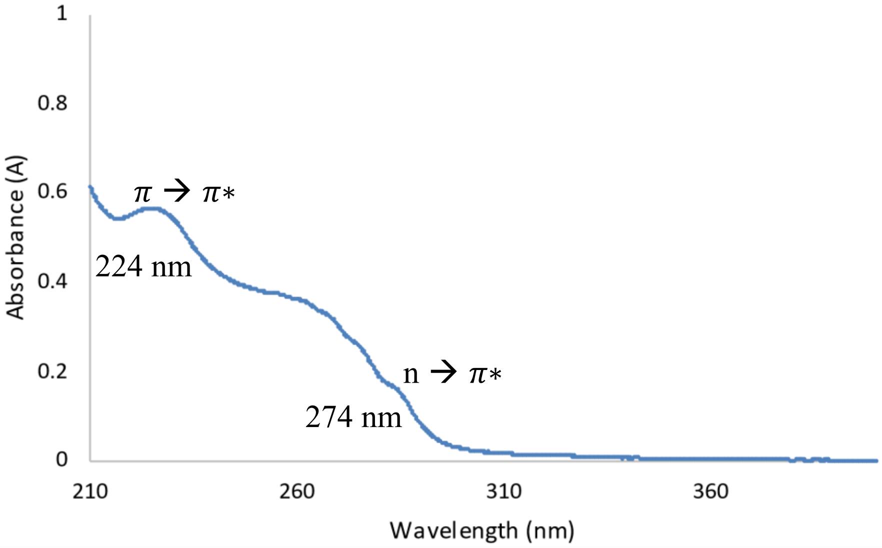
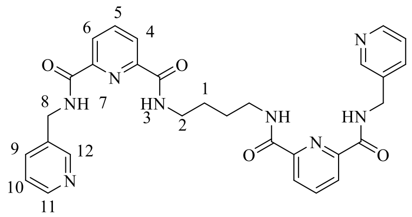


Figure 4. UV-vis spectrum of 1,2-bis[*N,N'*-6-(3-pyridylmethylamido)pyridyl-2-carboxyamido]butane.

At 1521 cm^{-1}, the (N-H) stretching was represented by a broad peak (Figure 3). C=C double bonds were indicated at 1234 cm^{-1} and 1028 cm^{-1}, and the region below 2000 cm^{-1} showed very sharp and intense peaks. The primary causes of vibrations in this area are the bending of C-H, N-H, and O-H bonds and the symmetrical deformations of CH_2 groups [17]. In contrast with the results for the reported compound having a propyl spacer [15], the N-H peaks were found to shift to lower frequencies due to the decreased electron density at the moieties [18]. The spectral region, commonly called the fingerprint region, is typically below 1000 cm^{-1}.

The UV-Vis spectrum of this compound (Figure 4) was similar to those of reported studies utilizing amide as a binding host [7, 15]. Two absorption peaks corresponding to the carbonyl and pyridine groups with electronic transitions of $n \rightarrow \pi*$ and $\pi \rightarrow \pi*$ were indicated at 274 nm and 224 nm, respectively. This phenomenon of electronic transitions in the pyridine group has been well-documented in the literature [19]. The 270-300 nm absorbance is common for molecules containing a C=O group, such as amides [20]. It is crucial to utilize a solvent that does not absorb UV light within the range where the sample absorbs to prevent any overlapping at the acetonitrile solvent cutoff point of 190 nm [21].

The ^{1}H NMR spectrum of 1,2-bis[*N,N*'-6-(3-pyridylmethylamido)pyridyl-2-carboxyamido]butane exhibited three distinct regions of resonance peaks: one corresponding to methyl groups, one for protons on the aromatic ring, and another for the N-H proton peak (Figure 5). Introducing a methyl substituent on the pyridine ring leads to magnetic inequivalence among certain hydrogen atoms. Specifically, the hydrogens positioned two carbons away from the methyl group, and the hydrogen opposite the methyl group across the ring differ magnetically from the hydrogens adjacent to the methyl group. In the ^{1}H NMR spectrum, the alkyl protons H1 and H2 were triplets at 1.68 ppm and 3.46 ppm, respectively. This observation is consistent with the results reported in previous research [7]. The C-H protons (H8) bonded to the pyridine and N-H group were observed as a multiplet at 4.71 ppm. The C-H protons of the pyridine (H9 and H10) at the pendant arms were observed as multiplets at 7.76 ppm - 7.81 ppm and 7.38 ppm to 7.44 ppm, respectively. The hydrogen of pyridine dicarboxamide (H4, H5, H6) appeared as a multiplet in the range of 8.21 ppm - 8.65 ppm. This aligns with findings from other studies [22]. Meanwhile, the electron-absorbing nitrogen in the pyridine ring caused the C-H protons of H11 and H12 to be slightly shifted downfield compared to the other aromatic protons, resulting in multiplets at 8.46 ppm - 8.54 ppm and 8.62 ppm - 8.65 ppm, respectively. The N-H proton (H3), bonded to the alkyl group, was observed as a triplet at 9.45 ppm. Nitrogen is recognized for possessing a nuclear spin with a value of unity (I=1), allowing it to assume three distinct spin states (+1, 0, and -1) [23]. Meanwhile, the N-H proton bonded to the carbonyl and CH_2 group was observed as a triplet at 10.00 ppm, appearing further downfield compared to the other aromatic protons due to the electron-absorbing ability of the nitrogen in the pyridine ring [24]. The obtained data is presented in Table 2.



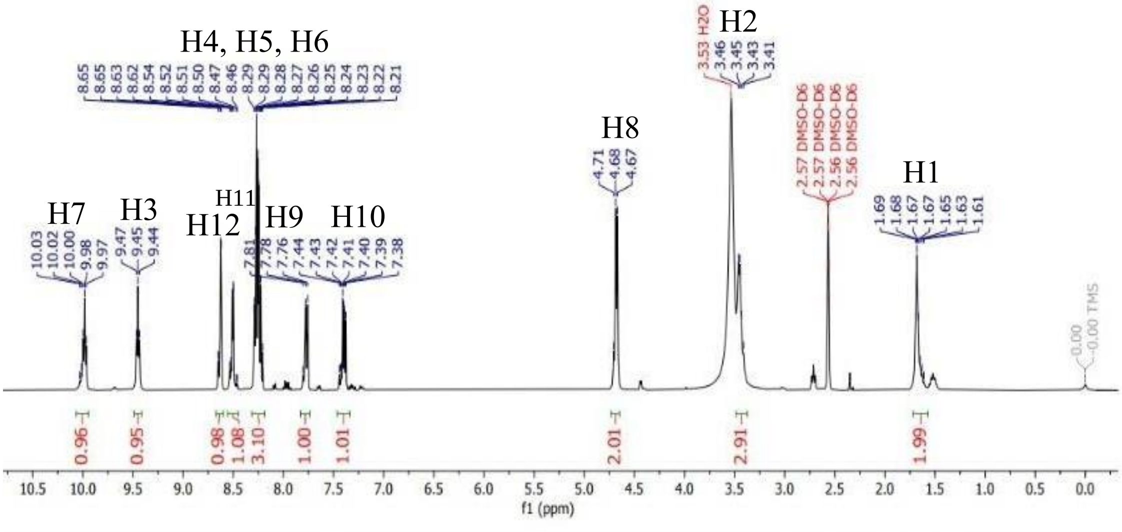


Figure 5. ^{1}H NMR spectrum of 1,2-bis[*N,N'*-6-(3-pyridylmethylamido)pyridyl-2-carboxyamido]butane.

Table 2. ^{1}H NMR data for 1,2-bis[*N,N'*-6-(3-pyridylmethylamido)pyridyl-2-carboxyamido]butane.

Assignment	Moiety, δ	Chemical shift, δ_H (ppm)
H1	m, 2H, CH_2	1.68
H2	m, 2H, CH_2	3.46
H8	t, 2H, CH_2	4.71
H10	m, 1H, CH	7.38 – 7.44
H9	m, 1H, CH	7.76 - 7.81
H4,H5,H6	m, 3H, CH_2	8.21 - 8.29
H11	m, 1H, CH	8.46 - 8.54
H12	m, 1H, CH	8.62 – 8.65
H3	t, 1H, NH	9.45
H7	t, 1H, NH	10.00

Aromatic compounds exhibit single lines in a benzene ring to increase a specific carbon setting together with any ^{13}C NMR spectrums (Figure 6). The resonance signals of δ(CH_2) were seen at 27.72 ppm and 28.99 ppm, respectively. The resonance signals associated with methyl (CH_2) carbons were found to fall within the expected range [25]. Due to the oxygen attached, the two carbonyl carbons (C=O) were predominantly deshielded, at 164.12 ppm and 163.52 ppm. This observation is consistent with results reported in another study [26]. At 124.09, 124.14 ppm, 124.92 ppm, 135.37 ppm, 135.40 ppm, 135.46 ppm, 140.09 ppm, 148.71 ppm, 148.88 ppm, 149.09 ppm and 149.32 ppm, the aromatic carbons (C-H, aromatic) appeared deshielded (Table 3).

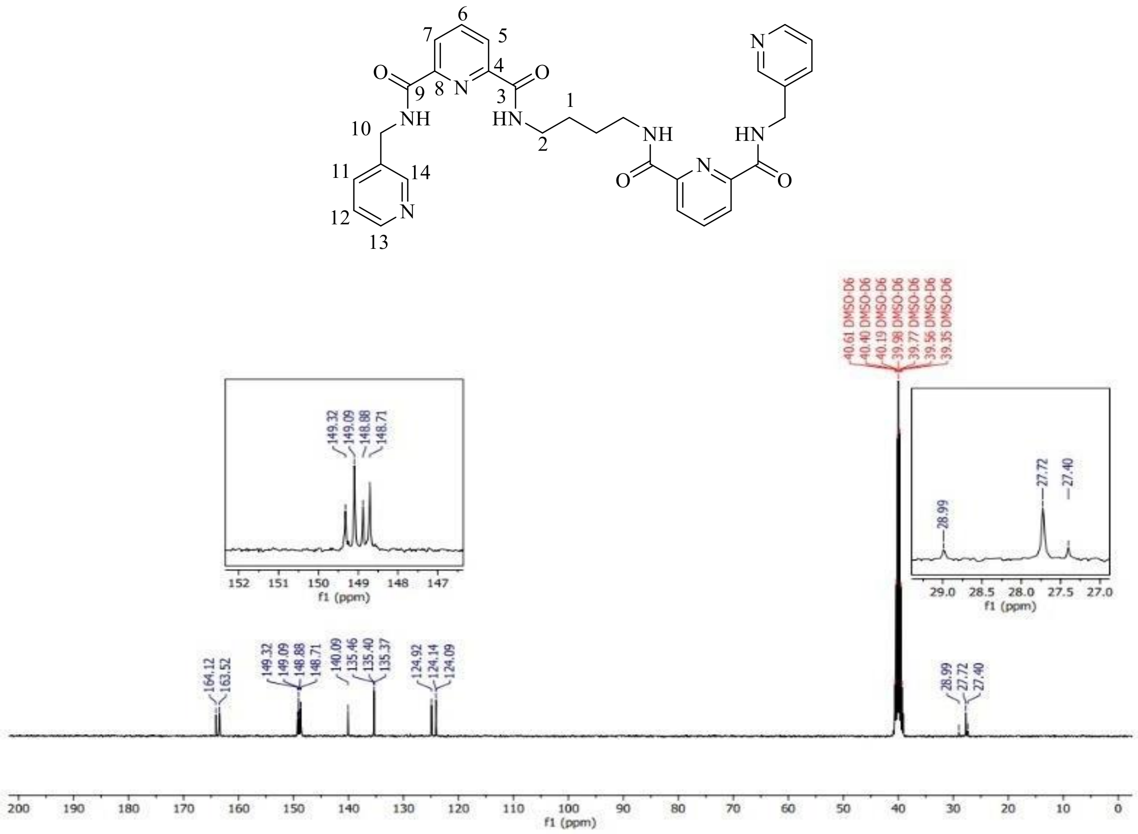


Figure 6. ^{13}C NMR spectrum of 1,2-bis[*N,N'*-6-(3-pyridylmethylamido)pyridyl-2-carboxyamido]butane

Table 3. ^{13}C NMR data for 1,2-bis[*N,N'*-6-(3-pyridylmethylamido)pyridyl-2-carboxyamido]butane

Assignment	Moiety, δ	Chemical shift, (ppm)
C1	CH_2	27.72
C2	CH_2	28.99
C5	C-H	124.14
C7	C-H	124.92
C13	C-H	135.37
C12	C-H	135.40
C11	C-H	135.46
C6	C-H	140.09
C14	C-H	148.71
C15	C-H	148.88
C4	C-H	149.09
C8	C-H	149.32
C3	C=O	163.52
C9	C=O	164.12

Anion Titration Studies

1,2-bis[*N,N*'-6-(3-pyridylmethylamido)pyridyl-2-carboxyamido]butane was found to bind with chromate, chloride and nitrate anions. The absorbance of the receptors shifted to the left from 224 nm to 222 nm, and shifted to the right from 364 nm to 367 nm, when titrated with chromate salts. A continuous shift in absorbance was observed following repeated injection of the chromate anion, indicating binding *via* hydrogen bonding interactions, as observed in a previous study [27]. As expected, the absorbance of the chromate anion at 364 nm increased with concentration. The ligand's absorbance at 222 nm steadily decreased in exchange, demonstrating significant contact or anticipated binding at the pyridine moieties (Figure 7).

When chloride was titrated with the host, the absorbance at 223 nm rose consistently and then sharply fell (Figure 8). The addition of more chloride anions caused a shift in the absorbance peak at 224 nm to 223 nm, indicating that the chloride anions interacted with the carbonyl moieties rather than the C=O chromophore and pyridine. In contrast, when 1,2-bis[*N,N'*-6-(3-pyridylmethylamido)pyridyl-2-carboxyamido]butane was titrated with nitrate anions (Figure 9), it produced inconsistently growing peaks at 223 nm. However, the absorbance peak at 224 nm disappeared when additional nitrate was added. According to a prior study, a low UV absorbance may indicate that the anion was highly concentrated and created a saturated anion complex, or that the anion-receptor contact was decreasing [28].

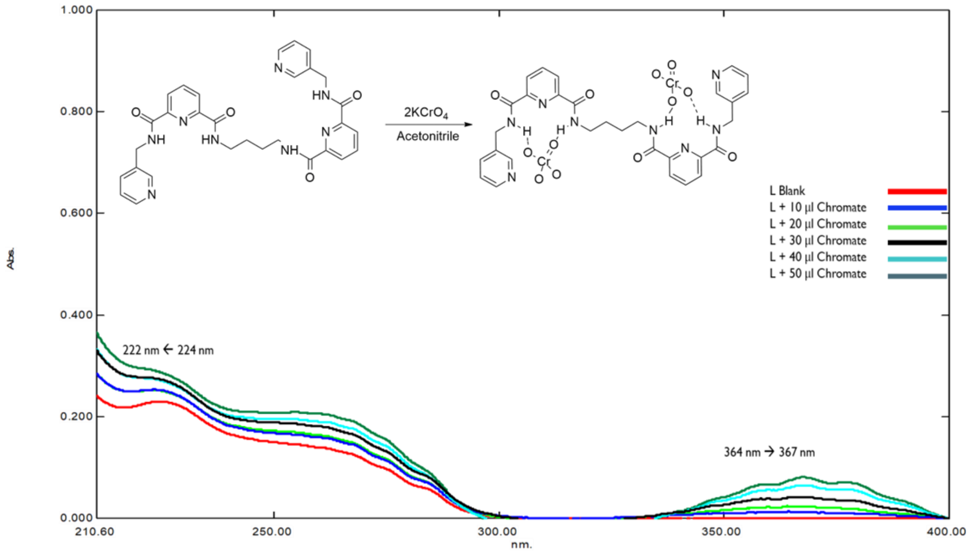


Figure 7. Titration study of the chromate anion.

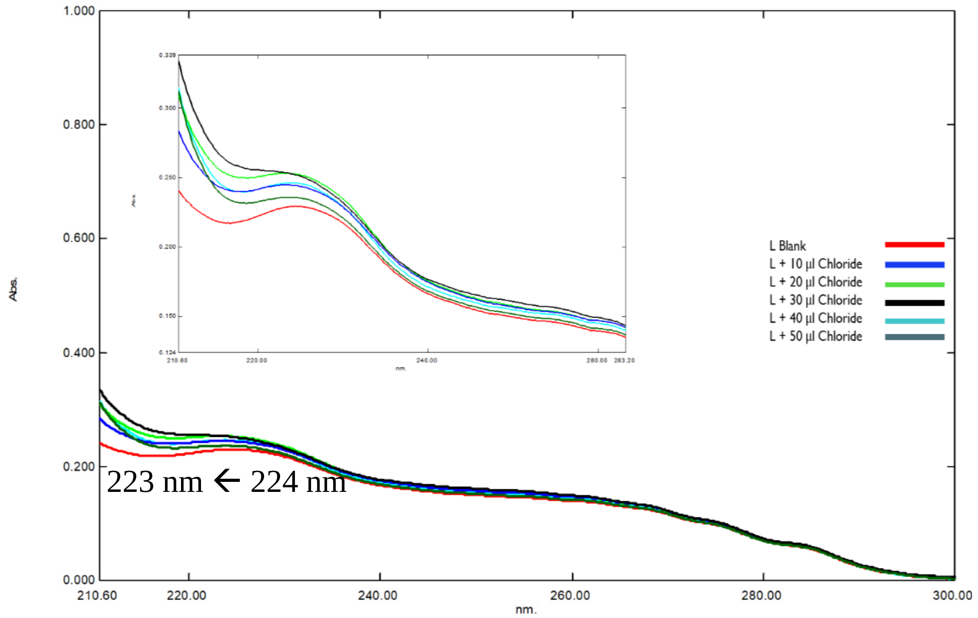


Figure 8. Titration study of the chloride anion.

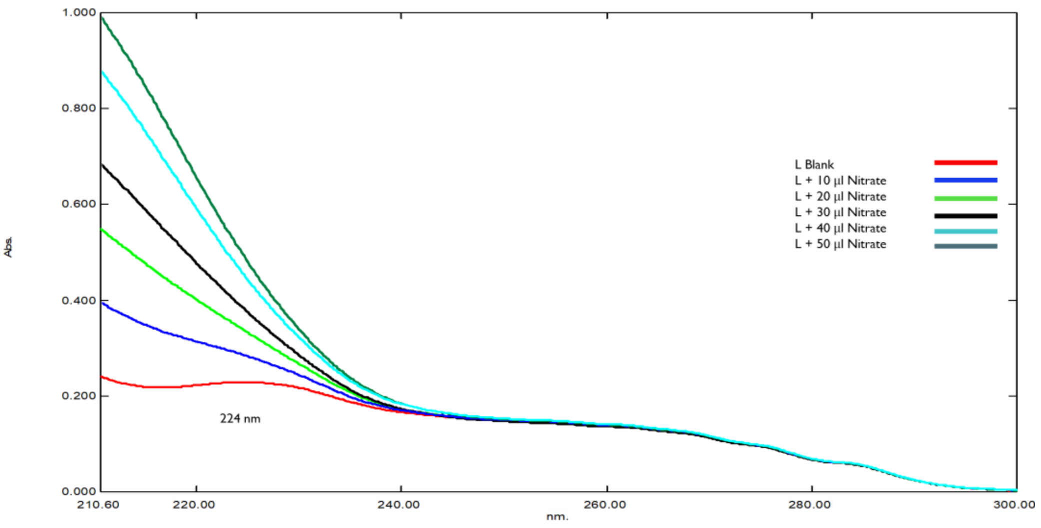


Figure 9. Titration study of the nitrate anion.

CONCLUSION

A new diamide compound composed of a butyl spacer and dicarboxamide moieties, namely, 1,2-bis[*N,N'*-6-(3-pyridylmethylamido)pyridyl-2-carboxyamido]butane was successfully synthesized and characterized by spectroscopic methods. The presence of non-covalent interactions such as hydrogen bonding was observed by common UV-Vis titration methods, and the results revealed that this compound had the potential to bind well with chromate anions compared to other investigated anion species. This is supported by consistent absorbance shifts in the UV spectrum of the NH amide region moieties. Due to this preliminary result, further anion recognition studies using NMR titrations and fluorescence titration methods are highly recommended. Further investigation should also focus on binding studies in different competitive solvents and the detection of low concentrations of anions.

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LIST OF ABBREVIATIONS

λ	Wavelength
λ_{max}	Maximum wavelength
δ	Chemical shift
v	Frequency
°C	Degree Celsius
%	Percentages
π	Pi
cm^{-1}	Per centimetre
CHN	Carbon, Hydrogen and Nitrogen
DMSO-d_6	Deuterated Dimethyl Sulfoxide
d	Doublet
FTIR	Fourier Transform Infrared
g	Gram
Hz	Hertz
IR	Infra-red
MHz	Megahertz
m	Multiplet/medium
mmol	Milimole
ml	Mililiter
µL	Microliter
NMR	Nuclear Magnetic Resonance
nm	Nanometer
ppm	Part per million
s	Strong/singlet
UV-vis	Ultraviolet-Visible

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