One-Pot Iodine-Mediated Synthesis of Cannabinol and Derivatives

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การสังเคราะห์แคนนาบินอลและอนุพันธ์แบบวันพอตโดยใช้ไอโอดีนเป็นตัวกลาง

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มงคลกรณ์ ธนากรเจริญวิทย์ : การสังเคราะห์แคนนาบินอลและอนุพันธ์แบบวันพอตโดยใช้ไอโอดีนเป็นตัวกลาง. (One-Pot Iodine-Mediated Synthesis of Cannabinol and Derivatives) อ.ที่ปรึกษาหลัก : ศ. ดร.ธีรยุทธ วิไลวัลย์, อ.ที่ปรึกษาร่วม : ผศ. ดร.ธนธรณ์ ขอทวีวัฒนา

แคนนาบินอล (CBN) เป็นสารจากพืชตระกูลกัญชาที่พบในปริมาณน้อยและแสดงฤทธิ์ทางชีวภาพที่หลากหลายโดยแสดงผลกระทบต่อระบบจิตประสาท วิธีหนึ่งที่ใช้ในการสังเคราะห์ CBN คือปฏิกิริยาระหว่างสารประกอบเทอร์พีนอยด์และอนุพันธ์ของรีซอร์สซินอลผ่านกระบวนการต่อเนื่องหลายขั้นตอนที่ประกอบด้วยปฏิกิริยาฟรีเดล-คราฟต์ แอลคิเลชัน, การปิดวง, และการเกิดอะโรมาไทซ์ ในการศึกษานี้ ผู้วิจัยคาดว่าไอโอดีนสามารถทำหน้าที่เป็นลิวอิสแอซิดซึ่งเป็นตัวเร่งปฏิกิริยาและตัวทำให้เกิดการอะโรมาไทซ์ ดังนั้นจึงได้พัฒนาปฏิกิริยาระหว่าง (1*S*,4*R*)-*p*-menthadienol และ 5-(1,1-dimethylheptyl)resorcinol ที่มีไอโอดีนเป็นตัวกลางในรูปแบบวันพอตสำหรับการสังเคราะห์ 3-(1,1-dimethylheptyl)cannabinol (DMH-CBN) โดยได้มีการประเมินตัวแปรต่าง ๆ ของปฏิกิริยา และศึกษาร้อยละผลได้ของผลิตภัณฑ์จากปฏิกิริยาโดย ^{1}H NMR พบว่า ภาวะที่เหมาะสมคือการใช้สารตั้งต้น 1.5 และ 1.0 อีควิวาเลนต์ และไอโอดีน 5.0 อีควิวาเลนต์ในตัวทำละลายโทลูอีนที่อุณหภูมิรีฟลักซ์เป็นเวลา 1 ชั่วโมง ให้ผลผลิตเป็น DMH-CBN ที่แยกได้ถึง 71% ปฏิกิริยาเดียวกันนี้ภายใต้ภาวะไมโครเวฟให้ผลิตภัณฑ์เดียวกันโดยมีร้อยละผลได้จาก NMR เป็น 30% วิธีเดียวกันนี้สามารถนำไปใช้ในการสังเคราะห์ CBN จากซิทรัลเป็นสารตั้งต้นเทอร์พีนอยด์ที่มีราคาถูก และโอลิฟทอล โดยให้ผลิตภัณฑ์ที่คาดไว้และแยกได้ถึง 22% การศึกษาขอบเขตของสารตั้งต้นพบกว่าวิธีการสังเคราะห์ CBN โดยใช้ไอโอดีนในรูปแบบวันพอตนี้สามารถให้ผลผลิตเป็นอนุพันธ์ CBN ที่มีหมู่แทนที่ชนิดแอลคิล รวมถึง CBN ที่ไม่มีหมู่แทนที่ที่ตำแหน่ง C3 ที่แยกได้ระหว่าง 28–63% สำหรับ CBN ที่ไม่มีหมู่แทนที่เมื่อทำปฏิกิริยากับ เอ็น-โบรโมซักซินิไดด์ 1.0 หรือ 2.0 อีควิวาเลนต์ให้ผลิตภัณฑ์เป็นอนุพันธ์ 4-โบรโม และ 2,4-ไดโบรโม โดยมีร้อยละผลผลิตที่แยกได้เป็น 63% และ 90% ตามลำดับ อย่างไรก็ตาม ภาวะนี้ไม่เหมาะสมกับรีซอร์สซินอลที่มีหมู่แทนที่แบบดึงอิเล็กตรอน โดยไม่ให้ผลิตภัณฑ์ที่ต้องการหรือได้ในปริมาณน้อยมาก อนุพันธ์ CBN ที่สังเคราะห์ได้บางชนิดได้นำมาประเมินประสิทธิภาพในการป้องกันการทำลายเซลล์เยื่อบุผิวผ่านการทดสอบ transepithelial electrical resistance (TER) โดยพบว่า 2,4-ไดเอทิล CBN และอนุพันธ์, 4-โบรโม และ 2,4-ไดโบรโม ของ CBN ที่ปราศจากหมู่แทนที่ที่ตำแหน่ง C3 แสดงฤทธิ์ที่ดีกว่า CBN ธรรมชาติ ดังนั้นสารเหล่านี้อาจเป็นตัวเลือกใหม่ของอนุพันธ์แคนนาบินอยด์ที่ใช้รักษาอาการบาดเจ็บของเซลล์เยื่อบุผิวได้

สาขาวิชา	เคมี	ลายมือชื่อนิสิต .....
ปีการศึกษา	2566	ลายมือชื่อ อ.ที่ปรึกษาหลัก .....
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Mongkonkorn Thanakorncharoenwit : One-Pot Iodine-Mediated Synthesis of Cannabinol and Derivatives. Advisor: Prof. TIRAYUT VILAIVAN, Ph.D. Co-advisor: Asst. Prof. TANATORN KHOTAVIVATTANA, Ph.D.

Cannabinol (CBN) is a minor cannabinoid that exhibits a broad range of medicinal activities without psychoactive effects. CBN derivatives have been typically synthesized from a terpenoid and a resorcinol derivative, through a multistep reaction including the Friedel-Crafts alkylation, cyclization, and aromatization. In this study, we envisioned that iodine could act as a Lewis acid catalyst and an aromatization agent. Consequently, we developed a one-pot iodine-mediated reaction between (1*S*,4*R*)-*p*-menthadienol and 5-(1,1-dimethylheptyl)resorcinol for the synthesis of 3-(1,1-dimethylheptyl)cannabinol (DMH-CBN). Various reaction parameters were evaluated, and the reaction yields were analyzed by quantitative ^{1}H NMR. The optimized reaction conditions were found to be 1.5 and 1.0 equivalent of starting materials with 5.0 equivalents of iodine in toluene at refluxing temperature for 1 hr, giving DMH-CBN in 71% isolated yield. This reaction under the microwave-assisted condition gave the same product in 30% NMR yield. The same method could be applied to the synthesis of natural CBN from citral as an inexpensive terpenoid starting material and olivetol, providing the expected product in 22% isolated yield. Substrate scope investigation revealed that this one-pot iodine-mediated synthesis of CBN could produce 28–63% isolated yields of alkyl-substituted CBN derivatives, including the C3-unsubstituted CBN. This unsubstituted CBN reacted with 1.0 or 2.0 equivalents of *N*-bromosuccinimide to yield 4-bromo and 2,4-dibromo derivatives at 63% and 90% isolated yield, respectively. However, this condition was incompatible with resorcinols bearing electron-withdrawing groups, yielding no or very small amounts of the desired product. Some synthesized CBN derivatives were evaluated for their protective role in the epithelial barrier through the transepithelial electrical resistance (TER) assay. The results indicated that 2,4-diethyl CBN, 4-bromo, and 2,4-dibromo derivatives of C3-unsubstituted CBN showed better activities than natural CBN. This suggests that they could potentially serve as new candidates from the cannabinoid series, aiding in the resealing of tight junctions.

Field of Study:	Chemistry	Student's Signature .....
Academic Year:	2023	Advisor's Signature .....
		Co-advisor's Signature .....

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CHAPTER I

INTRODUCTION

1.1 Cannabinol

Cannabis is a plant that got a lot of interest in the past few years, and the use of cannabis as a medicine was widely accepted in various countries. Consequently, there were many research which aimed to study its bioactive, such as tetrahydrocannabinol (THC) and cannabidiol (CBD), in terms of their structure, synthesis, biological mechanisms, applications, and so on. One of interesting bioactive compounds is cannabinol (CBN)[1] as chemical structure shown in **Figure 1.1** which is typically found in aged cannabis[2]. Normally, CBN is derived by the natural process from tetrahydrocannabinol (THC) through an oxidation reaction.[3]

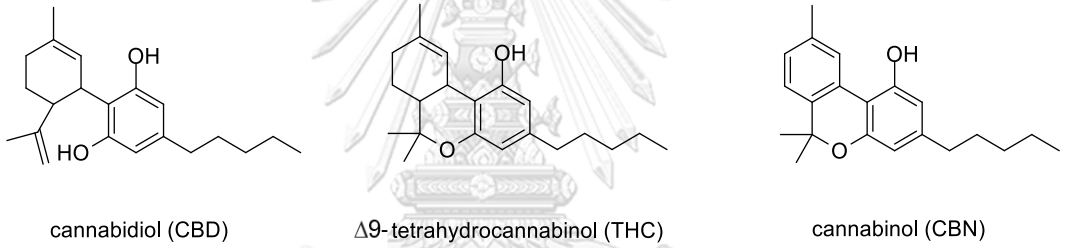


Figure 1.1 Structure of cannabidiol (CBD), Δ9-tetrahydrocannabinol (Δ9-THC) and cannabinol (CBN)

CBN has plenty of medicinal activities, some of them are similar to those of cannabidiol (CBD) and THC. For examples, it has an anticonvulsant effect,[4] increases appetite through binding to cannabinoid receptor type I (CB_1R),[5] stimulates the recruitment of mesenchymal stem cells (MSCs) from the bone marrow which are the cells that has a role in promoting of bone formation through the cannabinoid receptor type II (CB_2R) interaction.[6] protect intestinal barrier from *in vitro* inflammation.[7] Additionally, CBN has an advantage over THC that it is not a psychoactive compound meaning it does not cause any addictive effects.[8-10] Among the different structures of CBN, the synthetic analog of CBN called 3-(1,1-

dimethylheptyl)cannabinol (DMH-CBN) was found to be more effective in terms of binding to both CB_1R and CB_2R. It was also reported to show better activity in terms of inhibition of adenylyl cyclase (AC) than CBN which carries the normal pentyl substituent.[11]

1.2 Transepithelial electrical resistance (TER) essay, Ca^{2+} switch assay and effect of cannabinoids to tight junction resealing

As mentioned, cannabinoid compounds have various interesting biological activities, one of which involves the tight junction resealing of the intestinal epithelium barrier (**Figure 1.2**), leading to anti-inflammatory activity. In 2021, Veronica et al. found that cannabinoids, such as THC and CBD, have the potential to protect the intestinal epithelium barrier. They discovered that CBD was the most promising compound against intestinal inflammation when induced cell monolayer with $INF\gamma$+$TNF\alpha$, an inflammatory stimulus. The results showed that CBD at both 0.1 and 1 µg/mL could increase %TER compared to the control.[7] Based on this evidence, cannabinol derivatives could be another potential compound that can affect the tight junction in the intestinal epithelium barrier.

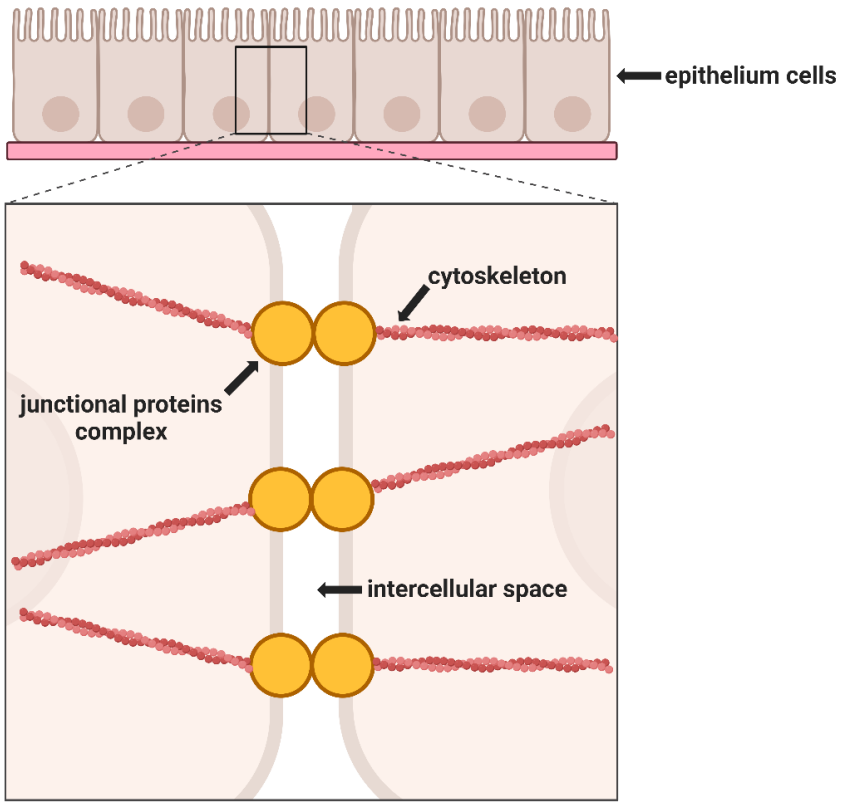


Figure 1.2 The sealing of the intercellular space between epithelial cells with tight junction

For this experiment, two assays are needed to evaluate this biological activity. The first is the transepithelial electrical resistance (TER) assay, which is used to investigate the %TER of the barrier tissue model. This involves determining the cells resistance through the measurement of intestinal barrier voltage with two electrodes from both sides of the cell monolayer. Normally, this intestinal barrier has its own resistance value, which refers to 100% TER. However, a reduction in the integrity of the intestinal epithelium barrier, which can be a cause of the penetration of inflammatory stimuli such as microbes, leading to inflammation or other diseases, can make a drop in %TER. This change can be used to detect junctional opening or the disaggregation of tight junctions.[12-14] In the intestinal barrier, tight junctions are used to seal the intercellular space between epithelial cells, and their formation is sensitive to Ca^{2+} ions. Lack of Ca^{2+} can lead to the opening of the junctional protein

complex.[13] From this point, Ca^{2+} switch assay can be used to simulate the conditions for tight junction tightness which can infer to junctional opening or resealing. To summarize, the experiment can start with exposing cell monolayer in Ca^{2+}-free medium and detect the junctional opening through measurement of the drop of %TER, then switch to normal medium with some and observe the change in %TER (**Figure 1.3**).[12]

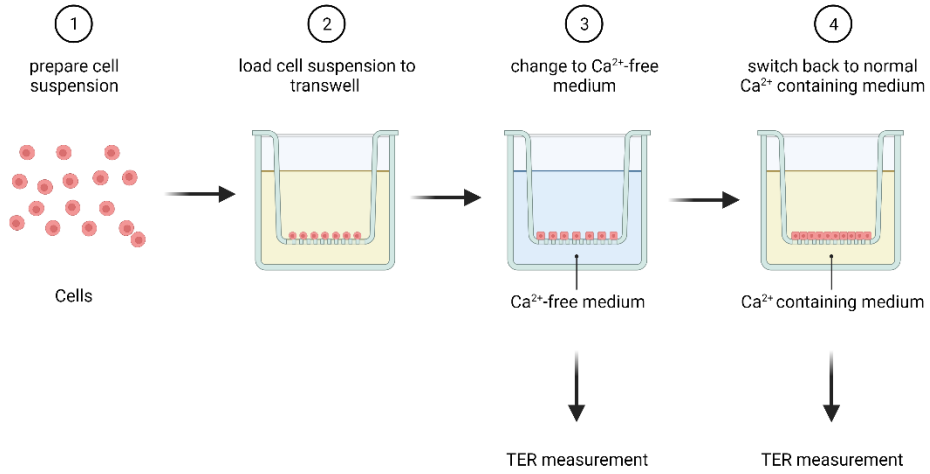


Figure 1.3 TER assay and Ca^{2+} switch assay for observation of tight junction opening and resealing

1.3 Previous synthesis of cannabinoids including CBN

Normally, extraction of products from the nature results in a mixture of various ingredients which need complicated isolation and gives only a small amount of each compound. That means, this process is not suitable for a huge production with high purity, so synthesis is a more appropriate and effective choice in terms of large-scale manufacturing and to save time in the process. On top of that, synthesis can facilitate structural modifications or functionalizations to produce various derivatives. In 2011, Bodwell and Nandaluru developed a method for synthesis of 6-dibenzo[b,d]pyran-6-one (DBP) through multicomponent reaction (MCR) and applied this method to total synthesis of CBN (**Figure 1.4**) by using 4-pentyl-6-methoxysalicylaldehyde, dimethyl glutaconate, pyrrolidine, and acetone to

synthesize DBP first, and then performing a few more steps to obtain a 3-23% overall yield of CBN.[15] This method required too many steps and a long time to complete, and also involved the use of transition metal compounds and organometallic reagents.

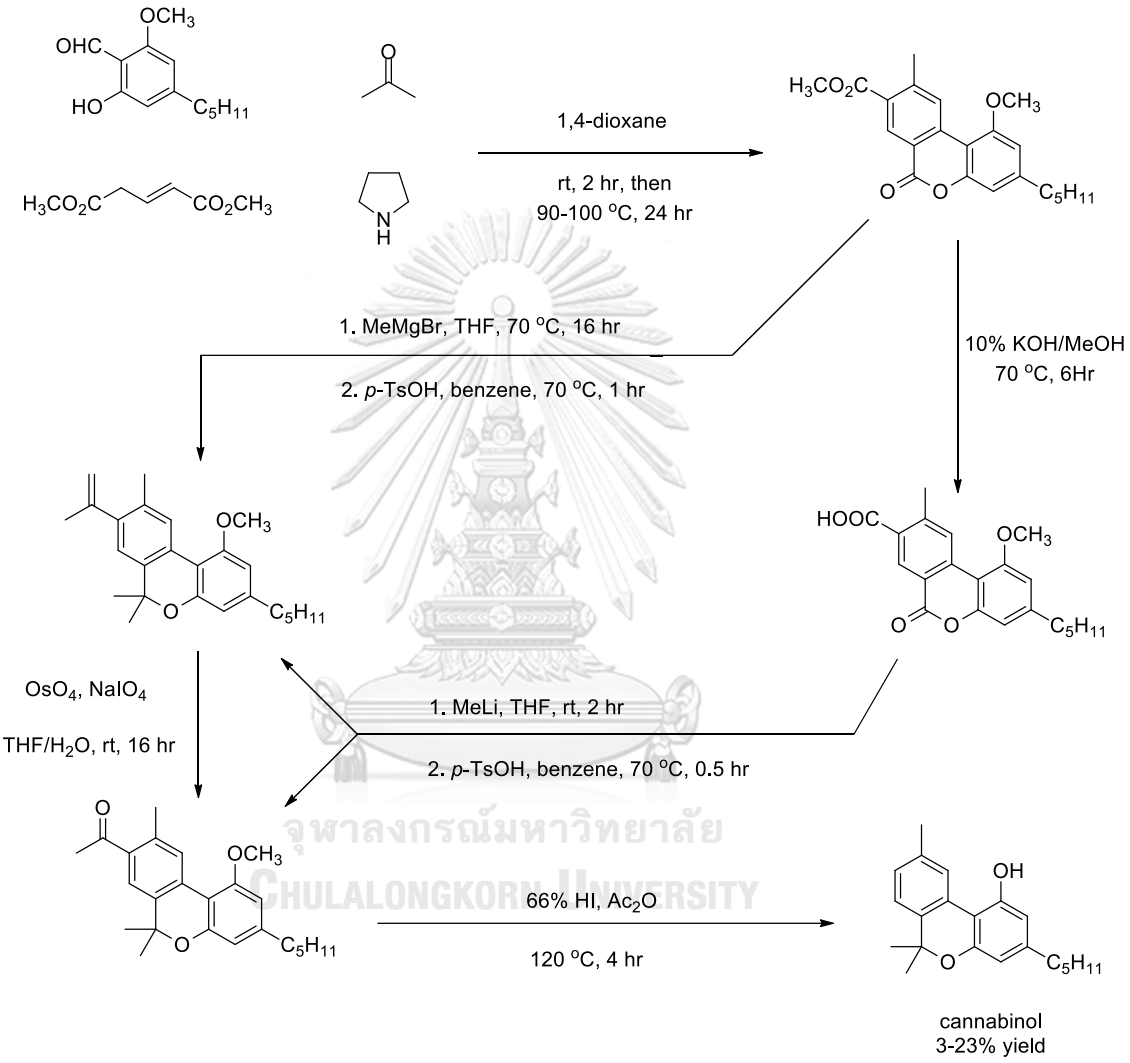


Figure 1.4 Synthesis of cannabinol from 6-dibenzo[b,d]pyran-6-one derivative[15]

Shortly thereafter, in 2018, Kloss et al. presented a more environmentally friendly method for the synthesis of biaryl compounds through a photosplicing reaction and applied to the synthesis of CBN (**Figure 1.5**). They condensed (2,6-

dimethoxy-4-pentylphenyl)methanamine with 2-cyano-5-methylbenzenesulfonyl chloride to form a sulfonamide intermediate. This intermediate was then irradiated with light to obtain a biaryl compound which can undergo further reactions and yielded a 15% overall yield of CBN.[16]

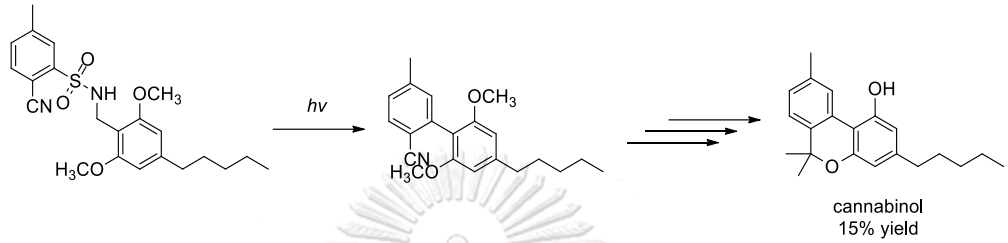


Figure 1.5 Synthesis of cannabinol via photosplicing reaction[16]

In another aspect, there was an interesting and popular strategy for synthesis of cannabinoid compounds using a condensation between terpenoids and resorcinol derivatives (**Figure 1.6**). This reaction could be used to obtain CBN without the use of metal compounds or organometallic reagents, and it also reduces the number of steps and is less time-consuming. Mechoulam et al. (1967), Petrzilka et al. (1969), and Razdan et al. (1974) successfully synthesized THC using this strategy with the help of some Lewis acids or Brønsted acid as a catalyst (such as BF_3 and p-TSA) to yield 19-53% yield of THC (**Figure 1.6a and 1.6b**). Normally, THC can be converted uncomplicatedly to CBN through aromatization reactions.[17-19] However, in 2018, Pollastro et al. presented a facile method that included the use of iodine as an aromatizing reagent (**Figure 1.6c**). This method is convenient to handle, safer than metal reagents and also inexpensive.[20] Then in 2019, Caprioglio et al. also aromatized THC to CBN with iodine after condensation of starting material with *n*-butylamine (**Figure 1.6d**).[21]

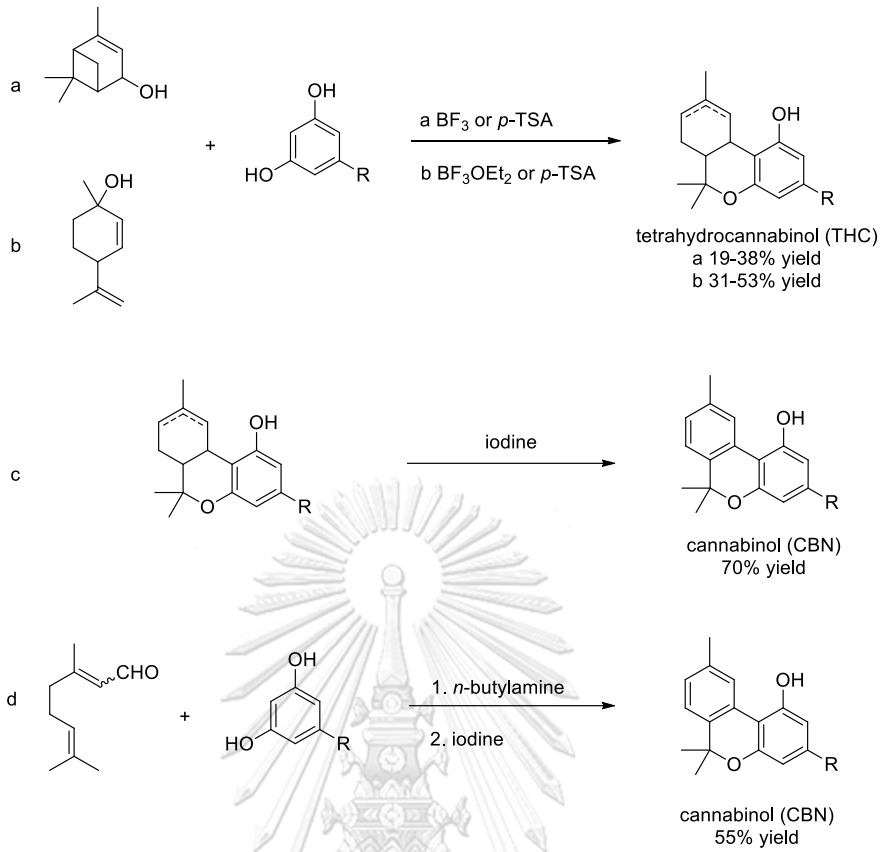


Figure 1.6 Synthesis of cannabinoid compounds from terpenoids and resorcinol derivatives[17-21]

1.4 Iodine-mediated synthesis of cannabinol and objective

Based on existing research, it can be observed that CBN is able to synthesize through condensation which is Friedel-Crafts followed by aromatization with iodine. However, iodine itself is a mild Lewis acid,[22] and it has the ability to catalyze Friedel-Crafts alkylation.[23] So, in this work, we presented the one-pot reaction for synthesis of CBN using iodine as both catalyst and aromatizing agent which was easier and faster way to obtain CBN without the use of metal or hard-to-handle compounds. Then, the protective role of cannabinol derivatives was investigated in the epithelial barrier by observing tight junction opening and resealing using the TER assay, assisted with the Ca^{2+} switch assay.

CHAPTER II

EXPERIMENTAL SECTION

2.1 Materials

Reagent-grade chemicals and solvents were purchased from standard suppliers (Fluorochem, Biosynth/Carbosynth, and RCI Lab Scan) and were used as received without further purification.

The resorcinol derivatives, including 5-isopropylresorcinol, 5-cyclohexylresorcinol, 5-(1-ethylpentyl)resorcinol, 4,6-dibromoresorcinol, and 4,6-dibromo resorcinol, were thankfully received from Mr.Phanomsak Yukhet (TV group research laboratory, department of chemistry, Chulalongkorn university). For other derivatives, they were synthesized through the below procedures.

5-Phenylresorcinol

5-Phenylcyclohexane-1,3-dione (94.1 mg, 0.5 mmol), iodine (25.4 mg, 0.1 mmol, 20 mol%) and DMSO (1.0 mL) were added into screw-cap glass tube, then heated at 80°C for 24 hr. The mixture was quenched with 10% w/v $Na_2S_2O_3$, then extracted with ethyl acetate, washed with H_2O, brine, and dried over anhydrous Na_2SO_4.[24] The crude reaction product was purified through silica gel column chromatography (eluting from 0% to 30% ethyl acetate/hexane). 5-Phenylresorcinol was obtained as a brown gum (78.7 mg, 82% yield) which crystallized into pale brown solid; ^{1}H NMR (500 MHz, DMSO-d_6) δ 9.37 (s, 1H), 7.53 – 7.48 (m, 2H), 7.44 – 7.39 (m, 2H), 7.35 – 7.30 (m, 1H), 6.45 (d J = 2.1 Hz, 2H), 6.21 (t J = 2.1 Hz, 1H).

5-Naphthylresorcinol

1-Bromo-3,5-dimethoxybenzene (525.3 mg, 2.42 mmol), 1-naphthylboronic acid (344.0 mg, 2.0 mmol), $Pd(PPh_3)_4$ (50.8 mg, 0.044 mmol, 2 mol%) Na_2CO_3 (500.3 mg, 4.72 mmol) and THF/H_2O 5:1 (10.0 mL) were added into screw-cap glass tube, then heated at 110 °C for 12 hr. The mixture was filtered through Celite and quenched with saturated NH_4Cl, then extracted with ethyl acetate, washed with

brine, and dried over anhydrous Na_2SO_4. The crude reaction product was purified through silica gel column chromatography (eluting from 0% to 3% ethyl acetate/hexane).[25] 1-(3,5-dimethoxyphenyl)naphthalene was obtained as a yellow gum (115.1 mg, 22% yield). This compound (0.44 mmol), glacial acetic acid (1.0 mL) and 48% HBr (1.0 mL) were added into screw-cap glass tube, then heated at 130 °C for 4 hr. The mixture was extracted with ethyl acetate, washed with brine, and dried over anhydrous Na_2SO_4.[26] The crude reaction product was purified through silica gel column chromatography (eluting from 0% to 30% ethyl acetate/hexane). 5-Naphthylresorcinol was obtained as a grey solid (88.2 mg, 86% yield); ^{1}H NMR (500 MHz, DMSO-d_6) **δ** 9.95 (s, 2H), 8.48 (dd *J* = 8.0, 1.6 Hz, 1H), 8.44–8.38 (m, 2H), 8.09–7.97 (m, 3H), 7.89 (dd *J* = 6.9, 1.2 Hz, 1H), 6.83–6.78 (m, 3H).

4,6-Diethylresorcinol

4,6-Diacetylresorcinol (970.9 mg, 5.0 mmol), concentrated hydrochloric acid (12.0 mL), zinc amalgam (freshly prepared from 5% $Hg(CH_3COO)_2$ w/v and 7.5 g of granulated zinc), and ethanol (3.0 mL) were added into rounded-bottom flask, then heated to reflux for 6 hr. The mixture was extracted with ethyl acetate, washed with brine, and dried over anhydrous Na_2SO_4.[27] The crude reaction product was purified through silica gel column chromatography (eluting from 0% to 20% ethyl acetate/hexane). 4,6-Diethylresorcinol was obtained as a pale brown solid (550.2 mg, 66% yield); ^{1}H NMR (500 MHz, $CDCl_3$) **δ** 6.86 (s, 1H), 6.31 (s, 1H), 5.00 (s, 2H), 2.54 (q *J* = 7.5 Hz, 4H), 1.19 (t *J* = 7.6 Hz, 6H).

5-Bromoresorcinol

1-Bromo-3,5-dimethoxybenzene (434.1 mg, 2.0 mmol), glacial acetic acid (2.0 mL) and 48% HBr (2.0 mL) were added into screw-cap glass tube, then heated at 130 °C for 4 hr. The mixture was extracted with ethyl acetate, washed with brine, and dried over anhydrous Na_2SO_4.[26] The crude reaction product was purified through silica gel column chromatography (eluting from 0% to 30% ethyl acetate/hexane). 5-

Bromoresorcinol was obtained as a white solid (362 mg, 92% yield); ^{1}H NMR (500 MHz, DMSO-d_6) **δ** 9.71 (s, 2H), 6.37 (d *J* = 2.2 Hz, 2H), 6.18 (t *J* = 2.1 Hz, 1H).

5-Decylamino(oxo)resorcinol

5-Carboxylresorcinol (77.1 mg, 0.5 mmol), *n*-decylamine (99.9 µL, 0.5 mmol), triethylamine (1.5 mmol), HATU (190.1 mg, 0.5 mmol) and DMF (2.0 mL) were added into screw-cap glass tube, then stirred at room temperature for overnight. The mixture was filtered and extracted with ethyl acetate and 2N hydrochloric acid, washed with brine, and dried over anhydrous Na_2SO_4. The crude reaction product was purified through silica gel column chromatography (eluting with hexane). 5-Decylamino(oxo)resorcinol was obtained as a pale brown solid (126.6 mg, 84% yield); ^{1}H NMR (500 MHz, DMSO-d_6) **δ** 9.45 (s, 2H), 8.20 (t *J* = 5.6 Hz, 1H), 6.63 (d *J* = 2.2 Hz, 2H), 6.32 (t *J* = 2.2 Hz, 1H), 3.16 (m, 2H), 1.46 (t *J* = 7.1 Hz, 2H), 1.24 (s, 14H), 0.85 (m, 3H).

2.2 Spectroscopic measurements

2.2.1 Nuclear magnetic resonance (NMR) spectroscopy

^{1}H NMR and spectra ^{13}C NMR were recorded on a JEOL JNM-ECZ500R/S1 spectrometer at 500 MHz using chloroform-d_1 ($CDCl_3$) as the solvent. Chemical shifts were reported in parts per million (ppm) relative to the ^{1}H signal of the residual $CHCl_3$ (δ_H 7.26 ppm) or the central peak of ^{13}C triplet signal of $CDCl_3$ (δ_C 77.16 ppm).

2.2.2 Quantitative NMR (qNMR)

^{1}H NMR spectra were recorded on a JEOL JNM-ECZ500R/S1 spectrometer at 500 MHz using chloroform ($CDCl_3$) as the solvent using methyl 3,5-dinitrobenzoate as the internal standard (I.S.) for quantitative method. Integration of the I.S. signal at 9.19 ppm was used as reference for calculation of the NMR yields according to equation (1).

$$\frac{n_C}{I_C} = \frac{n_{I.S.}}{I_{I.S.}} \quad (1)$$

Where n_C and $n_{I.S.}$ are moles of the product and the I.S. presented in the crude mixture, respectively, and I_C and $I_{I.S.}$ are integrations of the product and the I.S., which are equivalent to one proton, respectively.

2.2.3 Infrared (IR) spectroscopy

IR spectra were recorded on Bruker ALPHA II IR spectrometer using in ATR mode. Wave numbers were reported in cm^{-1}.

2.2.4 Mass Spectrometry (MS)

High resolution mass spectra were recorded on a JMS-T100LP (AccuTOF™ Dart) Mass Spectrometer in positive ESI mode (ESI+) using PEG 600 as an internal standard.

2.3 Melting point measurements

Melting point was determined on a Stuart SMP20 Melting point apparatus.

2.4 %Purity measurements

The purity of cannabinol derivatives was determined by Shimadzu HPLC (model RID-20A) with a photodiode array (PDA) detector using 90% MeCN in H_2O eluent system with 0.5 mL/min flow rate for 10 minutes and Hypersil™ BDS C18 HPLC Column (Dim. 150 x 4.6 mm) from Thermo Fisher Scientific. The peaks were monitored at 254 nm.

2.5 Reaction optimization

2.5.1 Optimization of DMH-CBN synthesis

For the model reaction, (1*S*,4*R*)-*p*-menthadienol (0.2 mmol) and 5-(1,1-dimethylheptyl)resorcinol (47.3 mg, 0.2 mmol) were dissolved in 2.0 mL of toluene in screw-cap glass tube, and heated in the presence of iodine (152.3 mg, 0.6 mmol) in

an oil bath preheated at 140 °C for 1 hr as the initial test conditions. Subsequently, several parameters were optimized, including the amounts of the starting material and iodine, followed by the iodine sources, reaction times, solvents, temperatures, and additives.

2.5.2 Optimization of CBN synthesis

For the synthesis of natural cannabinol, the terpenoid derivative (1*S*,4*R*)-*p*-menthadienol, citral, or geraniol) were reacted with olivetol under the same initial condition as above. Subsequently, some parameters were further optimized, including the amount of iodine, reaction times, and solvents.

2.6 Synthesis and characterization of cannabinol derivatives

2.6.1 Synthesis of alkyl-substituted cannabinol derivatives

General procedure: The terpenoid (0.3 mmol) and the resorcinol derivative (0.2 mmol) were added into a screw-capped glass tube followed by toluene (2.0 mL) and then iodine (1.0 mmol). The mixture was heated at 140 °C in an oil bath. After 1 hr, the reaction was cooled down, quenched with 10% w/v of $Na_2S_2O_3$, extracted with ethyl acetate, and concentrated under reduced pressure. The I.S. (methyl 3,5-dinitrobenzoate, 0.1 mmol) was added to the crude reaction mixture before dissolving in a deuterated solvent, and the yield was determined by qNMR. For isolated yield, the mixture was purified through column chromatography (ethyl acetate/hexane) and the yield was determined gravimetrically.

3-(1,1-Dimethylheptyl)cannabinol (DMH-CBN, **3a**)

Following the general procedure above using (1*S*,4*R*)-*p*-menthadienol (48.8 µL, 0.3 mmol) and 5-(1,1-dimethylheptyl)resorcinol (47.3 mg, 0.2 mmol), **3a** was obtained as a brown gum 53.8 mg (71% yield) with 99% purity; melting point: 103–104 °C (lit. 97–98 °C)[28]; ^{1}H NMR (500 MHz, $CDCl_3$) **δ** 8.16 (s, 1H), 7.15 (d *J* = 7.8 Hz, 1H), 7.07 (d *J* = 7.8 Hz, 1H), 6.56 (d *J* = 1.9 Hz, 1H), 6.41 (d *J* = 1.8 Hz, 1H), 2.39 (s, 3H), 1.60

(s, 6H), 1.57–1.51 (m, 2H), 1.25 (s, 6H), 1.23–1.15 (m, 6H), 1.11–1.02 (m, 2H), 0.83 (t *J* = 7.0 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) **δ** 154.4, 152.8, 151.9, 137.0, 127.7, 127.6, 126.5, 122.8, 108.8, 108.4, 107.8, 77.4, 44.6, 37.8, 31.9, 30.1, 28.8, 27.2, 24.8, 22.8, 21.7, 14.2; IR (ATR): 3435, 2926, 2855, 1800–2200, 1614 cm^{-1}; HRMS (ESI+): *m/z* calc. $C_{25}H_{35}O_2^+$ for 367.2632 $[M+H]^+$ found: 367.2618.

For the microwave-assisted reactions, (1*S*,4*R*)-*p*-menthadienol (48.8 µL, 0.3 mmol), 5-(1,1-dimethylheptyl)resorcinol (47.3 mg, 0.2 mmol) and toluene (3.0 mL) were added in 10 mL microwave reaction vial, then performed in the CEM microwave synthesis reactor (DISCOVER SYSTEM model) with of 140°C setting temperature and 300 W power for 1 hr. The reaction was then cooled down, quenched with 10% w/v of $Na_2S_2O_3$, extracted with ethyl acetate, and concentrated under reduced pressure. The I.S. (methyl 3,5-dinitrobenzoate, 22.6 mg, 0.1 mmol) was added to the crude reaction mixture before dissolving in a $CDCl_3$, and the yield was determined for 30% NMR yield.

Cannabinol (CBN, **3b**)

Following the general procedure using citral (255.5 µL, 1.5 mmol), olivetol (180.2 mg, 1.0 mmol), toluene (10.0 mL) and iodine (761.4 mg, 3.0 mmol), **3b** was obtained as a brown gum 68.5 mg (22% yield) with 80% purity; ^{1}H NMR (500 MHz, $CDCl_3$) ^{1}H NMR (500 MHz, $CDCl_3$) **δ** 8.20 (s, 1H), 7.15 (d *J* = 7.8 Hz, 1H), 7.08 (d *J* = 8.3 Hz, 1H), 6.45 (d *J* = 2.0 Hz, 1H), 6.29 (d *J* = 1.6 Hz, 1H), 5.51 (s, 1H), 2.54–2.45 (m, 2H), 2.39 (s, 3H), 1.61 (s, 6H), 1.64–1.58 (m, 2H), 1.34–1.31 (m, 4H), 0.90 (t *J* = 6.9 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) **δ** 154.7, 153.3, 144.6, 137.0, 136.9, 127.7, 126.6, 122.7, 110.8, 110.0, 108.8, 77.5, 35.7, 31.6, 30.6, 27.2, 22.7, 21.7, 14.2; IR (ATR): 3383, 2926, 2857, 1800–2200, 1620 cm^{-1}; HRMS (ESI+): m/z calc. $C_{21}H_{27}O_2^+$ for 311.2006 $[M+H]^+$ found: 311.1994.

3-Isopropylcannabinol (iPr-CBN, **3c**)

Following the general procedure using (1*S*,4*R*)-*p*-menthadienol (48.8 µL, 0.3 mmol) and 5-isopropylresorcinol (30.4 mg, 0.2 mmol), **3c** was obtained as a brown gum 17.3 mg (30% yield) with 86% purity; ^{1}H NMR (500 MHz, $CDCl_3$) **δ** 8.17 (s, 1H), 7.15 (d *J* = 7.9 Hz, 1H), 7.07 (d *J* = 8.1 Hz, 1H), 6.48 (d *J* = 1.5 Hz, 1H), 6.33 (d *J* = 1.7 Hz, 1H), 5.38 (s, 1H), 2.79 (m, 1H), 2.39 (s, 3H), 1.61 (s, 6H), 1.23 (d *J* = 6.9 Hz, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) **δ** 154.8, 153.2, 150.7, 137.0, 137.0, 127.7, 126.5, 122.7, 108.8, 108.1, 77.4, 33.9, 27.3, 23.8, 21.7; IR (ATR): 3387, 2924, 2855, 1800–2200, 1620 cm^{-1}; HRMS (ESI+): *m/z* calc. $C_{19}H_{23}O_2^+$ for 283.1693 $[M+H]^+$ found: 283.1700.

3-(1-Ethylpentyl)cannabinol (EtPe-CBN, **3d**)

Following the general procedure using (1*S*,4*R*)-*p*-menthadienol (48.8 µL, 0.3 mmol) and 5-ethylpentylresorcinol (41.7 mg, 0.2 mmol), **3d** was obtained as a brown gum 19.4 mg (28% yield) with 79% purity; ^{1}H NMR (500 MHz, $CDCl_3$) **δ** 8.18 (s, 1H), 7.15 (d *J* = 7.9 Hz, 1H), 7.07 (d *J* = 8.8 Hz, 1H), 6.40 (d *J* = 1.6 Hz, 1H), 6.25 (d *J* = 1.5 Hz, 1H), 5.29 (s, 1H), 2.39 (s, 3H), 2.26 (p *J* = 4.0 Hz, 1H), 1.69–1.55 (m, 2H), 1.60 (s, 6H), 1.54–1.46 (m, 2H), 1.33–1.21 (m, 2H), 1.19–1.09 (m, 2H), 0.83 (t *J* = 7.2 Hz, 3H), 0.79 (t *J* = 7.3 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) **δ** 154.6, 153.1, 148.1, 137.0, 137.0, 127.7, 127.7, 126.5, 122.7, 110.1, 109.4, 108.9, 77.41, 47.8, 36.1, 30.0, 27.2, 22.9, 21.7, 14.2, 12.4, 1.2; IR (ATR): 3374, 2917, 2852, 1800–2200, 1619 cm^{-1}; HRMS (ESI+): *m/z* calc. $C_{23}H_{31}O_2^+$ for 339.2319 $[M+H]^+$ found: 339.2333.

3-Cyclohexylcannabinol (Cy-CBN, **3e**)

Following the general procedure using (1*S*,4*R*)-*p*-menthadienol (48.8 µL, 0.3 mmol) and 5-cyclohexylresorcinol (38.5 mg, 0.2 mmol), **3e** was obtained as a brown gum 23.5 mg (36% yield) with 100% purity; melting point: 198–199 °C; ^{1}H NMR (500 MHz, $CDCl_3$) **δ** 8.15 (s, 1H), 7.14 (d *J* = 7.8 Hz, 1H), 7.06 (d *J* = 7.8 Hz, 1H), 6.46 (d *J* = 1.6 Hz, 1H), 6.31 (d *J* = 1.7 Hz, 1H), 5.20 (s, 1H), 2.40–2.36 (m, 1H), 2.38 (s, 3H), 1.91–1.86 (m, 2H), 1.86–1.80 (m, 2H), 1.59 (s, 6H), 1.41–1.36 (m, 2H), 1.36–1.32 (m, 2H), 1.27–1.21 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) **δ** 154.8, 153.1, 149.9, 137.0, 127.7, 126.5,

122.8, 109.3, 108.5, 77.4, 44.3, 34.2, 27.3, 26.9, 26.3, 22.5, 21.7, 14.2; IR (ATR): 3373, 2928, 2854, 1800–2200, 1620 cm^{-1}; HRMS (ESI+): *m/z* calc. $C_{22}H_{27}O_2^+$ for 323.2006 $[M+H]^+$ found: 323.1996.

2,4-Diethylcannabinol (diEt-CBN, **3f**)

Following the general procedure using (1*S*,4*R*)-*p*-menthadienol (48.8 µL, 0.3 mmol) and 4,6-diethylresorcinol (33.2 mg, 0.2 mmol), **3f** was obtained as a brown gum 30.0 mg (48% yield) with 78% purity; ^1H NMR (500 MHz, $CDCl_3$) **δ** 8.15 (s, 1H), 7.19 (d *J* = 7.8 Hz, 1H), 7.10 (d *J* = 7.7 Hz, 1H), 6.88 (s, 1H), 5.19 (s, 1H), 2.67–2.57 (m, 4H), 2.40 (s, 3H), 1.60 (s, 6H), 1.30 (t *J* = 7.6 Hz, 3H), 1.20 (t *J* = 7.5 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) **δ** 150.2, 148.8, 138.1, 137.0, 128.6, 128.3, 127.9, 126.3, 125.2, 122.9, 122.0, 111.1, 77.0, 27.0, 23.0, 22.9, 21.7, 15.0, 14.3; IR (ATR): 3274, 2925, 2870, 1800–2200, 1607 cm^{-1}; HRMS (ESI+): *m/z* calc. $C_{20}H_{25}O_2^+$ for 297.1849 $[M+H]^+$ found: 297.1855.

Unsubstituted cannabinol (CBN-C0, **3o**)

Following the general procedure using (1*S*,4*R*)-*p*-menthadienol (48.8 µL, 0.3 mmol) and 4,6-di-*tert*-butylresorcinol (44.5 mg, 0.2 mmol), **3o** was obtained as a brown gum 30.4 mg (63% yield) with 95% purity; ^1H NMR (500 MHz, $CDCl_3$) **δ** 8.25 (s, 1H), 7.17 (d *J* = 7.8 Hz, 1H), 7.11 (d *J* = 7.8 Hz, 1H), 7.03 (t *J* = 8.0 Hz, 1H), 6.61 (d *J* = 7.6 Hz, 1H), 6.46 (d *J* = 8.0 Hz, 1H), 5.74 (s, 1H), 2.41 (s, 3H), 1.63 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) **δ** 154.9, 153.6, 137.3, 137.0, 128.9, 128.1, 127.4, 126.9, 122.7, 111.4, 110.9, 109.9, 77.5, 27.2, 21.6; IR (ATR): 3363, 2924, 2853, 1800–2200, 1608 cm^{-1}; HRMS (ESI+): *m/z* calc. $C_{16}H_{17}O_2^+$ for 241.1233 $[M+H]^+$ found: 241.1245.

2.6.2 Synthesis of bromo-substituted cannabinol derivatives

4-Bromocannabinol (CBN-Br, **3s**)

Unsubstituted cannabinol (**3o**, 132.2mg, 0.55 mmol) was added into a screw-capped glass tube followed by acetonitrile (15.0 mL). *N*-bromosuccinimide (48.1 mg,

0.27 mmol) were divided into two portions and added at the beginning of reaction and 15 minutes. The mixture was heated at 90 °C for 0.5 hr. The reaction was cooled down, extracted with ethyl acetate, and concentrated under reduced pressure. Next, the crude reaction product was purified through silica gel column chromatography (eluting from 0% to 5% ethyl acetate/hexane). The product **3s** was obtained as a brown gum 54.4 mg (62% yield) with 92% purity; ^{1}H NMR (500 MHz, $CDCl_3$) **δ** 8.32 (s, 1H), 7.29 (d *J* = 8.7 Hz, 1H), 7.16 (d *J* = 7.9 Hz, 1H), 7.14 (d *J* = 8.3 Hz, 1H), 6.54 (d *J* = 8.7 Hz, 1H), 6.11 (s, 1H), 2.41 (s, 3H), 1.61 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) **δ** 154.4, 149.4, 137.1, 137.0, 130.8, 128.7, 127.5, 126.9, 122.6, 112.3, 112.0, 103.6, 77.8, 27.2, 21.7; IR (ATR): 3484, 2916, 2850, 1800–2200, 1598 cm^{-1}; HRMS (ESI+): *m/z* calc. $C_{16}H_{16}{}^{79}BrO_2{}^{+}$ for 319.0328 $[M+H]^+$ found: 319.0321.

2,4-Dibromocannabinol (CBN-Br_2, **3r**)

Unsubstituted cannabinol (**3o**, 36.0 mg, 0.15 mmol) and *N*-bromosuccinimide (53.4 mg, 0.3 mmol) were added into a screw-capped glass tube followed by the acetonitrile (4.0 mL). The mixture was heated at 90 °C for 0.5 hr. The reaction was cooled down, extracted with ethyl acetate, and concentrated under reduced pressure. Next, the crude reaction product was purified through silica gel column chromatography (eluting from 0% to 5% ethyl acetate/hexane). The product **3r** was obtained as a brown gum 54.2 mg (91% yield) with 87% purity; ^{1}H NMR (500 MHz, $CDCl_3$) **δ** 8.27 (s, 1H), 7.57 (s, 1H), 7.16 (s, 2H), 6.06 (s, 1H), 2.40 (s, 3H), 1.64 (s, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) **δ** 151.0, 148.8, 137.2, 137.1, 133.0, 129.3, 127.7, 126.5, 122.6, 113.4, 103.8, 103.6, 79.2, 27.2, 21.6; IR (ATR): 3481, 2917, 2850, 1800–2200, 1581 cm^{-1}; HRMS (ESI+): *m/z* calc. $C_{16}H_{15}{}^{79}Br_2O_2{}^{+}$ for 396.9433 $[M+H]^+$ found: 396.9415.

2.7 Ca^{2+} switch assay and measurement of transepithelial electrical resistance (TER)

All biological assays were performed by Ms. Pichayapa Sukmak from Chulabhorn Royal Academy under the supervision of Dr. Pawin Pongkorpsakol. T84 cell line at a density of approximately 70–80% confluency in T75 culture flask was trypsinized and seeded into 1.12 cm^2 Transwell insert (5 x 10^5 cells/Transwell) (Corning Life Sciences, Tewksbury, MA, USA) and cultured for 10 days or when the transepithelial electrical resistance (TER) reached $\geq$ 2,000 **Ω** cm^2. The Ca^{2+} switch (or Ca^{2+} depletion) assay was performed to induce the disassembly of intestinal tight junction in fully differentiated T84 cell monolayers. In brief, the culture medium of T84 cell monolayers was replaced by S-MEM, a Ca^{2+}-free medium, and incubated under 37 °C with 95% O_2/5% CO_2. At 16-hr post-incubation, the S-MEM was switched back to a regular medium (DMEM F-12) containing Ca^{2+} (Ca^{2+} switch) in the presence of cannabinol derivatives. TER measurement was performed using a Millicell ERS-2 Volt/Ohms meter from Sigma-Aldrich co. (St. Louis, Missouri, USA.) to evaluate intestinal tight junction integrity in T84 cell monolayers.

CHAPTER III

RESULTS AND DISCUSSION

3.1 Optimization of DMH-CBN synthesis

Due to the absence of regioselectivity problem, 3-(1,1-dimethylheptyl)cannabinol (DMH-CBN, **3a**) was initially chosen as a model target compound. The synthesis of DMH-CBN was performed using 5-(1,1-dimethylheptyl)resorcinol (**1a**) and (1*S*,4*R*)-*p*-menthadienol (**2a**) as the initial coupling partners in the presence of molecular iodine. The product yield was calculated by using integration of the CBN derivatives characteristic peak, which is the most deshielded singlet peak around 8.1–8.3 ppm ($\mathbf{H_a}$) compared with the integration of methyl-3,5-dinitrobenzoate at 9.22 ppm ($\mathbf{H_{I.S.}}$) that was added as internal standard (I.S.) as shown in **Figure 3.1**.

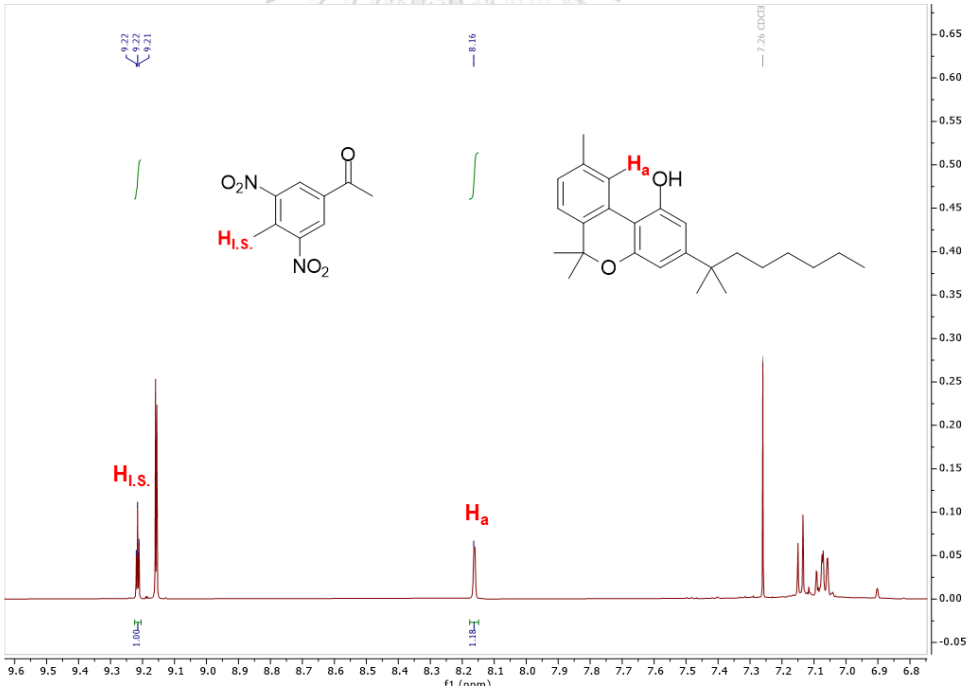


Figure 3.1 qNMR analysis (500 MHz, $CDCl_3$) of **3a** compared to I.S.

Encouragingly, the reaction produced the desired product **3a** with a yield of 38% when 3 equiv. of iodine were present. The product **3a** was then isolated and its structure was confirmed by ^{1}H NMR, which showed a 1H singlet peak at 8.16 ppm of

the aromatic proton, four 1H doublet peaks at 7.15, 7.07, 6.56, and 6.41 ppm of the aromatic protons, a 3H singlet peak at 2.39 ppm of the methyl protons, a 6H singlet peak at 1.60 ppm of the dimethyl protons on the pyran ring, and a 6H singlet peak at 1.25 ppm of the dimethyl protons on the DMH group. In addition, there were also three multiplet signals (3H, 5H, and 2H) at 1.58–1.49, 1.21–1.16, and 1.10–1.03 ppm of the methylene protons on the DMH chain, and a 3H triplet peak at 0.83 ppm of the terminal methyl proton on the DMH chain as shown in **Figure 3.2**. This result was consistent with the reference spectrum from a previous report as shown in **Table 3.1**.[21]

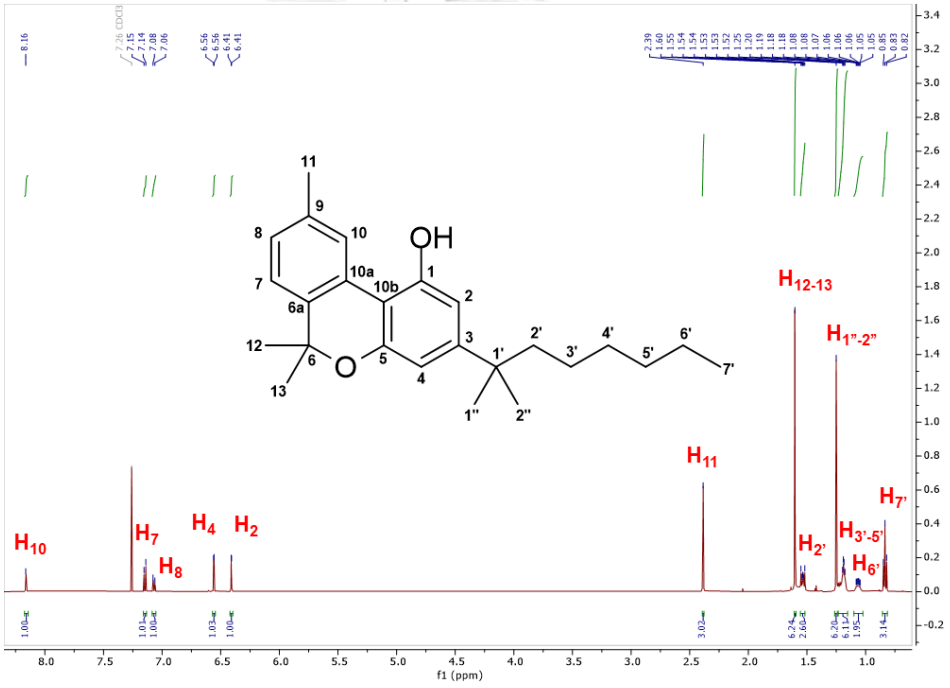
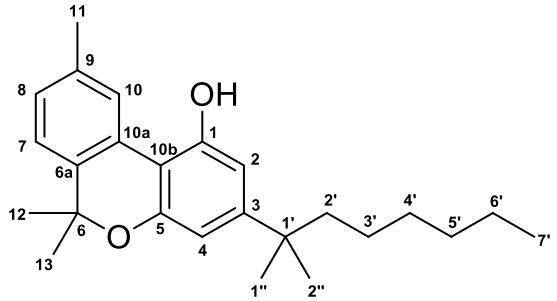


Figure 3.2 ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **3a**

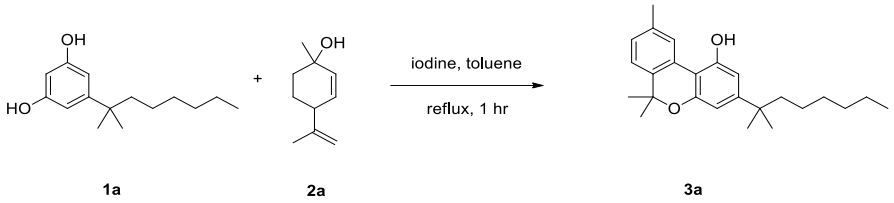
Table 3.1 ^{1}H NMR chemical shifts of **3a** compared with the literature data[21]



position	^{1}H NMR of **3a**	^{1}H NMR from reference[21]
2	6.41 (d, 1H)	6.41 (s, 1H)
4	6.56 (d, 1H)	6.56 (s, 1H)
7	7.15 (d, 1H)	7.15 (d, 1H)
8	7.07 (d, 1H)	7.07 (d, 1H)
10	8.16 (s, 1H)	8.18 (s, 1H)
11	2.39 (s, 3H)	2.39 (s, 6H)
12–13	1.60 (s, 6H)	1.61 (s, 6H)
2’	1.51–1.57 (m, 2H)	1.51–1.56 (m, 2H)
3’–5’	1.15–1.23 (m, 6H)	1.19 (m, 6H)
6’	1.02–1.11 (m, 2H)	1.06–1.09 (m, 2H)
7’	0.83 (t, 3H)	0.82–0.86 (t, 3H)
1’’–2’’	1.25 (s, 6H)	1.25 (s, 6H)

The preliminary optimizations involved adjusting the quantities of the starting materials and reagents as detailed in **Table 3.2**. Increasing the amount of iodine to 5 equiv. was found to enhance the product yield (**Table 3.2**, entries 1–3). Due to the rapid decomposition of the terpenoid component in the presence of iodine, as shown by the presence of residual **1a** in the reaction mixtures according to ^{1}H NMR analyses, the amount of **2a** was increased to 1.5 equiv. This adjustment resulted in an increase in the product yield from 50% to 61% (**Table 3.2**, entry 4). Accordingly, the condition from entry 4 were chosen for further optimization.

Table 3.2 Preliminary optimization of the quantities of starting materials and reagent



1a **2a** **3a**

Entry	**1a** (equiv)	**2a** (equiv)	Reagent (equiv)	Yield[a] (%)
1	1.0	1.0	I_2 (3.0)	38
2	1.0	1.0	I_2 (5.0)	50
3	1.0	1.0	I_2 (7.0)	48
4	1.0	1.5	I_2 (5.0)	61
5	1.0	2.0	I_2 (5.0)	65
6	1.0	2.5	I_2 (5.0)	65

[a] NMR yield

Next, the reaction time was optimized. The product formation was almost complete within 1 hr. Thus, prolonging the reaction time to 2 hr proved unnecessary (**Table 3.3**, entries 1–3). To verify the roles and mechanism of iodine, several experiments were conducted. In the absence of iodine, no product was formed, confirming its crucial role in this reaction as both the Friedel-Crafts alkylation catalyst and the aromatizing agent (**Table 3.3**, entry 4). In addition to molecular iodine, potassium iodide (KI), iodine monochloride (ICl), and *N*-iodosuccinimide (NIS) were evaluated as alternative iodine sources. Among these, only the use of ICl provided **3a** in 35% yield (**Table 3.3**, entries 5–7). The yield from ICl was slightly lower in comparison to the reaction utilizing molecular iodine. These experiments confirm that the Friedel-Crafts alkylation reaction requires iodine in its molecular form as shown in **Figure 3.3**, as opposed to the hidden Brønsted acid catalysis mode, which involves ionized forms of iodine.[29]

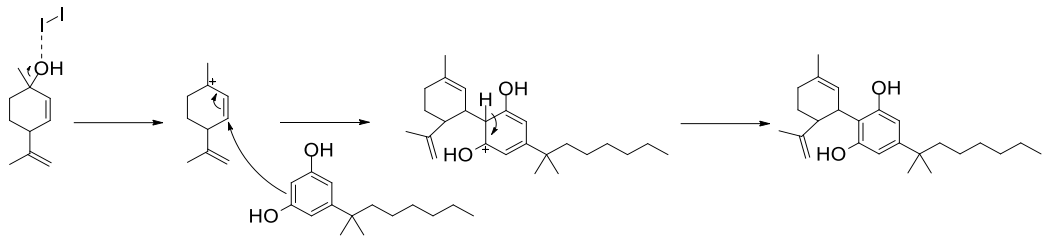


Figure 3.3 Proposed mechanism of iodine-catalyzed Friedel-Craft alkylation

Regarding the aromatization reaction, it was hypothesized that this process occurs through an addition-elimination mechanism as shown in **Figure 3.4**.[20] The results from the solvent optimization experiments indicated the effectiveness of nonpolar solvents such as toluene, *p*-xylene, and carbon tetrachloride (CCl_4). Among these, toluene was found to be the most suitable solvent, giving a higher yield, and having lower toxicity compared to CCl_4 (**Table 3.3**, entries 8–9). In the case of other solvents, it was apparent that iodine could potentially be deactivated through a strong halogen bond with the solvent. Consequently, it was likely to further decompose into hydroiodic acid (HI), thereby unable to fulfill the catalytic role of molecular iodine (**Table 3.3**, entries 10–13).[23] When the reaction was performed at a lower temperature, the yield of **3a** dropped dramatically (**Table 3.3**, entries 14–15). To decrease the amount of I_2 used for the reaction, dimethyl sulfoxide (DMSO) was added to the reaction mixture as an I_2 regeneration reagent through an oxidation reaction.[30] However, no product formation was observed when a large amount of DMSO was present (**Table 3.3**, entry 16). This indicates that the reaction is not compatible with polar solvents which is in line with the previous observation. Nevertheless, a small amount of DMSO could be tolerated, although the reaction was not as effective as under normal conditions without DMSO (**Table 3.3**, entries 17–19). After purification, this method could achieve a 73% isolated yield of **3a** in 1 hr.

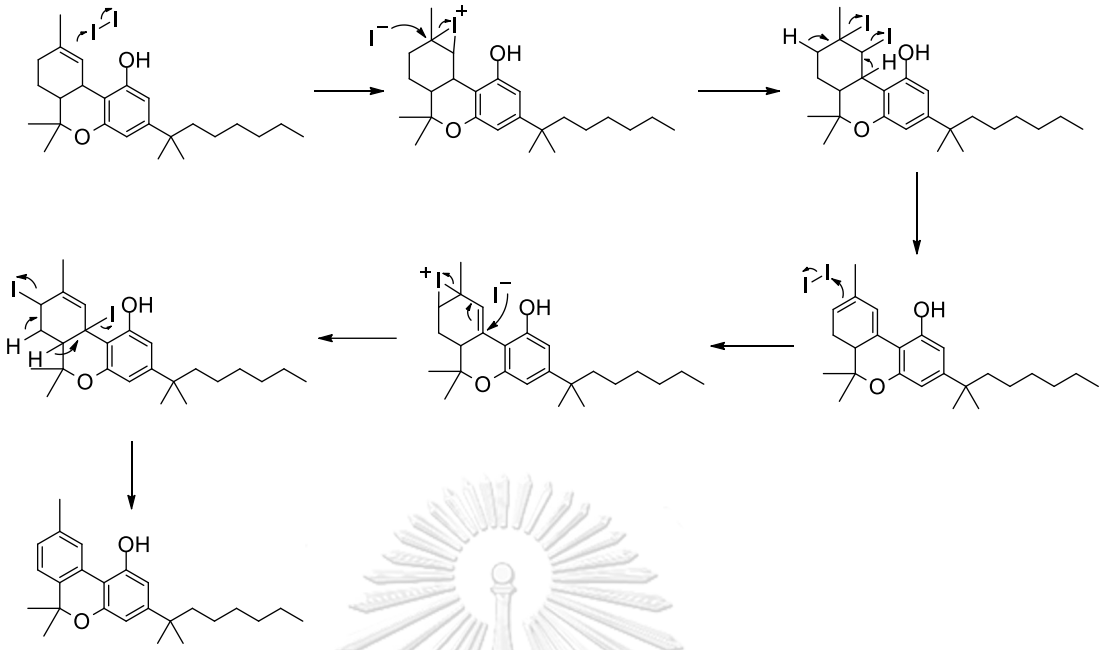
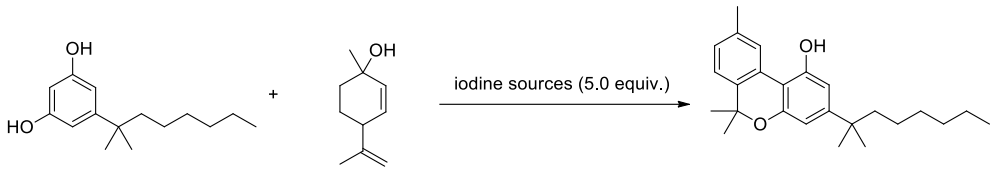


Figure 3.4 Proposed mechanism of iodine-mediated aromatization through addition-elimination process[20]

Table 3.3 Effects of additional reaction parameters including reaction times, iodine sources, solvents, temperatures and additive in synthesis of DMH-CBN (**3a**)



1a (1.0 equiv) **2a** (1.5 equiv) **3a**

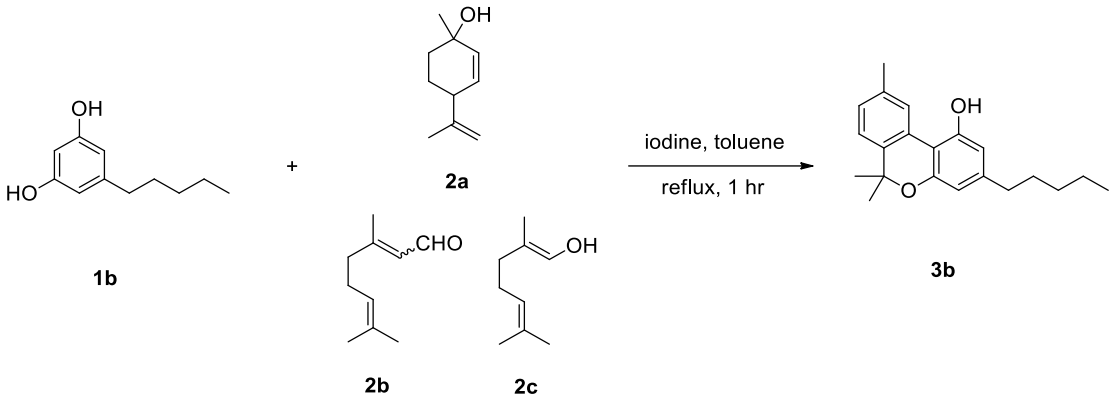
Entry	Reagent (equiv)	Additive (equiv)	Solvent[a]	Temperature (°C)	Time (hr)	Yield[b] (%)
1	I_2 (5.0)	-	toluene	110	1	61
2	I_2 (5.0)	-	toluene	110	0.5	56
3	I_2 (5.0)	-	toluene	110	2	64
4	-	-	toluene	110	1	0
5	KI (5.0)	-	toluene	110	1	0
6	ICl (5.0)	-	toluene	110	1	35
7	NIS (5.0)	-	toluene	110	1	0
8	I_2 (5.0)	-	*p*-xylene	120	1	55
9	I_2 (5.0)	-	CCl_4	77	1	66
10	I_2 (5.0)	-	acetonitrile	82	1	14
11	I_2 (5.0)	-	DMSO	120	1	0
12	I_2 (5.0)	-	HFIP	58	1	trace
13	I_2 (5.0)	-	DCM	40	1	trace
14	I_2 (5.0)	-	toluene	100	1	31
15	I_2 (5.0)	-	toluene	rt	1	0
16	I_2 (5.0)	DMSO (50.0)	toluene	110	1	0
17	I_2 (5.0)	DMSO (0.5)	toluene	110	1	52
18	I_2 (3.0)	DMSO (1.0)	toluene	110	1	56
19	I_2 (3.0)	DMSO (0.5)	toluene	110	1	55

[a] 2.0 mL of solvent, [b] NMR yield

3.2 Optimization of CBN synthesis

After achieving the optimized conditions for the synthesis of DMH-CBN, the conditions were applied to the synthesis of CBN (**3b**) which is the natural bioactive compound found in the Cannabis plant. This reaction was investigated further to determine alternative ways or the more suitable of condition for the synthesis of **3b**. For the terpenoid starting material, in addition to (1*S*,4*R*)-*p*-menthadienol (**2a**), simpler terpenoid starting materials including citral (a mixture of geranial and neral, **2b**) and geraniol (**2c**) were also tested. The resorcinol component required for the synthesis of **3b** was olivetol (5-pentylresorcinol). The structures of all compounds involved are as shown in **Table 3.4**. After experimenting with various starting materials under the previously obtained conditions for the synthesis of DMH-CBN, the results showed that geraniol didn't yield the desired product **3b**. On the other hand, citral gave **3b** in 17% yield which was comparable to those from (1*S*,4*R*)-*p*-menthadienol (18% yield). For the formation of **3b**, it was proposed that olivetol acts as a nucleophile and then attacks the carbonyl which is activated with iodine. Consequently, the intermediate undergoes cyclization through a hetero Diels-Alder reaction to form THC (**Figure 3.5**), which is later aromatized to become **3b**. This is a very promising result since citral is an inexpensive starting material (0.78 USD/mL, Sigma-Aldrich) compared to (1*S*,4*R*)-*p*-menthadienol (32.27 USD/mL, Biosynth Carbosynth). Consequently, we focused next on the optimization of the reaction between **1b** and **2b** for the synthesis of **3b**.

Table 3.4 Investigation of terpenoids as starting material for synthesis of **3b**



entry	**1** (equiv)	**2** (equiv)	Reagent (equiv)	%Yield[a]
1	**1b** (0.2)	**2a** (0.3)	I_2 (5.0)	18
2	**1b** (0.2)	**2b** (0.3)	I_2 (5.0)	17
3	**1b** (0.2)	**2c** (0.3)	I_2 (5.0)	0

[a] NMR yield

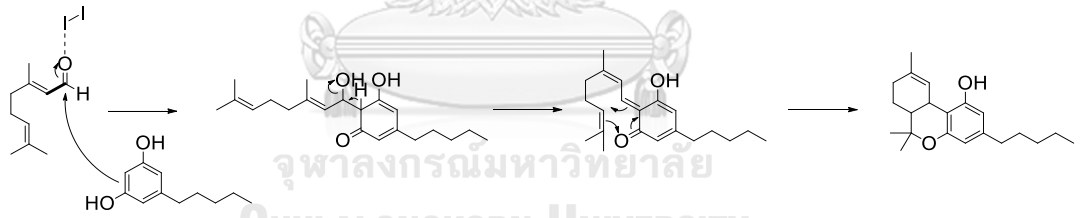


Figure 3.5 A proposed mechanism of iodine-mediated reaction between **1b** and **2b**[21, 31]

The product **3b** was then isolated and its structure was confirmed by ^{1}H NMR, which showed a 1H singlet peak at 8.20 ppm of the aromatic proton, four 1H doublet peaks at 7.15, 7.08, 6.45, and 6.29 ppm of the aromatic protons, a 3H singlet peak at 2.39 ppm of the methyl protons, a 6H singlet peak at 1.61 ppm of the dimethyl protons, a 2H multiplet peak at 2.45–2.54 ppm of benzylic protons on the pentyl chain, and a 3H triplet peak at 0.90 ppm of the terminal methyl protons on the pentyl side chain. In addition, there were also two multiplet signals (2H and 4H) at

1.58–1.64 and 1.28–1.37 ppm of the methylene protons on the pentyl side chain as shown in **Figure 3.6** This result was consistent with the reference spectrum from a previous report as shown in **Table 3.5**.[32]

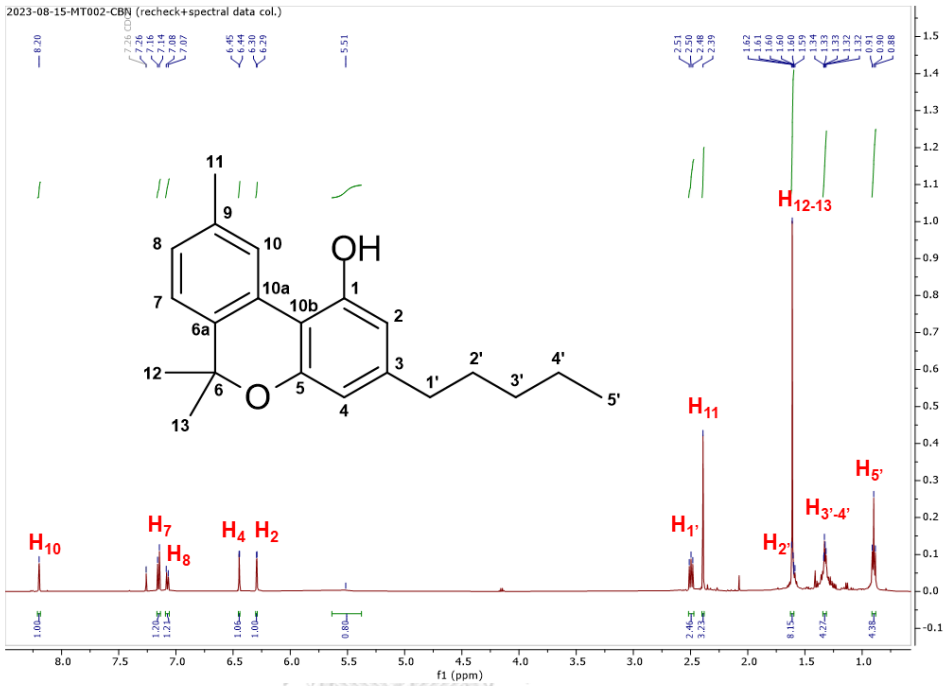
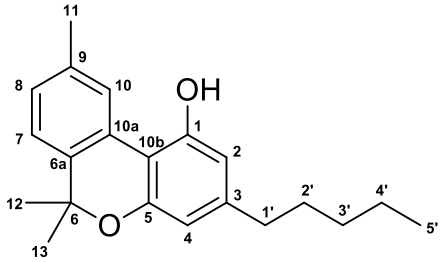


Figure 3.6 ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **3b**

Table 3.5 ^{1}H NMR chemical shifts of **3b** compared with reference[32, 33]



position	^{1}H NMR of **3b**	^{1}H NMR from reference[32]
2	6.29 (d, 1H)	6.22 (s, 1H)
4	6.45 (d, 1H)	6.36 (s, 1H)
7	7.15 (d, 1H)	7.04–7.14 (d, 1H)
8	7.08 (d, 1H)	6.97–7.03 (d, 1H)
10	8.20 (s, 1H)	8.10 (s, 1H)
11	2.39 (s, 3H)	2.31 (s, 3H)
12–13	1.61 (s, 6H)	1.55 (s, 6H)
1’	2.45–2.54 (m, 2H)	2.40–2.50 (t, 2H)
2’	1.58–1.64 (m, 2H)	1.61–1.64 (m, 2H)
3’–4’	1.34–1.31 (m, 4H)	1.15–1.31 (m, 4H)
5’	0.90 (t, 3H)	0.81 (t, 3H)

position	^{13}C NMR of **3b**	^{1}H NMR from reference[32]
1	154.7	155.6
2,4	110.0	109.8
3	144.6	144.6
5	153.3	153.0
6	77.5	79.9
6a	136.9	136.9
7	122.7	122.6
8	127.7	127.5
9	137.0	136.9
10	126.6	126.4
10a	108.8	108.7
10b	110.8	111.8
11	21.7	21.6
12–13	27.2	27.1
1’	35.7	35.6
2’	30.6	30.5
3’	31.6	31.5
4’	22.7	22.6
5’	14.2	14.1

The reaction time was next optimized (**Table 3.6**, entries 1 and 2), and the results indicated no significant effect on the yield. Consequently, the amount of iodine was decreased from 5 equiv. to 3 equiv. (**Table 3.6**, entries 3 and 4), which led to slightly higher yields. Lastly, some other nonpolar solvents were tested in this reaction (**Table 3.6**, entries 5 and 6), but based on the yields toluene remained the most suitable solvent. The relatively low yield (18% compared to 61% from model reaction) might suggest that citral was sensitive to the excessive amounts of iodine, which exceeded the theoretical requirement (2 equiv. for the aromatization, and catalytic amounts for the Friedel-Crafts reaction and ring closure). This could result in easier decomposition under these conditions. Furthermore, the intermediate from the condensation could produce another side product, cannabichromene (CBC), through the hetero Diels-Alder reaction of the isomerized intermediate (**Figure 3.7**).[21, 31]

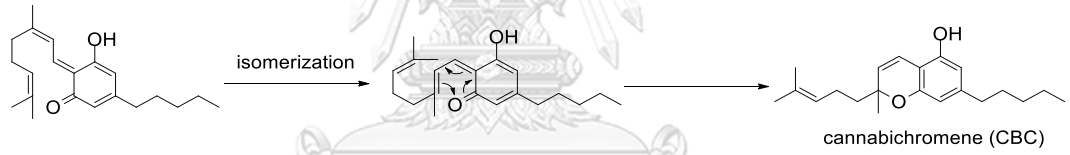


Figure 3.7 A hetero Diels-Alder reaction of intermediate leading to CBC[21, 31]

Additionally, in term of resorcinol derivatives, **1b** exhibits less steric hindrance compared to **1a**, which could lead to the formation of another THC isomer (abnormal THC) before aromatization (**Figure 3.8**).[32] This method provided only 18% of CBN, which was lower than the previous report that achieved 55% of CBN. However, this work's method could be accomplished with only one reagent in 1 hr and in one pot, compared to the previous report that required both *n*-butylamine and iodine, a filtration step before aromatization, and an overnight reaction for condensation, along with an additional 3 hr for aromatization. It should be noted that in previous work, it was reported that the direct treatment of these two starting materials with only iodine in their method resulted in a complex mixture and the

two step reaction was necessary to produce CBN. Moreover, they only demonstrated the synthesis of CBN from terpenoid and resorcinol derivatives, excluding others that started at the iodine treatment step of CBC derivatives.[21]

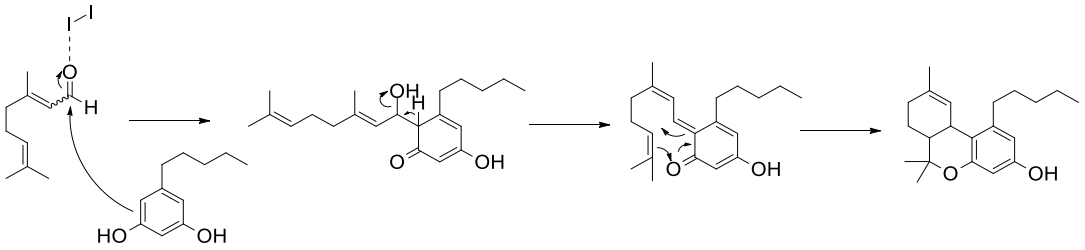
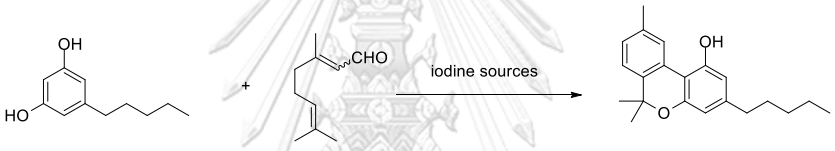


Figure 3.8 An alternative reaction of **1b** with citral leading to abnormal THC

Table 3.6 Effects of additional reaction parameters including amount of iodine, reaction times and solvents in synthesis of **3b**



1b (1.5 equiv) **2b** (1.0 equiv) **3b**

Entry	Reagent (equiv)	Solvent[a]	Temperature (°C)	Time (hr)	Yield[b] (%)
1	I_2 (5.0)	toluene	110	1	17
2	I_2 (5.0)	toluene	110	2	15
3	I_2 (3.0)	toluene	110	1	23
4	I_2 (3.0)	toluene	110	2	24
5	I_2 (3.0)	benzene	110	1	10
6	I_2 (3.0)	CCl_4	110	1	9

[a] 2.0 mL of solvent, [b] NMR yield

3.3 Substrate scope

The optimized conditions obtained from both DMH-CBN and natural CBN were subsequently tested with other substrates, as depicted in **Table 3.7**. The

formation of cannabinol derivatives was confirmed by three characteristic signals with a 1:3:6 integration ratio: the most characteristic deshielded peak ($\mathbf{H_a}$) typically found around 8.1–8.3 ppm, a 3H singlet peak ($\mathbf{H_b}$) around 2.4 ppm for the methyl protons, and a 6H singlet peak ($\mathbf{H_c}$) around 1.6 ppm for the dimethyl protons on the pyran ring. For qNMR analysis in substrate scope experiments, characteristic peak ($\mathbf{H_a}$) and I.S. peak ($\mathbf{H_{I.S.}}$) were used for the calculation of yields, as shown in the example in **Figure 3.9**.

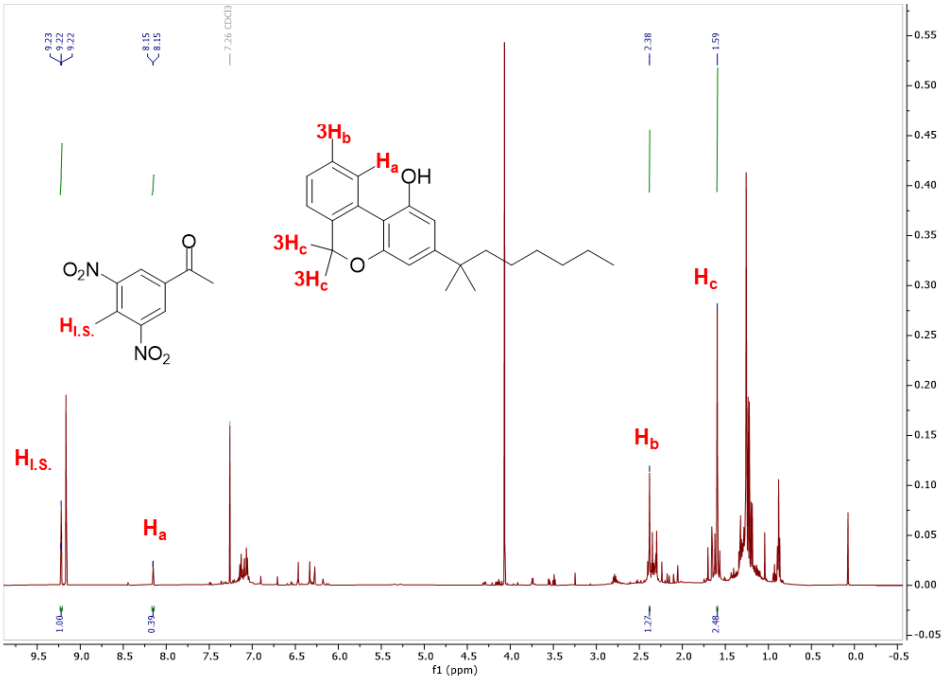
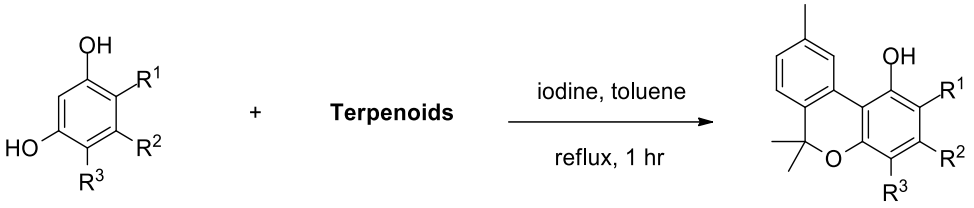


Figure 3.9 Example of preliminary characterization and qNMR analysis (500 MHz, $CDCl_3$) from a crude reaction mixture

To evaluate the scope of the one-pot iodine-mediated synthesis of cannabinol derivatives, the reaction was tested with various combinations of resorcinol derivatives and (1*S*,4*R*)-*p*-menthadienol or citral as shown in **Table 3.7**. The model reaction of **1a** with (1*S*,4*R*)-*p*-menthadienol provided the optimized yields of DMH-CBN at 61%. Another model reaction of **1b** with citral gave natural CBN in 23% yield (**Table 3.7**, entries 1–2). The reactions between (1*S*,4*R*)-*p*-menthadienol with other 5-substituted alkyl resorcinol derivatives, including isopropylresorcinol

(**1c**), 5-(1-ethyl)pentylresorcinol (**1d**), and 5-cyclohexylresorcinol (**1e**), produced 20%, 26%, and 24% yields of the desired products (**Table 3.7**, entries 3–5). These yields were comparable to that of **1b** but lower than **1a**, which may indicate the impact of steric hindrance at the 5-position of the resorcinol derivatives. Specifically, 5-substituted resorcinol derivatives possessing small steric hindrance could lead to the formation of other isomers of cannabinoid compounds, such as abnormal cannabinoids, which may result from an attack at C4 or C6 on the resorcinol ring (**Figure 3.10**) rather than C2 (the carbon atom located between the two hydroxy groups).[32]

Table 3.7 Substrate scope of resorcinol derivatives (**1a**–**q**) and terpenoids (**2a**–**b**)



1a-q (1.5 equiv) **2a-b** (1.0 equiv) **3a-q**

Entry	Resorcinol derivatives				Terpenoids		product
	1	R^1	R^2	R^3	**2a**[a]	**2b**[b]	
1	**1a**	H	$C(CH_3)_2C_7H_{15}$	H	61	37	**3a**
2	**1b**	H	C_5H_{11}	H	18	23	**3b**
3	**1c**	H	$CH(CH_3)_2$	H	20	-[c]	**3c**
4	**1d**	H	$CH(C_2H_5)C_4H_9$	H	26	-	**3d**
5	**1e**	H	C_6H_{11}	H	24	-	**3e**
6	**1f**	C_2H_5	H	C_2H_5	22	trace	**3f**
7	**1g**	$C(CH_3)_3$	H	$C(CH_3)_3$	47[d]	trace	**3o**
8	**1h**	H	Br	H	trace	trace	**3h**
9	**1i**	Br	CH_3	Br	trace	-	**3i**
10	**1j**	Br	C_5H_{11}	Br	trace	-	**3j**
11	**1k**	H	C_6H_5	H	trace	trace	**3k**
12	**1l**	H	$C_{10}H_7$ (Naph)	H	trace	trace	**3l**
13	**1m**	H	$CONHC_{10}H_{21}$	H	0	0	**3m**
14	**1n**	$COCH_3$	H	$COCH_3$	0	-	**3n**
15	**1o**	H	H	H	0	-	**3o**
16	**1p**	H	CH_3	H	trace	-	**3p**
17	**1q**	H	OH	H	trace	-	**3q**

[a] NMR yield of cannabinol derivatives from 1 h reaction with 5 equiv. of iodine in toluene, [b] NMR yield of cannabinol derivatives from 1 h reaction with 3 equiv. of iodine in toluene, [c] experiments that were not performed, [d] de-*tert*-butylation product (**3o**)

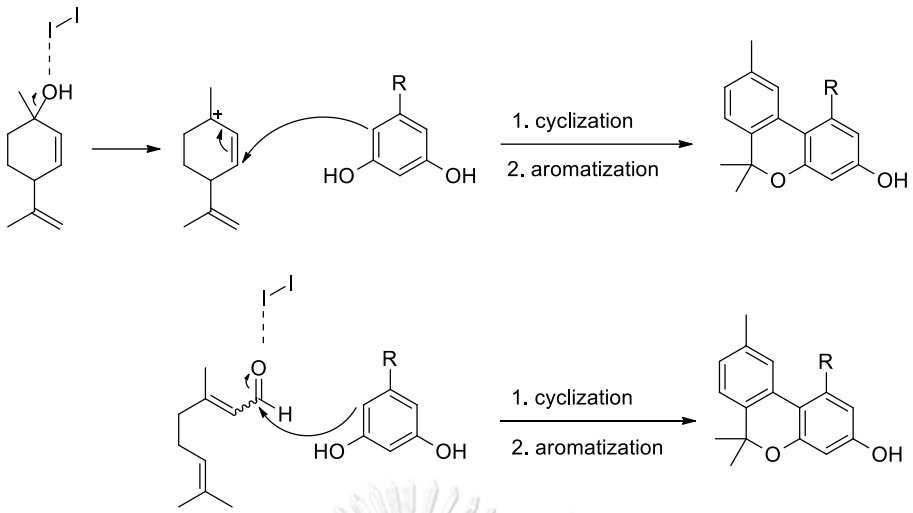


Figure 3.10 The Friedel-Craft alkylation at C4 or C6 of a resorcinol derivative leading to abnormal CBN

Two 4,6-disubstituted alkyl resorcinol derivatives, 4,6-diethylresorcinol (**1f**) and 4,6-di-*tert*-butylresorcinol (**1g**), yielded similar results, giving 22% and 47% of the CBN derivatives (**Table 3.7**, entries 6–7). However, for the resorcinol substrate **1g**, it was observed that both *tert*-butyl groups were eliminated leading to the formation of unsubstituted cannabinol (**3o**) instead of the expected di-*tert*-butylated compound **3g**. The removal of the *tert*-butyl groups was evident as the 18H singlet peak of the two *tert*-butyl group protons at 1.39 ppm could not be observed in the ^{1}H NMR spectrum. In addition, there are five aromatic protons, rather than four, appearing in the range of 6.44–7.13 ppm. Another notable observation is the appearance of a triplet peak at 7.02 ppm ($\mathbf{H_{Ar}}$), confirming the presence of two adjacent protons at the 2 and 4 positions as shown in **Figure 3.11**.

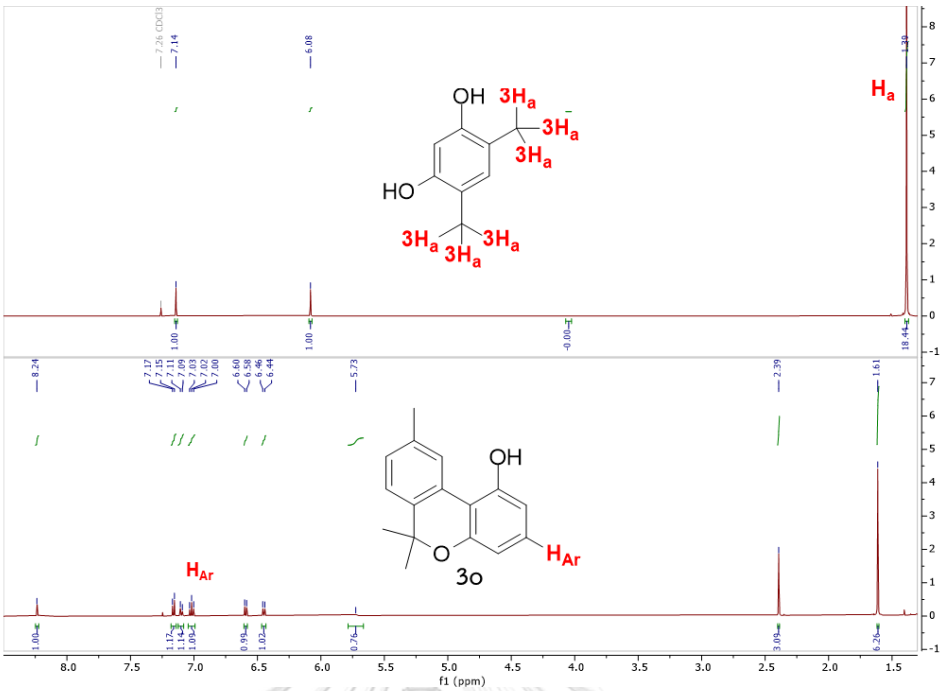


Figure 3.11 ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of confirming the de-*tert*-butylation through absence of 4,6-di-*tert*-butyl groups protons in compound **3o**

It was proposed that the two *tert*-butyl groups were eliminated under the acidic conditions[34] caused by the by-product of the aromatization step, HI, after the formation of the cannabinol derivative as shown in **Figure 3.12**. As the yield of the product was significantly high, it was believed that the 4,6-*tert*-butyl groups should still present in the resorcinol rings at the beginning of the reaction. If the groups had been eliminated before undergoing the Friedel-Craft alkylation, **1g** would have been converted to **1o** which did not give any CBN product, as indicated in **Table 3.7**, entry 15. Accordingly, the reaction between **1g** and **2a** leading to the product **1o** is proposed as shown in **Figure 3.12**.

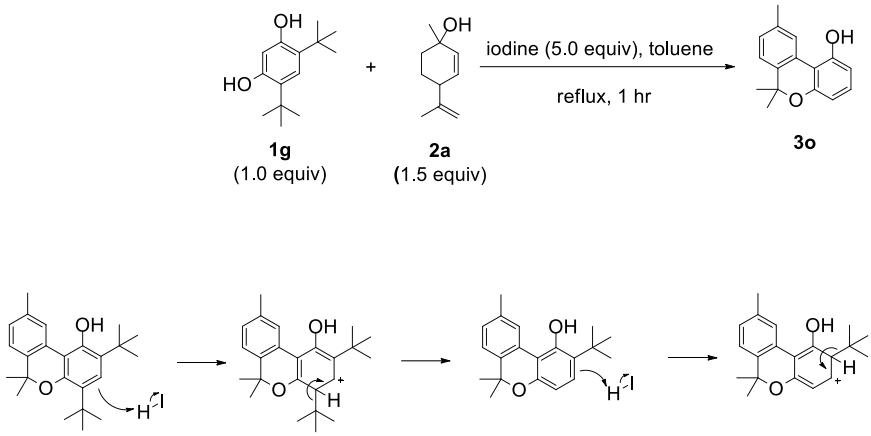


Figure 3.12 A proposed mechanism for the formation of **3o** through the de-*tert*-butylation reaction

After purification, this method could yield **3o** in a 63% isolated yield in 1 hr and in one pot. This result is higher and more efficient than a recent report (**Figure 3.13**),[32] which achieved a 36% overall yield from a three-step reaction which included the synthesis of unsubstituted cannabidiol (CBD-C0, 51%), acid-catalyzed cyclization of CBD-C0 to unsubstituted tetrahydrocannabinol (THC-C0, 82%), and aromatization to produce **3o** (CBN-C0, 87%). The overall process took 27 hr, not including purification, compared to the developed one-pot method which required only 1 hr.

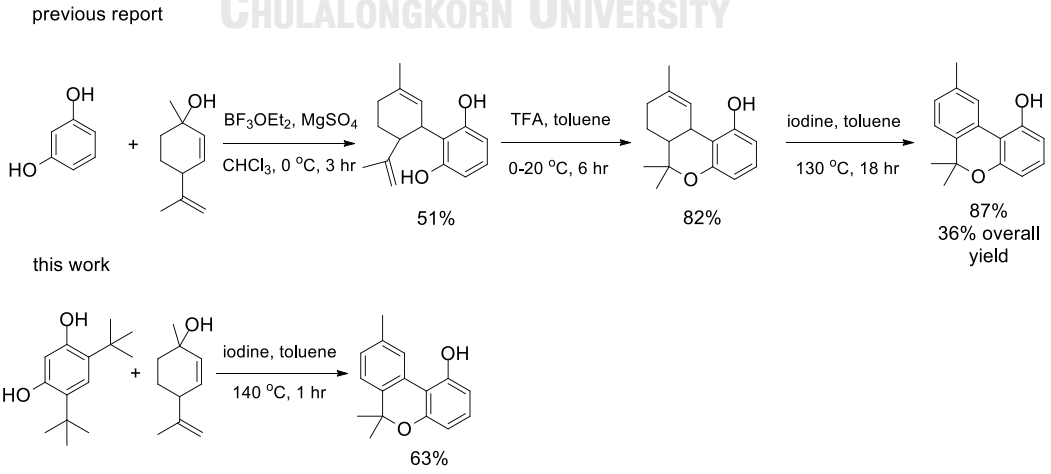


Figure 3.13 Comparison of synthesis of unsubstituted CBN (**3o**) between previous report and this work[32]

To further verify the origin of the de-*tert*-butylation, the reaction between **1g** and 5.0 equiv. HI was attempted. The results showed the complete conversion of **1g** to **1o** (**Figure 3.14**) within 1 hr of the reaction with HI in toluene, suggesting the possible role of HI for the de-*tert*-butylation. Iodine, on the other hand, was not effective for the de-*tert*-butylation as shown by very little removal of the *tert*-butyl group after heating **1g** with iodine (5 equiv.) in toluene for 1 hr. The results showed that most of them still have one *tert*-butyl group remaining on the ring, confirming that de-*tert*-butylation could be performed at the very end of the reaction when there is a significant amount of HI in the mixture.

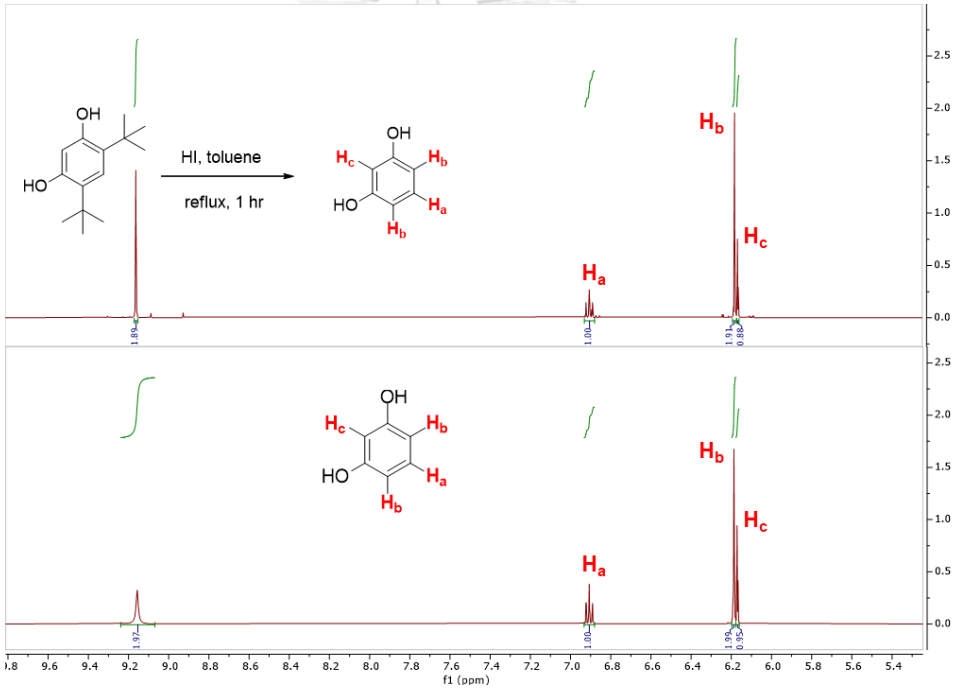


Figure 3.14 ^{1}H NMR (500 MHz, $CDCl_3$) spectra of reaction crude between **1g** and HI compared to **1o**

Unfortunately, resorcinol derivatives carrying an electron-withdrawing group instead of the simple alkyl group failed to give the desired products. As seen with 5-bromoresorcinol (**1h**), 4,6-dibromoorcinol (**1i**), and 4,6-dibromoolivetol (**1j**), these compounds with electron-withdrawing substituents through inductive effects produced only trace amounts of the target products. Likewise, resorcinol derivatives

bearing an electron-withdrawing group with mesomeric effects, such as 5-phenylresorcinol (**1k**) and 5-naphthylresorcinol (**1l**), also generated trace amounts of the cannabinol derivatives. Similarly, 5-decylamino(oxo)resorcinol (**1m**) and 4,6-diacetylresorcinol (**1n**), yielded no product (**Table 3.7** entries 8–14). The results could be explained by the decreased nucleophilicity of the resorcinol derivatives caused by the electron-withdrawing groups might slow down the attack in the Friedel-Crafts alkylation step. Since the terpenoids themselves could not withstand the concentrated iodine conditions for such an extended period, decomposition could occur and depleting the electrophiles in the reaction. Resorcinol itself (**1o**), as well as other derivatives including orcinol (**1p**) and phloroglucinol (**1q**), also did not yield the CBN products (**Table 3.7** entries 15–17). For these substrates, it was hypothesized that the reaction was unsuccessful because of the low solubility of these polar resorcinol derivatives in toluene.

Next, the reaction of citral as the terpenoid coupling partner instead of (1*S*,4*R*)-*p*-menthadienol was attempted with selected resorcinol derivatives including **1a**, **1b**, **1f**–**h**, and **1k**–**l**. However, apart from the resorcinol substrates **1a** and **1b**, other resorcinol derivatives only produced trace amounts of products, approximately 10% or less, which were also lower than those obtained under the (1*S*,4*R*)-*p*-menthadienol condition. Therefore, it was considered unnecessary to screen the remaining resorcinol derivatives. Finally, seven products which were obtained in more than 20% yield as shown in **Figure 3.15**, were synthesized at 0.2 mmol scale of resorcinol derivatives and purified to obtain isolated yields. Most of these products were obtained in consistent yields comparable to or higher than those determined by ^{1}H NMR.

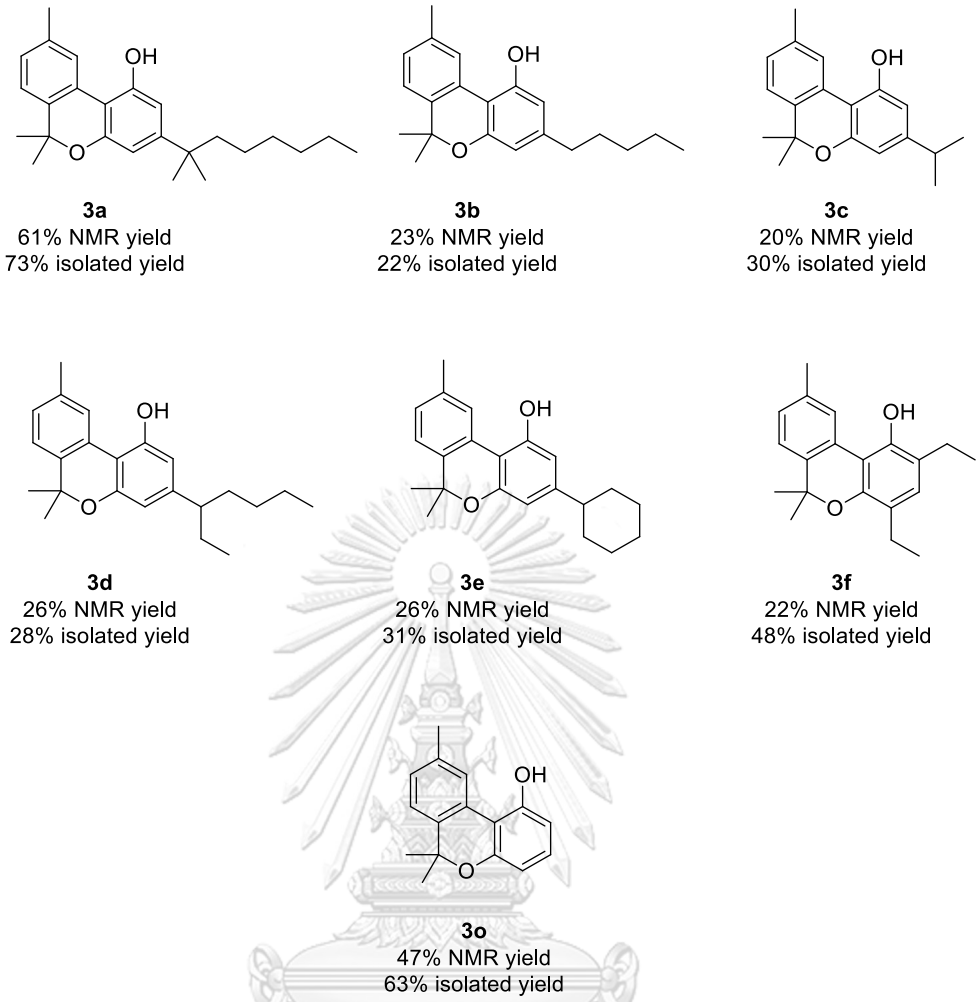


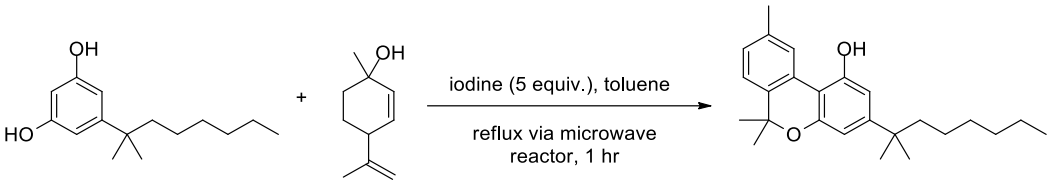
Figure 3.15 Structure, NMR yields and isolated yields of cannabinol derivatives

3.4 Synthesis of DMH-CBN via microwave-assisted reaction

With an attempt to improve the yield further, the model reaction between **1a** and **1b** was further examined under a microwave-assisted condition with 140°C of setting temperature and 300 W of power (**Table 3.8**, entry 2). The results indicated a production of **3a** in 30% yield, which was lower than that obtained using the classical protocol with an oil bath (**Table 3.8**, entry 1). The %conversion of **2a** showed a similar trend, demonstrating that **2a** could not endure the concentrated iodine conditions. Nevertheless, the critical difference was observed in the

%conversion of **1a**, which decreased dramatically to 44%. This decrease could be attributed to the fact that only small amounts of **1a** were utilized.

Table 3.8 Synthesis of **3a** under microwave-assisted conditions



Entry	Temperature (°C)	%conversion of **1a**	%conversion of **2a**	Yield[c] (%)
1	110[a]	88	100	61
2	110[b]	44	100	30

[a] heated in an oil bath, [b] heated in a microwave reactor, [c] NMR yield

Based on this evidence, it was proposed that **2a** decomposes more rapidly under the microwave conditions than the classical reflux. Consequently, only a small amount of **2a** could react with **1a**, leading to a larger quantity of **1a** remaining in the reaction. However, it was confirmed that **3a** could also be synthesized under the microwave condition which is easy to handle, quick to set up, and reduces the risk associated with refluxing temperatures.

3.5 Synthesis of unsubstituted cannabinol and bromo derivatives as key intermediates for further derivatization

The C3-unsubstituted CBN (CBN-C0, **3o**) should serve as a versatile intermediate for the synthesis of other cannabinol derivatives. For instance, this compound could be brominated and then used as a starting material in various well-known reactions, such as the Suzuki reaction and the Heck reaction. The first batch of **3o** bromination was attempted using 2 equiv. of *N*-bromosuccinamide (NBS) in acetonitrile (MeCN), resulting in the formation of 2,4-dibromocannabinol (CBN-Br_2, **3r**) in 90% isolated yield. Its ^{1}H NMR spectrum (**Figure 3.16**) clearly showed, in the

aromatic region, that only four protons remained in the structure out of the initial six protons. This suggests that bromination occurred twice on the aromatic ring. In the aromatic region, it showed two 1H singlet peaks at 8.27 and 7.57 ppm, a 2H singlet peak at 7.16 ppm, and a 1H singlet peak at 6.06 ppm of the hydroxy protons. Upon careful examination, the apparent singlet peak at 7.16 ppm with integration of 2H were in fact two proton signals that are strongly coupled to each other. Thus, the structure that is consistent with this spectral characteristic should be the 2,4-dibromo derivative of **1o**. A similar substitution pattern was previously reported in the study of bromination of natural CBN.[35] Moreover, high-resolution mass spectrometry (HRMS) was used to further confirm the elemental composition of **3r**. The mass spectrum showed a peak at m/z 396.9415 $[M-H]^+$, close to 396.9433 (calc.), and also revealed the characteristic mass patterns of the dibrominated product, including peaks of M, M+2, and M+4, as shown in **Figure 3.17**.

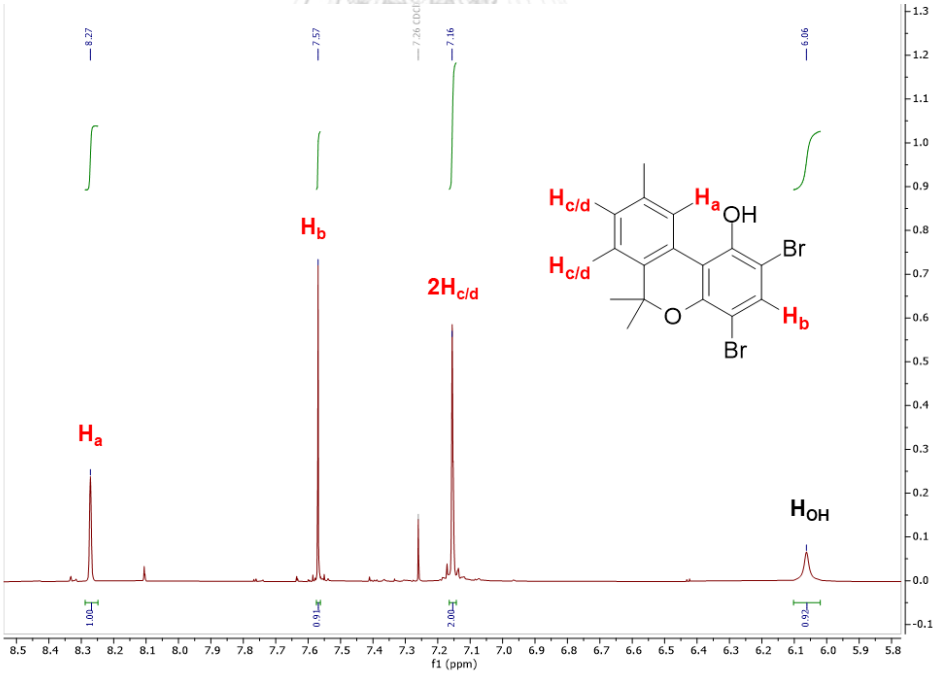


Figure 3.16 ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of the dibromination product **3r**

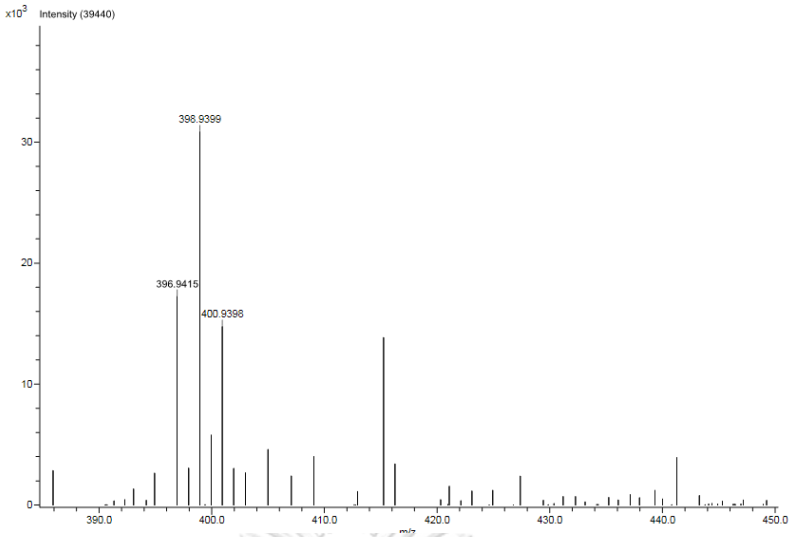


Figure 3.17 DART-TOF HRMS spectrum of **3r**

In an attempt to control the bromination rate in order to obtain only the monobrominated product, the amount of NBS was reduced to 0.5 equiv. and it was added in two portions at 0 and 15 minutes. After refluxing for 30 minutes, **3o** was transformed into 4-bromocannabinol (CBN-Br, **3s**) in 63% isolated yield. The ^{1}H NMR spectrum (**Figure 3.18**) showed five protons in the aromatic region: one characteristic peak at 8.30 ppm and four 1H doublet peaks at 7.29, 7.16, 7.13, and 6.53 ppm of the other aromatic peaks. This spectrum confirmed that only one bromination occurred, and the bromine atom should attach to the carbon on the more electron rich phenol-containing ring resulting in four doublet peaks. To determine the position of the bromine atom in relation to the hydroxy group (either ortho or para), 2D NMR analysis was required. The ^{1}H-^{13}C HMBC spectrum (**Figure 3.19**) revealed a signal between the 1H singlet peak at 6.09 ppm of the hydroxy proton and the carbon peak at 112 ppm. This carbon corresponds to the one that bears an aromatic proton at 6.54 ppm, as observed in the ^{1}H-^{13}C HSQC spectrum (**Figure 3.20**). Based on this evidence, it can be inferred that the bromine is located at the para- position of the hydroxy group or the 4-position on the CBN scaffold. HRMS was used to confirm the structure of **3s**. Its mass was found at m/z 319.0321

$[M-H]^+$, close to 319.0328 (calc.), and also showed characteristic mass patterns of the monobrominated product, including peaks of M and M+2, as shown in **Figure 3.21**. These two reactions could be summarized as shown in **Figure 3.22**.

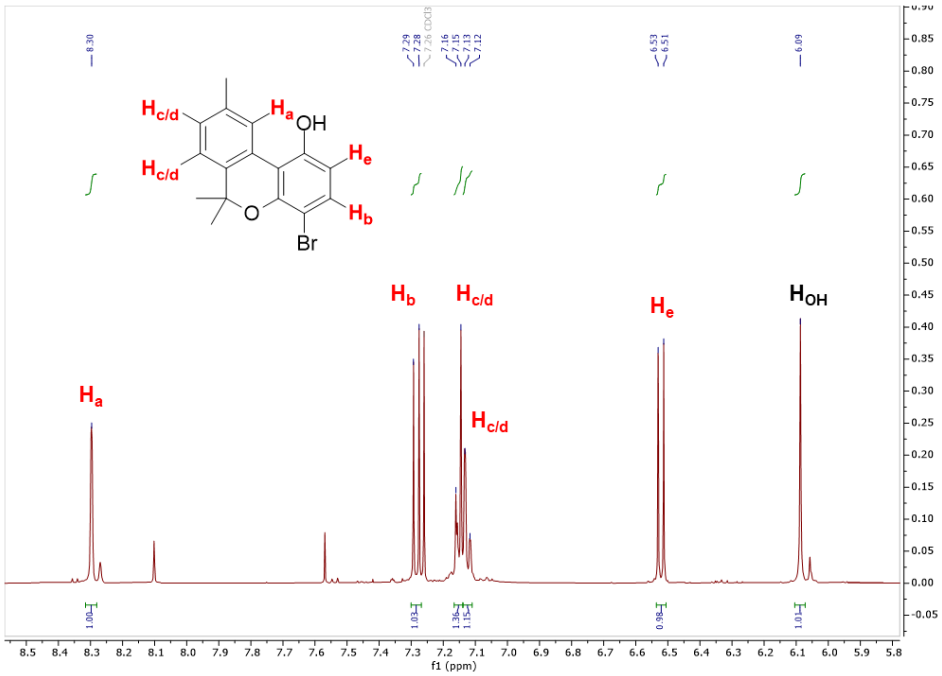


Figure 3.18 ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of **3s**

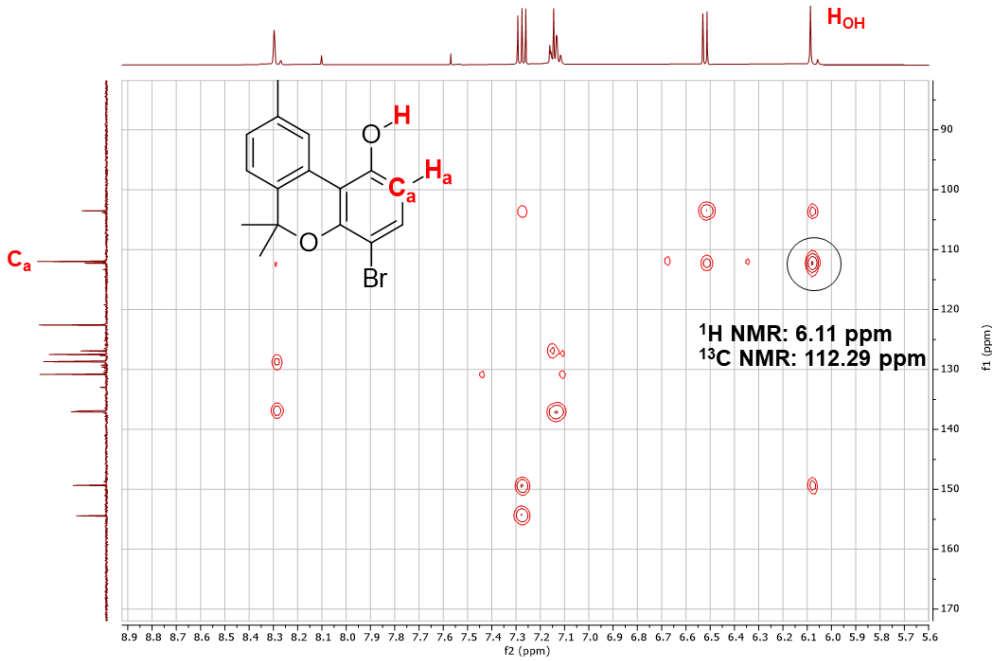


Figure 3.19 ^{1}H-^{13}C HMBC NMR spectrum of **3s**

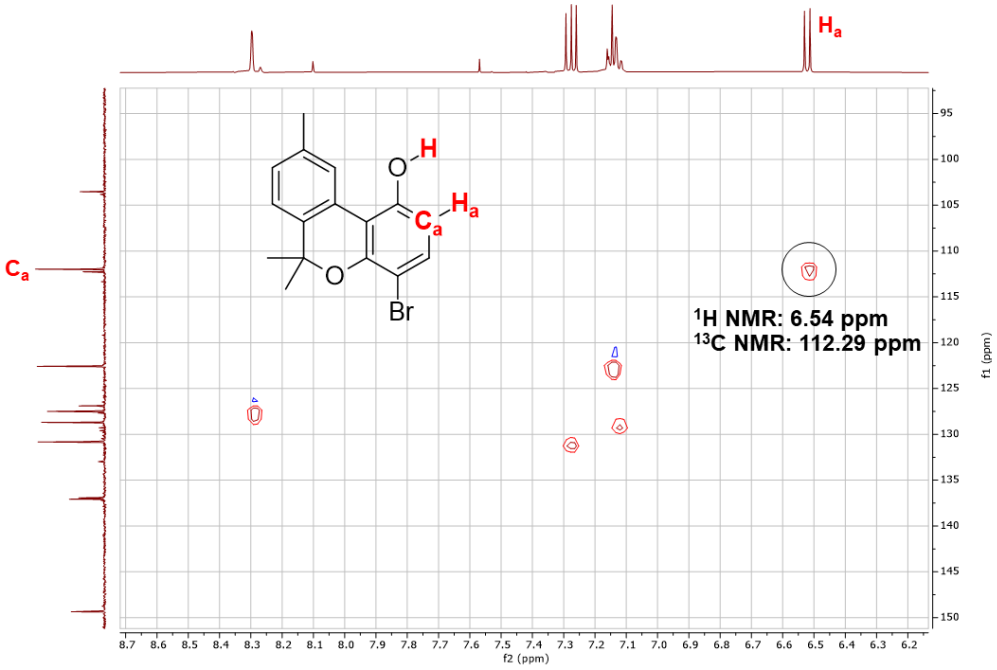


Figure 3.20 ^{1}H-^{13}C HSQC NMR spectrum of **3s**

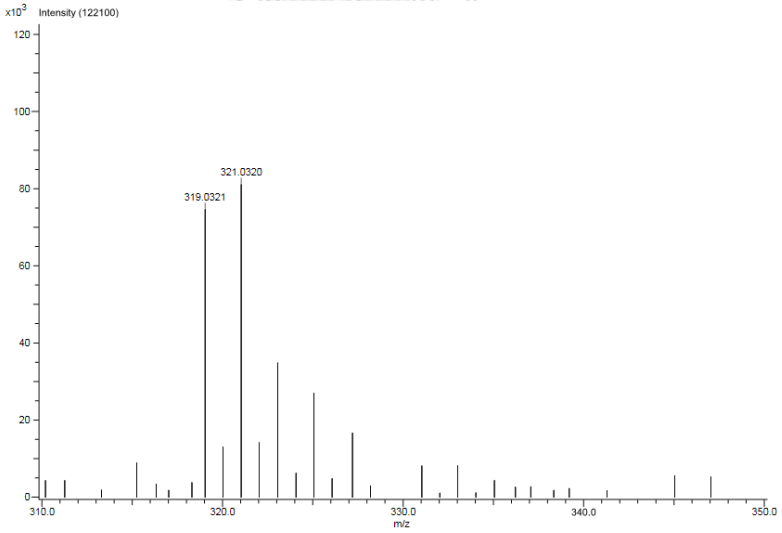
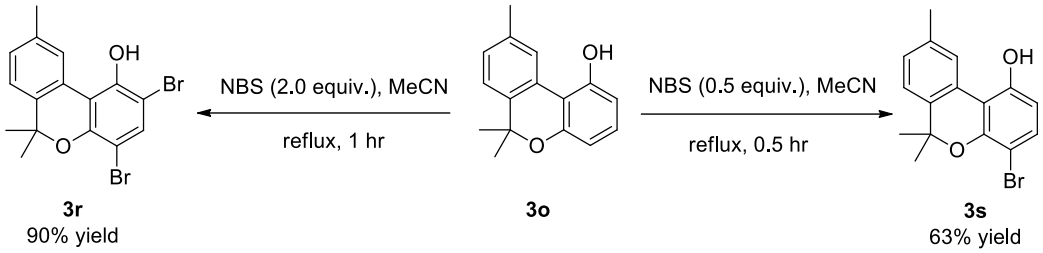


Figure 3.21 DART-TOF HRMS spectrum of **3s**



3r
90% yield

3o

3s
63% yield

Figure 3.22 Bromination of **3o** to give **3s** and **3r**

The introduction of two additional portions of NBS over the course of 1 hr resulted in the formation of another brominated product, which was identified as **3r** that is identical to the product from the first bromination reaction as shown in **Figure 3.23**.

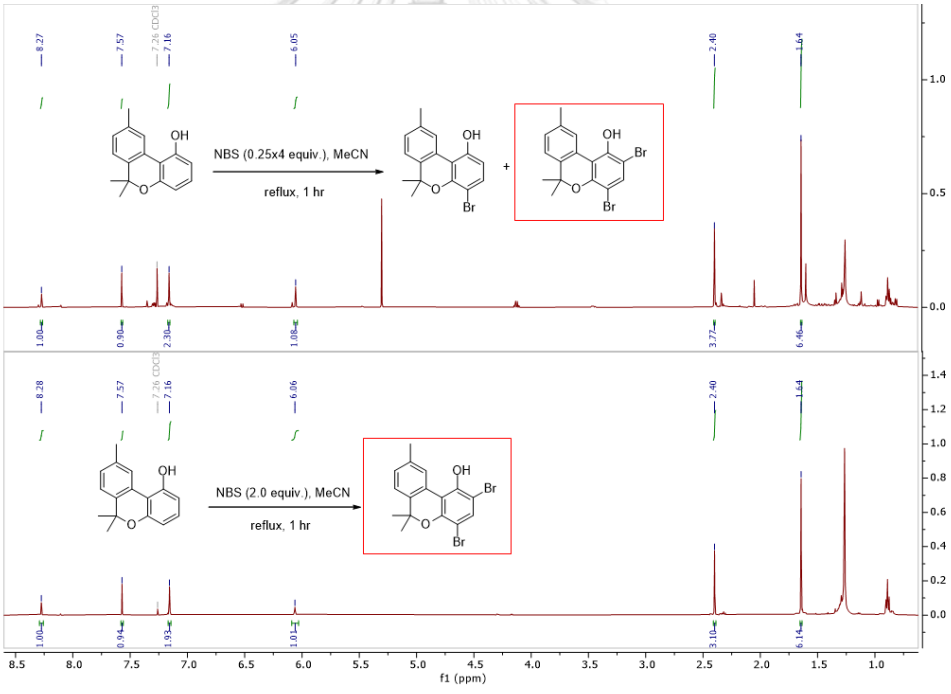


Figure 3.23 Comparison of ^{1}H NMR spectra of **3r** obtained from different bromination methods

3.6 Measurement of transepithelial electrical resistance (TER)

Cannabinoid compounds have been reported to have the ability to protect the intestinal epithelium barrier from *in vitro* inflammation, resulting in the recovery

of junctional permeability and the maintenance of epithelial function. They reported that THC and CBD, especially CBD, showed a protective role on the intestinal epithelium barrier.[7] Therefore, in this work, we are interested in exploring whether CBN derivatives, belonging to the same cannabinoid family, might give promising results. Herein, transepithelial electrical resistance (TER) assay with Ca^{2+} assay as a method for tight junction opening and resealing,[12-14] were used to evaluate the activity of CBN derivatives synthesized in this work. The %TER was measured from the barrier tissue model through the recording of voltage with electrodes from both sides of the cell monolayer as shown the results in **Figure 3.24**

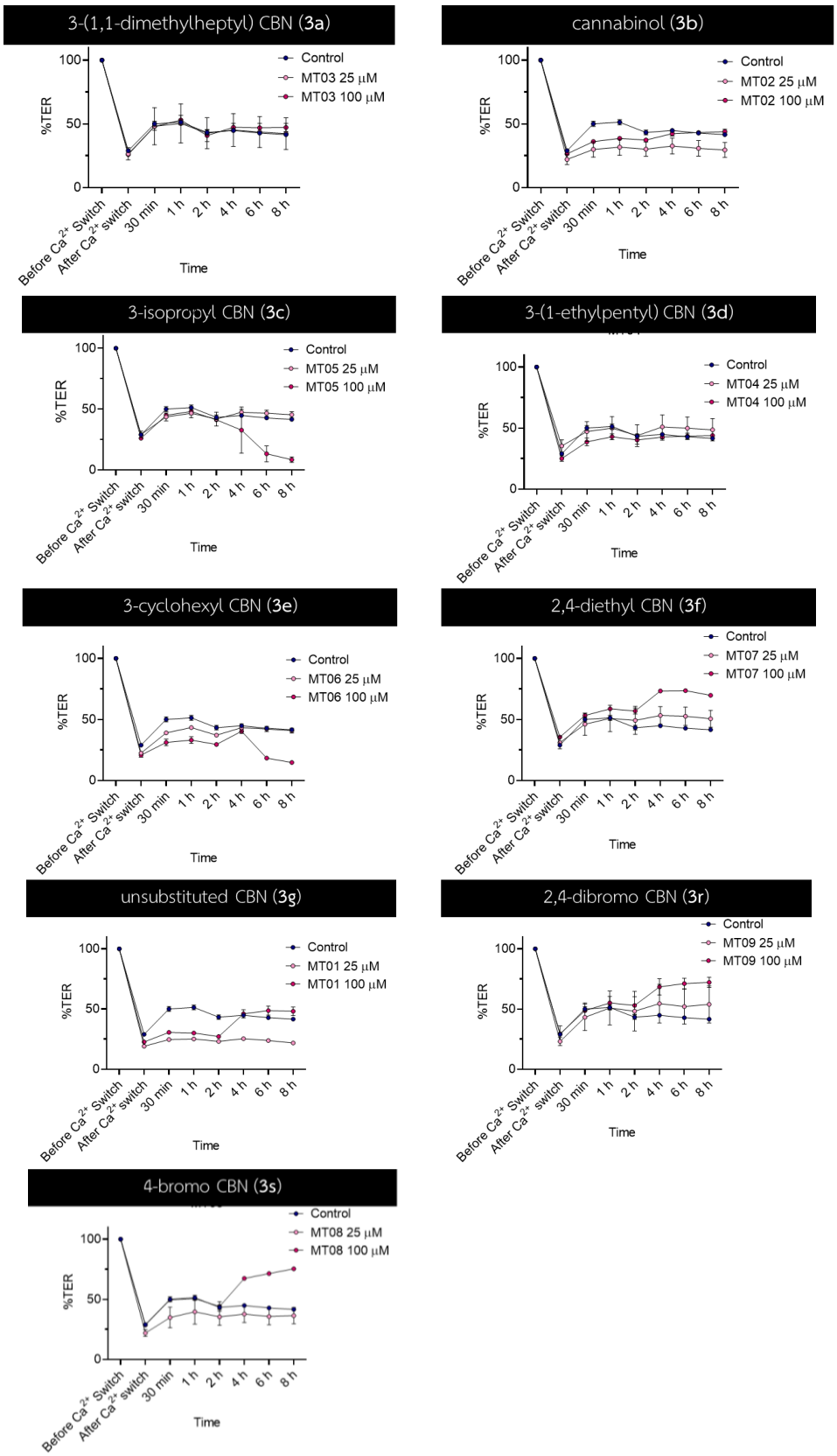
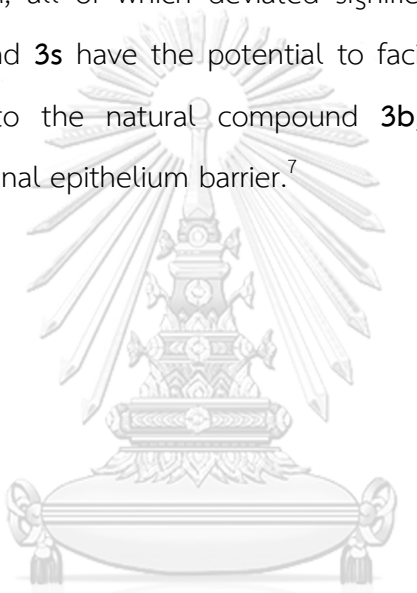


Figure 3.24 Effect of cannabinol derivatives to change in %TER for 25 and 100 μM

For **3b**, the natural cannabinol, the %TER values at both 25 μM and 100 μM showed lower %TER compared to the control (**Figure 3.24**, **3b**). These results indicated a lower potential in terms of tight junction resealing. The DMH derivative **3a** seemed to perform better, as shown in **Figure 3.24**, **3a**, where both concentrations showed an equivalent %TER compared to the control at all time spots. However, **3f**, the diethyl derivative, and **3r** and **3s**, the bromo derivatives, showed promising results at a concentration of 100 μM, with **3r** and **3f** also showing good results at 25 μM, all of which deviated significantly from the control. This suggests that **3f**, **3r**, and **3s** have the potential to facilitate better in tight junction formation compared to the natural compound **3b**, which might lead to the protection of the intestinal epithelium barrier.[7]



CHAPTER IV

CONCLUSION

Herein, we present a facile one-pot method for the synthesis of CBN through a cascade reaction between terpenoids and resorcinol derivatives. It was proposed that the reaction occurred through a Friedel-Crafts alkylation, cyclization, and aromatization reaction sequence, all of which were mediated by molecular iodine which is inexpensive and relatively safe as the only reagent. The reaction proceeded best in toluene and was completed in less than 1 hr. Both conventional and microwave heating could be used, although conventional heating gave a better yield. The reaction is applicable for the synthesis of natural CBN, DMH-CBN, and many other derivatives of CBN carrying different alkyl groups by varying the resorcinol part. However, the method did not work with resorcinol bearing an electron-withdrawing group as well as resorcinol derivatives that were poorly soluble in toluene. Better yields were observed with sterically hindered alkyl substituent on the resorcinol such as in the case of DMH-CBN.

For the terpenoid part, (1*S*,4*R*)-*p*-menthadienol and citral could participate in the reaction. While (1*S*,4*R*)-*p*-menthadienol gave better yields in most cases, the use of citral was attractive because it is an inexpensive starting material and gave acceptable results with natural CBN and DMH-CBN. Interestingly, the reaction between (1*S*,4*R*)-*p*-menthadienol or citral and 4,6-di-*tert*-butylresorcinol provided unsubstituted cannabinol instead of the expected product. Apparently, the *tert*-butyl group was removed under the reaction conditions, possibly by HI generated during the aromatization step. This compound is a potential starting material for the synthesis of other cannabinol derivatives.

The CBN derivatives were tested for biological activities. The transepithelial electrical resistance assay indicated that 2,4-diethylcannabinol, 4-bromocannabinol, and 2,4-dibromocannabinol, appeared to have potential in terms of protecting intestinal epithelial barrier higher than the natural CBN.

To conclude, the developed iodine-mediated cascade reaction between (1*S*,4*R*)-*p*-menthadienol or citral and resorcinol derivatives offers a convenient and safe approach for the synthesis of CBN derivatives in one pot under a short reaction time. However, the study of reaction by-products, improvement of product yields, and adjustment of conditions to accommodate a more diverse range of substrates are required for future work.

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APPENDIX

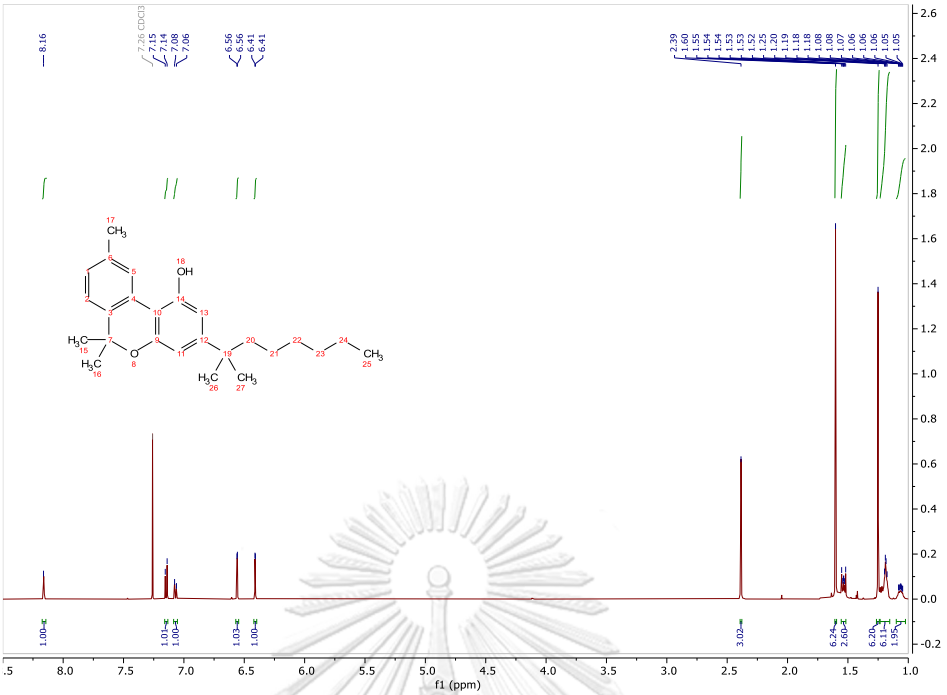


Figure A1. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of 3-(1,1-dimethylheptyl)cannabinol (DMH-CBN, **3a**)

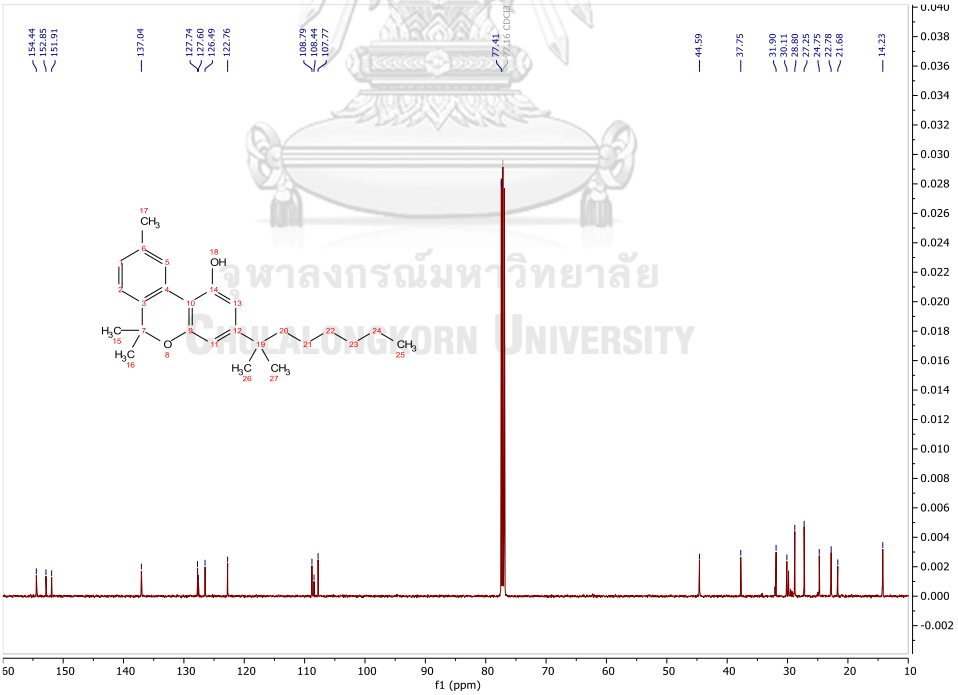


Figure A2. ^{13}C NMR (126 MHz, $CDCl_3$) spectrum of 3-(1,1-dimethylheptyl)cannabinol (DMH-CBN, **3a**)

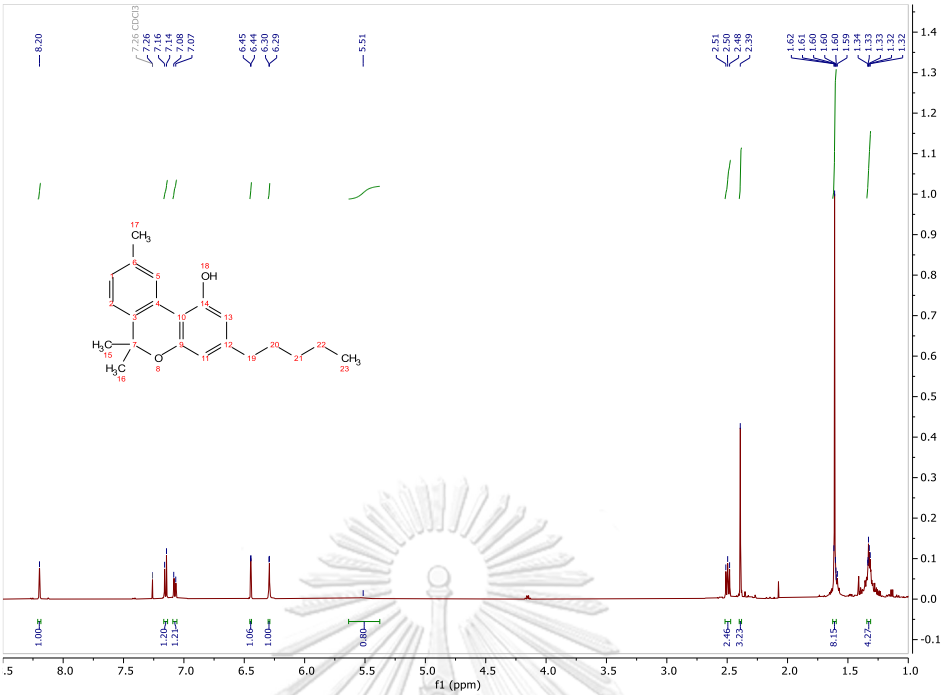


Figure A3. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of cannabinol (CBN, **3b**)

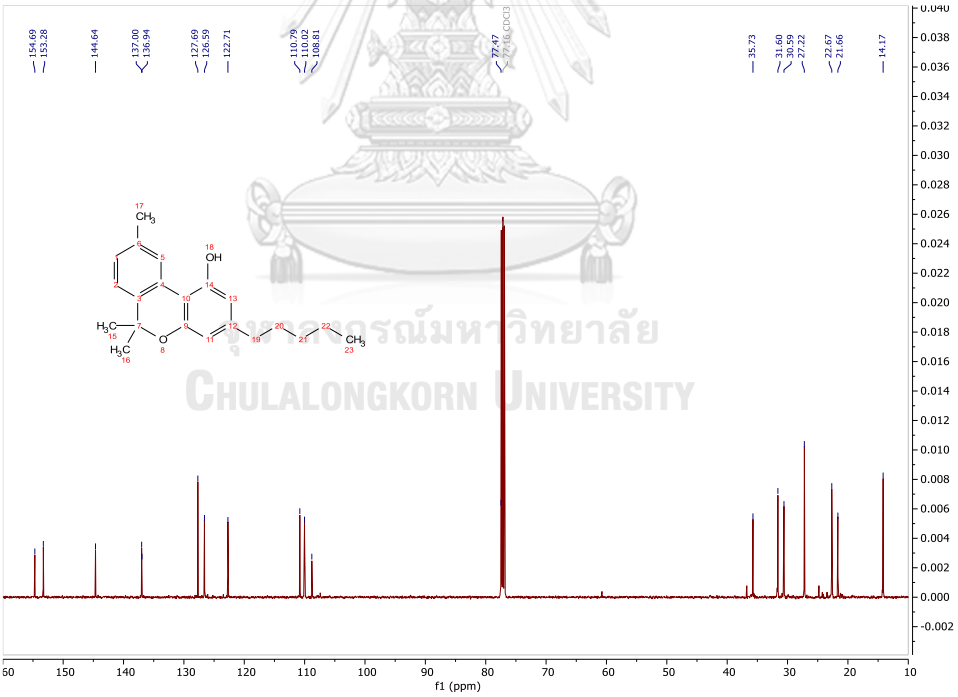


Figure A4. ^{13}C NMR (126 MHz, $CDCl_3$) spectrum of cannabinol (CBN, **3b**)

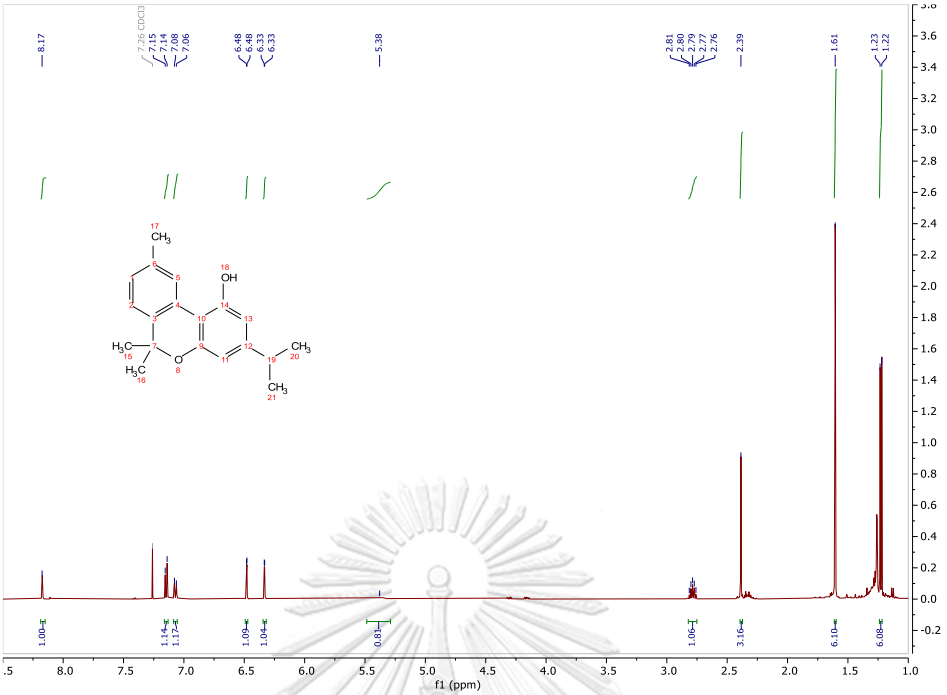


Figure A5. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of 3-isopropylcannabinol (iPr-CBN, **3c**)

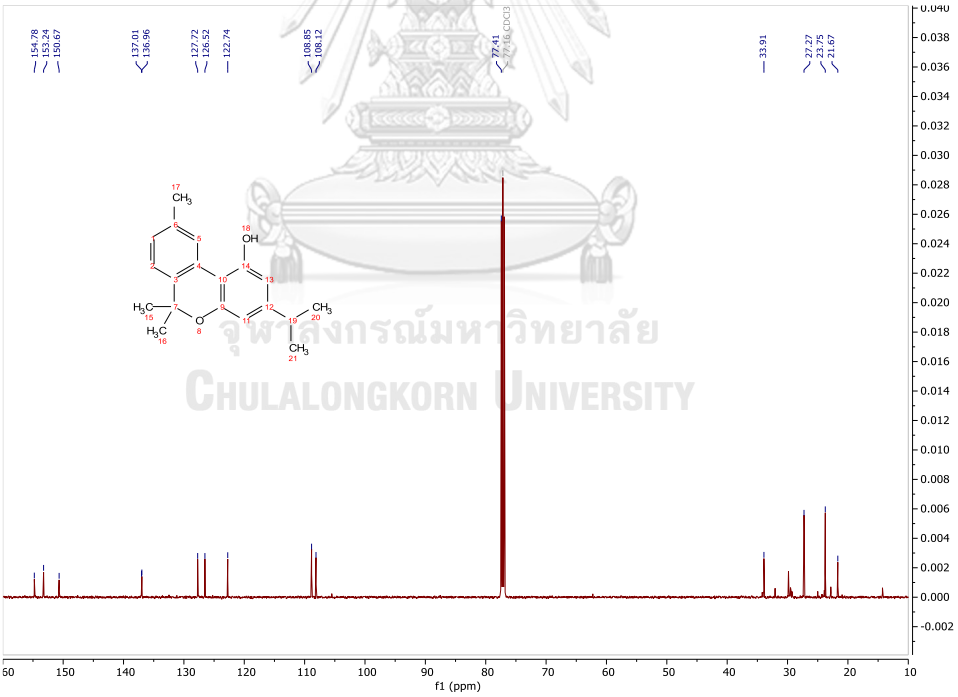


Figure A6. ^{13}C NMR (126 MHz, $CDCl_3$) spectrum of 3-isopropylcannabinol (iPr-CBN, **3c**)

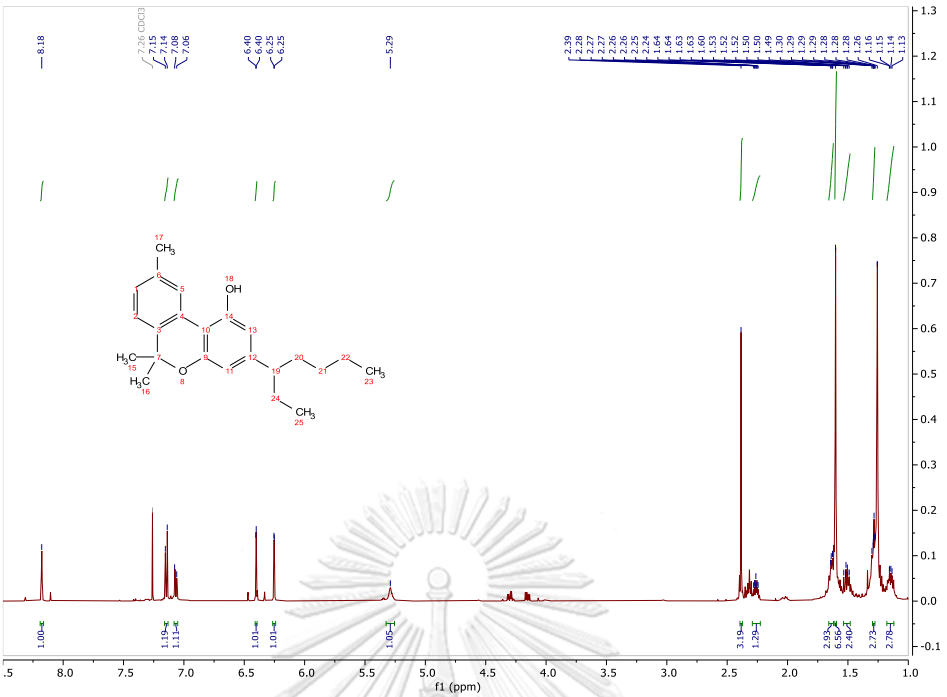


Figure A7. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of 3-(1-ethylpentyl)cannabinol (EtPe-CBN, **3d**)

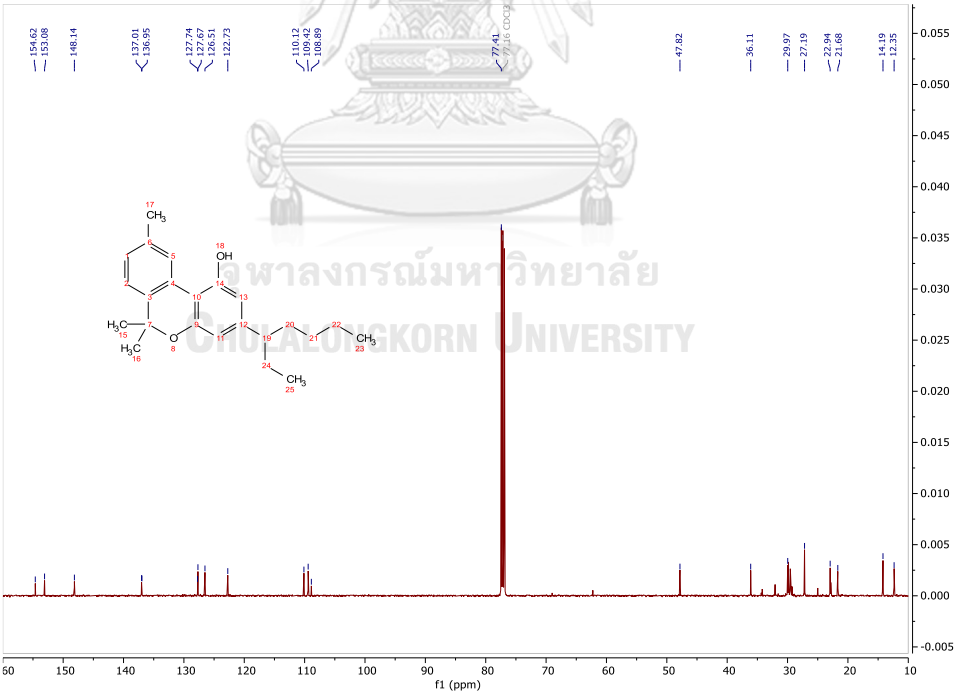


Figure A8. ^{13}C NMR (126 MHz, $CDCl_3$) spectrum of 3-(1-ethylpentyl)cannabinol (EtPe-CBN, **3d**)

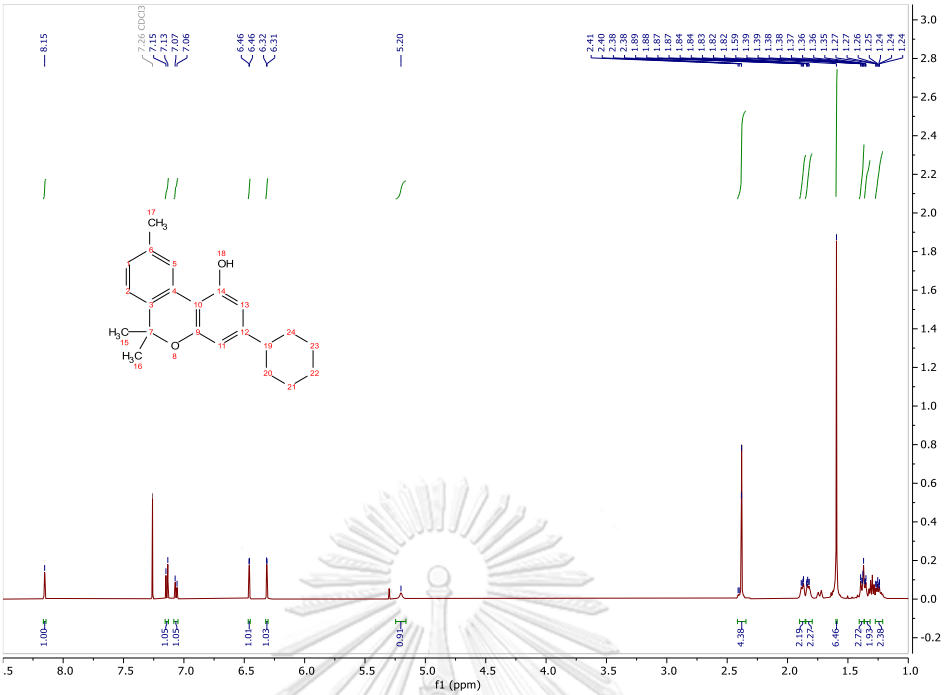


Figure A9. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of 3-cyclohexylcannabinol (Cy-CBN, **3e**)

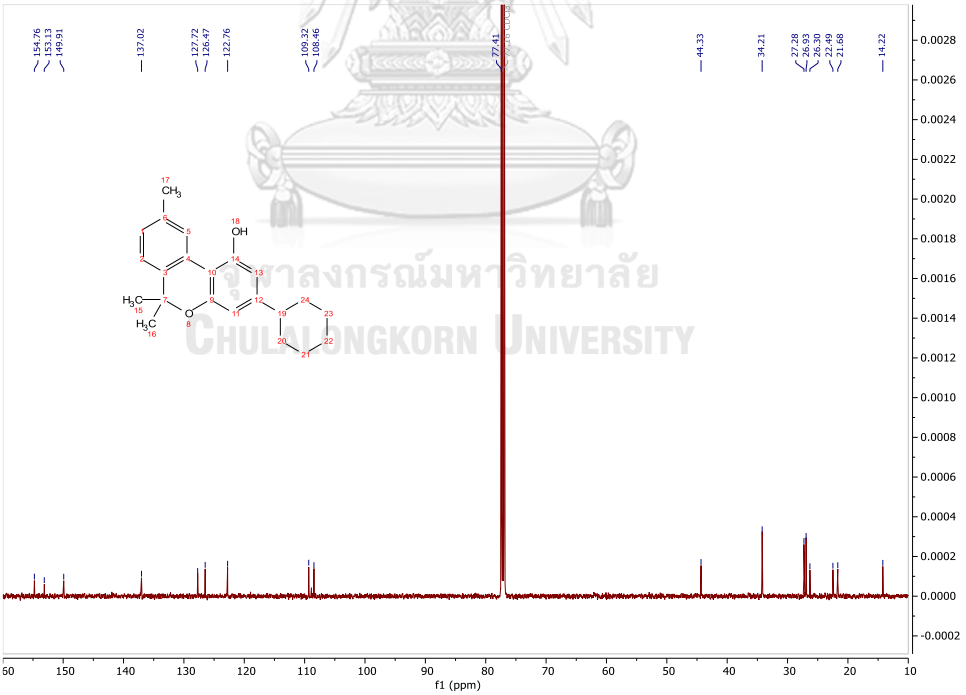


Figure A10. ^{13}C NMR (126 MHz, $CDCl_3$) spectrum of 3-cyclohexylcannabinol (Cy-CBN, **3e**)

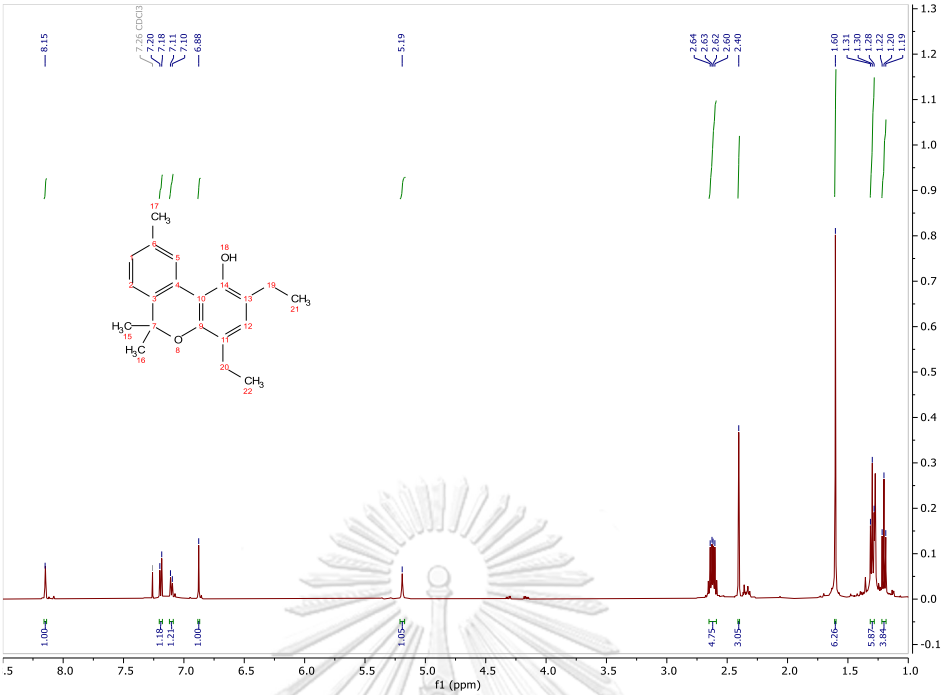


Figure A11. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of 2,4-diethylcannabinol (diEt-CBN, **3f**)

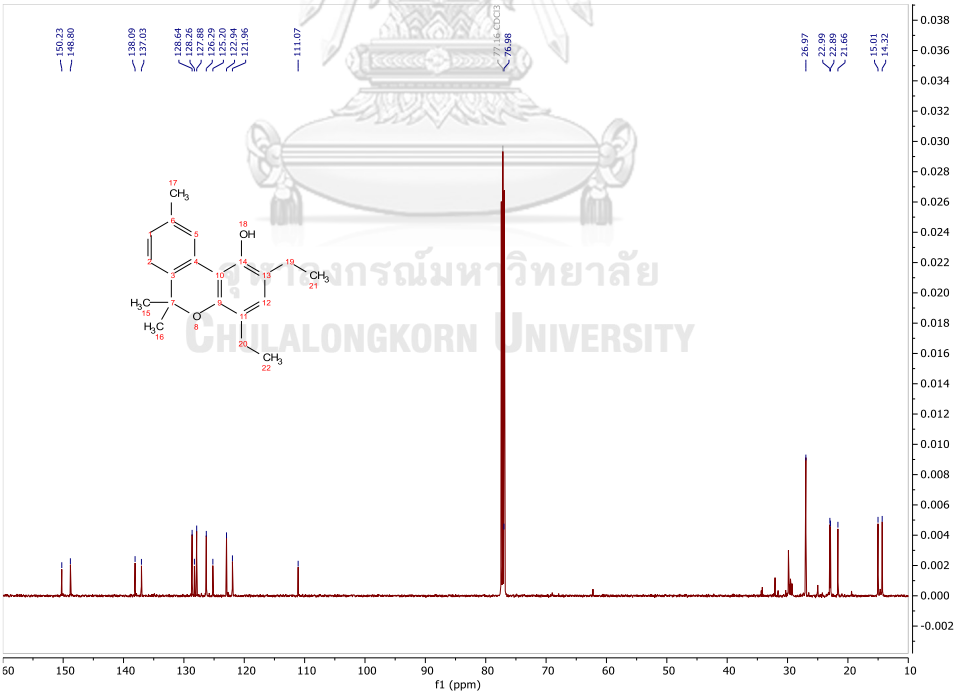


Figure A12. ^{13}C NMR (126 MHz, $CDCl_3$) spectrum of 2,4-diethylcannabinol (diEt-CBN, **3f**)

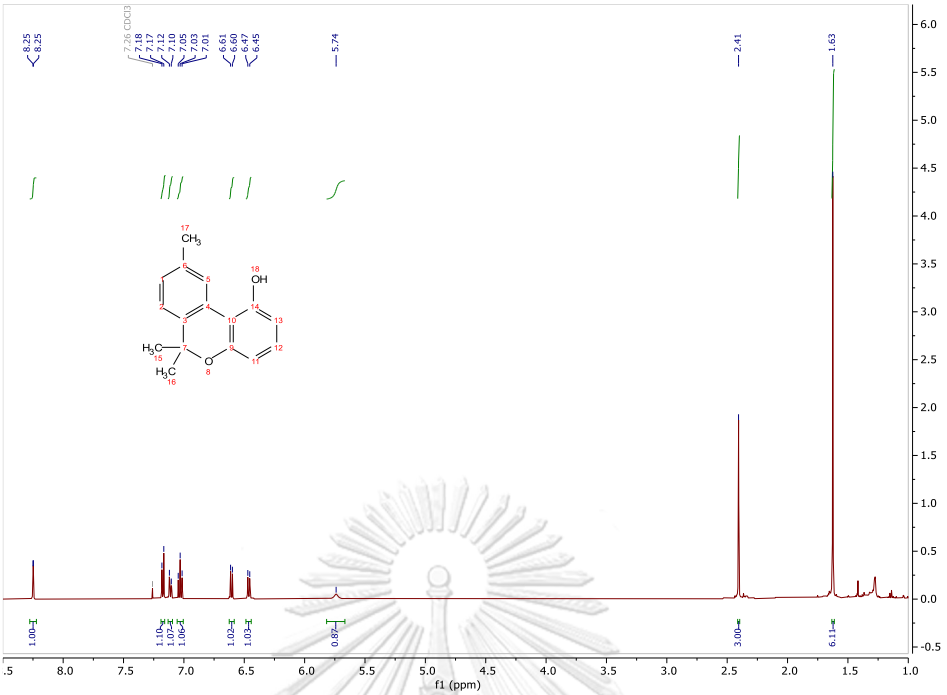


Figure A13. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of unsubstituted cannabinol (CBN-C0, **3o**)

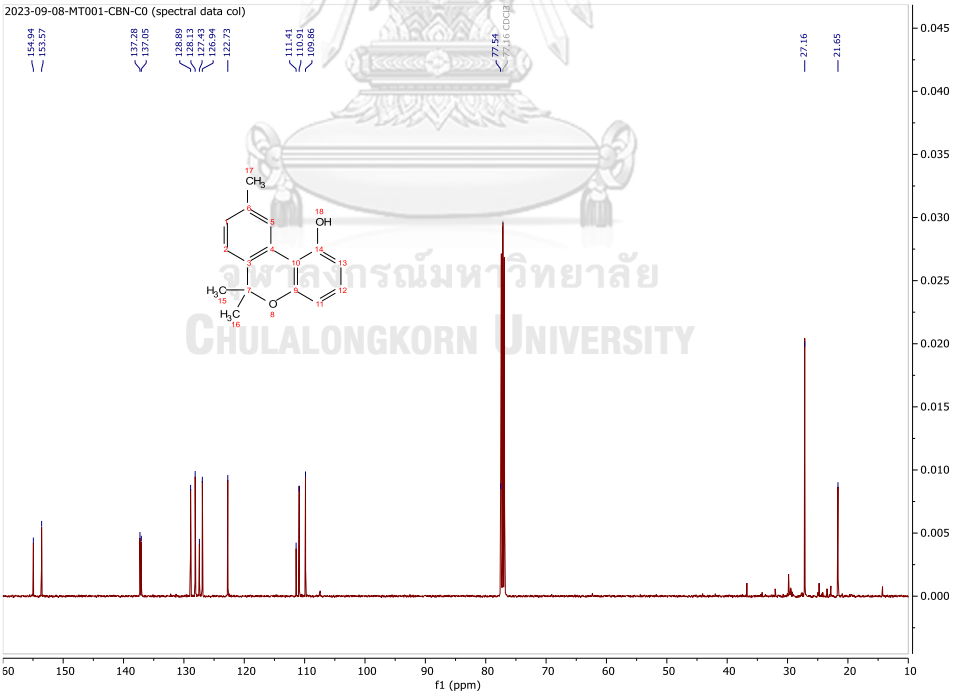


Figure A14. ^{13}C NMR (126 MHz, $CDCl_3$) spectrum of unsubstituted cannabinol (CBN-C0, **3o**)

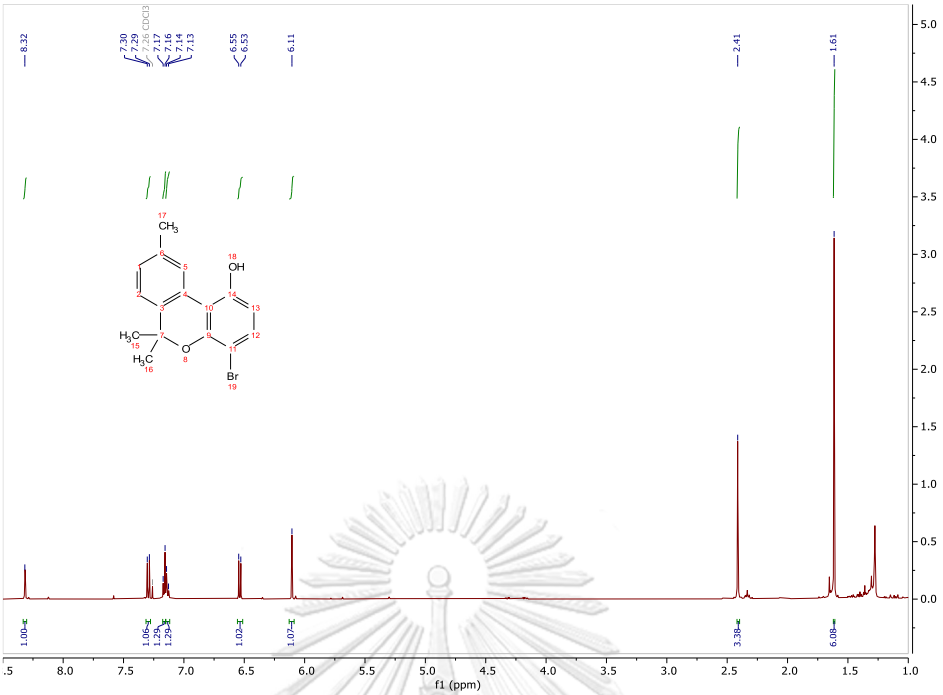


Figure A15. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of 4-bromocannabinol (CBN-Br, **3s**)

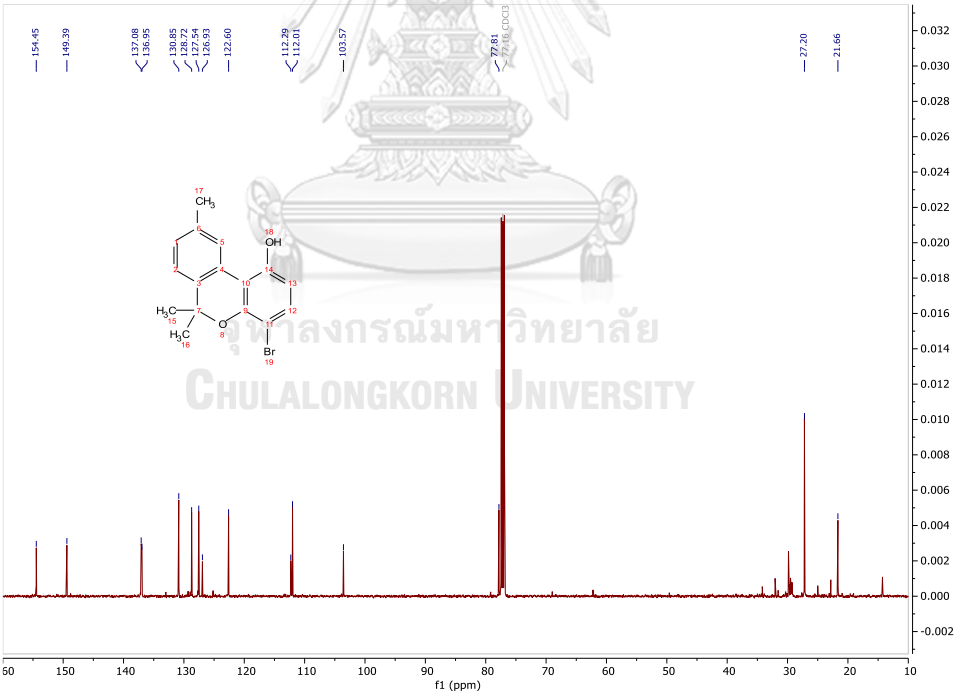


Figure A16. ^{13}C NMR (126 MHz, $CDCl_3$) spectrum of 4-bromocannabinol (CBN-Br, **3s**)

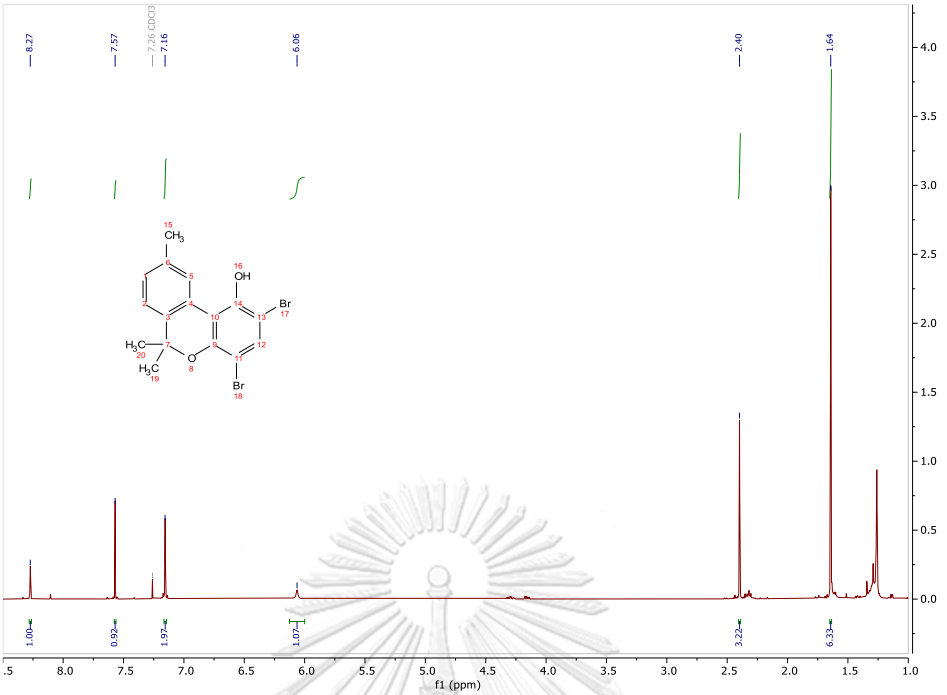


Figure A17. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of 2,4-dibromocannabinol (CBN-Br_2, **3r**)

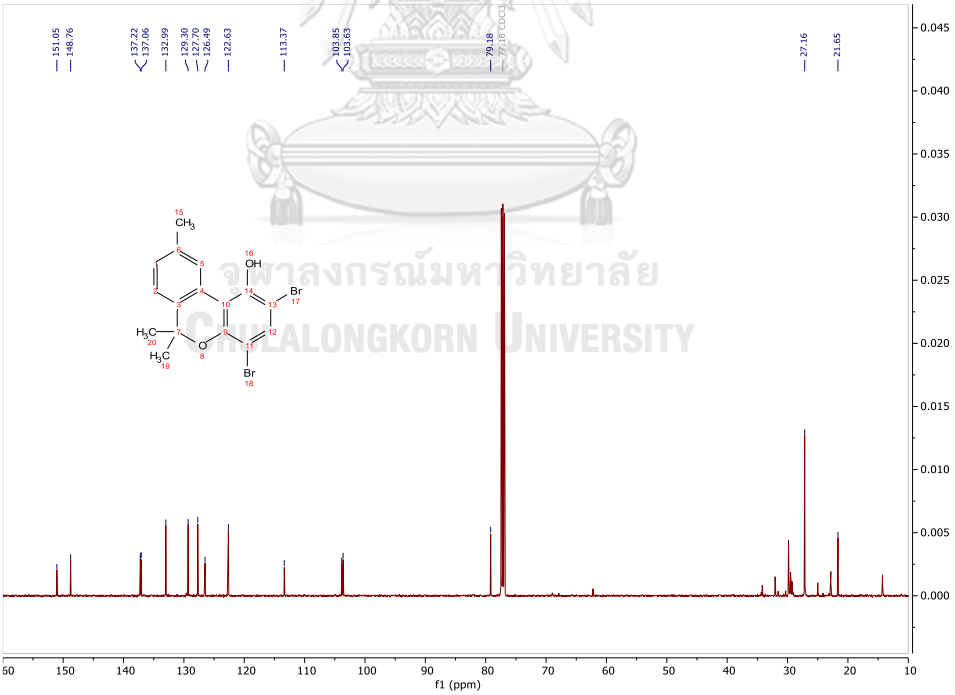


Figure A18. ^{13}C NMR (126 MHz, $CDCl_3$) spectrum of 2,4-dibromocannabinol (CBN-Br_2, **3r**)

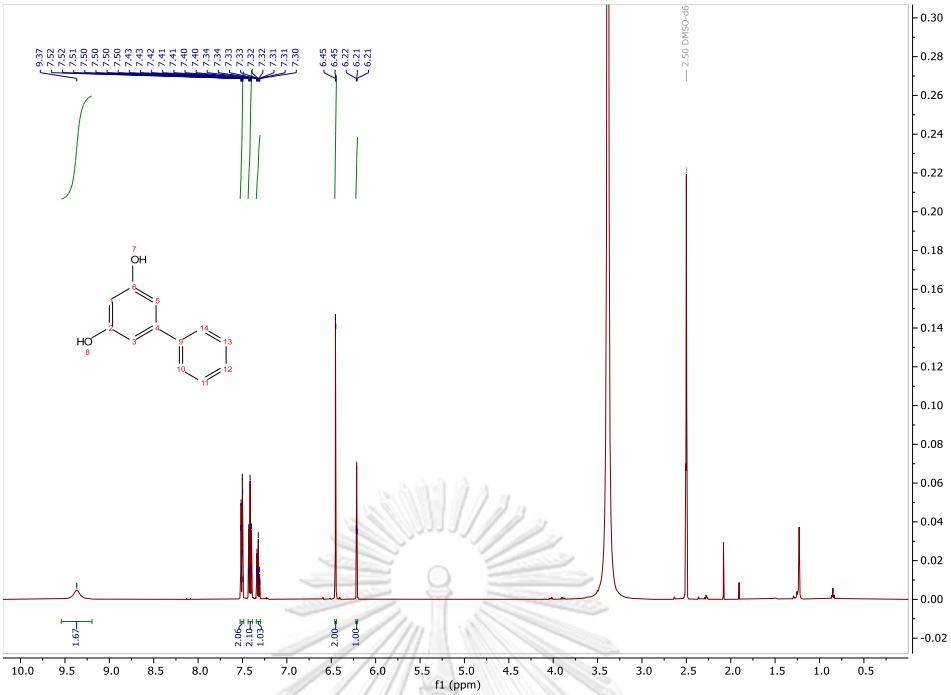


Figure A19. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of 5-phenylresorcinol

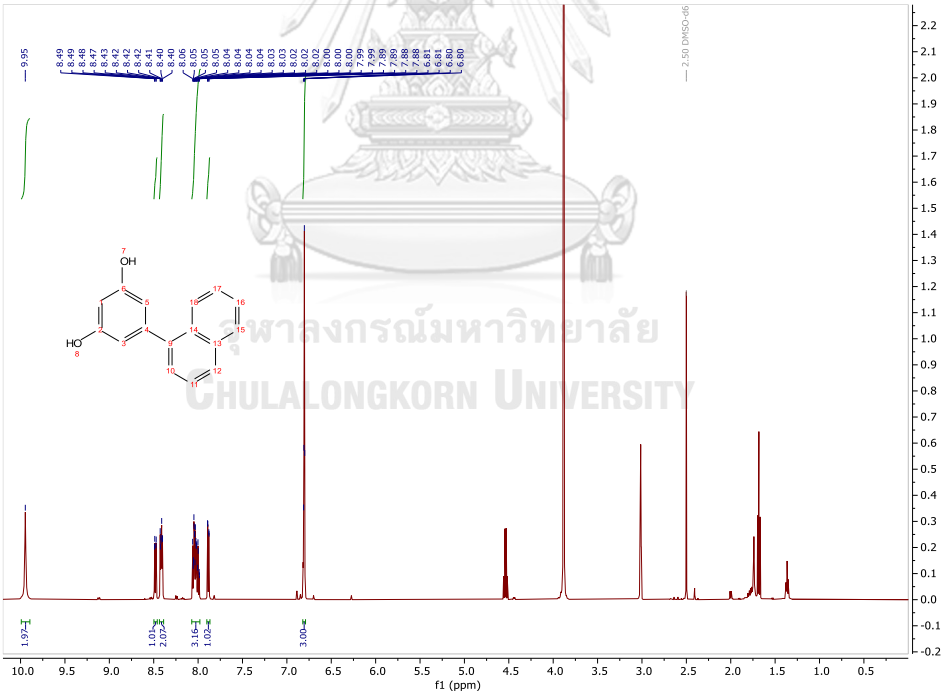


Figure A20. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of 5-naphthylresorcinol

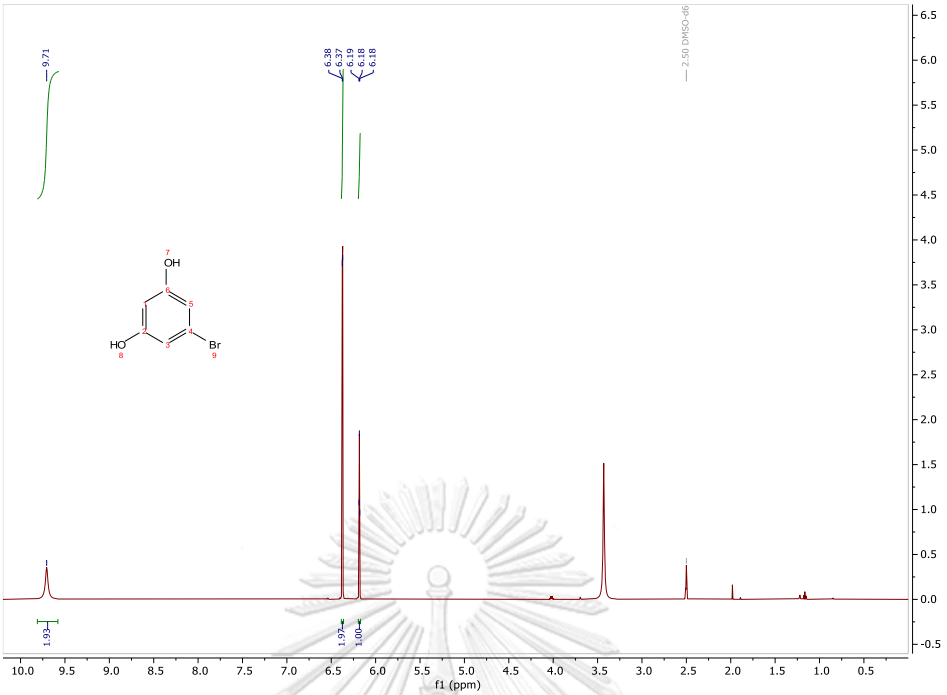


Figure A21. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of 5-bromoresorcinol

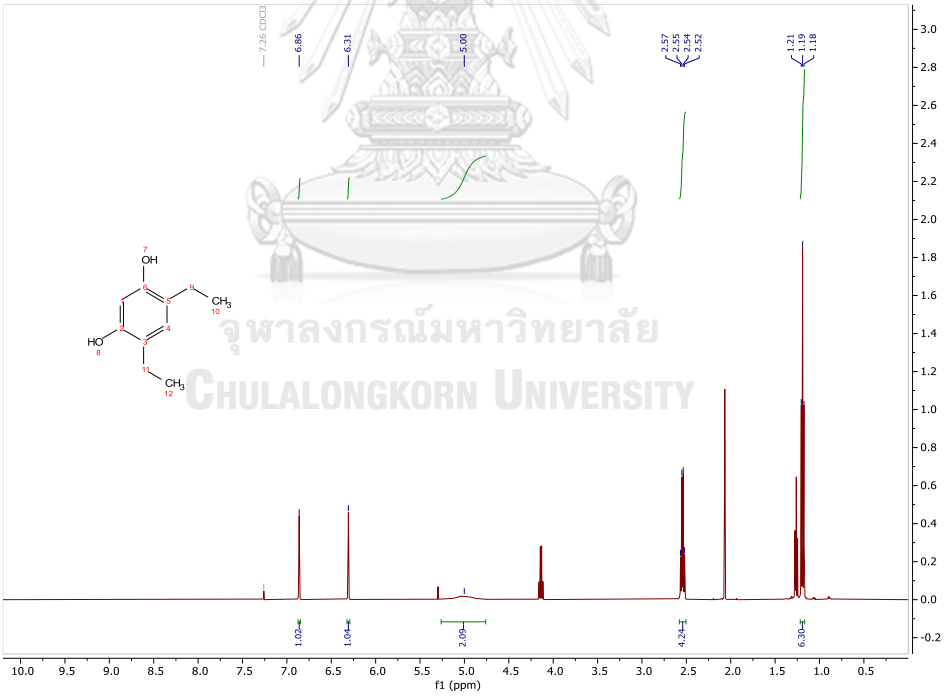


Figure A22. ^{1}H NMR (500 MHz, $CDCl_3$) spectrum of 4,6-diethylresorcinol

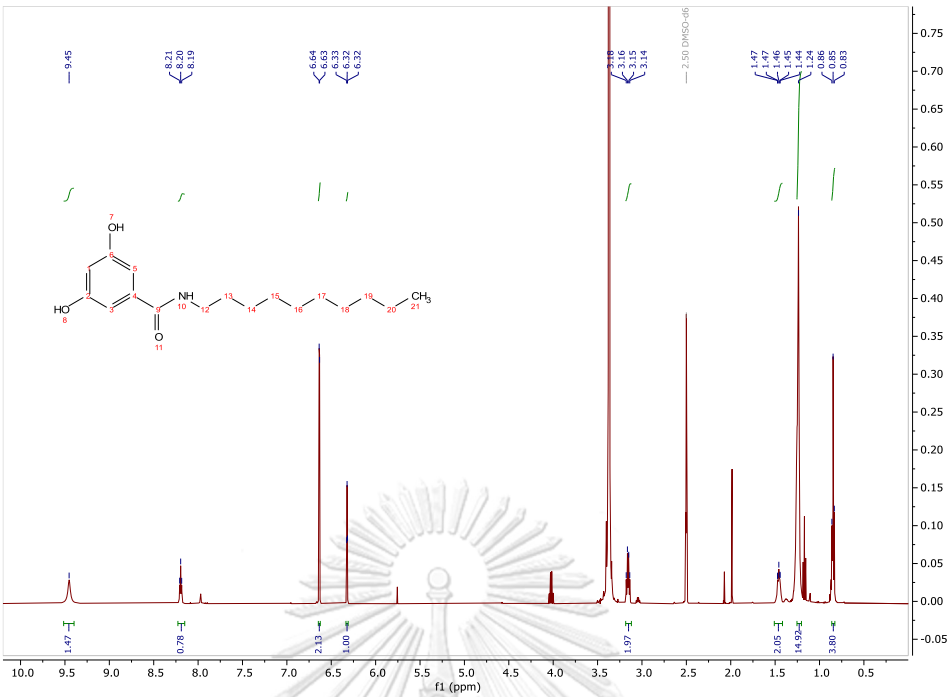
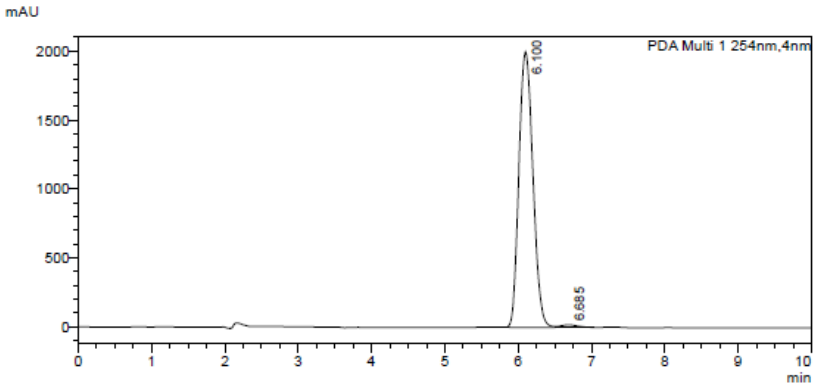


Figure A23. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of 5-decylamino(oxo)resorcinol

Table A1. %Purity of cannabinol derivatives

entry	compound	%purity
1	3-(1,1-dimethylheptyl)cannabinol	99
2	cannabinol	80
3	3-isopropylcannabinol	86
4	3-(1-ethylpentyl)cannabinol	79
5	3-cyclohexylcannabinol	100
6	2,4-diethylcannabinol	78
7	unsubstituted cannabinol	95
8	4-bromocannabinol	92
9	2,4-dibromocannabinol	87

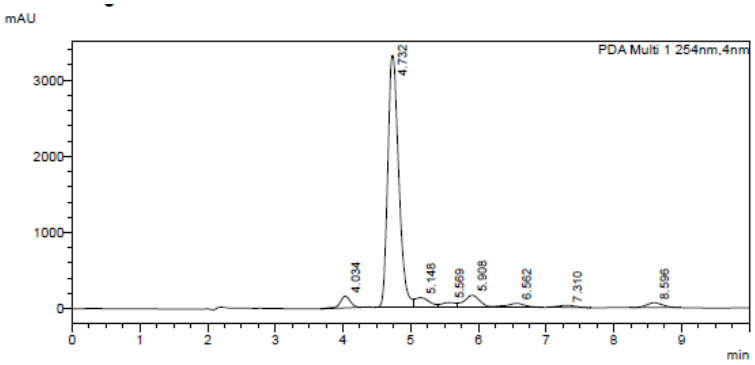


<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	6.100	26579995	2000645	98.905			
2	6.685	294351	18198	1.095		V	
Total		26874346	2018843				

Figure A24. HPLC chromatogram and %purity of 3-(1,1-dimethylheptyl)cannabinol (DMH-CBN, **3a**); analytical HPLC (Shimadzu, model RID-20A); Hypersil™ BDS C18 HPLC Column Dim. 150 x 4.6 mm (Thermo Fisher Scientific); eluent 90% MeCN in H_2O, 0.5 mL/min flow rate for 10 minutes; PDA UV detector

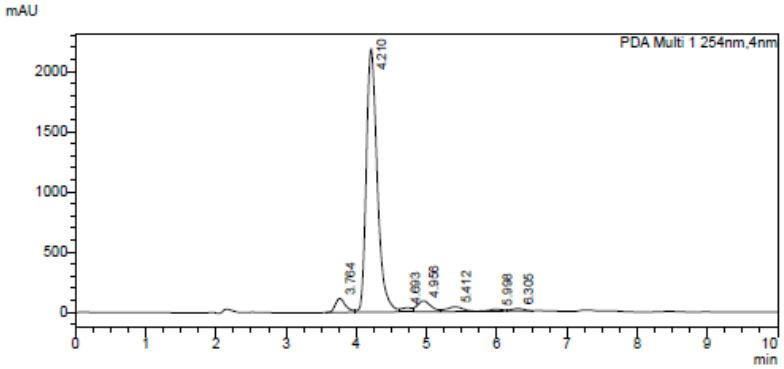


<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	4.034	1619438	153577	3.472			
2	4.732	37153339	3307473	79.655			
3	5.148	1936812	126462	4.152		V	
4	5.569	980075	62565	2.101		V	
5	5.908	2509921	158218	5.381		V	
6	6.562	1033232	53079	2.215		V	
7	7.310	434982	27137	0.933			
8	8.596	975000	60508	2.090		M	
Total		46642799	3949019				

Figure A25. HPLC chromatogram and %purity of cannabinol (CBN, **3b**); analytical HPLC (Shimadzu, model RID-20A); Hypersil™ BDS C18 HPLC Column Dim. 150 x 4.6 mm (Thermo Fisher Scientific); eluent 90% MeCN in H_2O, 0.5 mL/min flow rate for 10 minutes; PDA UV detector

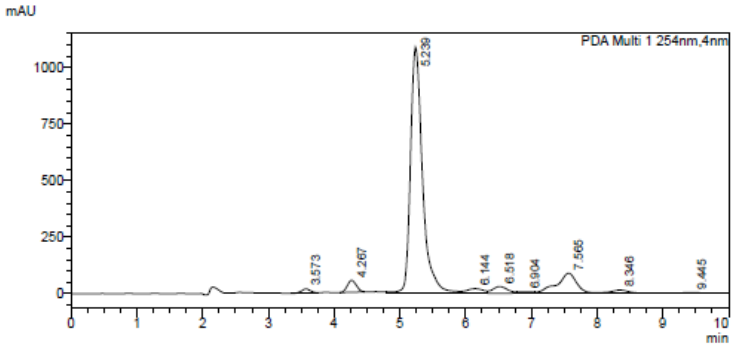


<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	3.764	1188031	115632	4.307			
2	4.210	23749754	2181743	86.108		V	
3	4.693	378094	32791	1.371		V	
4	4.956	1198192	89901	4.344		V	
5	5.412	614976	38693	2.230		V	
6	5.998	212937	16346	0.772		V	
7	6.305	239410	19639	0.868		V	
Total		27581394	2494746				

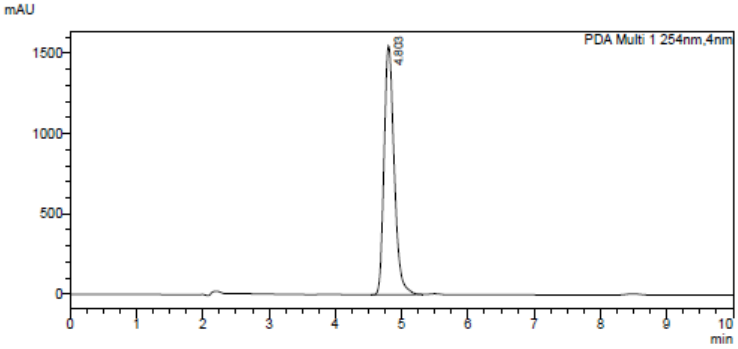
Figure A26. HPLC chromatogram and %purity of 3-isopropylcannabinol (iPr-CBN, **3c**); analytical HPLC (Shimadzu, model RID-20A); Hypersil™ BDS C18 HPLC Column Dim. 150 x 4.6 mm (Thermo Fisher Scientific); eluent 90% MeCN in H_2O, 0.5 mL/min flow rate for 10 minutes; PDA UV detector



<Peak Table>
PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	3.573	206370	20150	1.169			
2	4.267	504374	52786	2.856		M	
3	5.239	13910596	1088950	78.772			
4	6.144	366825	20412	2.077		V	
5	6.518	465488	29691	2.636		V	
6	6.904	89354	6311	0.506		V	
7	7.565	1839808	88552	10.418		V	
8	8.346	254487	13892	1.441		V	
9	9.445	22122	1589	0.125			
Total		17659424	1322313				

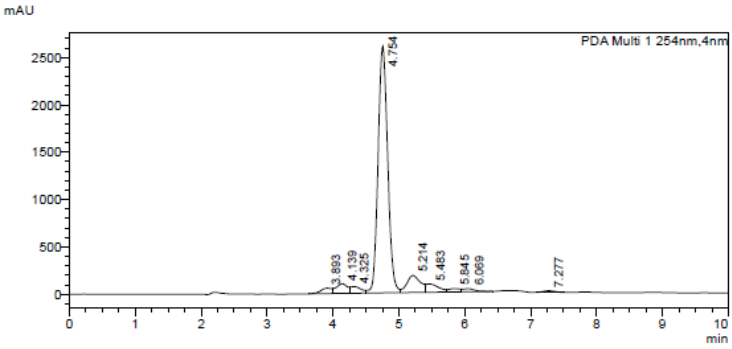
Figure A27. HPLC chromatogram and %purity of 3-(1-ethylpentyl)cannabinol (EtPe-CBN, **3d**) ; analytical HPLC (Shimadzu, model RID-20A); Hypersil™ BDS C18 HPLC Column Dim. 150 x 4.6 mm (Thermo Fisher Scientific); eluent 90% MeCN in H_2O, 0.5 mL/min flow rate for 10 minutes; PDA UV detector



<Peak Table>
PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	4.803	16421152	1547043	100.000			
Total		16421152	1547043				

Figure A28. HPLC chromatogram and %purity of 3-cyclohexylcannabinol (Cy-CBN, **3e**); analytical HPLC (Shimadzu, model RID-20A); Hypersil™ BDS C18 HPLC Column Dim. 150 x 4.6 mm (Thermo Fisher Scientific); eluent 90% MeCN in H_2O, 0.5 mL/min flow rate for 10 minutes; PDA UV detector

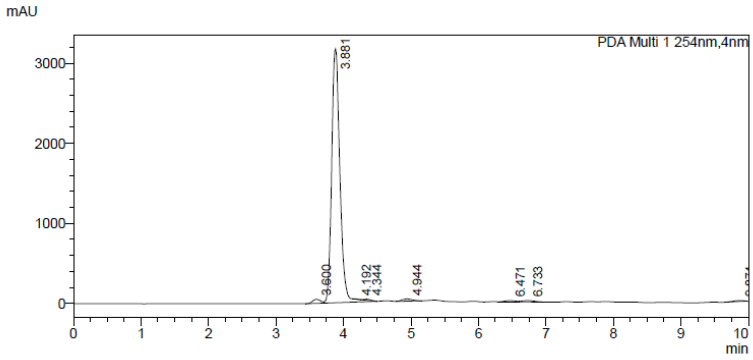


<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	3.893	670277	55159	1.985			
2	4.139	1193067	101761	3.533		V	
3	4.325	766320	69566	2.270		V	
4	4.754	26370644	2604165	78.101		V	
5	5.214	2602343	178721	7.707		V	
6	5.483	1111941	85463	3.293		V	
7	5.845	436532	35667	1.293		V	
8	6.069	413871	31033	1.226		V	
9	7.277	199815	16820	0.592			
Total		33764809	3178354				

Figure A29. HPLC chromatogram and %purity of 2,4-diethylcannabinol (diEt-CBN, **3f**); analytical HPLC (Shimadzu, model RID-20A); Hypersil™ BDS C18 HPLC Column Dim. 150 x 4.6 mm (Thermo Fisher Scientific); eluent 90% MeCN in H_2O, 0.5 mL/min flow rate for 10 minutes; PDA UV detector

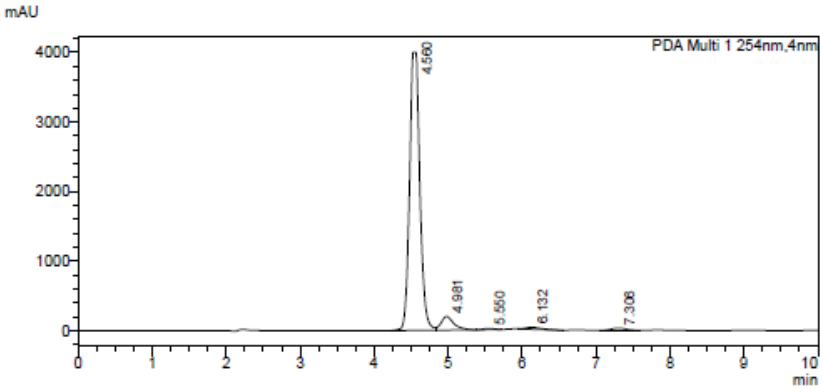


<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	3.600	421277	51086	1.493			
2	3.881	26858018	3169397	95.207		SV	
3	4.192	15058	4196	0.053		T	
4	4.344	94659	14778	0.336		TV	
5	4.944	289553	27581	1.026			
6	6.471	179187	16119	0.635			
7	6.733	235476	18819	0.835		V	
8	9.874	116873	10703	0.414			
Total		28210102	3312678				

Figure A30. HPLC chromatogram and %purity of unsubstituted cannabinol (CBN-C0, **3o**); analytical HPLC (Shimadzu, model RID-20A); Hypersil™ BDS C18 HPLC Column Dim. 150 x 4.6 mm (Thermo Fisher Scientific); eluent 90% MeCN in H_2O, 0.5 mL/min flow rate for 10 minutes; PDA UV detector

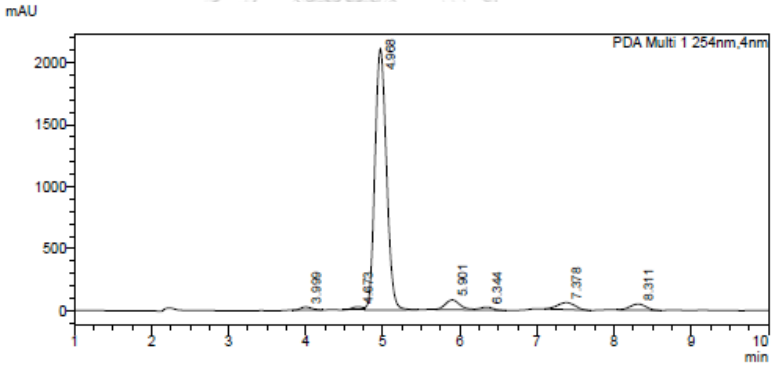


<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	4.560	38490010	3994687	91.955			
2	4.981	2343924	197049	5.600		V	
3	5.550	155425	15720	0.371			
4	6.132	441411	32190	1.055			
5	7.306	426505	30815	1.019			
Total		41857275	4270461				

Figure A31. HPLC chromatogram and %purity of 4-bromocannabinol (CBN-Br, **3s**); analytical HPLC (Shimadzu, model RID-20A); Hypersil™ BDS C18 HPLC Column Dim. 150 x 4.6 mm (Thermo Fisher Scientific); eluent 90% MeCN in H_2O, 0.5 mL/min flow rate for 10 minutes; PDA UV detector



<Peak Table>

PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	3.999	248673	26188	0.982			
2	4.673	250081	25406	0.988			
3	4.968	21908311	2103302	86.536		V	
4	5.901	1045668	78909	4.130			
5	6.344	287805	24794	1.137		V	
6	7.378	886490	56324	3.502			
7	8.311	689817	48739	2.725			
Total		25316846	2363662				

Figure A32. HPLC chromatogram and %purity of 2,4-bromocannabinol (CBN-Br_2, **3r**); analytical HPLC (Shimadzu, model RID-20A); Hypersil™ BDS C18 HPLC Column Dim. 150 x 4.6 mm (Thermo Fisher Scientific); eluent 90% MeCN in H_2O, 0.5 mL/min flow rate for 10 minutes; PDA UV detector

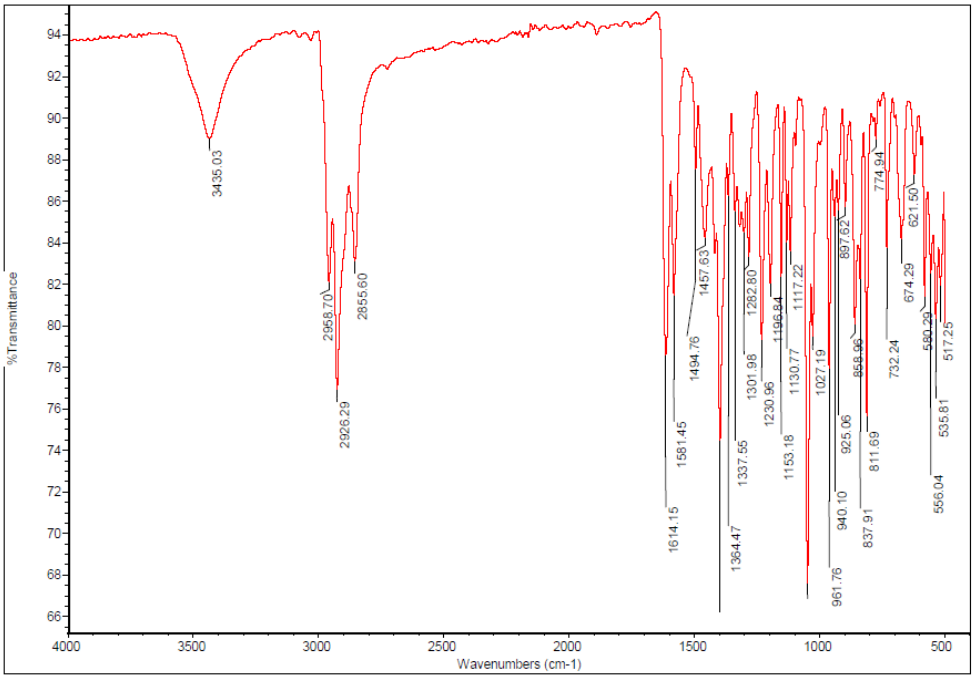


Figure A33. ATR-IR spectrum of 3-(1,1-dimethylheptyl)cannabinol (DMH-CBN, **3a**)

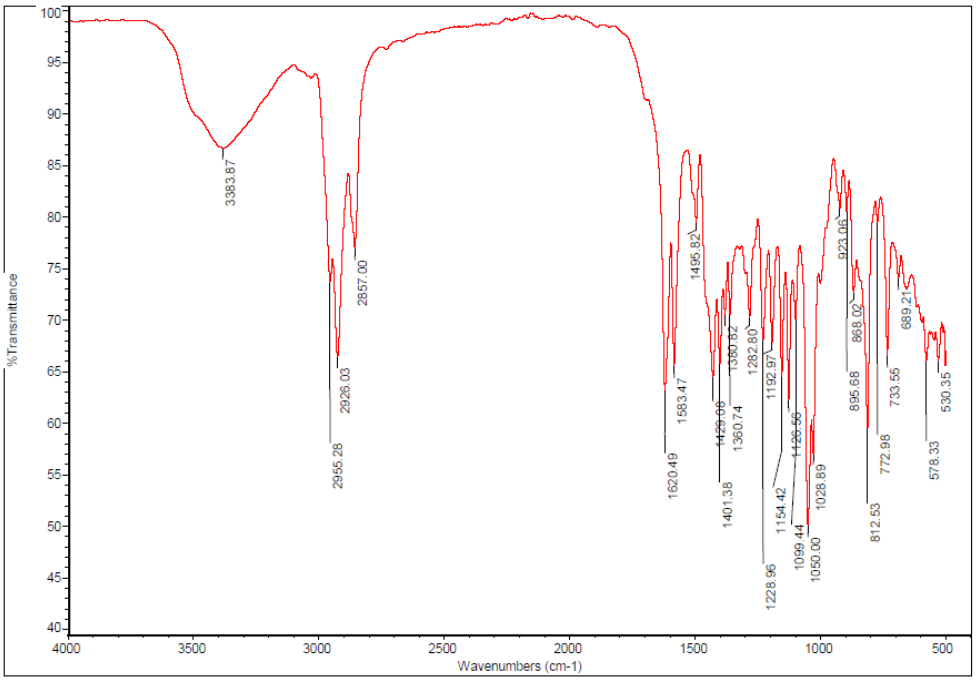


Figure A34. ATR-IR spectrum of cannabinol (CBN, **3b**)

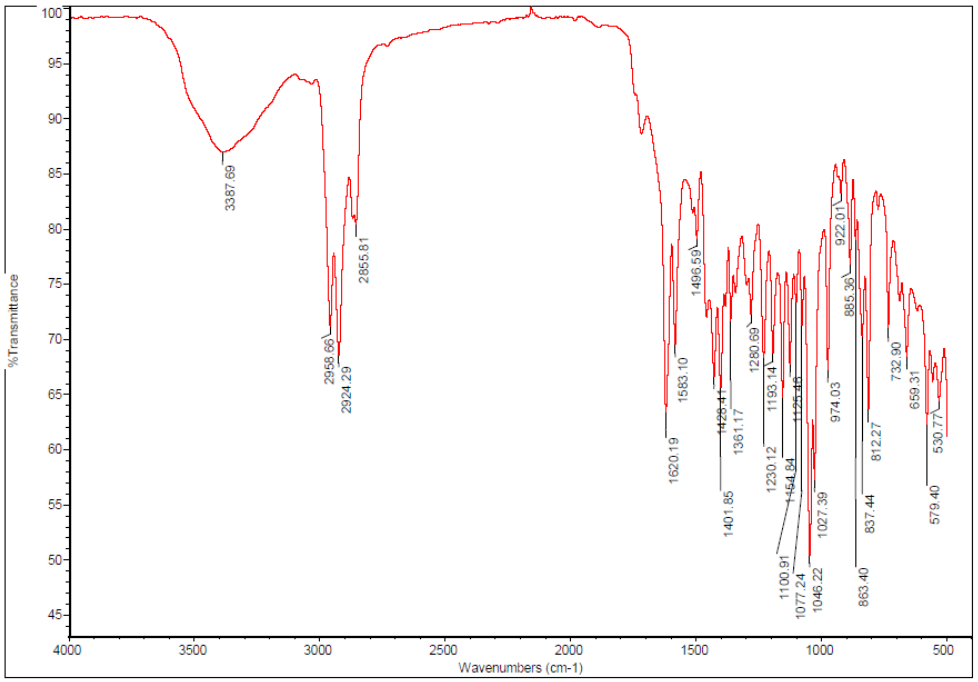


Figure A35. ATR-IR spectrum of 3-isopropylcannabinol (iPr-CBN, **3c**)

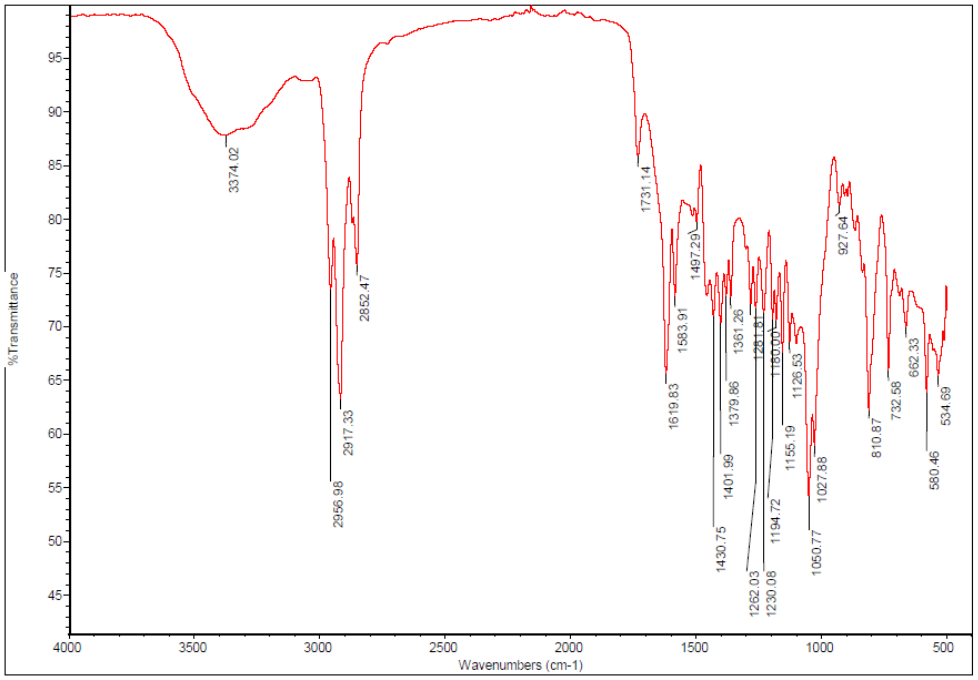


Figure A36. ATR-IR spectrum of 3-(1-ethylpentyl)cannabinol (EtPe-CBN, **3d**)

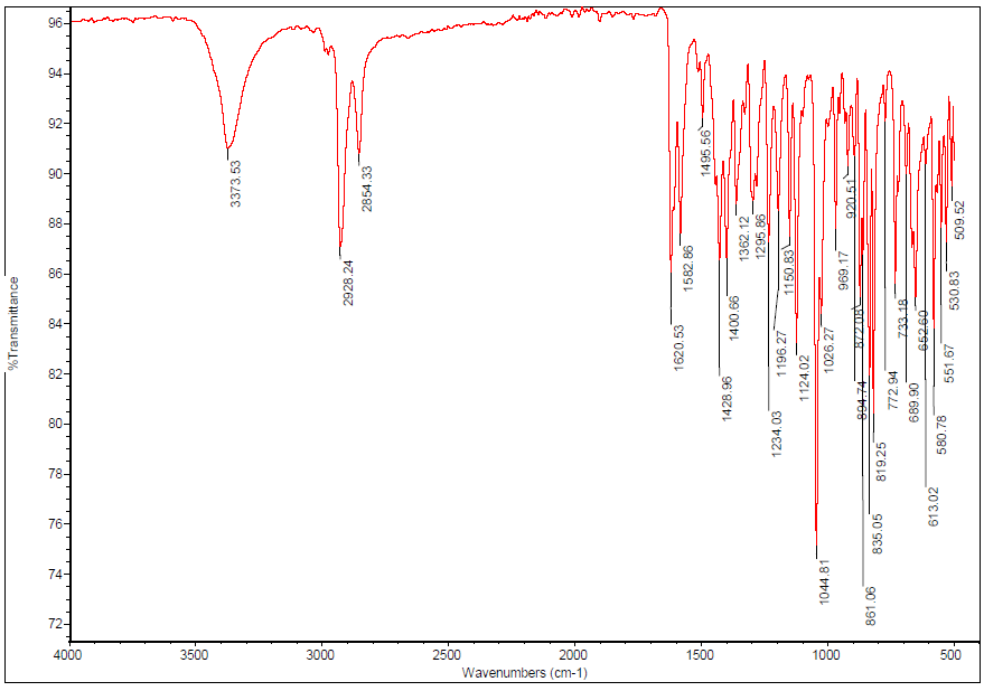


Figure A37. ATR-IR spectrum of 3-cyclohexylcannabinol (Cy-CBN, **3e**)

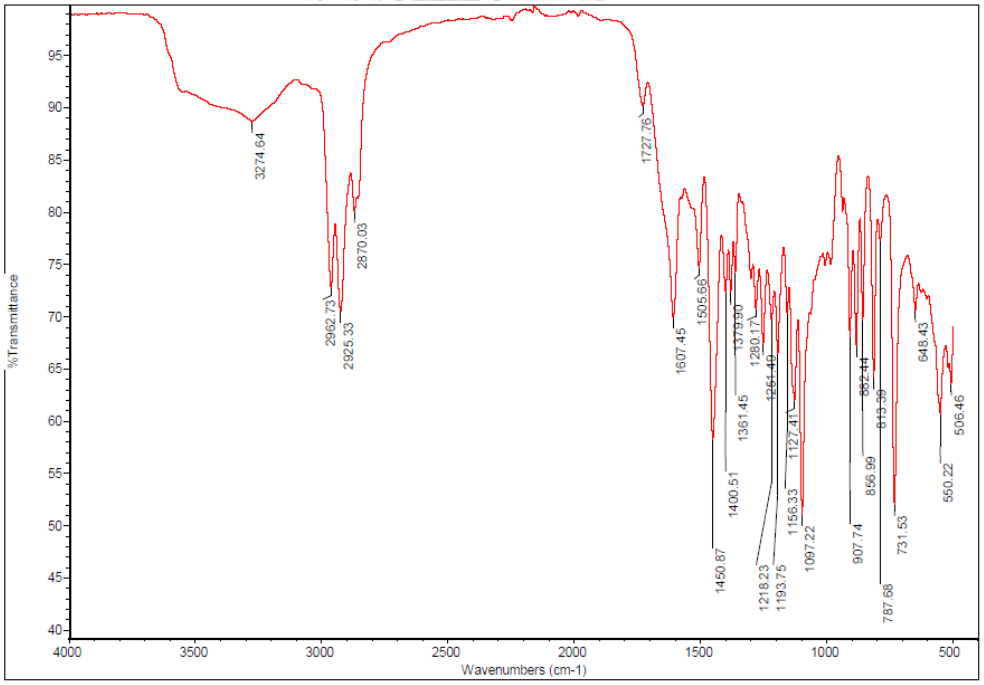


Figure A38. ATR-IR spectrum of 2,4-diethylcannabinol (diEt-CBN, **3f**)

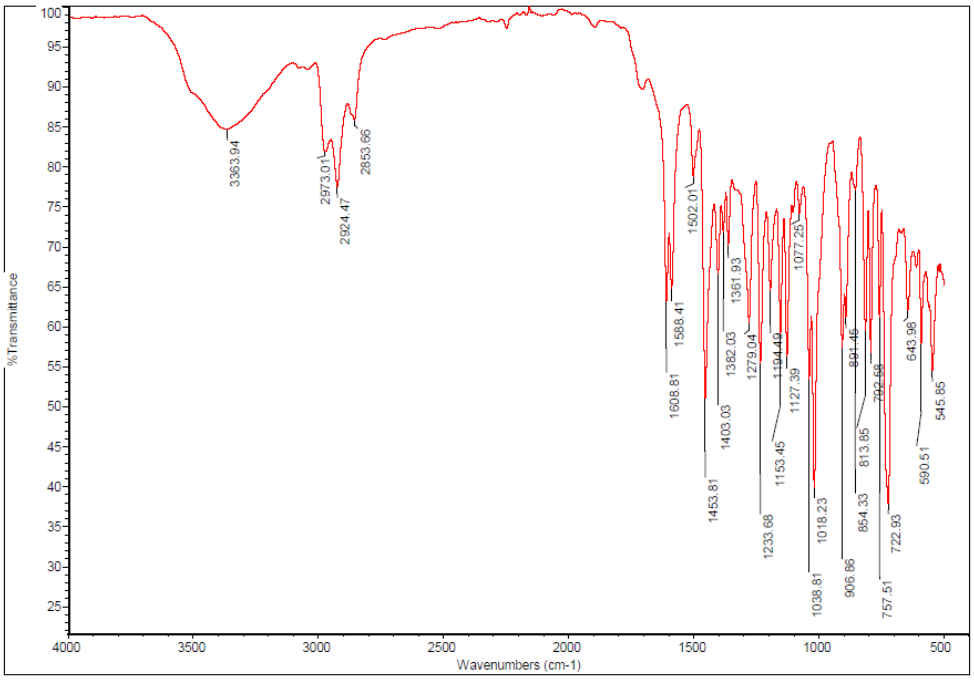


Figure A39. ATR-IR spectrum of unsubstituted cannabinol (CBN-C0, **3o**)

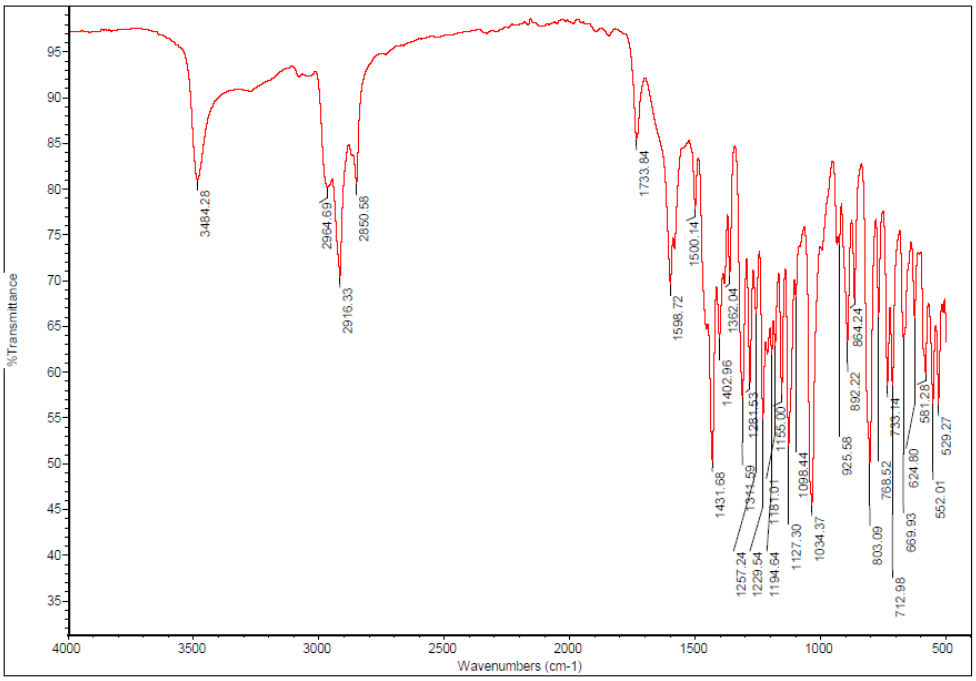


Figure A40. ATR-IR spectrum of 4-bromocannabinol (CBN-Br, **3s**)

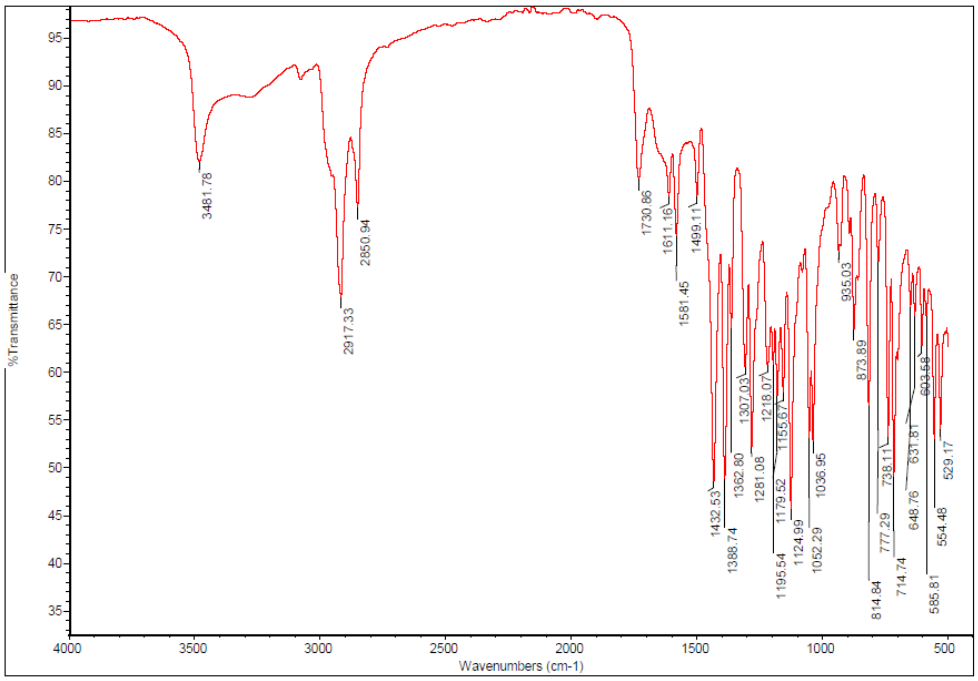


Figure A41. ATR-IR spectrum of 2,4-dibromocannabinol (CBN-Br_2, **3r**)

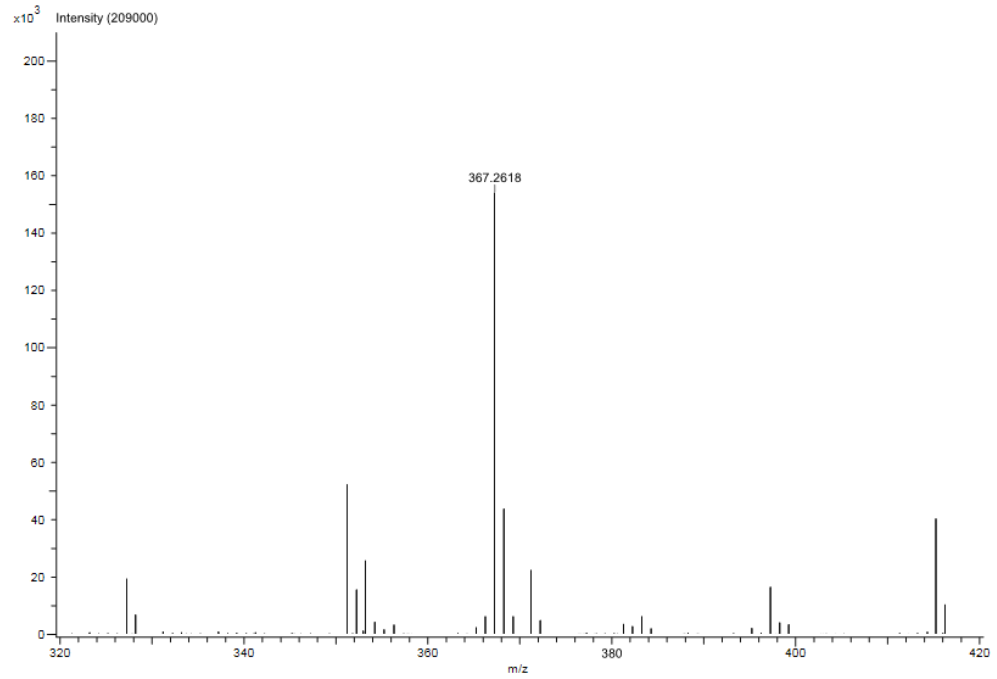


Figure A42. DART-TOF HRMS spectrum of 3-(1,1-dimethylheptyl)cannabinol (DMH-CBN, **3a**)

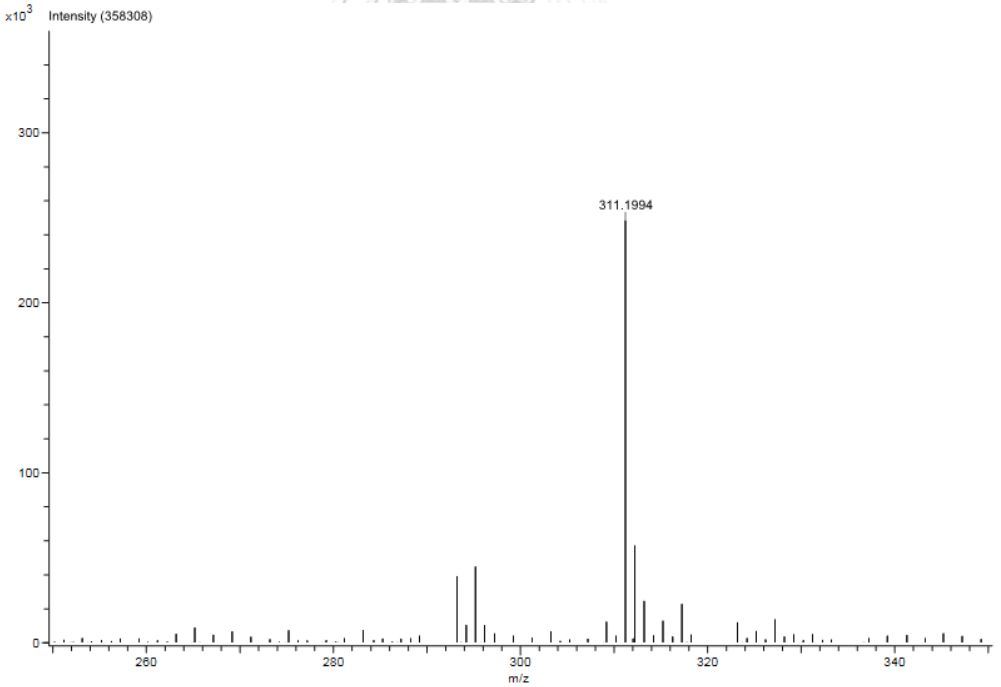


Figure A43. DART-TOF HRMS spectrum of cannabinol (CBN, **3b**)

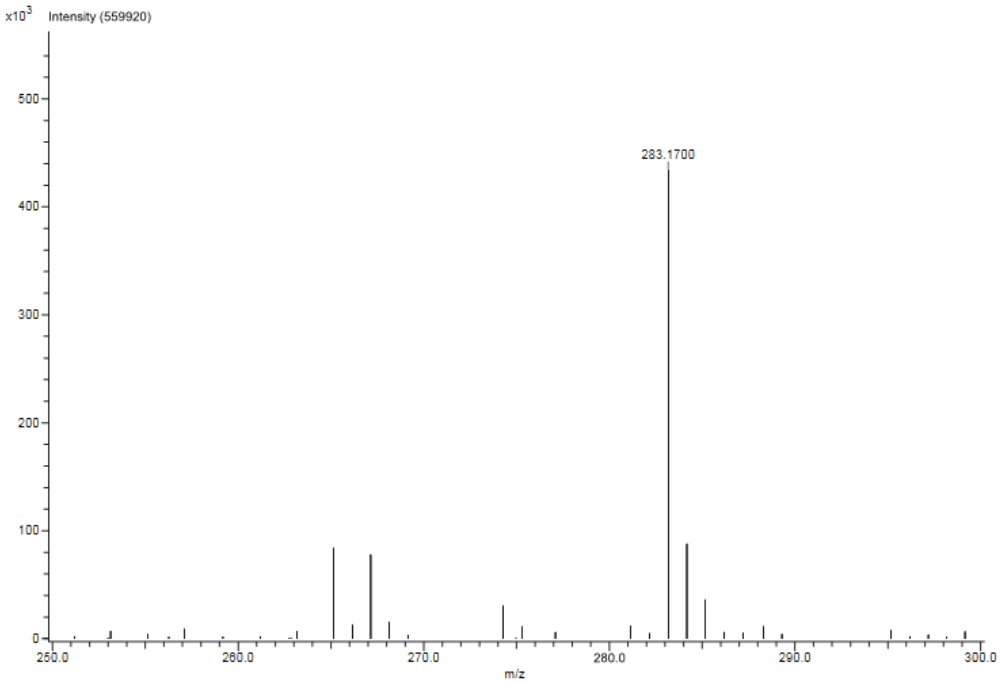


Figure A44. DART-TOF HRMS spectrum of 3-isopropylcannabinol (iPr-CBN, **3c**)

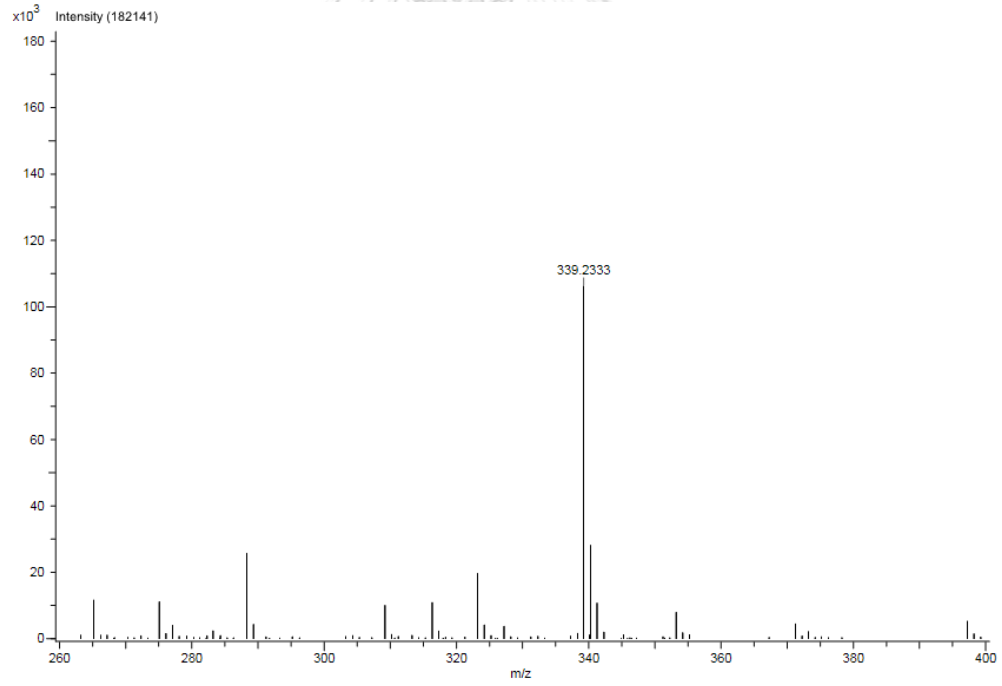


Figure A45. DART-TOF HRMS spectrum of 3-(1-ethylpentyl)cannabinol (EtPe-CBN, **3d**)

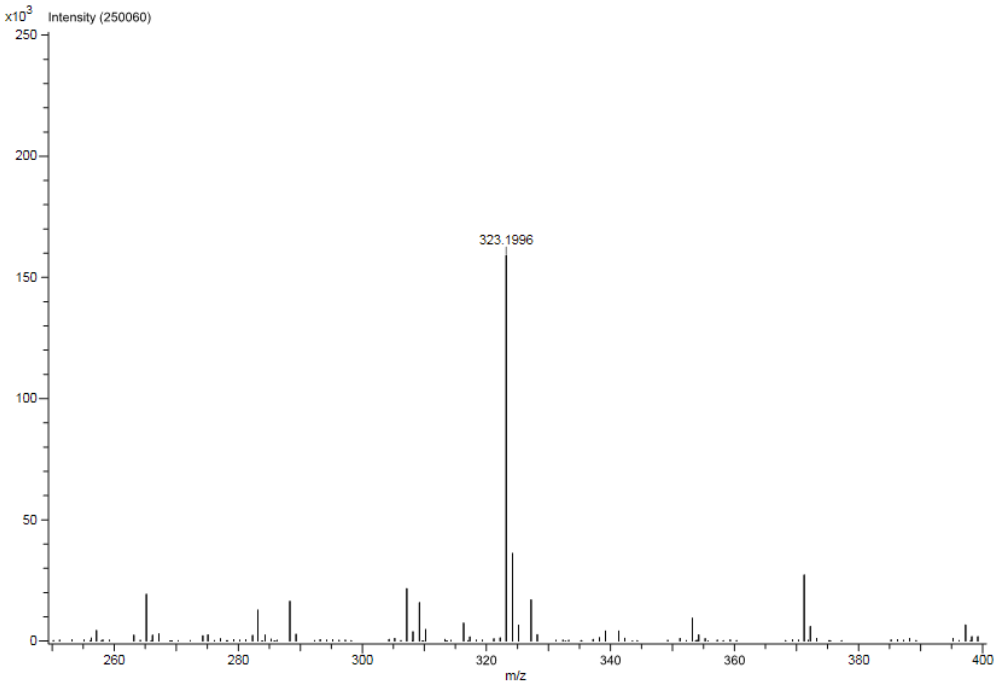


Figure A46. DART-TOF HRMS spectrum of 3-cyclohexylcannabinol (Cy-CBN, **3e**)

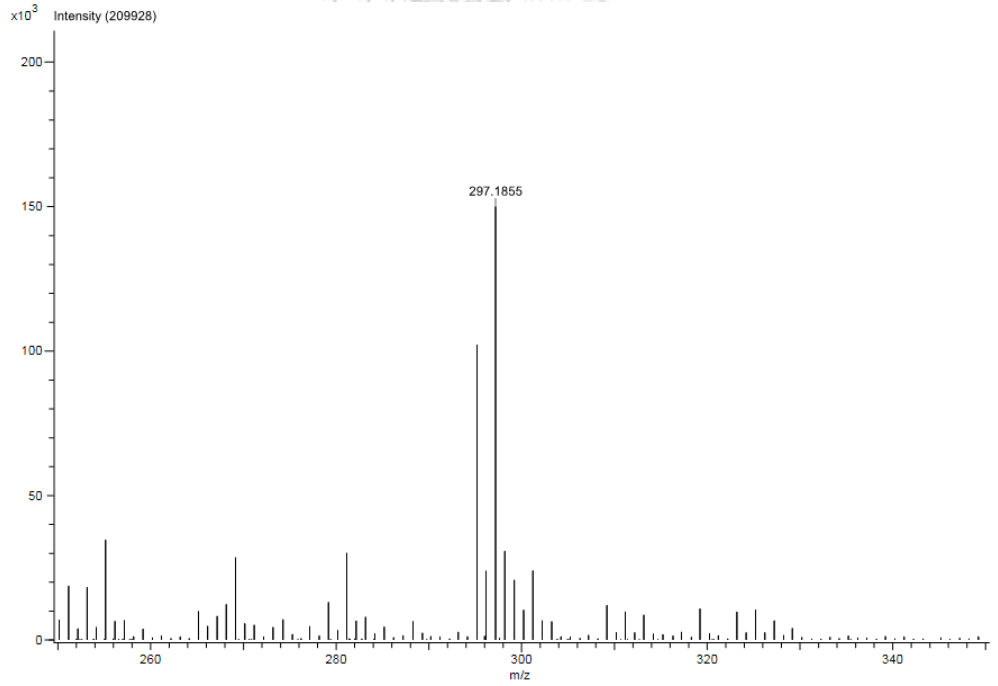


Figure A47. DART-TOF HRMS spectrum of 2,4-diethylcannabinol (diEt-CBN, **3f**)

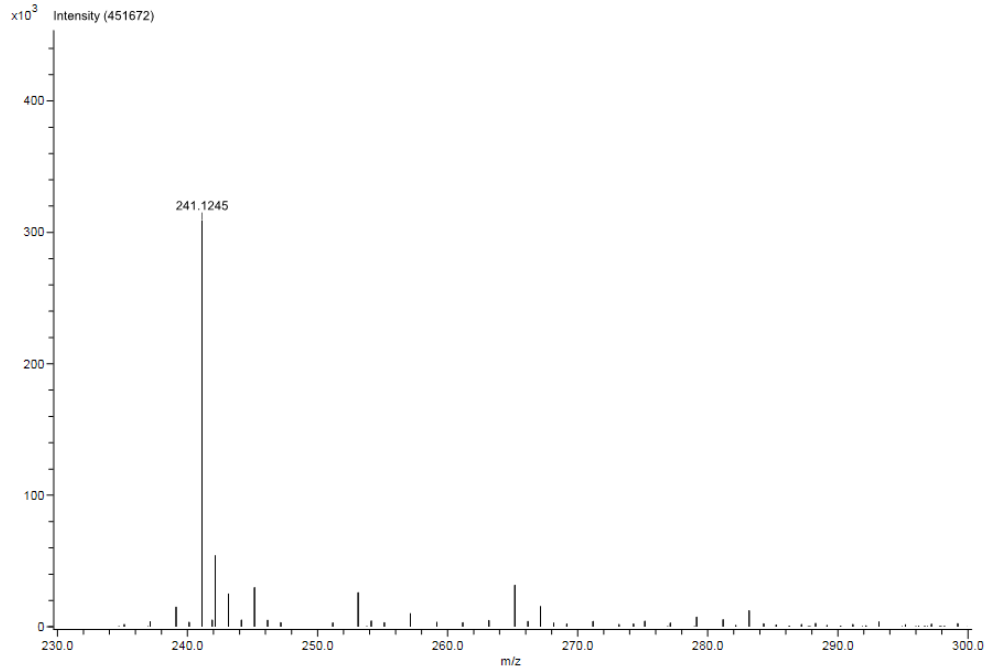


Figure A48. DART-TOF HRMS spectrum of unsubstituted cannabinol (CBN-C0, **3o**)

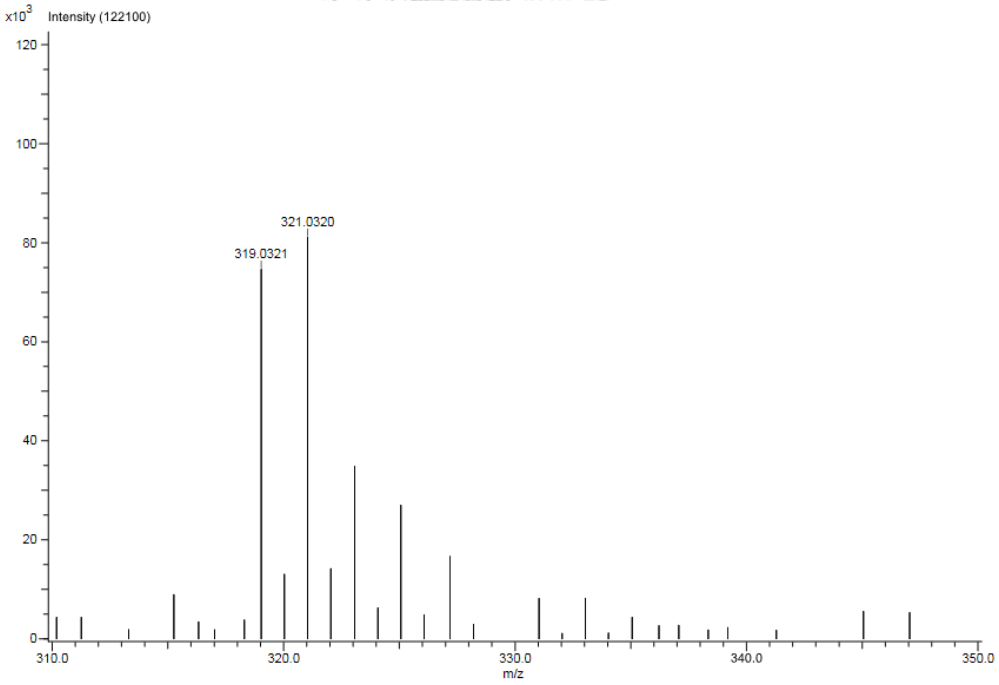


Figure A49. DART-TOF HRMS spectrum of 4-bromocannabinol (CBN-Br, **3s**)

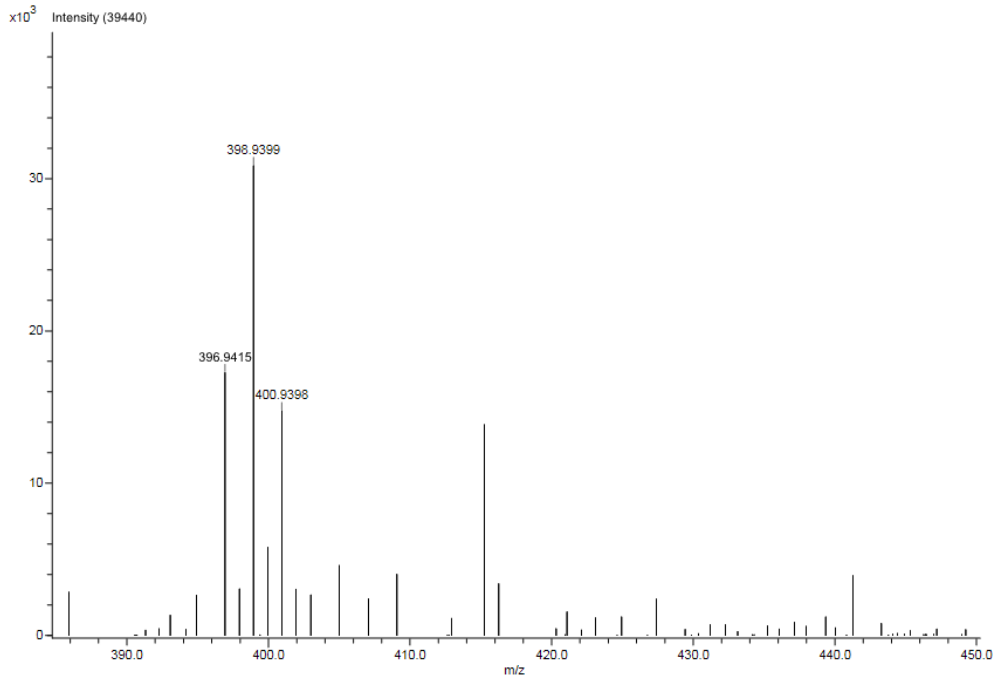


Figure A50. DART-TOF HRMS spectrum of 2,4-dibromocannabinol (CBN-Br_2, **3r**)

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