

Green Synthesis, Characterization and Photophysical Properties of Rhodamine Derivatives

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Abstract

Rhodamine 6G-chromone-derived compounds **RD1 - RD4** have been synthesized by condensation of rhodamine 6G hydrazide and substituted chromone aldehydes in ethanol using microwave-assisted reaction. The structures of these synthesized rhodamine 6G derivatives were confirmed by proton nuclear magnetic resonance (¹H-NMR), carbon nuclear magnetic resonance (¹³C-NMR), and high-resolution mass spectra data (HRMS). Colorimetric and photophysical studies show the synthesized compounds selectively detect copper (II) ion in aqueous acetonitrile solution.

Keywords

Rhodamine 6G, Microwave, Synthesis, Sensor, Absorbance, Fluorescence

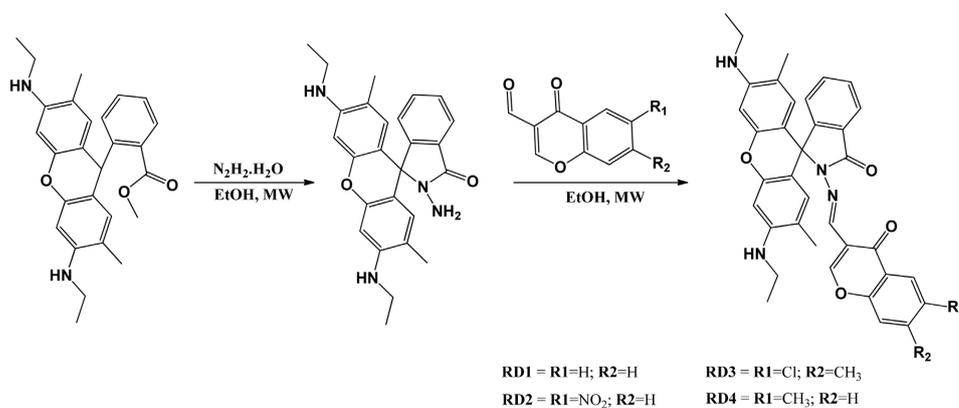
1. Introduction

The development of green chemistry and environmentally friendly organic synthetic methods progressed rapidly in recent years. It has been known for many years that rhodamine derivatives are a widely used class of compounds due to their versatile applications in various fields [1] [2]. Because of their strong fluorescence and photostability, rhodamine derivatives have been found to have applications in sensors [3] [4] [5], imaging [6], fluorescent markers [7], and in laser applications [8]. The rhodamine skeleton, which has excellent photophysical properties like long absorption and emission wavelength, high molar extinction coefficient, high fluorescence quantum yield, good solubility, and photostability, has attracted great interest [9] [10]. These compounds have been well established as fluorescent sensors for cations due to their ability to trigger the change in structure between the spirocyclic and ring-open spirolactam forms, which result

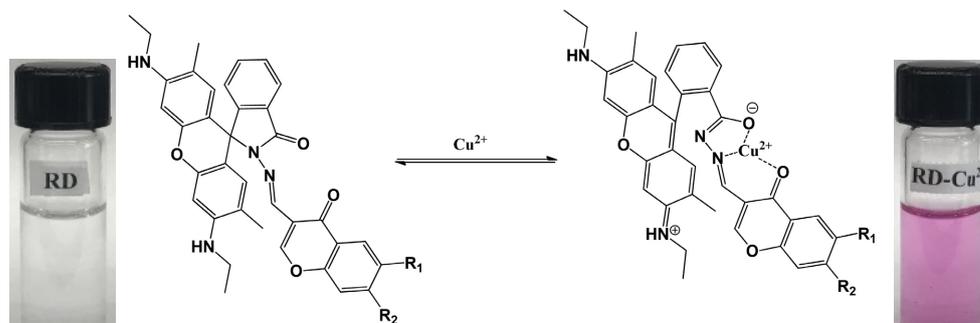
in different optical properties [11]. Several studies have shown that the type of the substituent group on the lactam ring has influence on the properties of rhodamine derivatives [12] [13].

Microwave application in organic synthesis has had exponential growth over the last twenty years due to its easy experimental conditions, rapid turnaround, and easy workups. Moreover, it is considered environmentally friendly and typically offers high yields, along with simplified processing and handling compared to conventional synthesis methods [14] [15] [16]. Microwave irradiation technique possesses several green attributes such as increased energy efficiencies, less purification process, cleanliness, and use of green solvents. More efficient ways of synthesizing rhodamine derivatives are needed so that a variety of products with various chemical and spectral properties can be made available to thoroughly investigate their potential use as optical sensors. The power of microwave synthesis is utilized for speeding the reaction and providing an efficient and convenient way of obtaining these rhodamine variations or related compounds [17].

In this work, we present an efficient, clean, and straightforward synthetic procedure to prepare four rhodamine-derived compounds. These target compounds are synthesized in a two-step reaction (Scheme 1), using mild conditions and inexpensive reagents under green conditions with relatively high yields.



Scheme 1. Microwave-assisted synthesis of compound **RD1 - RD4**.



Scheme 2. A possible proposed binding mechanism of compounds **RD1 - RD4** towards Cu^{2+} .

2. Experimental

2.1. Reagents and Instruments

All chemicals used in this study were commercially purchased and used as received. Hydrazine monohydrate (79%), Rhodamine 6G, Chrome-3- carboxaldehyde, 3-Formyl-6-nitrochromone, 6-Chloro-3-formyl-7-methylchromone, 3-Formyl-6-methylchromone, acetonitrile, ethanol, DMSO (d_6), and nitrate salts were used in this experiment. Microwave irradiation reactions were carried out in a CEM reactor. The CEM microwave irradiation system is developed to enhance the capacity to perform reactions under controlled conditions. The reactions were performed safely at a maximum temperature of 100°C. However, reactions can safely be performed at pressures up to 20 bar and temperatures ranging from 40°C to 300°C. $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ spectra were recorded at 400 MHz NMR on a Bruker Avance spectrometer with tetramethyl silane (TMS) as an internal standard and DMSO- d_6 as solvent. MALDI-HRMS analysis was recorded using Bruker 12T solarix FT-ICR-MS. All optical experiments were recorded using Agilent Cary 60 Ultraviolet -Visible (UV-Vis's) spectrometer and Varian Cary Eclipse fluorescence spectrophotometer respectively.

2.2. Microwave-Assisted Synthesis of RD1

Rhodamine 6G hydrazide intermediate was synthesized according to Yang's method [18]. A mixture of Rhodamine hydrazide intermediate (100 mg, 0.219 mmol), Chrome-3-carboxaldehyde (22 mg, 0.0406 mmol) and 2 ml of ethanol was placed in a microwave vessel. The resulting mixture was stirred and placed in a reactor. The reaction vessel was then run under pressure and irradiation at a specific temperature and time shown in **Table 1** and then the reaction vessel was cooled in ice bath. The reaction mixture was filtered out and washed three times with cold ethanol. After drying, the solid product was isolated, and obtained a higher yield ranging from 77% - 80%. $^1\text{H-NMR}$ (d_6 -DMSO), δ (ppm): 8.59 (s, 1H), 8.41 (s, 1H), 8.00 (m, 1H), 7.90 (m, 1H), 7.71 (m, 1H), 7.60 (m, 3H), 7.50 (m, 1H), 7.00 (d, 1H), 6.40 (s, 2H), 6.26 (s, 2H), 5.01 (s, 2H, -NH), 3.14 (q, 4H, NCH_2CH_3), 1.87 (s, 6H, $-\text{CH}_3$), 1.21 (t, 6H, NCH_2CH_3). $^{13}\text{C-NMR}$ (DMSO), δ (ppm): 165.23, 152.07, 151.33, 147.35, 132.31, 129.48, 128.00, 127.01, 123.43, 122.13, 117.79, 104.99, 95.85, 64.96, 55.99, 37.45, 18.53, 17.06, 14.20. HRMS (MALDI): m/z Calcd for **RD1** ($M + 1$): 585.2496; Found: 585.2504.

Table 1. Microwave-assisted irradiation reaction method for the preparation of **RD1** (phase II).

Trials	Method/Solvent	Temperature (°C)	Time (mins)	Yield (%)
T1	Microwave/Ethanol	100	30	78.5
T2	Microwave/Ethanol	100	20	80.5
T3	Microwave/Ethanol	80	30	77.7
T4	Microwave/Ethanol	80	20	82

2.3. Microwave-Assisted Synthesis of RD2

A mixture of Rhodamine hydrazide derivative (100 mg, 0.219 mmol), 3-Formyl-6-nitrochromone (22 mg, 0.0511 mmol) and 2 ml of solvent was placed in a microwave vessel. The resulting mixture was stirred and placed in a reactor. The reaction vessel was then run under pressure and irradiation at a specific temperature and time as shown in **Table 2**. The reaction mixture was filtered out and washed three times with cold ethanol. After drying, the solid product was isolated, and obtained a higher yield ranging from 64% - 84%. ¹H-NMR (d₆-DMSO), δ (ppm): 8.68 (s, 1H), 8.50 (m, 2H), 7.90 (m, 2H), 7.62 (m, 2H), 7.00 (m, 2H), 6.27 (s, 2H), 6.10 (s, 2H), 5.11 (s, 2H, -NH), 3.14 (q, 4H, NCH₂CH₃), 1.86 (s, 6H, -CH₃), 1.22 (t, 6H, NCH₂CH₃). ¹³C-NMR (DMSO), δ (ppm): 164, 153, 152, 145, 1321, 130, 127.02, 127.01, 122.43, 120.23, 116.79, 103.99, 94.85, 63.96, 54.99, 36.44, 18.43, 17.05, 14.20. HRMS (MALDI): m/z Calcd for **RD2** (M + 1): 630.2347; Found: 630.2364.

2.4. Microwave-Assisted Synthesis of RD3

A mixture of Rhodamine hydrazide derivative (100 mg, 0.219 mmol), 6-Chloro-3-formyl-7-methylchromone (22 mg, 0.0519 mmol) and 2 ml of ethanol was placed in a microwave vessel. The resulting mixture was stirred and placed in a reactor. The reaction vessel was then run under pressure and irradiation at a specific temperature and time as shown in **Table 3**. The reaction mixture was filtered out and washed three times with cold ethanol. After drying, the solid product was isolated, and obtained a higher yield ranging from 78% - 84%. ¹H-NMR (d₆-DMSO), δ (ppm): 8.62 (s, 1H), 8.46 (s, 1H), 7.88 (m, 2H), 7.60 (s, 1H), 7.58 (m, 2H), 7.00 (m, 1H), 6.40 (s, 2H), 6.23 (s, 2H), 5.04 (s, 2H, -NH), 3.15 (q, 4H, NCH₂CH₃), 1.89 (s, 6H, -CH₃), 1.22 (t, 6H, NCH₂CH₃). ¹³C-NMR

Table 2. Microwave-assisted irradiation reaction method for the preparation of **RD2** (phase II).

Trials	Method/Solvent	Temperature (°C)	Time (mins)	Yield (%)
T1	Microwave/Ethanol	100	30	65
T2	Microwave/Ethanol	100	20	64
T3	Microwave/Ethanol	80	30	84
T4	Microwave/Ethanol	80	20	84.6

Table 3. Microwave-assisted irradiation reaction method for the preparation of **RD3** (phase II).

Trials	Method/Solvent	Temperature (°C)	Time (mins)	Yield (%)
T1	Microwave/Ethanol	100	30	80
T2	Microwave/Ethanol	100	20	78
T3	Microwave/Ethanol	80	30	82
T4	Microwave/Ethanol	80	20	84

(DMSO), δ (ppm): 165.23, 152.07, 151.33, 147.35, 132.31, 129.48, 128.00, 127.01, 123.43, 122.13, 117.79, 104.99, 95.85, 64.96, 55.99, 37.45, 18.53, 17.06, 14.20. HRMS (MALDI): m/z Calcd for **RD3** ($M + 1$): 633.2263; Found: 633.2263.

2.5. Microwave-Assisted Synthesis of RD4

A mixture of Rhodamine hydrazide derivative (100 mg, 0.219 mmol), 3-Formyl-6-methylchromone (22 mg, 0.0439 mmol) and 2 ml of solvent was placed in a vessel. The resulting mixture was stirred and placed in a reactor. The reaction vessel was then run under pressure and irradiation at a specific temperature and time as shown in **Table 4**. The reaction mixture was filtered out and washed three times with cold ethanol. After drying, the solid product was isolated, and obtained a higher yield ranging from 81% - 89%. $^1\text{H-NMR}$ (d_6 -DMSO), δ (ppm): 8.62 (s, 1H), 8.40 (s, 1H), 7.88 (m, 1H), 7.70 (s, 1H), 7.56 (m, 4H), 7.02 (m, 2H), 6.37 (s, 2H), 6.20 (s, 2H), 5.08 (s, 2H, -NH), 3.20 (q, 4H, NCH_2CH_3), 2.40 (s, 3H, - CH_3), 1.86 (s, 6H, - CH_3), 1.21 (t, 6H, NCH_2CH_3). $^{13}\text{C-NMR}$ (DMSO), δ (ppm): 165.23, 152.07, 151.33, 147.35, 132.31, 129.48, 128.00, 127.01, 123.43, 122.13, 117.79, 104.99, 95.85, 64.96, 55.99, 37.45, 18.53, 17.06, 14.20. HRMS (MALDI): m/z Calcd for **RD4** ($M + 1$): 599.2652; Found: 599.2655.

3. Results and Discussion

3.1. Microwave-Assisted Synthesis

Novel green microwave-assisted synthesis methods using ethanol as a solvent for the synthesis of rhodamine 6G-Chromone imines have been established. A total of four rhodamine 6G derived imine derivatives were synthesized using a controlled CEM microwave heating reactor under closed-vessel conditions. The microwave system is equipped with a magnetic stirrer as well as temperature and power controls. Microwave-assisted synthesis of Rhodamine hydrazide with various substituted chromone compounds such as Chrome-3-carboxyaldehyde, 3-formyl-6-Nitrochromone, 6-Chloro-3-formyl-7-methylchromone, 3-formyl-6-methylchromone, and 2-amino-3-formylchromone in ethanol results all target products in high yield, as illustrated in **Scheme 1**. In the first phase, the intermediate compound was synthesized by microwave irradiation of Rhodamine 6G and excess hydrazine hydrate in ethanol, and in the second phase, the target products (**RD1 - RD4**) were synthesized by microwave irradiation and condensation of the resulting rhodamine 6G hydrazone and corresponding aldehydes in

Table 4. Microwave-assisted irradiation reaction method for the preparation of **RD4** (phase II).

Trials	Method/Solvent	Temperature ($^{\circ}\text{C}$)	Time (mins)	Yield (%)
T1	Microwave/Ethanol	100	30	86
T2	Microwave/Ethanol	100	20	81.6
T3	Microwave/Ethanol	80	30	83.3
T4	Microwave/Ethanol	80	20	89

1:1 molar ratio in ethanol. The microwave-assisted synthesis results in a green and fast synthesis time, minimum solvent use, easy operation, and scalability. The reaction conditions for the microwave-assisted synthesis of rhodamine 6G imine derivatives **RD1** - **RD4** are summarized in **Tables 1-4**. The optimized conditions for each rhodamine 6G-Chromone based imine derivatives were determined by conducting extensive temperature and time studies. It was observed that microwave-assisted reaction with temperature 80 °C afforded the best result with 82% - 89% product yield for 20 - 30 minutes (**Tables 1-4**, entry 3 and 4). The results indicate that microwave irradiation method accelerates synthesis of Rhodamine 6G-Chromone imine products in minutes. Due to our interest in green chemistry, minimum solvent conditions were mainly exploited. The target products required no rigorous purification, and pure solid products were isolated from the reaction mixture. Therefore, this irradiation method offers an easy and practical access for the synthesis of a series of rhodamine 6G-based imine products. Structural analysis of these rhodamine 6G-Chromone imine derivatives were performed with ¹H-NMR, ¹³C-NMR, and high-resolution mass spectrometry, and all data are in accordance with the proposed structure (**Figures S1-S12**).

3.2. Optical Properties

The rhodamine 6G component has been demonstrated as an attractive sensor with emission turn on effect [18] [19]. In this work, chrome-3-carboxaldehyde, 3-Formyl-6-nitrochromone, 6-Chloro-3-formyl-7-methylchromone, and 3-Formyl-6-methylchromone were used to modify rhodamine component, hoping to optimize a suitable structure for sensing application. All the photophysical studies were performed in aqueous acetonitrile in which rhodamine 6G-chromone compounds **RD1** - **RD4** formed colorless solutions. In addition, the absorption spectra showed no peak above 400 nm due to the predominant ring-closed spirolactum (**Figure 1(a)**). This was further confirmed by the ¹³C NMR signal around δ 66 corresponding to bridging carbon, C-1 (**Figure S2**). Similarly, compounds were very weakly fluorescent at 565 nm ($\lambda_{\text{ex}} = 488$ nm) in the absence of any analyte due to the predominant ring-closed spirolactum. The fluorescence spectrum of target compounds showed a peak at 565 nm upon the addition of Cu²⁺ corresponding to the delocalization in the xanthene moiety of rhodamine 6G (**Figure 2**). Several studies show that substituents can affect the optical properties of compounds [20]-[25]. As shown in **Figure 2(a)**, Rhodamine 6G derivative **RD1** with no substituent on the chromone unit exhibits excellent selectivity and sensitivity towards Cu²⁺. Rhodamine 6G derivatives **RD3** and **RD4** were designed to study the effect of an electron-donating group at the chromone ring. These compounds showed less fluorescence emission upon binding with copper (II) ion and some interference from Ni²⁺ and Pb²⁺ ions. The presence of a strong electron-withdrawing group on the chromone unit in compound **RD2** can significantly affect the solubility and optical properties. The results indicate different electronic distributions among the compound's structure

have an influence on their optical sensing properties. Furthermore, Job's plot experiment was used to investigate the stoichiometry of rhodamine-copper (II) ion binding. In an aqueous acetonitrile solution, the rhodamine derivative **RD1** is coordinated to copper (II) ion with a 1:1 stoichiometry, **Figure 3**. As demonstrated in **Scheme 2**, a monomeric system forms a 1:1 complex, which is a reversible process.

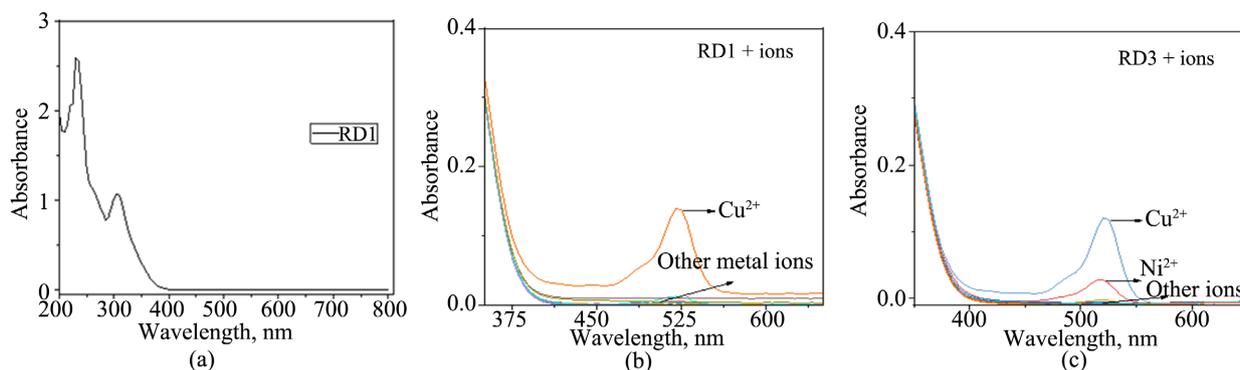


Figure 1. UV-Vis's spectra of 20 μM (a) free **RD1**, (b) **RD1** with 20 mM metal ions in $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (7:3 v/v) HCl-tris buffer solution and (c) **RD3** with 20 mM metal ions in $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (7:3 v/v) HCl-tris buffer solution.

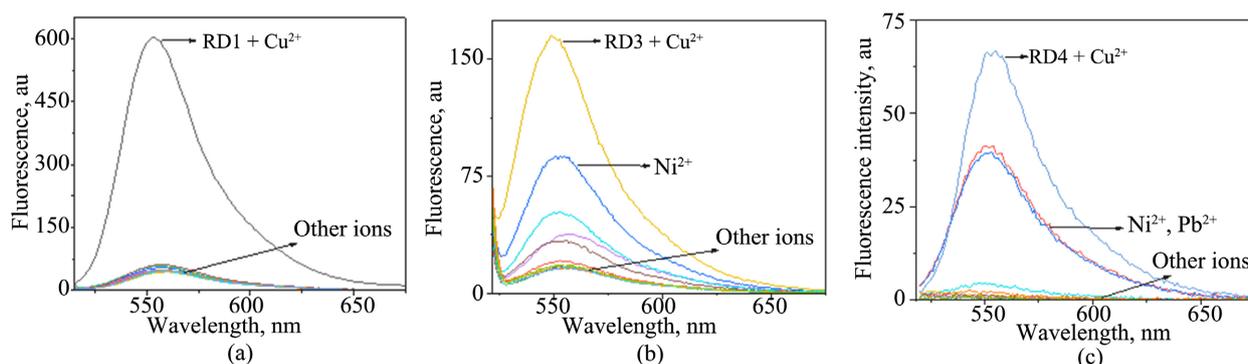


Figure 2. Fluorescence spectra of 20 μM (a) **RD1**, (b) **RD3** (c) **RD4** with 20 mM metal ions in $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (7:3 v/v) HCl-tris buffer solution.

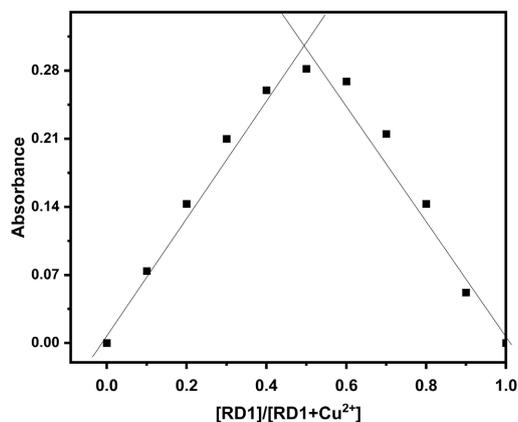


Figure 3. Binding stoichiometry and Job's plot experiment for compound **RD1** and Cu^{2+} in acetonitrile solution.

4. Conclusion

The microwave irradiation synthesis method described here is the most convenient way to synthesize the rhodamine 6G derivatives. The CEM single mode microwave irradiation system has provided substantially decreased reaction time, high yield, simple experimental procedure, and environmental friendliness. Upon binding, copper (II) ion triggers the formation of a highly fluorescent ring-open spirolactam system, while other ions showed no significant change. The recognition ability of compounds **RD1** - **RD4** was investigated by absorbance and fluorescence spectroscopy.

Acknowledgements

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Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplyment

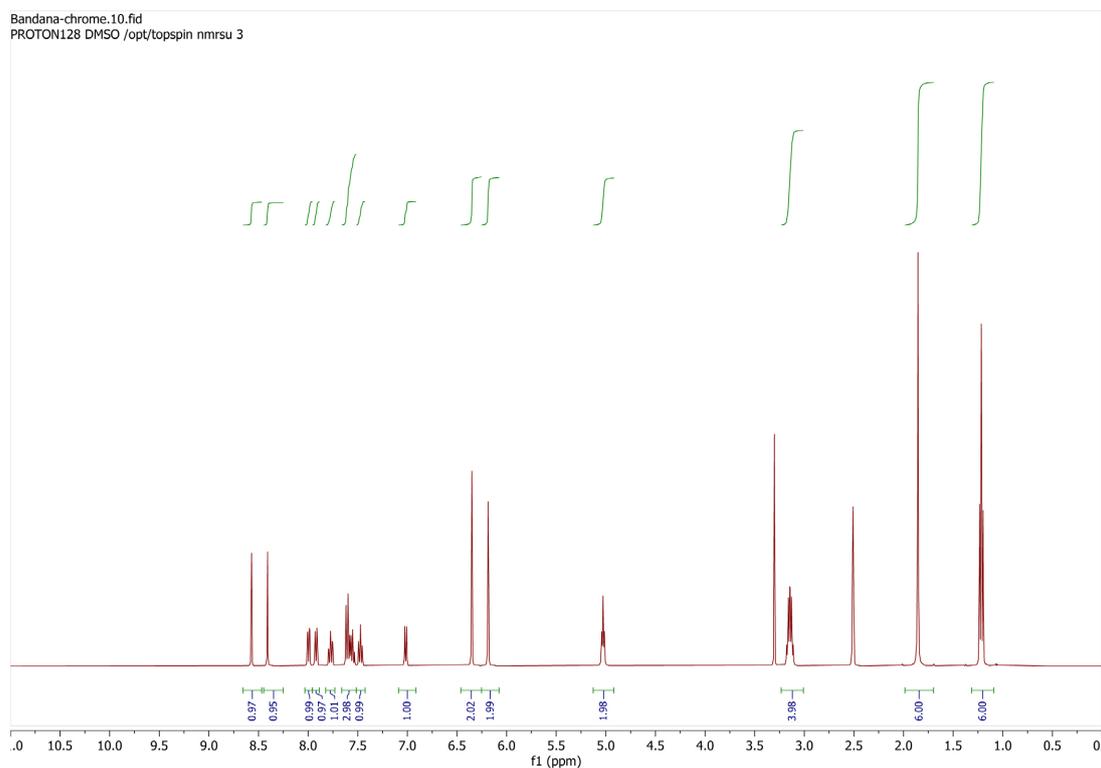


Figure S1. Proton NMR spectrum of compound **RD1** (DMSO-d₆, 400 MHz).

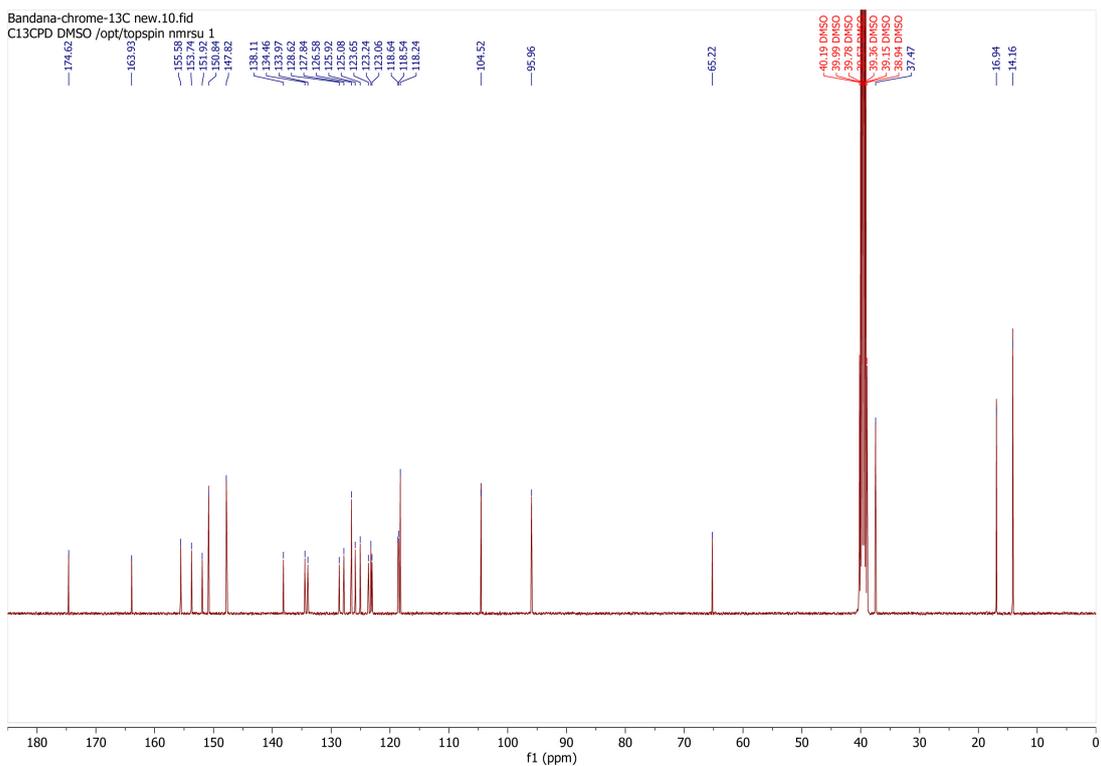


Figure S2. Carbon NMR spectrum of compound **RD1** (DMSO-d₆, 400 MHz).

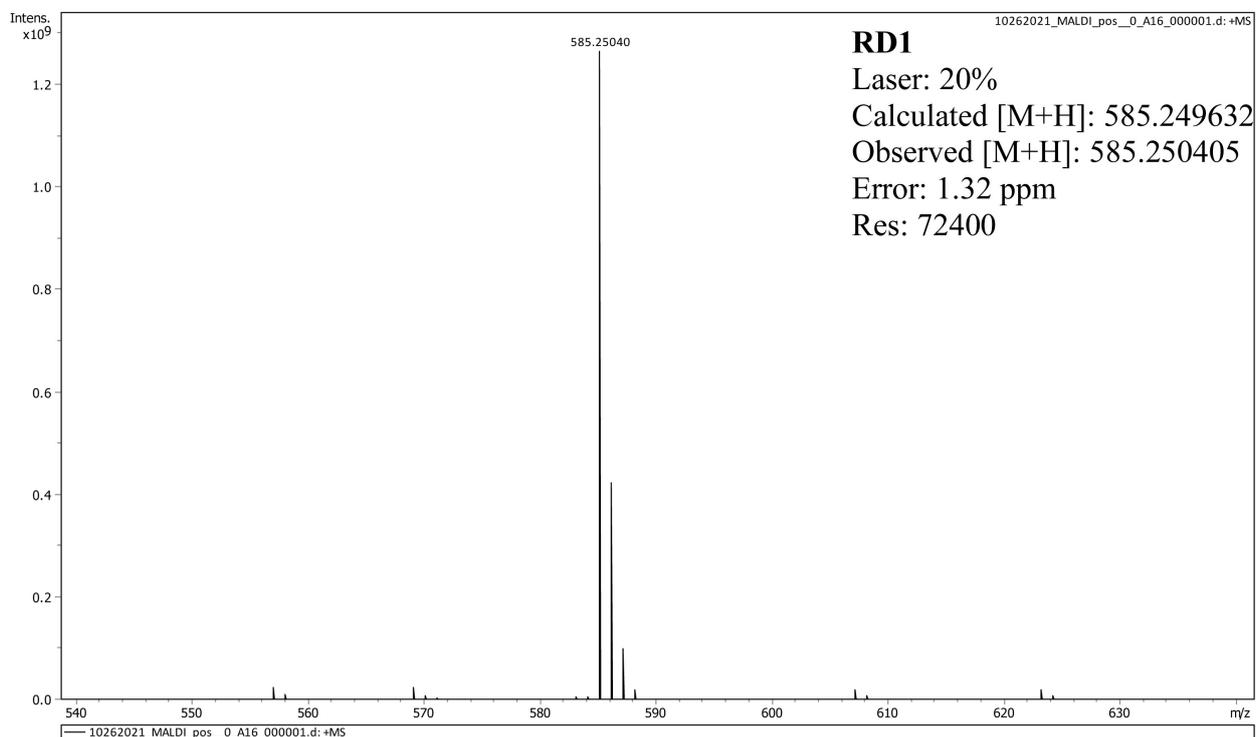


Figure S3. HRMS spectra of compound **RD1** (MALDI positive mode).

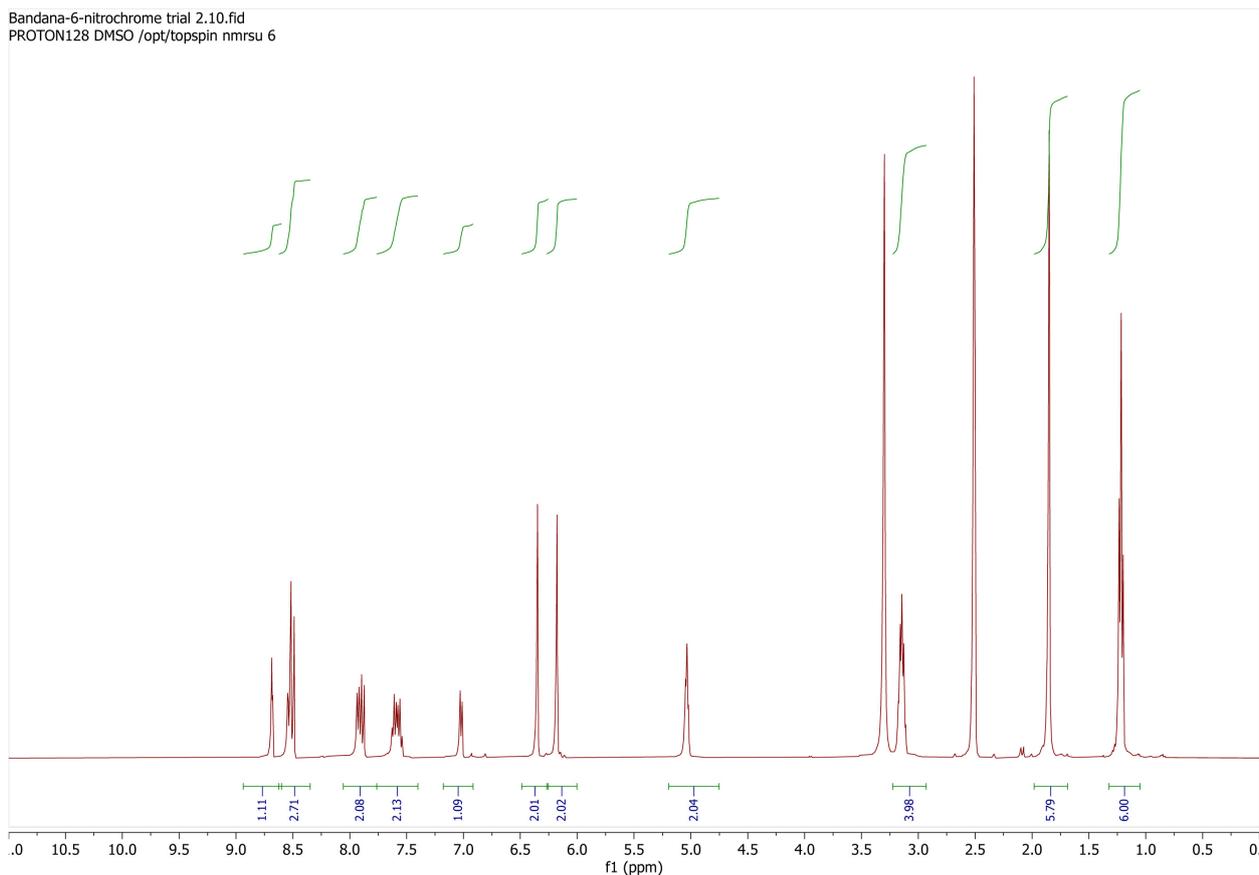


Figure S4. Proton NMR spectrum of compound **RD2** (DMSO-d₆, 400 MHz).

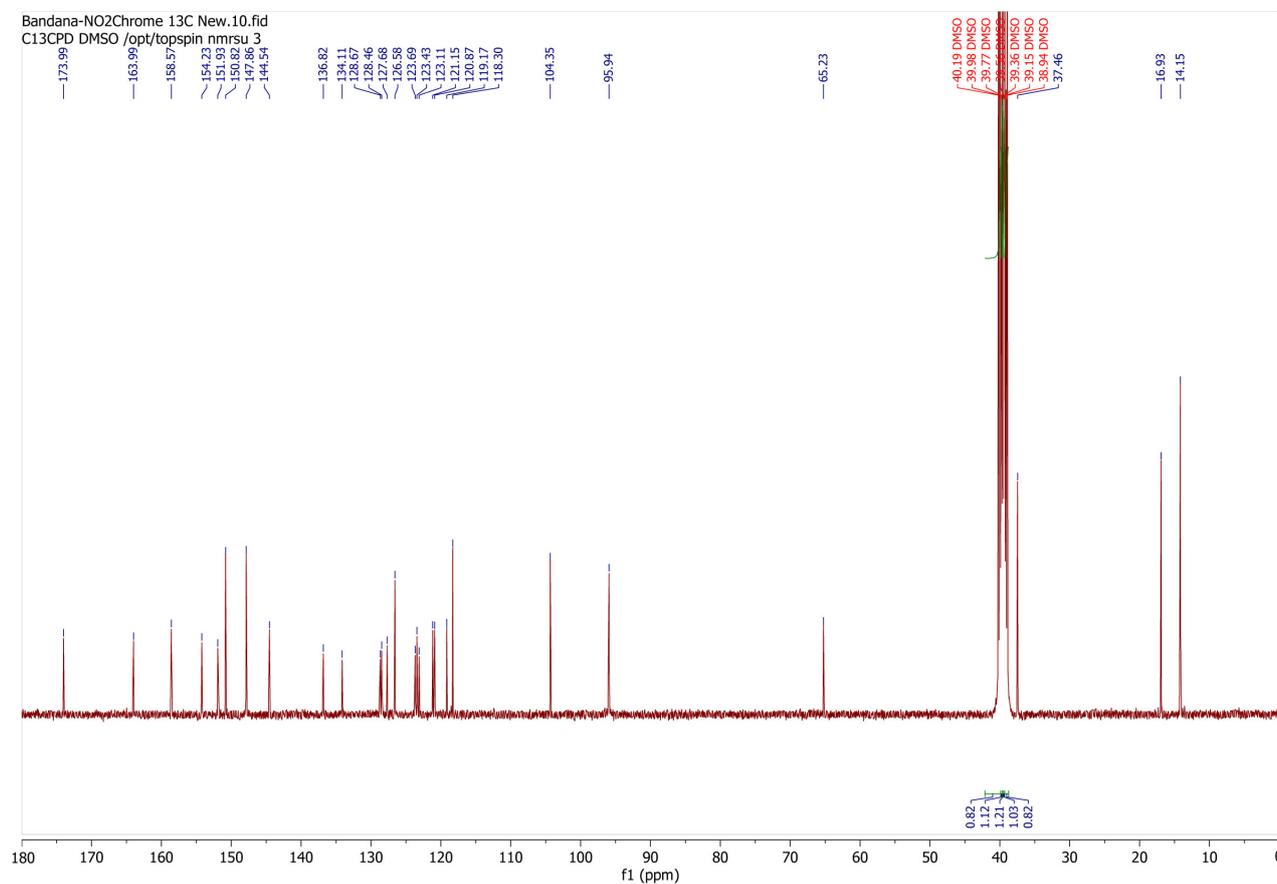


Figure S5. Proton NMR spectrum of compound RD2 (DMSO-d₆, 400 MHz).

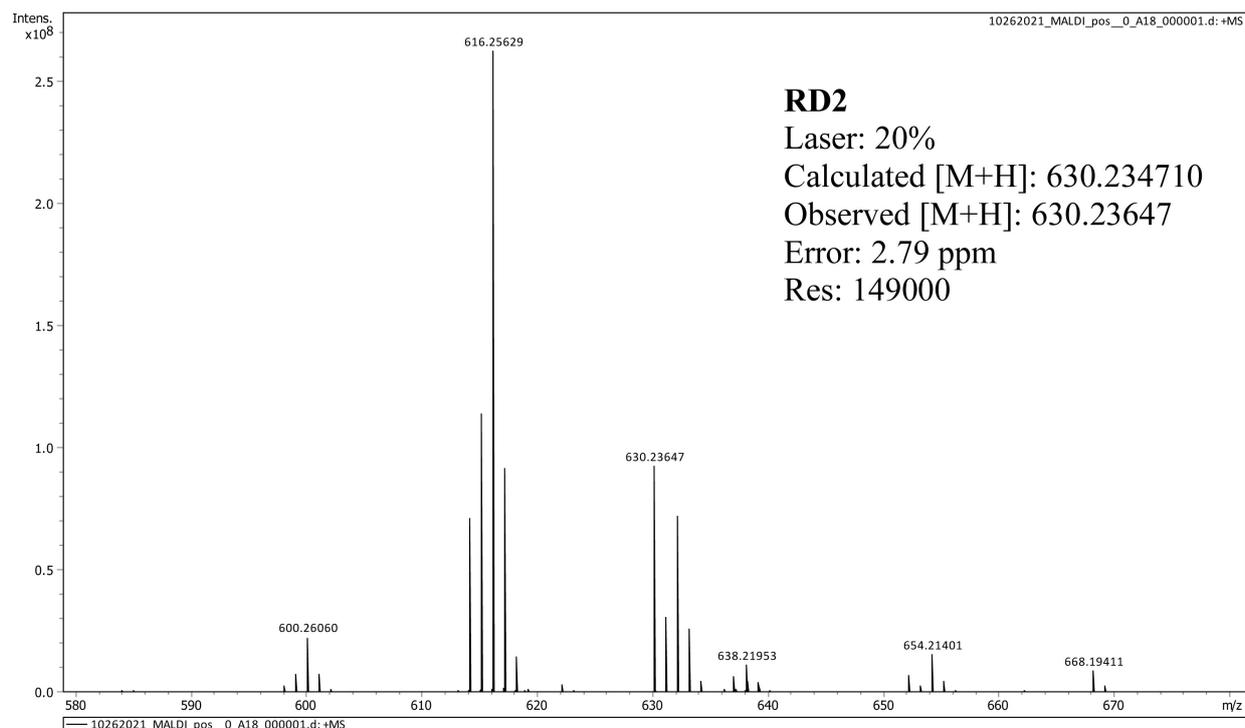


Figure S6. HRMS spectra of compound RD2 (MALDI positive mode).

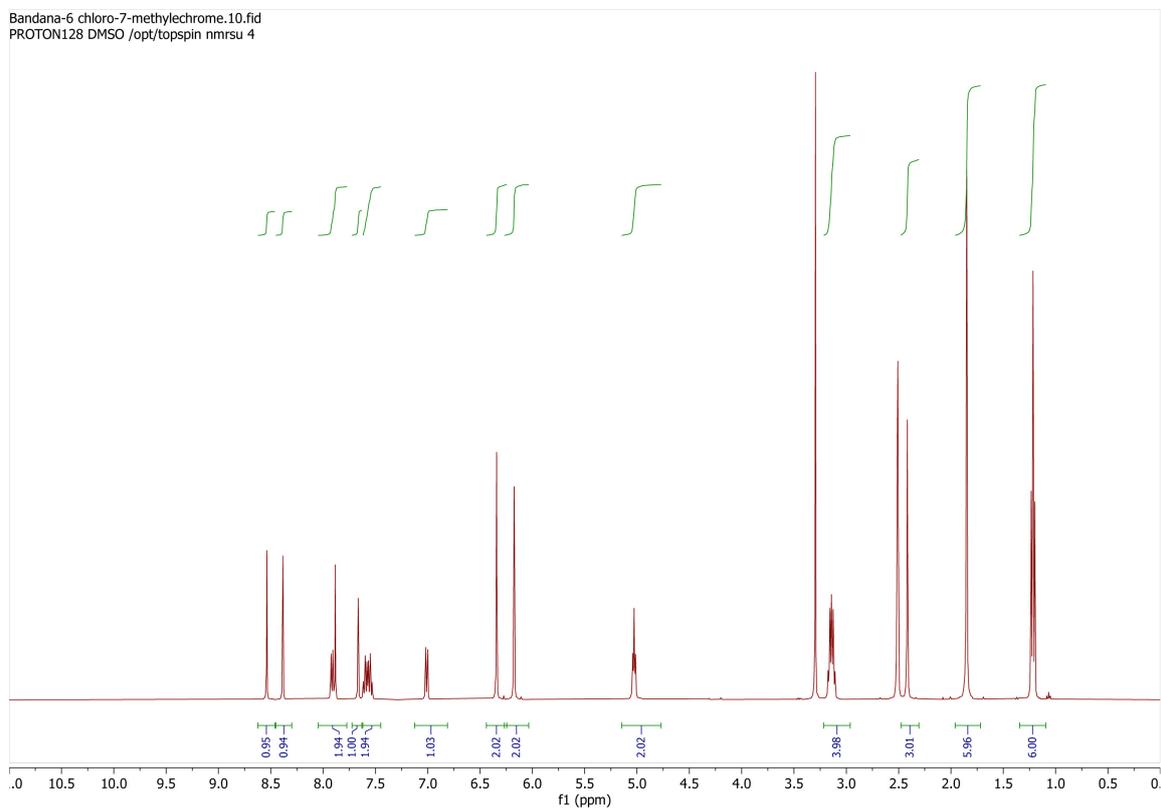


Figure S7. Proton NMR spectrum of compound **RD3** (DMSO-d₆, 400 MHz).

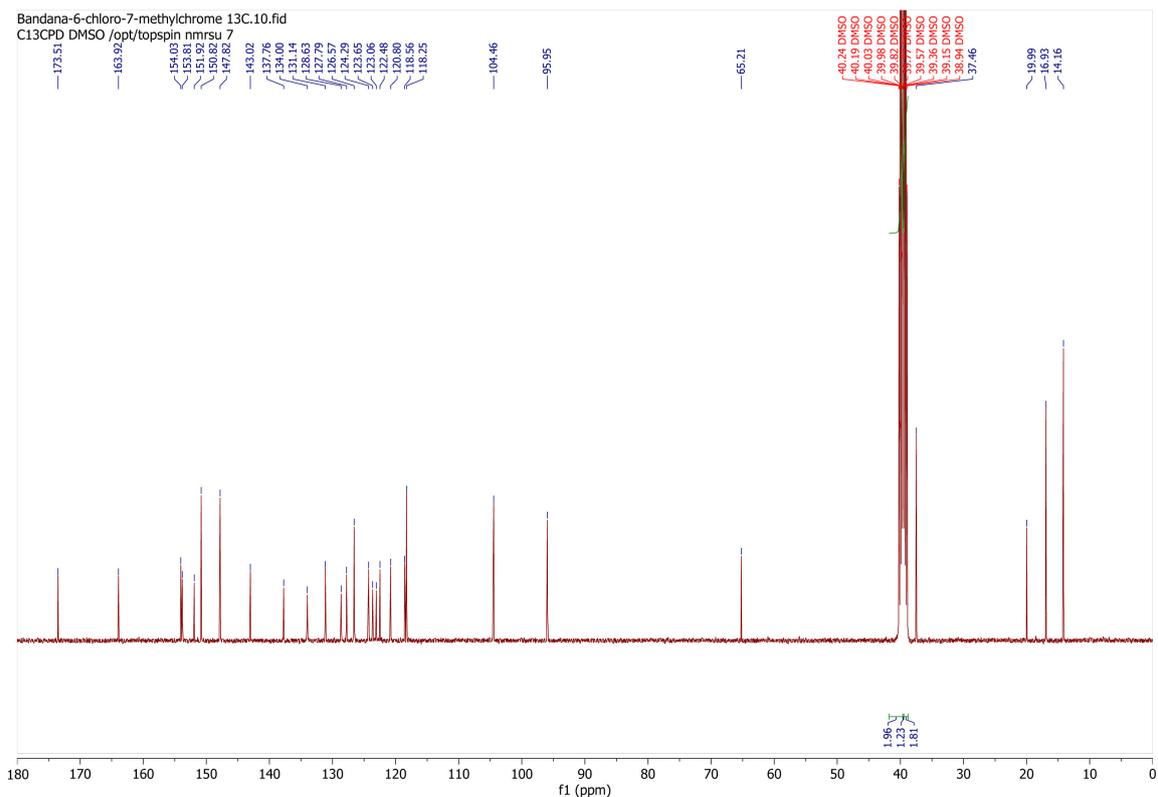


Figure S8. Carbon NMR spectrum of compound **RD3** (DMSO-d₆, 400 MHz).

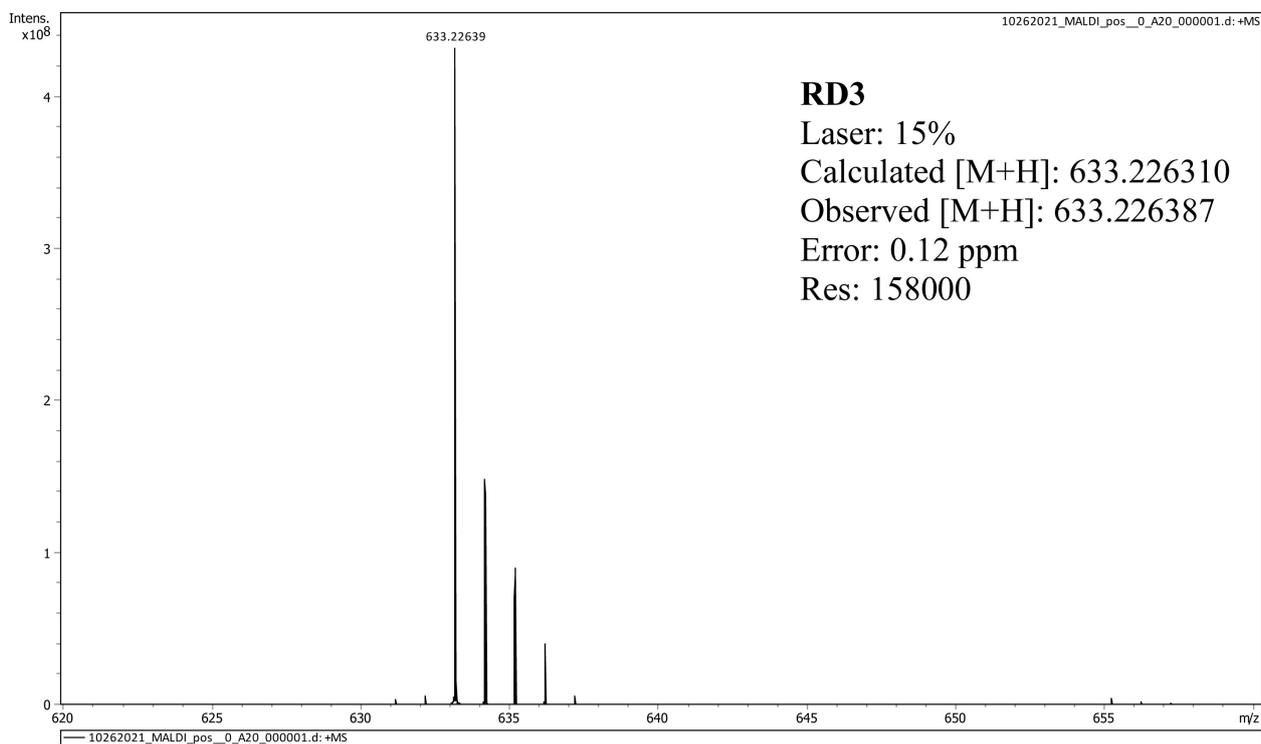


Figure S9. HRMS spectra of compound **RD3** (MALDI positive mode).

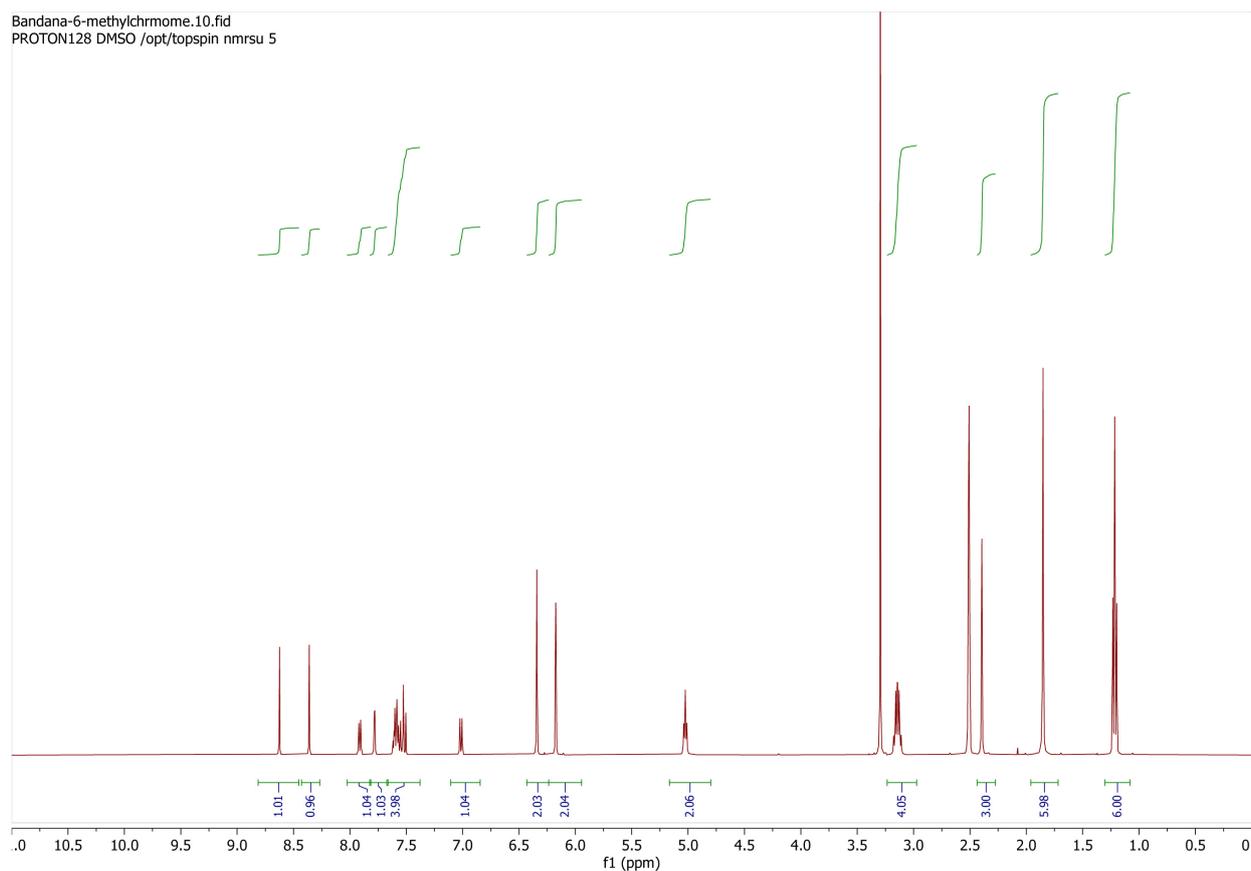


Figure S10. Proton NMR spectrum of compound **RD4** (DMSO-d₆, 400 MHz).

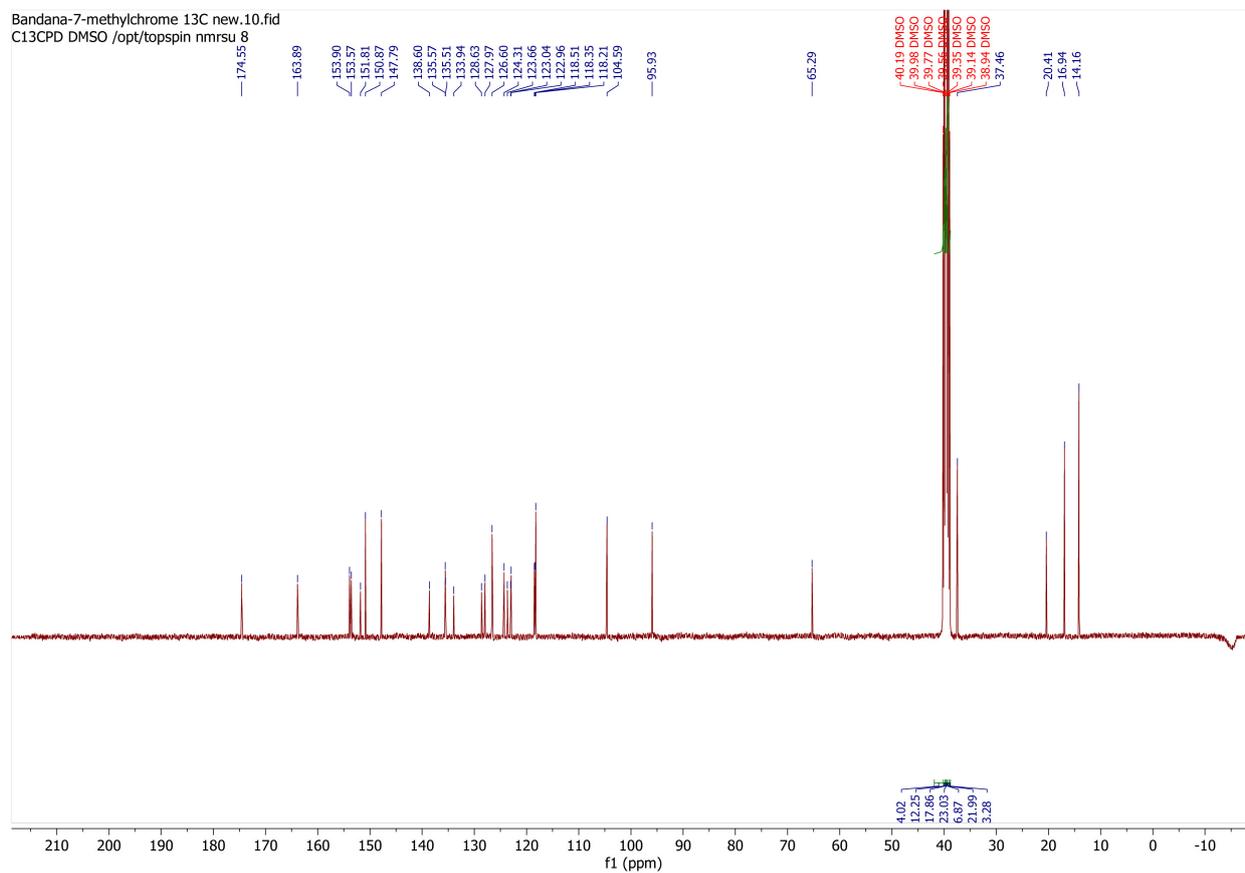


Figure S11. Carbon NMR spectrum of compound **RD4** (DMSO-d₆, 400 MHz).

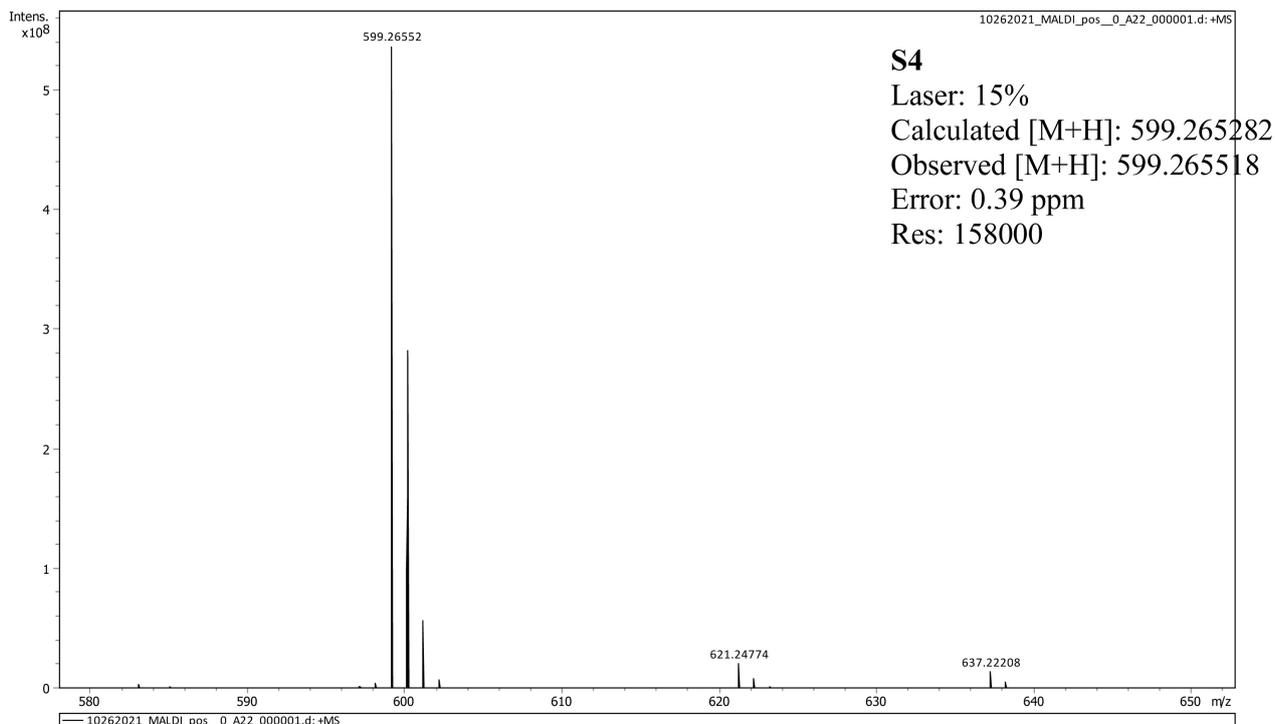


Figure S12. HRMS spectra of compound **RD4** (MALDI positive mode).