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LAMPIRAN

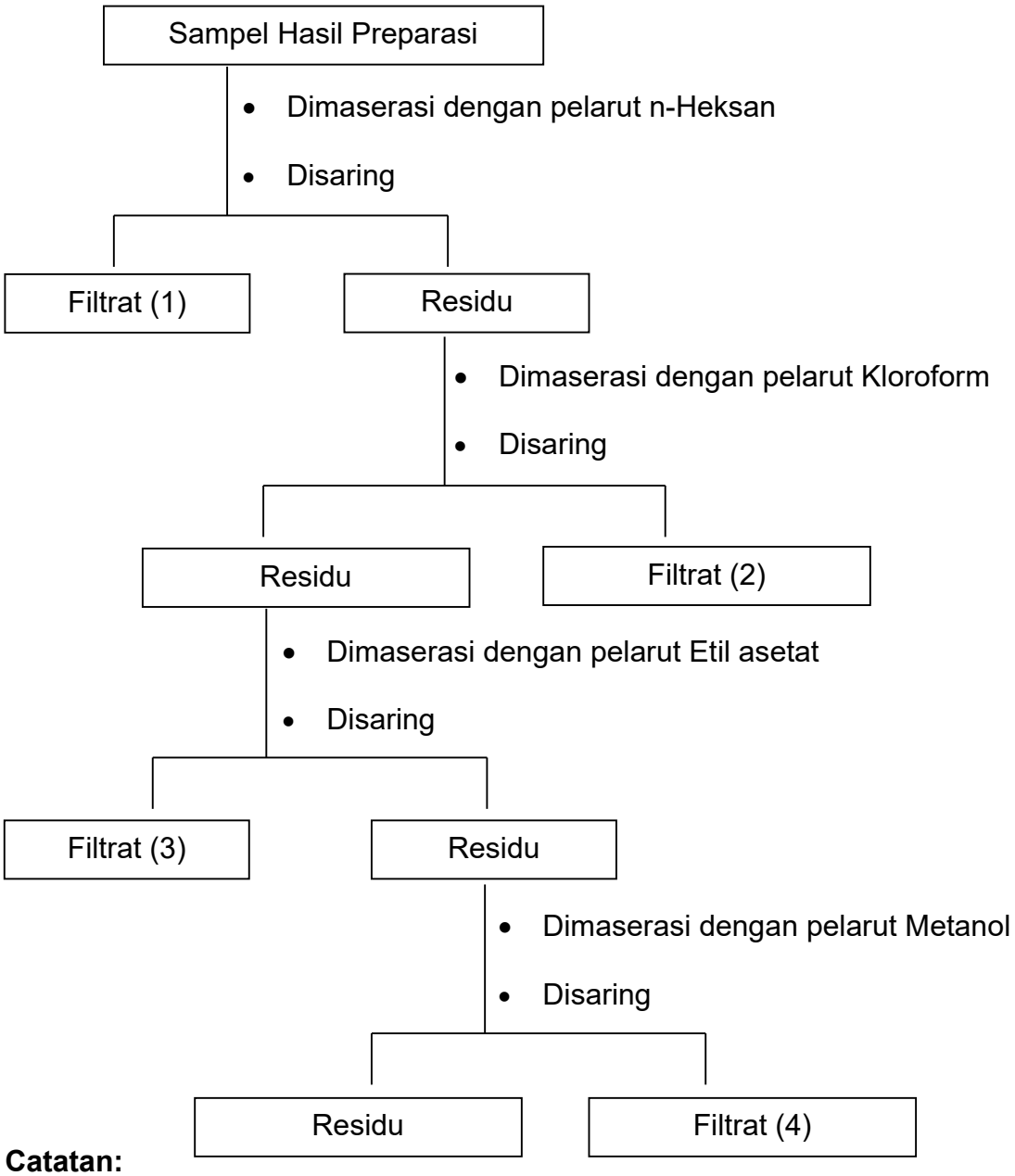
Lampiran 1. Bagan Kerja Preparasi Sampel

Sampel Makroalga

- Dibilas menggunakan air laut
- Dibilas dengan air tawar
- Ditiriskan
- Dikering-anginkan di tempat yang ternaungi dari sinar matahari
- Sampel kering
- Dipotong-potong
- Diserbukkan menggunakan mesin penggiling

Sampel Hasil Preparasi

Lampiran 2. Bagan Kerja Ekstraksi Sampel



Catatan:

Masing-masing filtrat yang diperoleh dievaporasi sehingga diperoleh ekstrak kental dengan keterangan sebagai berikut:

Filtrat (1) : diperoleh ekstrak n-Heksan

Filtrat (2) : diperoleh ekstrak Kloroform

Filtrat (3) : diperoleh ekstrak Etil asetat

Filtrat (4) : diperoleh ekstrak Metanol

Lampiran 3. Bagan Kerja Uji Aktivitas Antibakteri

Inokulum Bakteri Uji

- Disuspensi dalam akuades steril dengan OD 0,08-0,1 (10^7 CFU.mL^{-1})
- Diusapkan pada permukaan media Nutrien Agar (NA) dengan menggunakan usapan steril
- Kertas cakram steril (diameter 6 mm) yang telah direndam masing-masing dalam larutan ekstrak dan kontrol diletakkan pada permukaan media NA yang telah diusapkan bakteri uji
- Diinkubasi selama 24 jam pada suhu 37^oC
- Diukur diameter zona bening yang dihasilkan di sekitar kertas cakram dengan menggunakan jangka sorong

Data Diameter Zona Bening

Lampiran 4. Bagan Kerja Uji Aktivitas Antioksidan

1. Pembuatan Reagen DPPH 0,4 mM

Serbuk DPPH

- Ditimbang sebanyak 0,0156 g
- Dilarutkan dengan 100 mL metanol p.a
- Dihomogenkan

Larutan DPPH 0,4 mM

2. Penentuan Aktivitas Antioksidan

Larutan Induk Ekstrak/Isolat 500 ppm

- Dipipet masing-masing 0,1; 0,2; 0,4; 0,8 dan 1,6 mL ke dalam tabung reaksi.
- Ditambahkan masing-masing 1 mL larutan DPPH 0,4 mM
- Ditambahkan metanol p.a sehigga didapatkan volume total 5 mL untuk masing-masing deret standar (konsentrasi 10; 20; 40; 80 dan 160 ppm)
- Diinkubasi selama 30 menit pada suhu 37 ˚C.
- Diukur absorbansinya dengan menggunakan spektrofotometer UV-Vis pada panjang gelombang maksimum larutan DPPH.

Absorbansi

Catatan:

1. Penentuan panjang gelombang maksimum larutan DPPH dilakukan pada rentang panjang gelombang 500-520 nm
2. Pengukuran absorbansi deret menggunakan larutan DPPH 0,4 mM + metanol p.a (1:4) sebagai kontrol dan metanol p.a sebagai blanko
3. Data absorbansi deret standar digunakan dalam perhitungan % aktivitas antioksidan dengan menggunakan persamaan berikut:

$$\% \text{ Aktivitas antioksidan} = \frac{(\text{Abs kontrol} - \text{Abs sampel})}{\text{Abs kontrol}} \times 100\ \%$$

4. Aktivitas antioksidan dinyatakan dalam IC_{50} yang diperoleh dari persamaan regresi pada kurva konsentrasi Vs % aktivitas antioksidan

$$y = ax + b$$

y = 50 (nilai % aktivitas antioksidan)

x = IC_{50} ($\mu g.mL^{-1}$)

a = slope

b = intercept

Lampiran 5. Bagan Kerja Uji Toksisitas dengan Metode BSLT

1. Persiapan larva udang *Artemia salina* Leach

Telur udang *Artemia salina* Leach

- Ditimbang sebanyak 0,25 gram
- Dimasukkan ke dalam wadah penetasan yang berisi 500 mL air laut
- Diaerasi selama 48 jam yang disertai dengan pencahayaan lampu pijar
- Larva yang telah berumur 48 jam dipisahkan dari telurnya

Larva udang *Artemia salina* Leach

2. Persiapan sampel

Ekstrak/Isolat

- Ditimbang sebanyak 1 mg
- Dilarutkan dengan 10 µL DMSO
- Diencerkan dengan air laut hingga volume 1 mL

Larutan Induk Ekstrak/Isolat 1000 ppm

3. Penentuan Aktivitas Toksisitas

Larutan Induk Ekstrak/Isolat 1000 ppm

- Dipipet sebanyak 150 µL
- Ditambahkan air laut sebanyak 150 µL (diperoleh larutan ekstrak/isolat 300 µL dengan konsentrasi 500 ppm)
- Dipipet sebanyak 100 µL ke dalam mikroplate baris A
- Ditambahkan air laut 100 µL ke dalam mikroplate baris A-D
- Dipipet sebanyak 100 µL dari baris A ke dalam baris B dan dari baris B dipipet sebanyak 100 µL ke dalam baris C dan seterusnya
- Terakhir dari baris D dipipet sebanyak 100 µL ke dalam baris E
- Ditambahkan 100 µL air laut dengan larva sebanyak 10-15 ekor masing-masing ke dalam mikroplate baris A-E sehingga diperoleh konsentrasi akhir masing-masing 125; 62,50; 31,25; 15,63 dan 7,81 µg.mL^{-1}
- Diinkubasi selama 24 jam yang disertai dengan pencahayaan lampu pijar

Data

Catatan:

1. Pengerjaan dilakukan dengan 3 kali pengulangan (triplo)
2. Data kematian larva yang diperoleh dikonversi menjadi % kematian larva dengan menggunakan persamaan berikut:

$$\text{Kematian (\%)} = \frac{\text{(Kematian sampel – Kematian kontrol)}}{\text{Jumlah Larva}} \text{ x } 100\%$$

3. Data % kematian larva ditentukan nilai probitnya dengan menggunakan tabel transformasi % kematian ke nilai probit (tabel terlapir pada Lampiran 8)
4. Toksisitas ekstrak/isolat dinyatakan dengan LC_{50} ($\mu g.mL^{-1}$) yang diperoleh melalui persamaan regresi linear dari log konsentrasi sampel terhadap nilai probit

$$y = ax + b$$

y = 5 (nilai probit untuk kematian 50%)

x = log LC_{50} ($\mu g.mL^{-1}$)

a = slope

b = intercept

Lampiran 6. Bagan Kerja Uji Sitotoksisitas

Sel uji

- Didistribusikan ke dalam *microplate 96 well*
- Diinkubasi selama 24 jam.
- Ditambahkan seri konsentrasi ekstrak/isolat, kontrol sel dan kontrol media ke dalam masing-masing sumuran
- Diinkubasi lagi selama 24 jam
- Larutan uji kemudian dibuang
- Sumuran dibilas dengan 100 µL larutan PBS sebanyak 3 kali tiap sumuran.
- Setelah sumuran bersih kemudian ditambahkan 10 µl MTT 5 $mg.mL^{-1}$.
- Kemudian diinkubasi lagi selama 4 jam pada suhu 37°C
- Reaksi MTT dihentikan dengan *reagen stopper* SDS 10% dalam 0,01% HCl sebanyak 100 µL,
- Diinkubasi pada suhu kamar dengan ditutup dengan aluminium foil pada tempat gelap selama 24 jam.
- Serapan dibaca dengan ELISA *reader* pada panjang gelombang 550 nm.

Data

Catatan:

1. Data absorbansi digunakan dalam perhitungan % viabilitas sel dengan menggunakan persamaan berikut:

$$\% \text{ Viabilitas sel} = \frac{(\text{Abs perlakuan} - \text{Abs kontrol media})}{(\text{Abs kontrol sel} - \text{Abs kontrol media})} \times 100\%$$

2. Data % viabilitas sel ditentukan nilai probitnya dengan menggunakan tabel transformasi % viabilitas sel ke nilai probit (tabel terlapir pada Lampiran 8)
3. Toksisitas ekstrak/isolat dinyatakan dengan LC_{50} ($\mu g.mL^{-1}$) yang diperoleh melalui persamaan regresi linear dari log konsentrasi sampel terhadap nilai probit

$$y = ax + b$$

y = 5 (nilai probit untuk kematian 50%)

x = log LC_{50} ($\mu g.mL^{-1}$)

a = slope

b = intercept

Lampiran 7. Bagan Kerja Analisis *Molecular Docking*

1. Preparasi struktur senyawa isolat

Struktur Isolat

- Digambar dengan menggunakan program Chem Draw Ultra Professional 15.0
- Diconvert menjadi struktur 3D
- Dioptimasi dengan metode MMFF94 menggunakan Chem Draw Ultra 3D

Struktur Isolat Teroptimasi

2. Preparasi struktur Protein Target

Struktur Kompleks Protein Target

- Diunduh dari database *Protein Data Bank* (http://www.rcsb.org/-pdb)
- Dipisahkan dari molekul air dan ligan nativenya dengan menggunakan program AutoDock Tools 1.5.6
- Ditambahkan atom H polar
- Ditambahkan muatan *(Kollman Charges)*
- Disimpan dalam format PDBQT

Struktur Protein Target

3. Proses *Docking*

Struktur Protein Target dan Isolat Teroptimasi

- Diatur area pencarian konformasi struktur senyawa isolat *(grid box)*
- Parameter *grid* disimpan dalam format file gpf
- Diatur parameter *docking* dan disimpan dalam format file dpf
- Running AutoGrid dengan Autogrid4
- Running AutoDock dengan Autodock4
- Hasil docking divisualisasi dengan menggunakan program Discovery Studio Visualizer 2021

Data

Lampiran 8. Tabel Transformasi Nilai Probit

%	0	1	2	3	4	5	6	7	8	9
0	-	2,67	2,96	3,12	3,25	3,36	3,45	3,52	3,59	3,66
10	3,72	3,77	3,82	3,87	3,92	3,96	4,01	4,05	4,08	4,12
20	4,16	4,19	4,23	4,26	4,29	4,33	4,36	4,39	4,42	4,45
30	4,48	4,50	4,53	4,55	4,59	4,61	4,64	4,67	4,69	4,72
40	4,75	4,77	4,80	4,82	4,85	4,87	4,90	4,92	4,95	4,97
50	5	5,03	5,05	5,08	5,10	5,13	5,15	5,18	5,20	5,23
60	5,25	5,28	5,31	5,33	5,36	5,39	5,41	5,44	5,47	5,50
70	5,52	5,55	5,58	5,61	5,64	5,67	5,71	5,74	5,77	5,81
80	5,84	5,88	5,92	5,95	5,99	6,04	6,08	6,13	6,18	6,23
90	6,28	6,34	6,41	6,48	6,55	6,64	6,75	6,88	7,05	7,33
-	0,0	0,1	0,2	0,3	0,4	0,5	0,6	0,7	0,8	0,9
99	7,33	7,37	7,41	7,46	7,51	7,58	7,65	7,75	7,88	8,09

Lampiran 9. Hasil Identifikasi Sampel

DIREKTORAT PENGELOLAAN LABORATORIUM,
FASILITAS RISET, DAN KAWASAN SAINS TEKNOLOGI
Gedung B.J. Habibie Jalan M.H. Thamrin Nomor 8,
Jakarta Pusat 10340
Telepon/WA: 0811 8612 392; E-mail: dit-plfrkst@brin.go.id
www.brin.go.id

No. ID ELSA *Transaction Number*	**:** 34232
Metode *Method*	**:Identifikasi secara morfologi**
Nama Laboratorium *Name of Laboratory*	**:** Laboratorium Oseanografi - BRIN
Alamat Laboratorium *Laboratory Address*	**:** Jl. Pasir Putih 1, Ancol Timur (Gedung Pusat Riset Oseanografi- BRIN) Jakarta - Indonesia 11048 Email : layanan@mail.lipi.go.id ; Telp +62 811-1391-617

Kondisi Pengukuran/Parameter Pengujian *Measurement Conditions/Testing Parameters:*
Identifikasi dengan menggunakan karakter morfologi

Hasil Pengujian *Testing Results* : *Bornetella nitida* Munier-Chalmas ex Sonder, 1880 (Kode sampel 1346-34232-1) dan *Gracilaria salicornia* (C.Agardh) E.Y.Dawson, 1954 (Kode sampel 1346-34232-2)

https://data.lipi.go.id/privateurl.xhtml?token=5d984285-a42b-4ca5-b7ac-089298d1d4e4

Catatan *Note:*
Daftar sampel yang dilakukan pengujian terdapat di lembar pengesahan.
Penamaan sampel sesuai dengan penamaan pada saat permohonan pengajuan layanan.

Terima kasih sudah melakukan pengujian/ penyewaan alat/ proses riset dengan fasilitas yang tersedia di Laboratorium Oseanografi. Jika dikemudian hari, hasil pengujian atau analisis ini akan dipublikasikan, mohon kiranya bisa menambahkan dalam Ucapan Terima Kasih atau Acknowledgement di dalam publikasi Anda,

seperti dalam contoh format berikut:

Dalam bahasa Indonesia : "Penelitian ini didukung oleh fasilitas riset, dan dukungan ilmiah serta teknis dari Laboratorium Oseanografi di Badan Riset dan Inovasi Nasional".
Dalam bahasa Inggris : "The authors acknowledge the facilities, and the scientific and technical assistance of the Oceanography Laboratories at the National Research and Innovation Agency

Keterangan Identifikasi

Kelompok Riset Ekosistem Pesisir Bervegetasi, Pusat Riset Oseanografi – BRIN Jakarta, menerangkan bahwa material makroalga yang dikirim ke kantor kami:

ID Transaksi : 34232

Telah diidentifikasi/diberikan nama ilmiah makroalga tersebut adalah sebagai berikut:

Kode sampel 1346-34232-1: *Bornetella nitida* Munier-Chalmas ex Sonder, 1880

Filum/Divisio : Chlorophyta
Kelas/Class : Ulvophyceae
Bangsa/Ordo : Dasycladales
Suku/Famili : Dasycladaceae
Marga/Genus : *Bornetella*
Jenis/Spesies : *Bornetella nitida* Munier-Chalmas ex Sonder, 1880

Kode sampel 1346-34232-2: *Gracilaria salicornia* (C.Agardh) E.Y.Dawson, 1954

Filum/Divisio : Rhodophyta
Kelas/Class : Florideophyceae
Bangsa/Ordo : Gracilariales
Suku/Famili : Gracilariaceae
Marga/Genus : *Gracilaria*
Jenis/Spesies : *Gracilaria salicornia* (C.Agardh) E.Y.Dawson, 1954

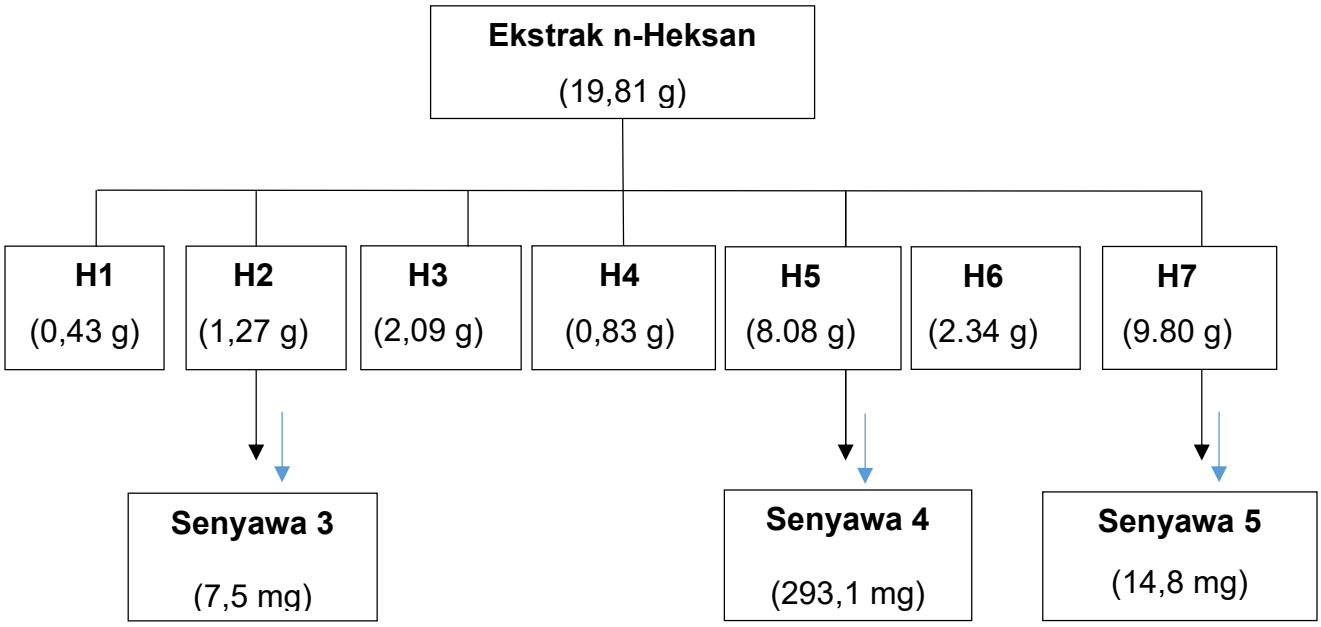
Demikian surat keterangan ini dibuat untuk dapat dipergunakan sebagaimana mestinya.

Jakarta, 03 Juni 2022

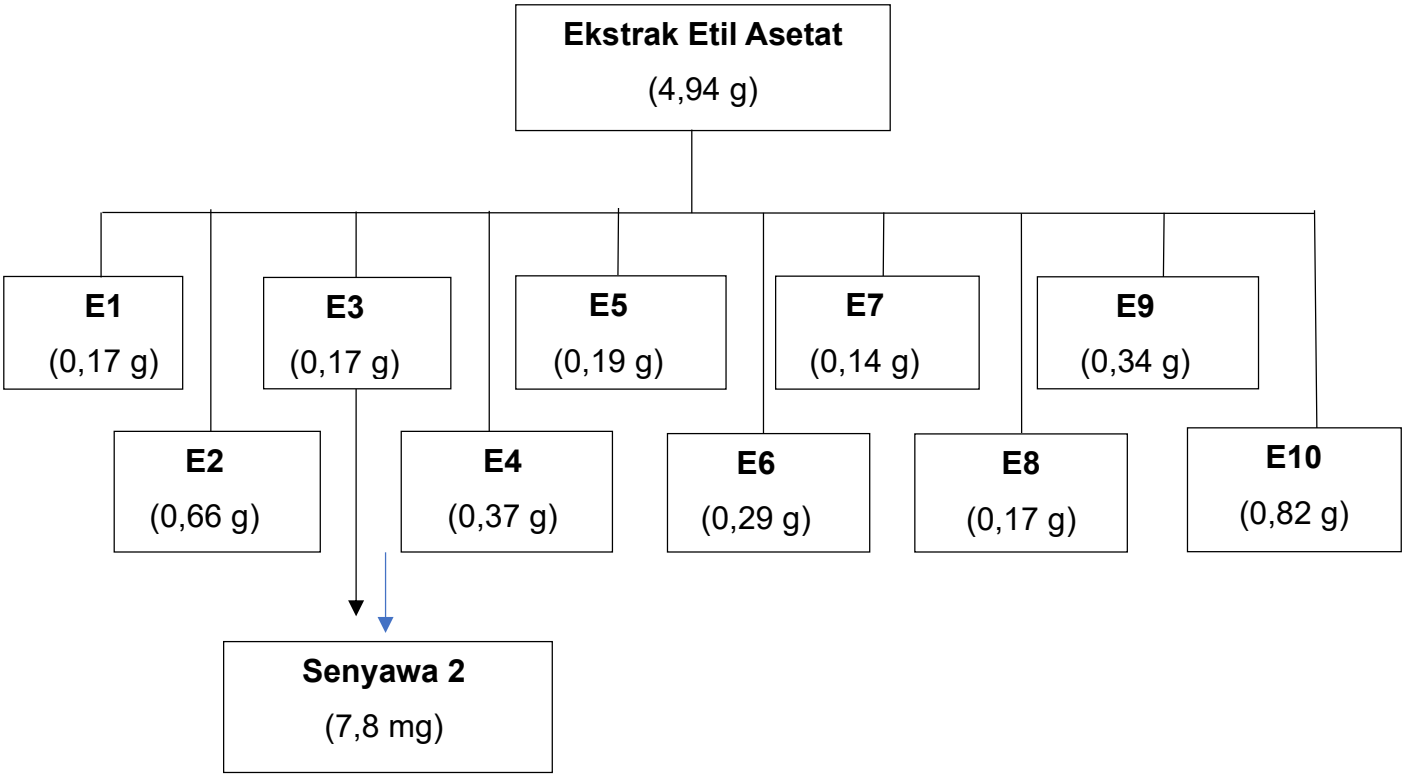
Peneliti Makroalga

Tri Handayani, M.Si.
NIP. 198009032006042003

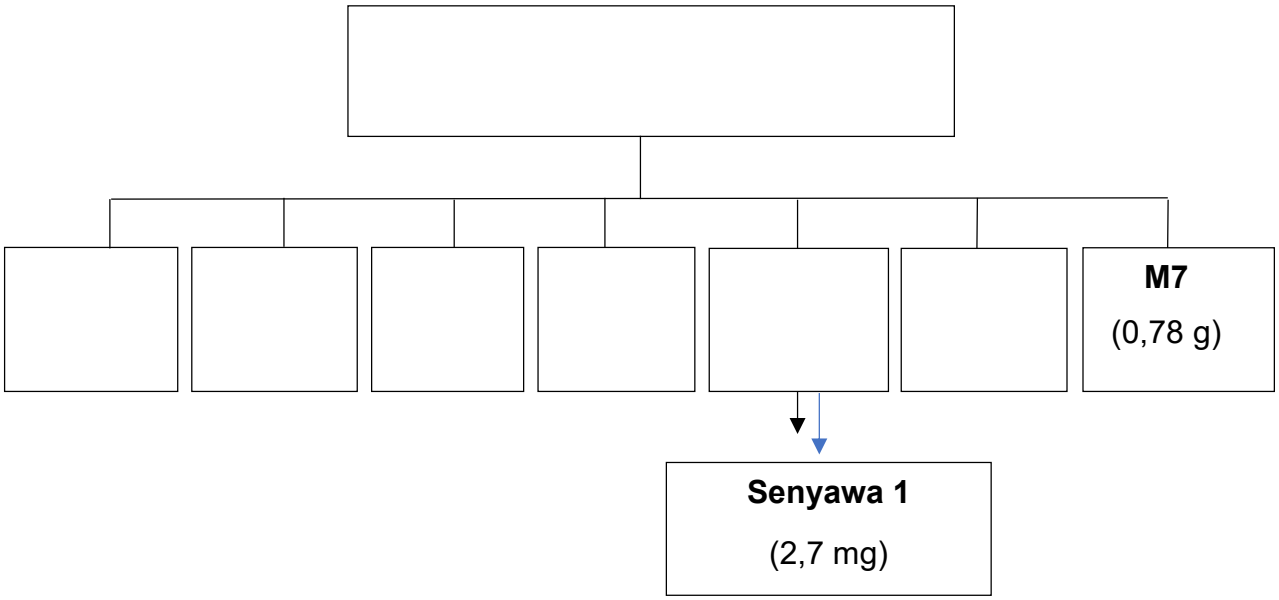
Lampiran 10. Bagan Isolasi Senyawa dari ekstrak n-Heksan *B. nitida*



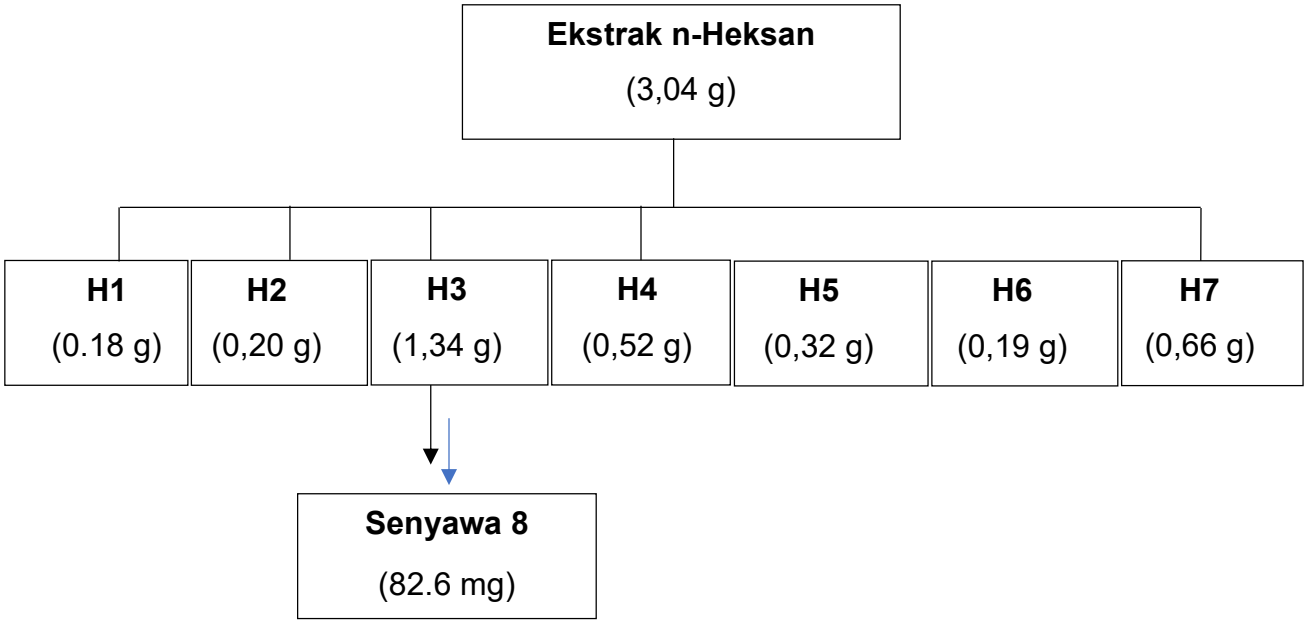
Lampiran 11. Bagan Isolasi Senyawa dari ekstrak etil asetat *B. nitida*



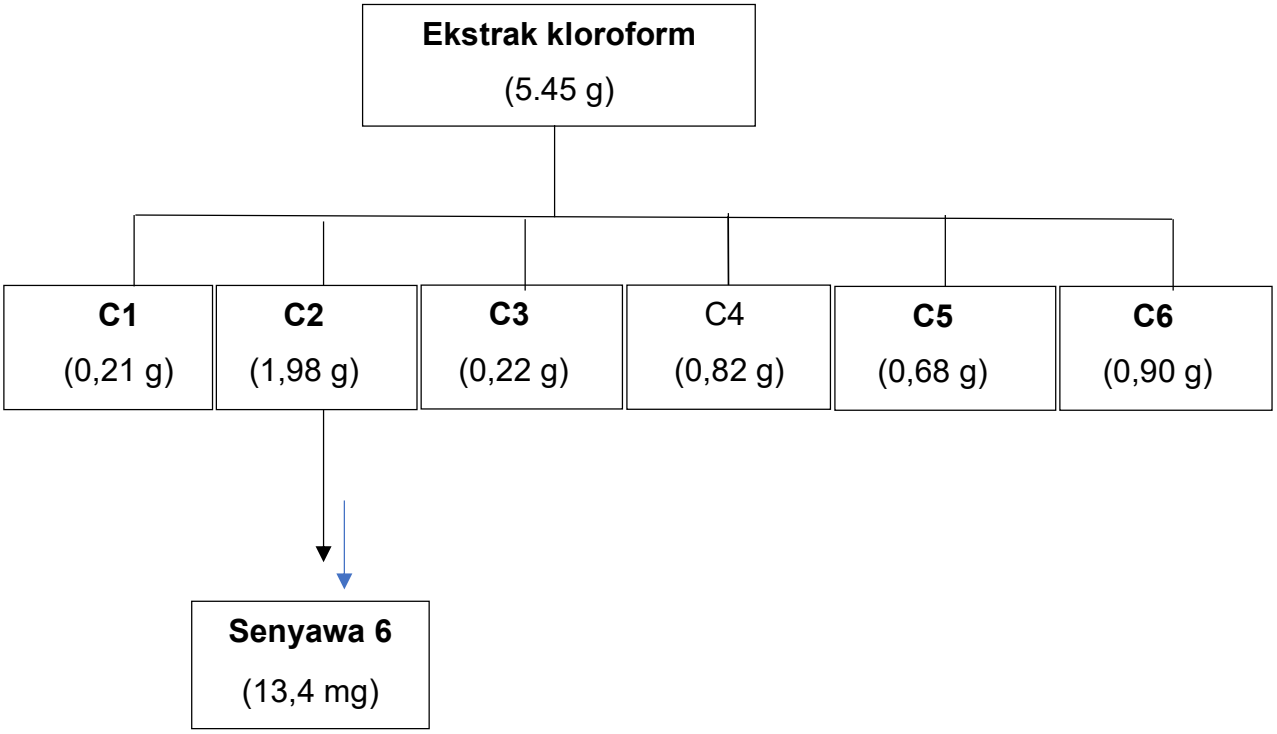
Lampiran 12. Bagan Isolasi Senyawa dari ekstrak metanol *B. nitida*



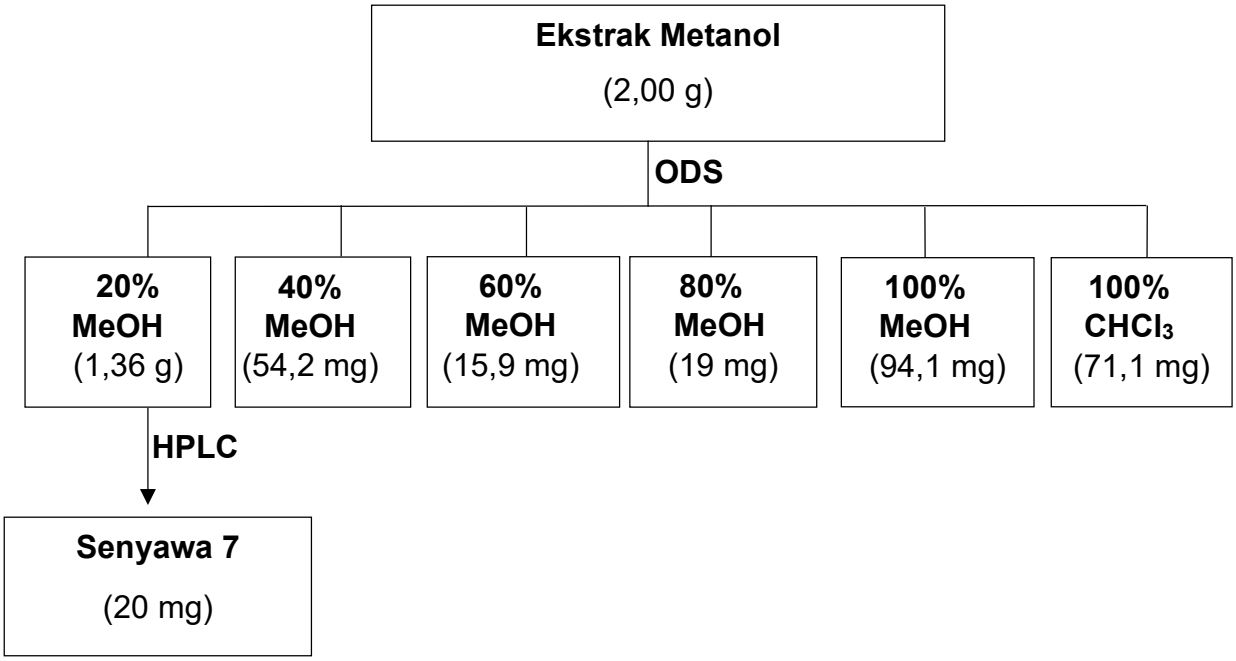
Lampiran 13. Bagan Isolasi Senyawa dari ekstrak n-heksan *G. salicornia*



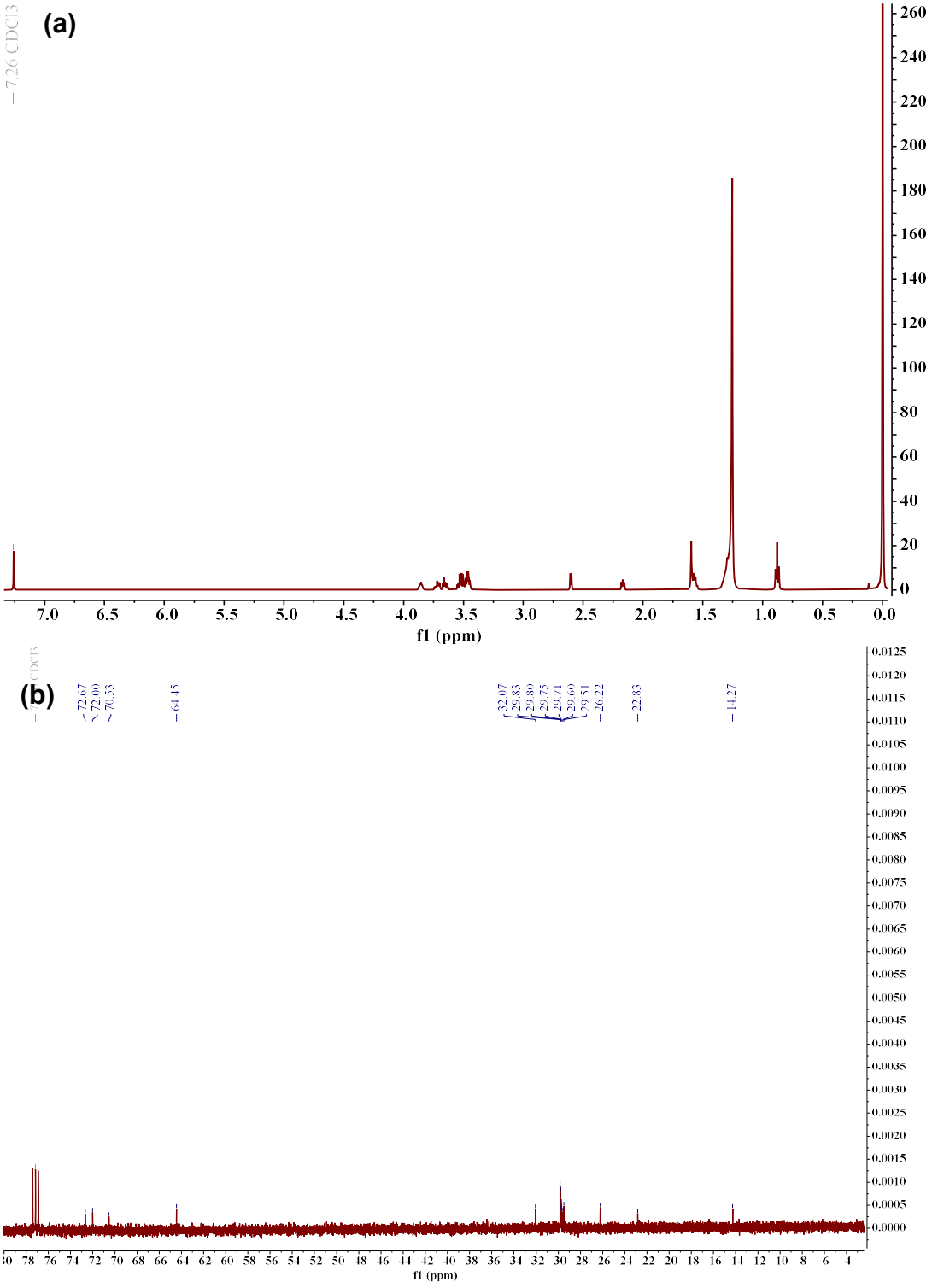
Lampiran 14. Bagan Isolasi Senyawa dari ekstrak kloroform *G. salicornia*



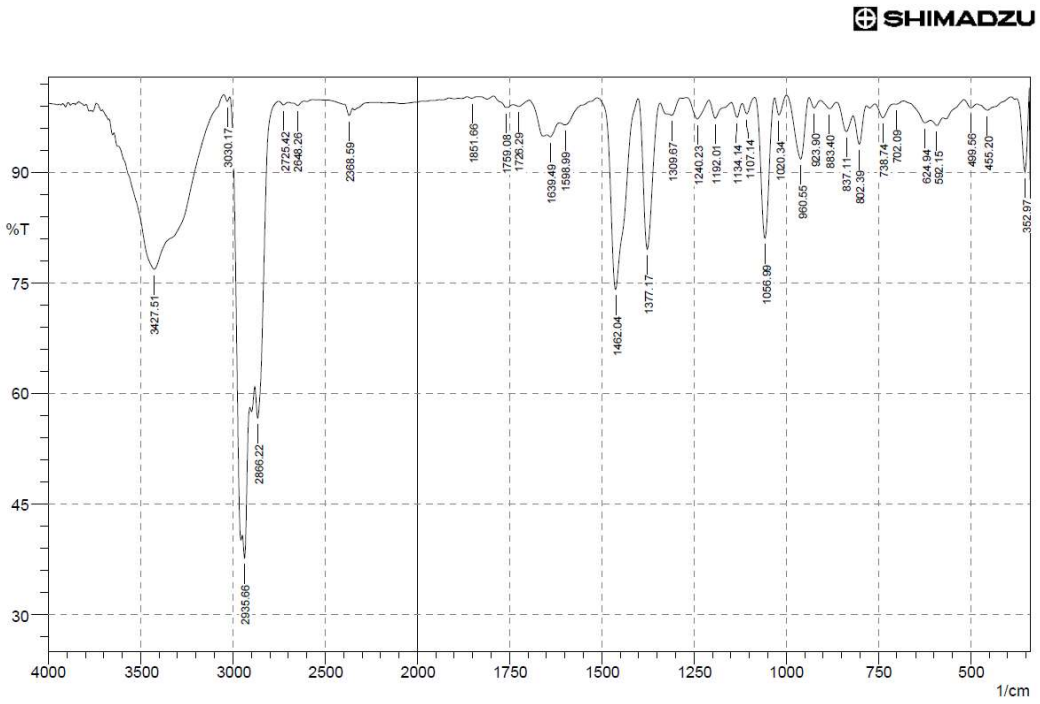
Lampiran 15. Bagan Isolasi Senyawa dari ekstrak metanol *G. salicornia*



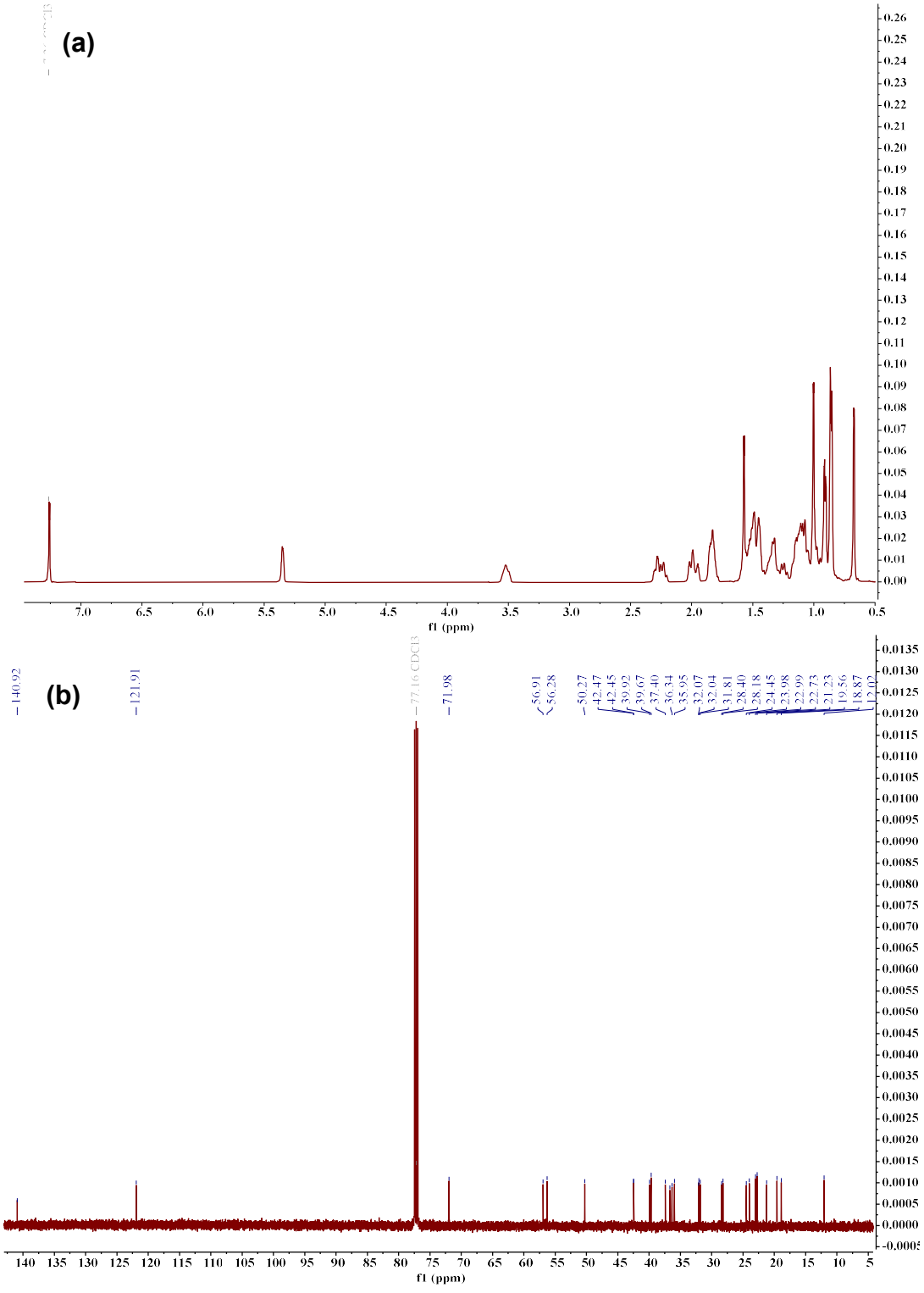
Lampiran 16. Spektrum ^{1}H-NMR (a) dan ^{13}C-NMR (b) Senyawa (**1**)



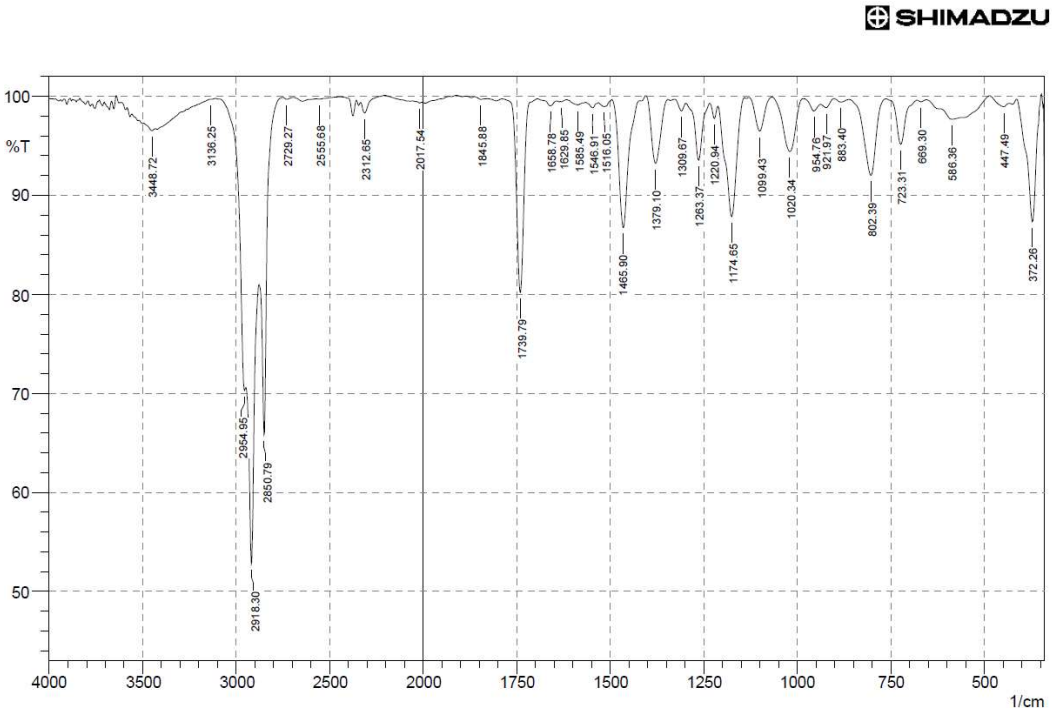
Lampiran 17. Spektrum FT-IR Senyawa Kolesterol **(2)**



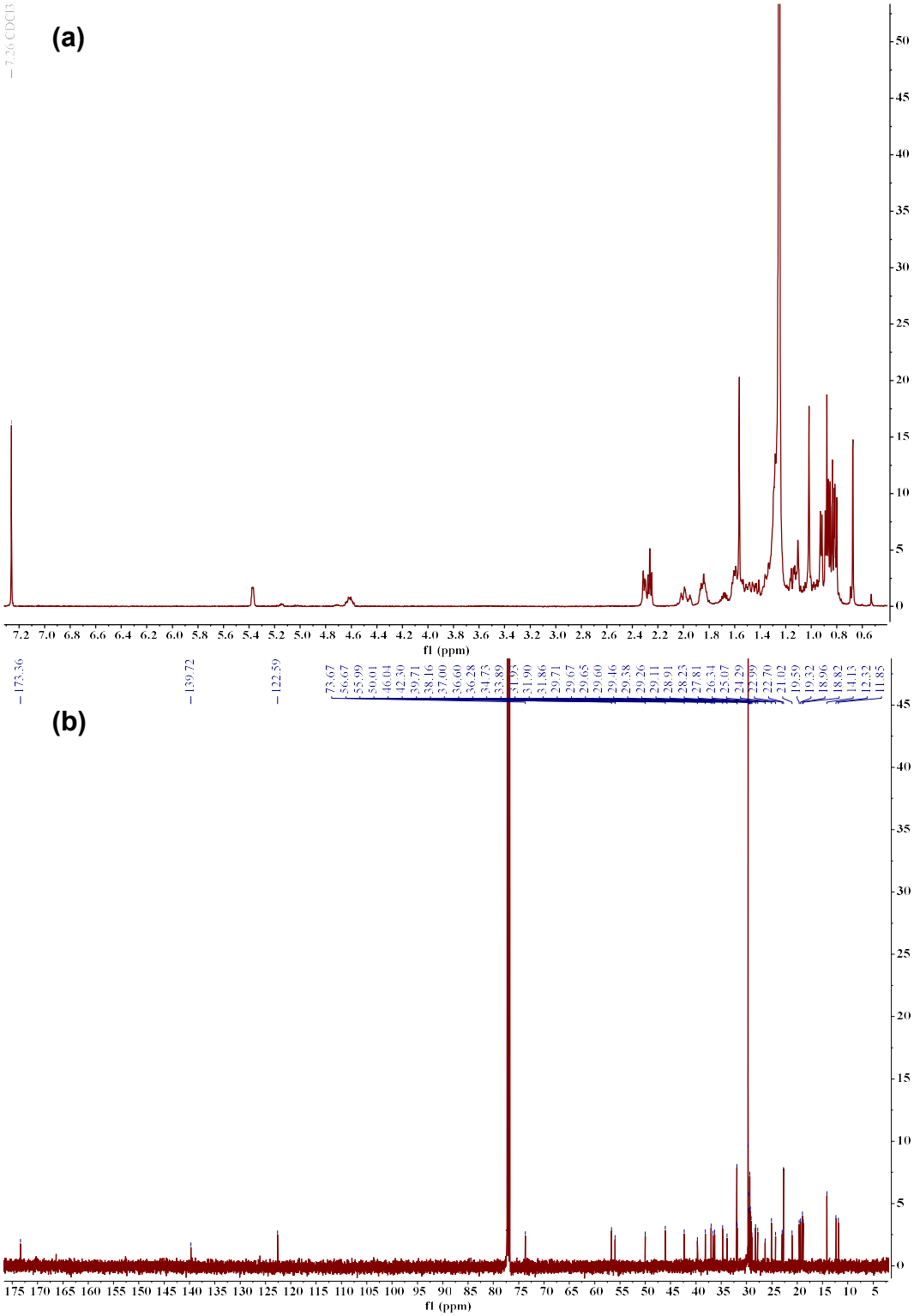
Lampiran 18. Spektrum ^{1}H-NMR (a) dan ^{13}C-NMR (b) Senyawa **(2)**



Lampiran 19. Spektrum FT-IR Senyawa β-Sitosteroltetrakosanoat (**3**)

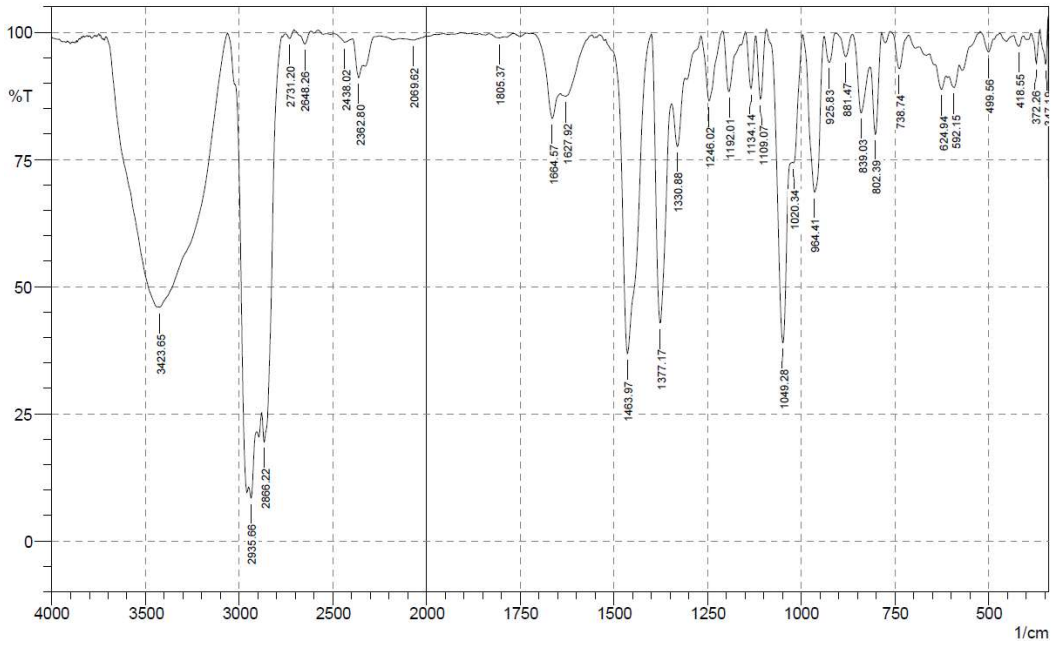


Lampiran 20. Spektrum ^{1}H-NMR (a) dan ^{13}C-NMR (b) Senyawa **(3)**

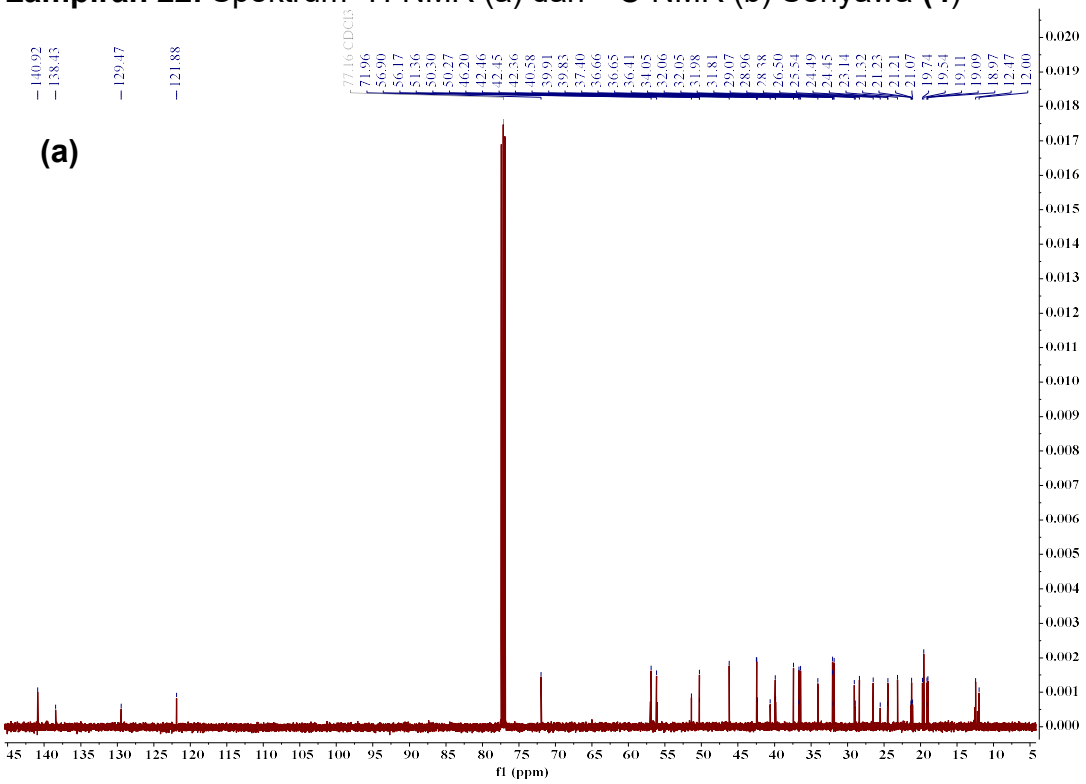


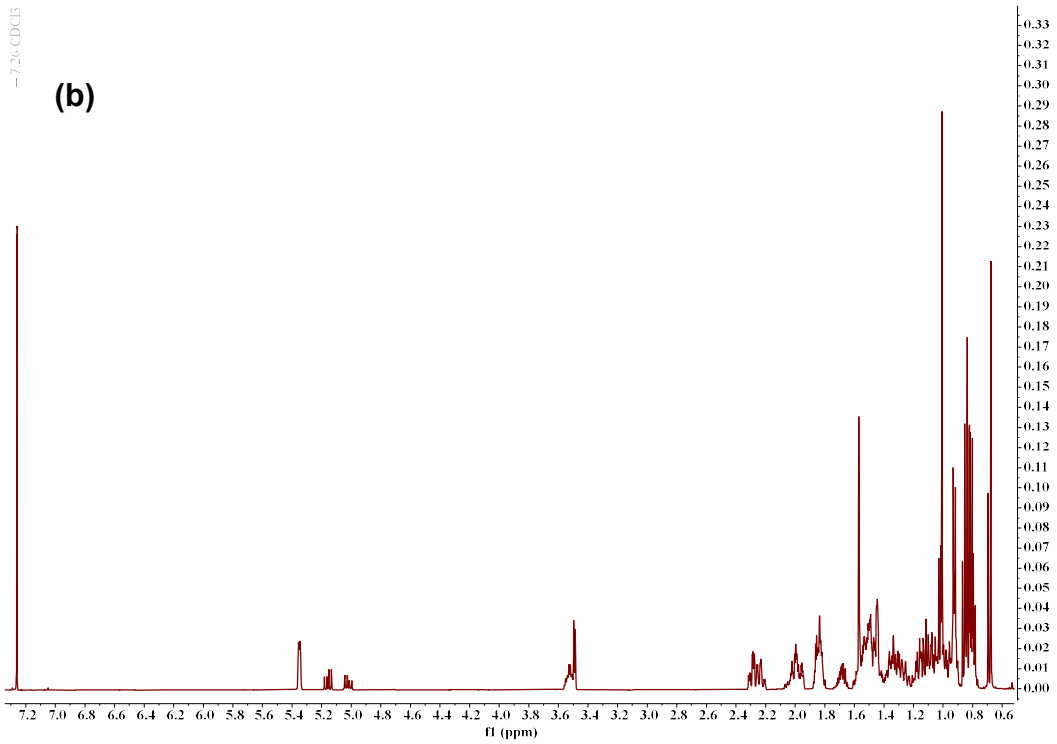
Lampiran 21. Spektrum FT-IR Senyawa Stigmasterol (**4**)

SHIMADZU

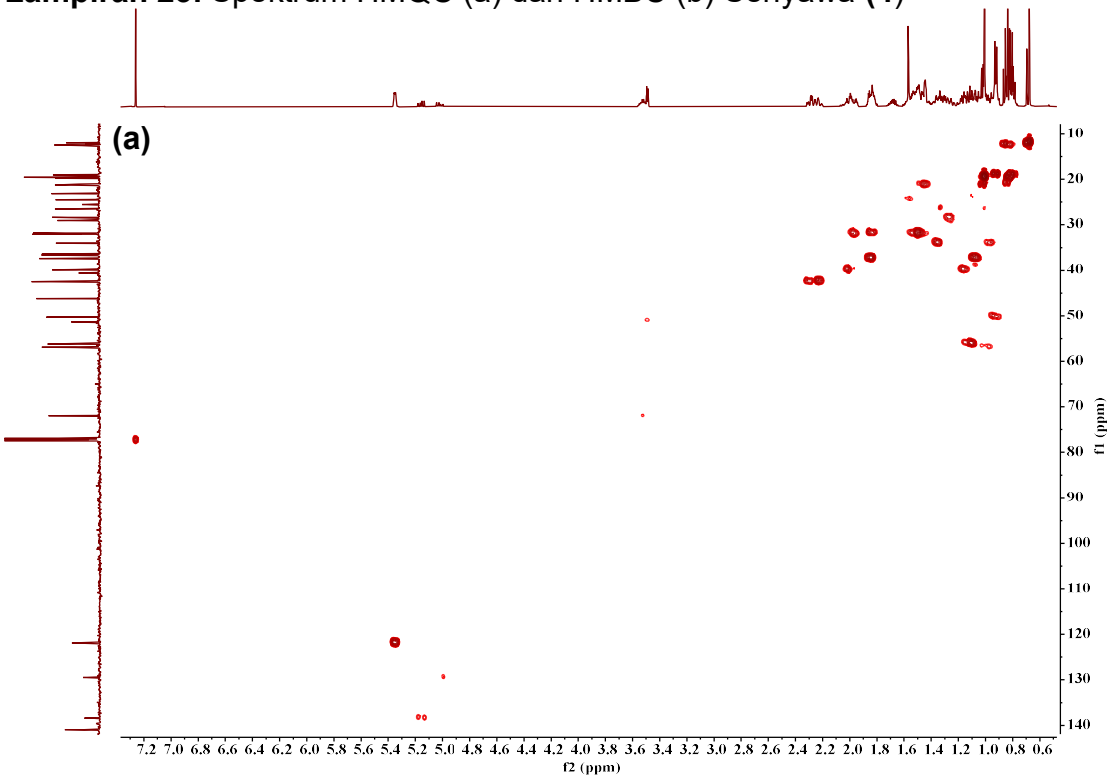


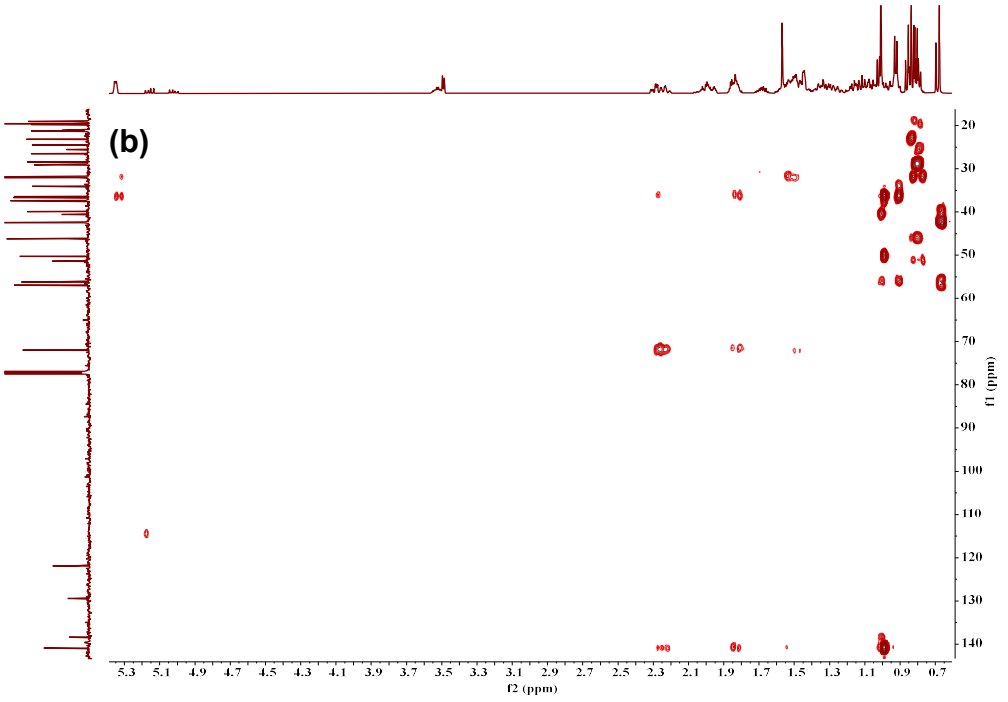
Lampiran 22. Spektrum ^{1}H-NMR (a) dan ^{13}C-NMR (b) Senyawa **(4)**



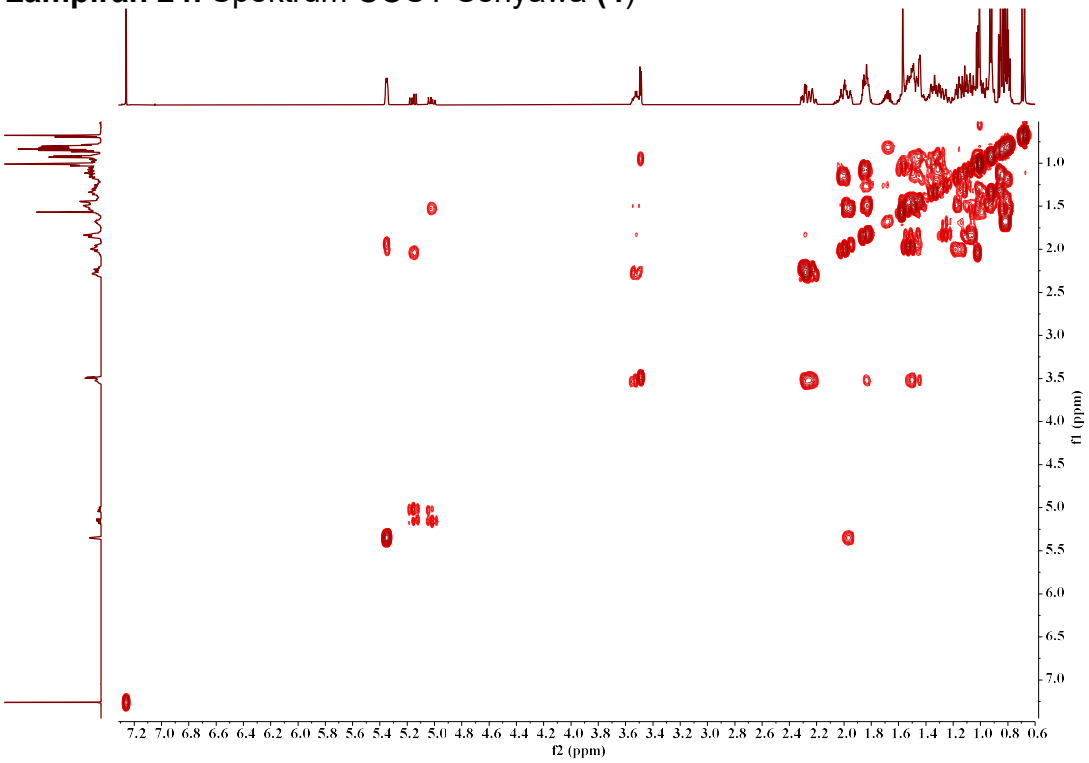


Lampiran 23. Spektrum HMQC (a) dan HMBC (b) Senyawa **(4)**

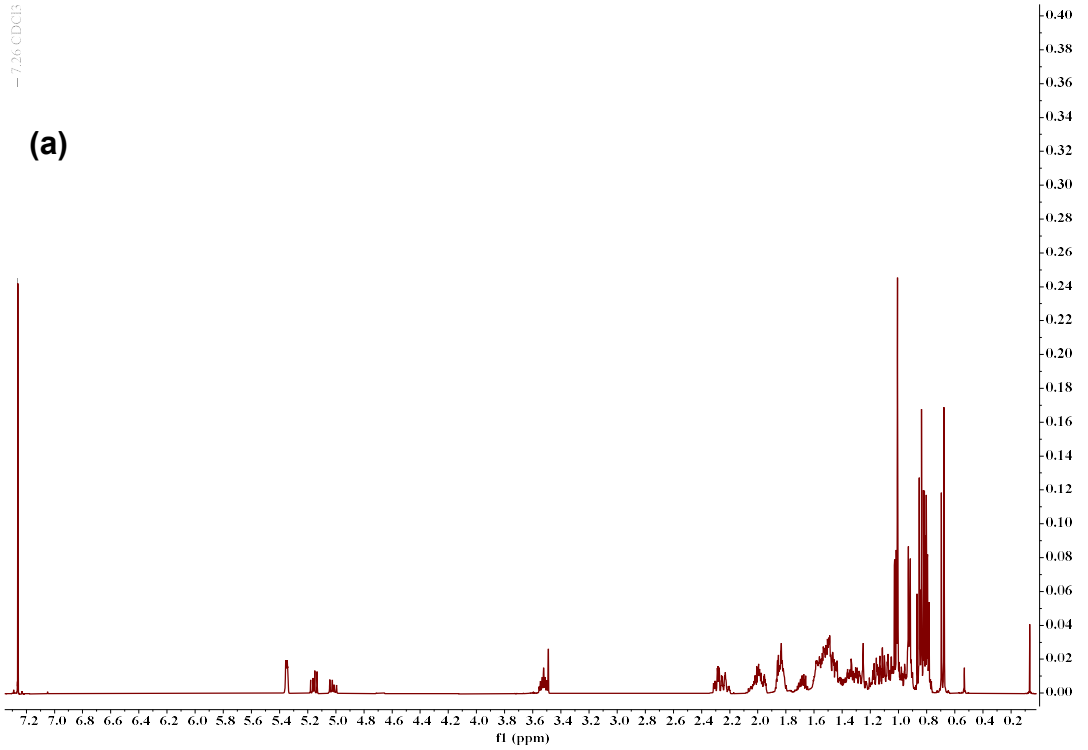


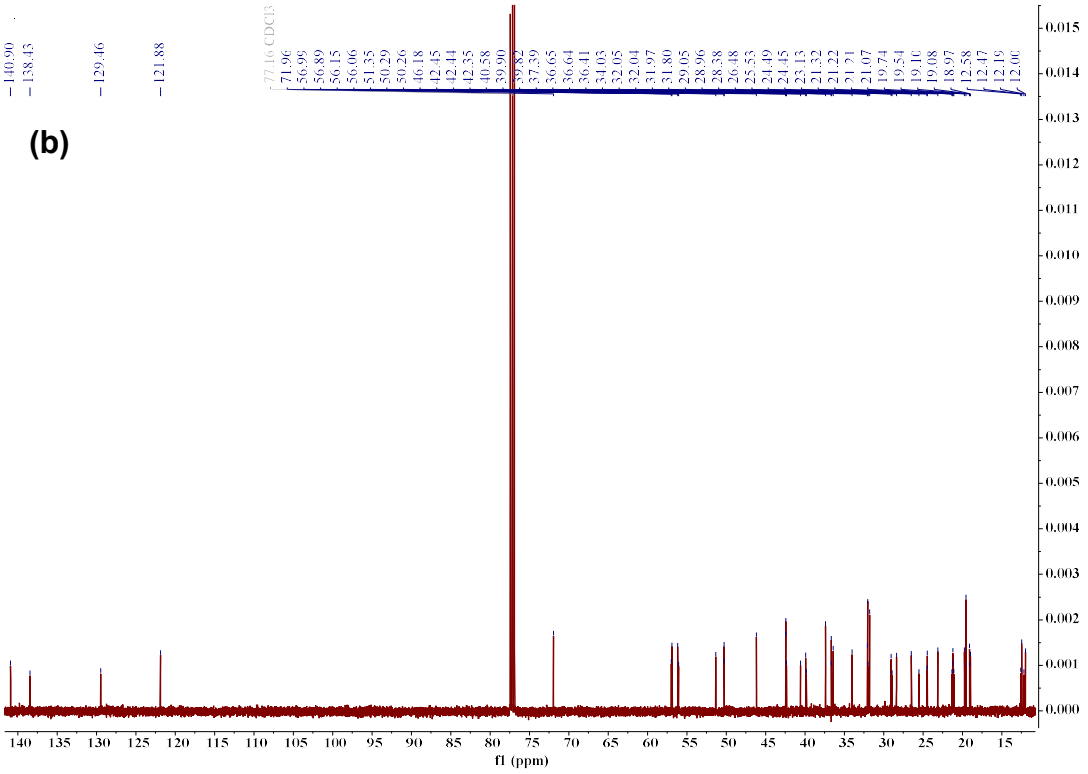


Lampiran 24. Spektrum COSY Senyawa (**4**)

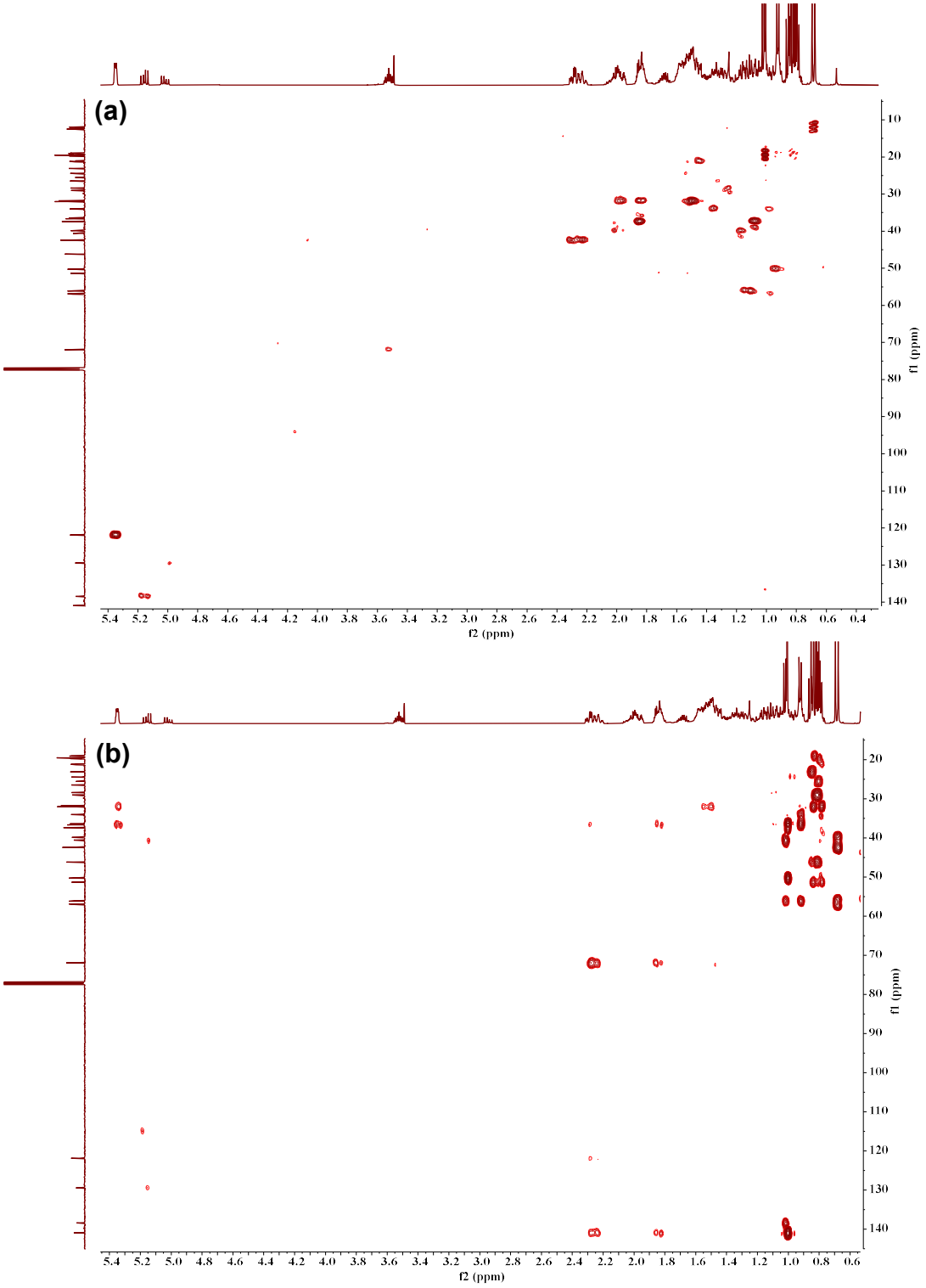


Lampiran 25. Spektrum ^{1}H-NMR (a) dan ^{13}C-NMR (b) Senyawa (**5**)

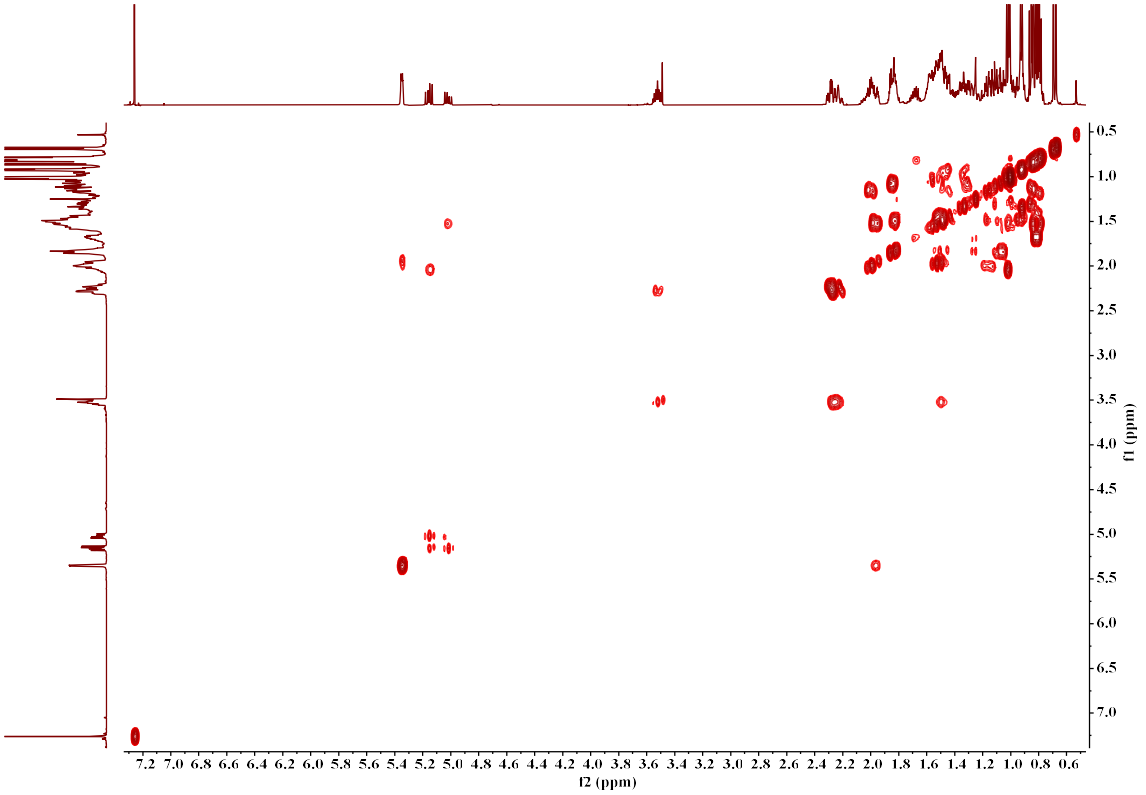




Lampiran 26. Spektrum HMQC (a) dan HMBC (b) Senyawa **(5)**

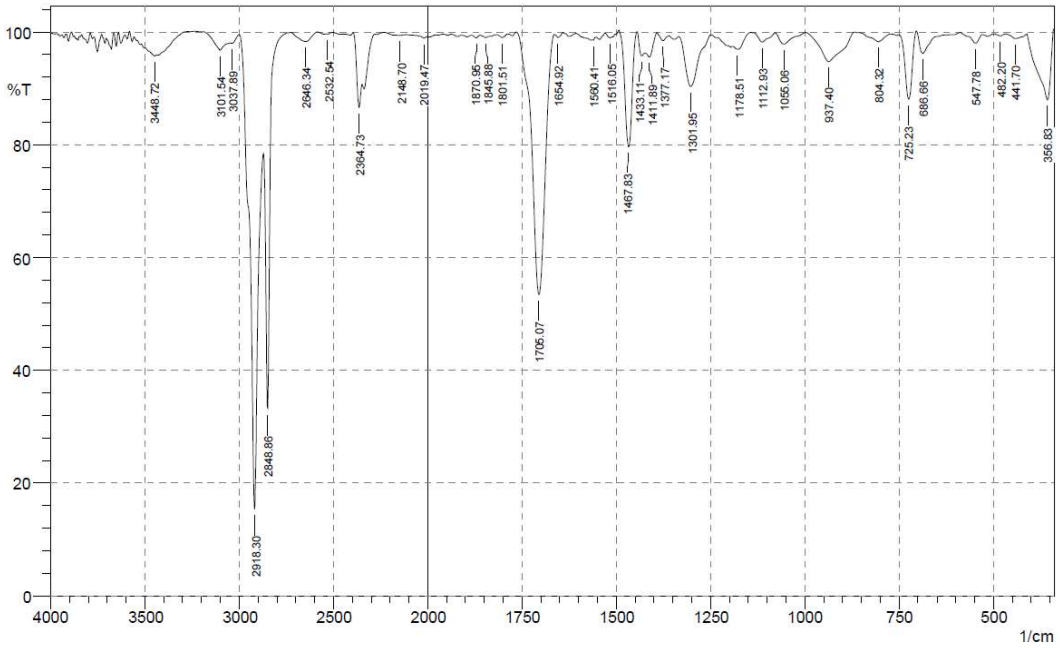


Lampiran 27. Spektrum COSY Senyawa (**5**)

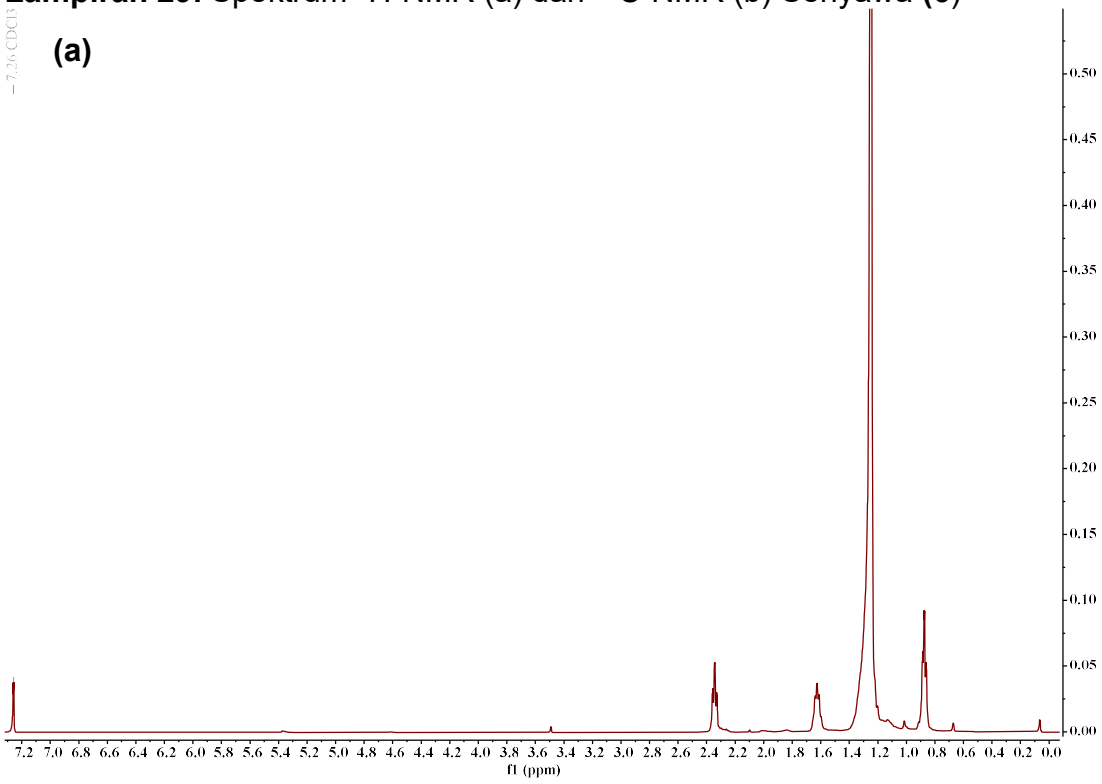


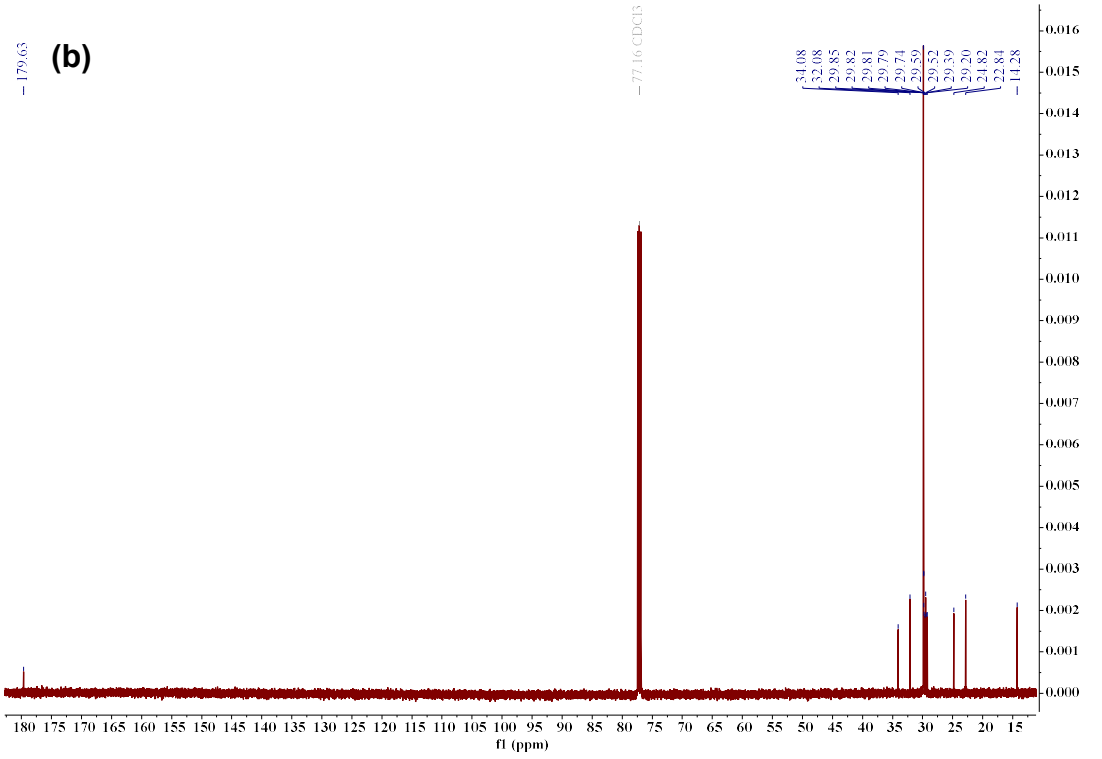
Lampiran 28. Spektrum FT-IR Senyawa Asam Behenat (**6**)

SHIMADZU

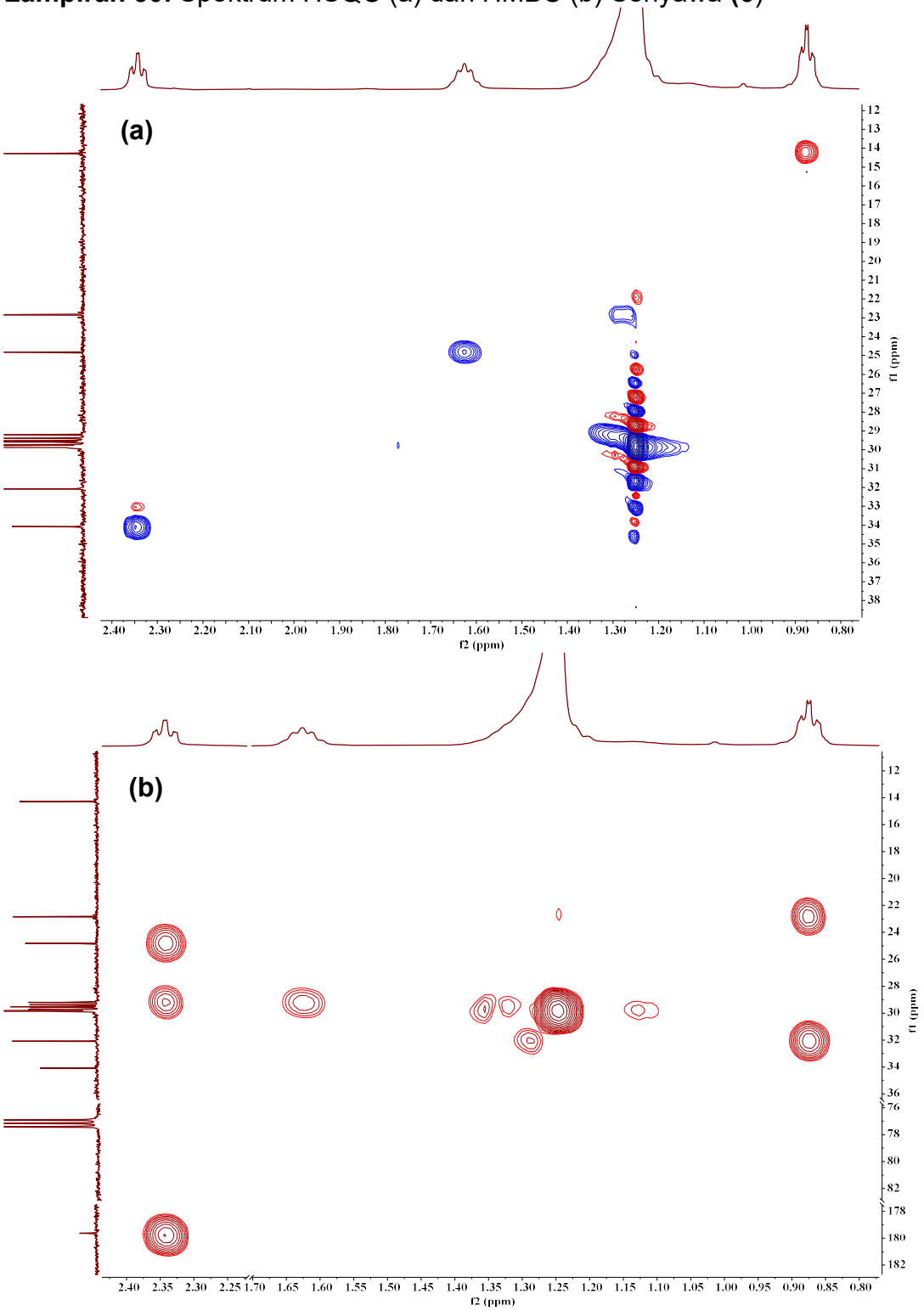


Lampiran 29. Spektrum ^{1}H-NMR (a) dan ^{13}C-NMR (b) Senyawa **(6)**

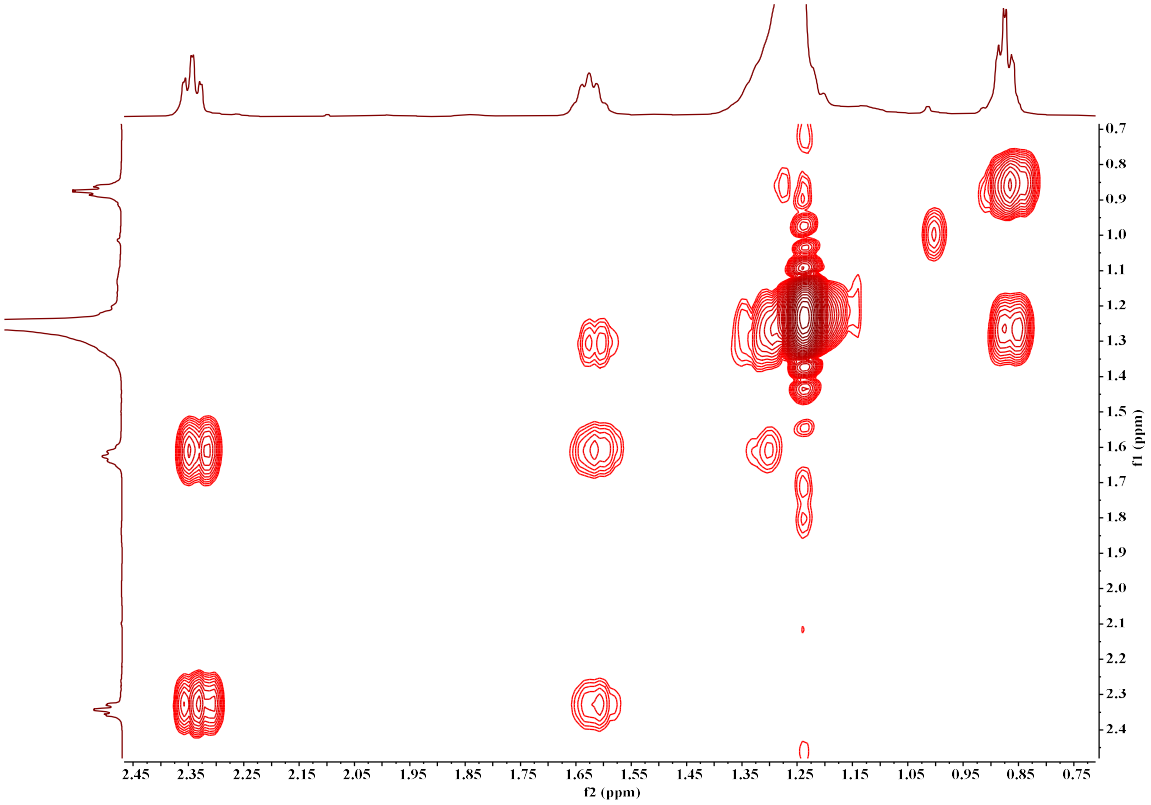




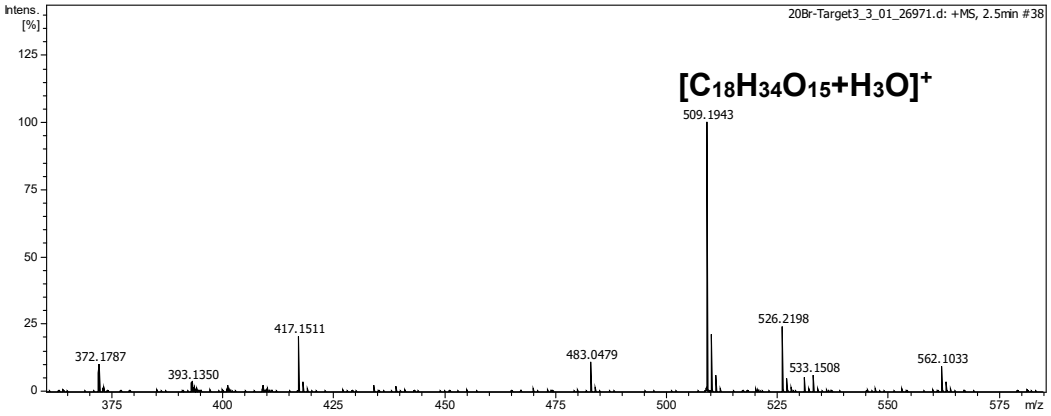
Lampiran 30. Spektrum HSQC (a) dan HMBC (b) Senyawa **(6)**

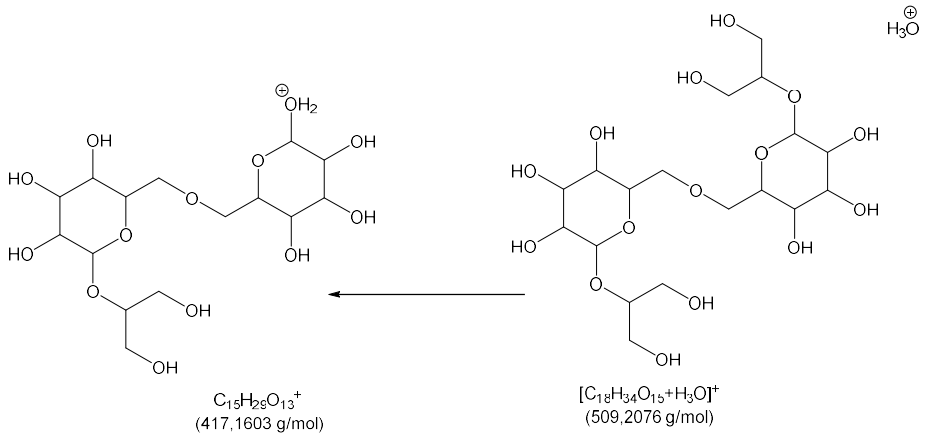


Lampiran 31. Spektrum COSY Senyawa **(6)**



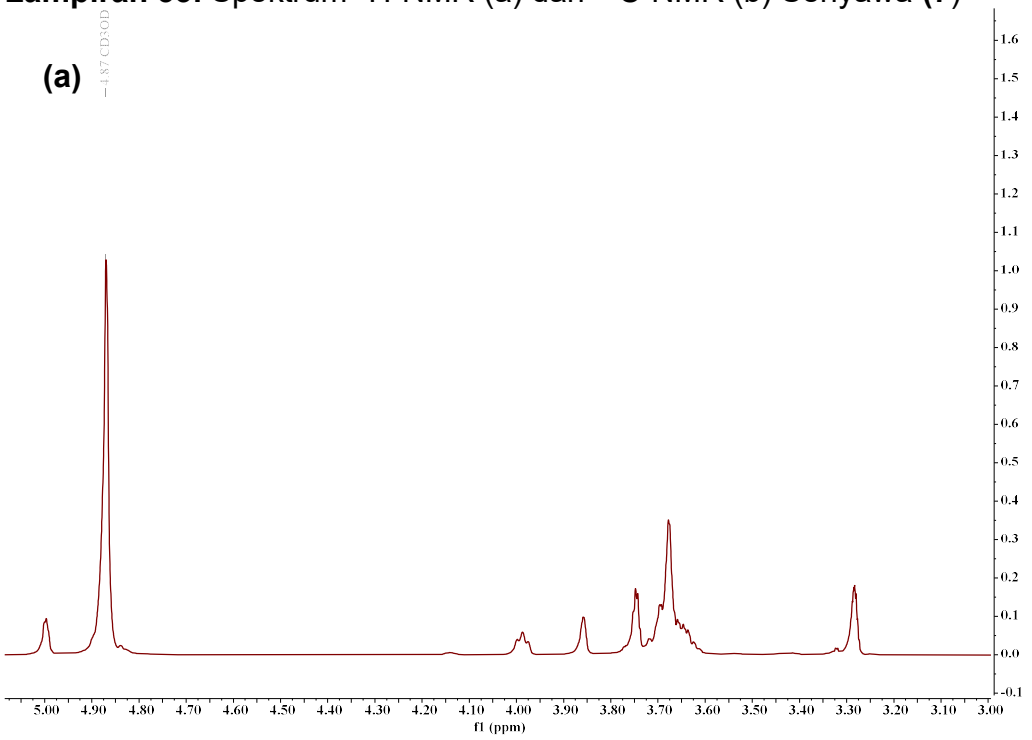
Lampiran 32. Spektrum ESI-MS Senyawa **(7)**



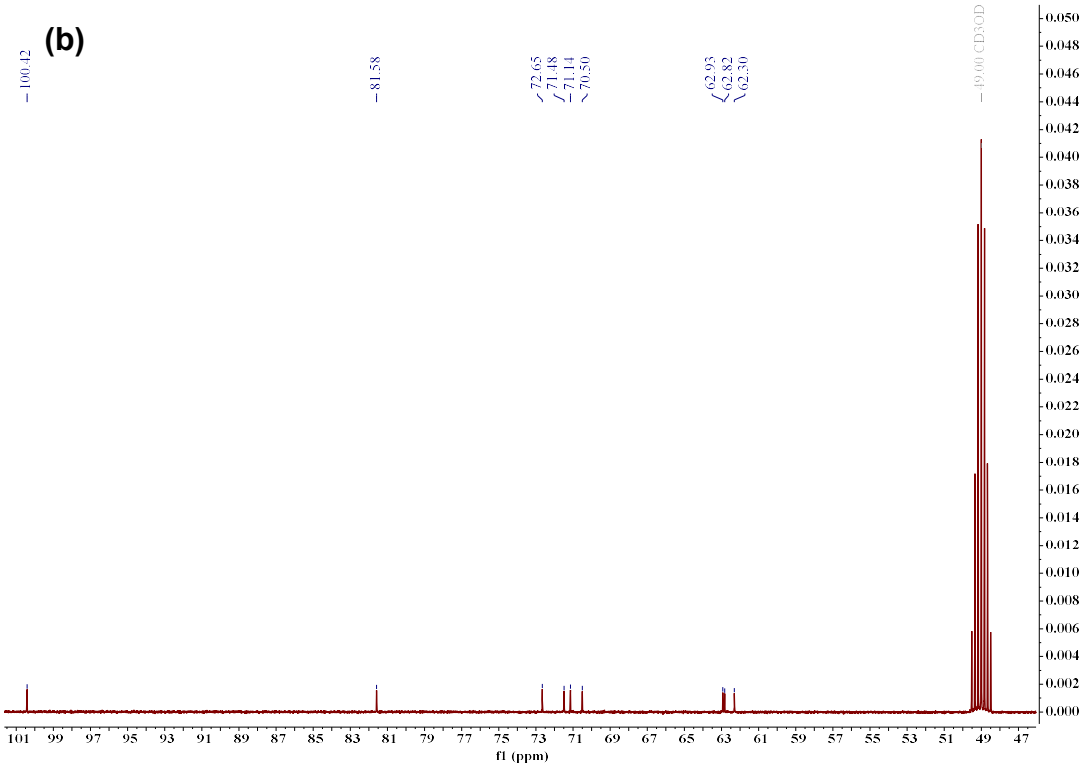


Lampiran 33. Spektrum ^{1}H-NMR (a) dan ^{13}C-NMR (b) Senyawa **(7)**

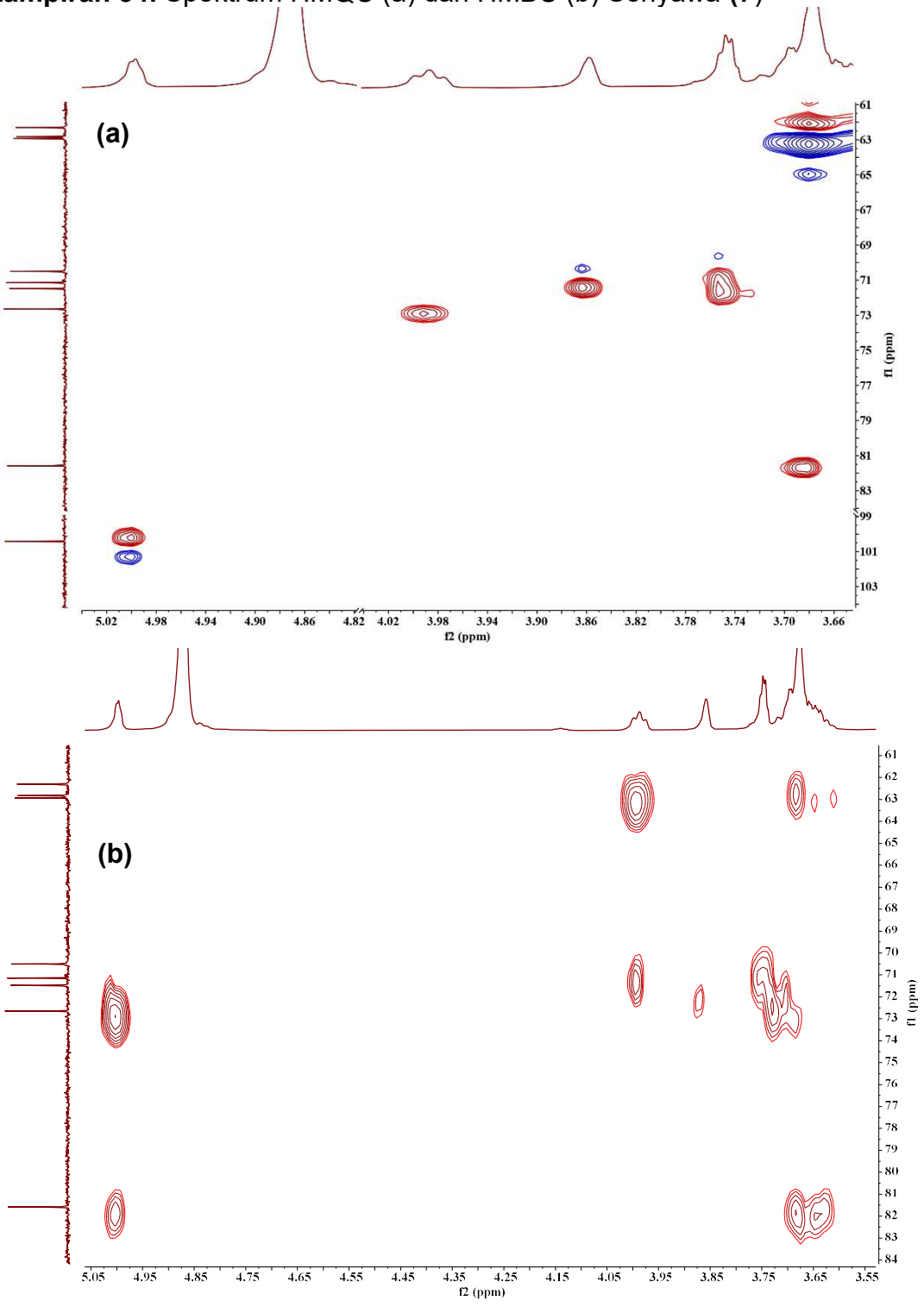
(a)



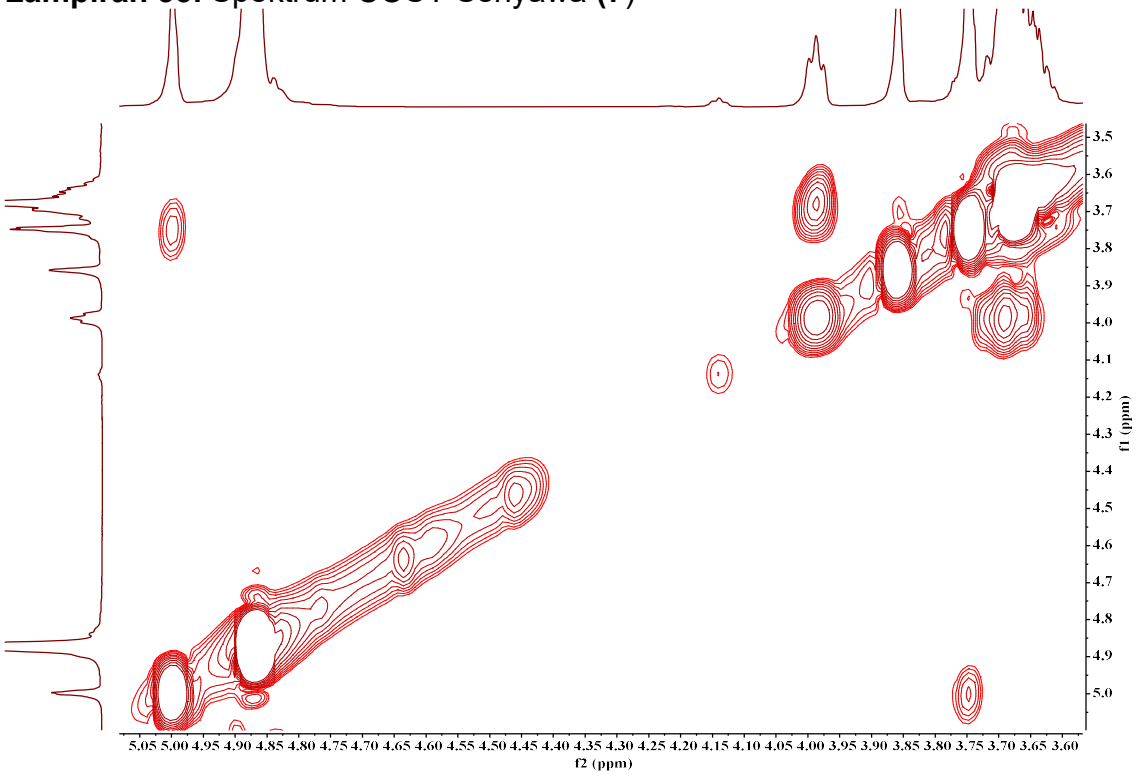
(b)



Lampiran 34. Spektrum HMQC (a) dan HMBC (b) Senyawa **(7)**

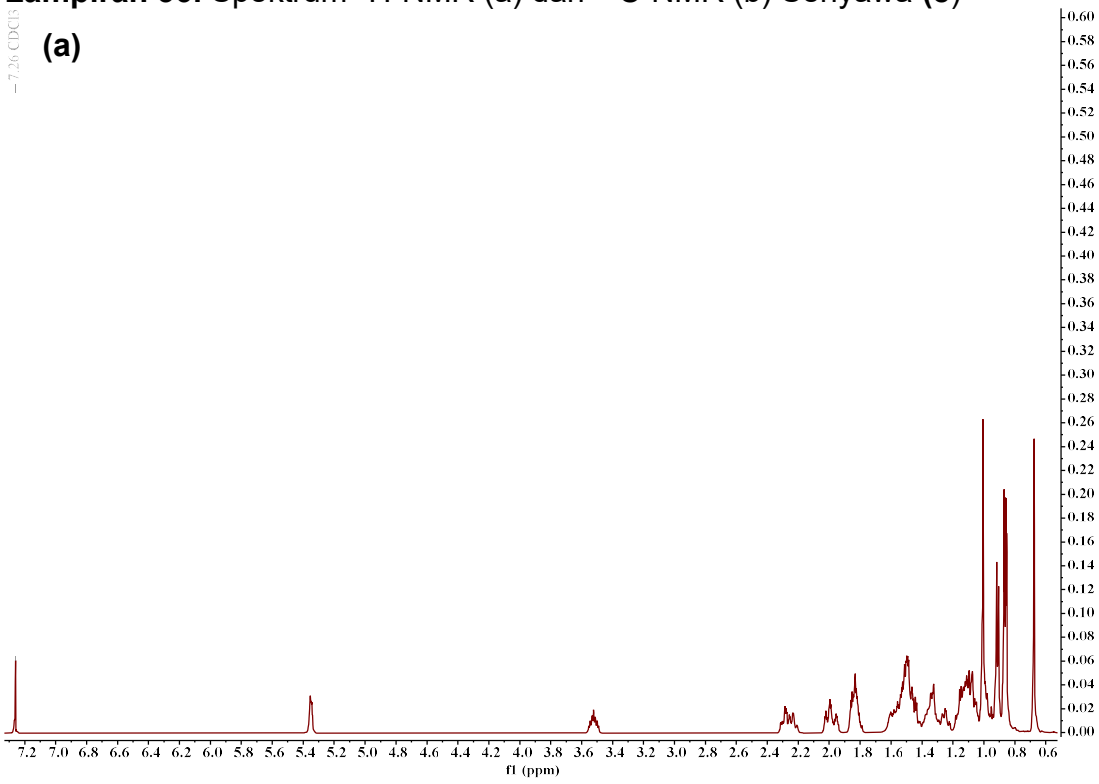


Lampiran 35. Spektrum COSY Senyawa (**7**)

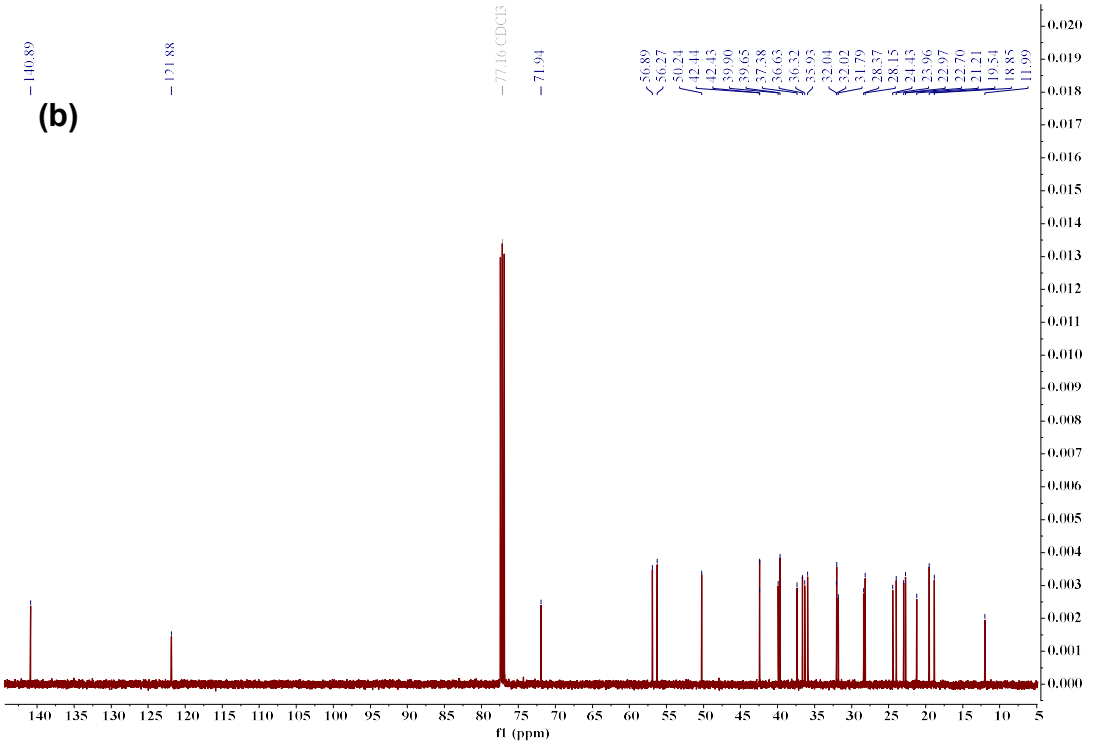


Lampiran 36. Spektrum ^{1}H-NMR (a) dan ^{13}C-NMR (b) Senyawa **(8)**

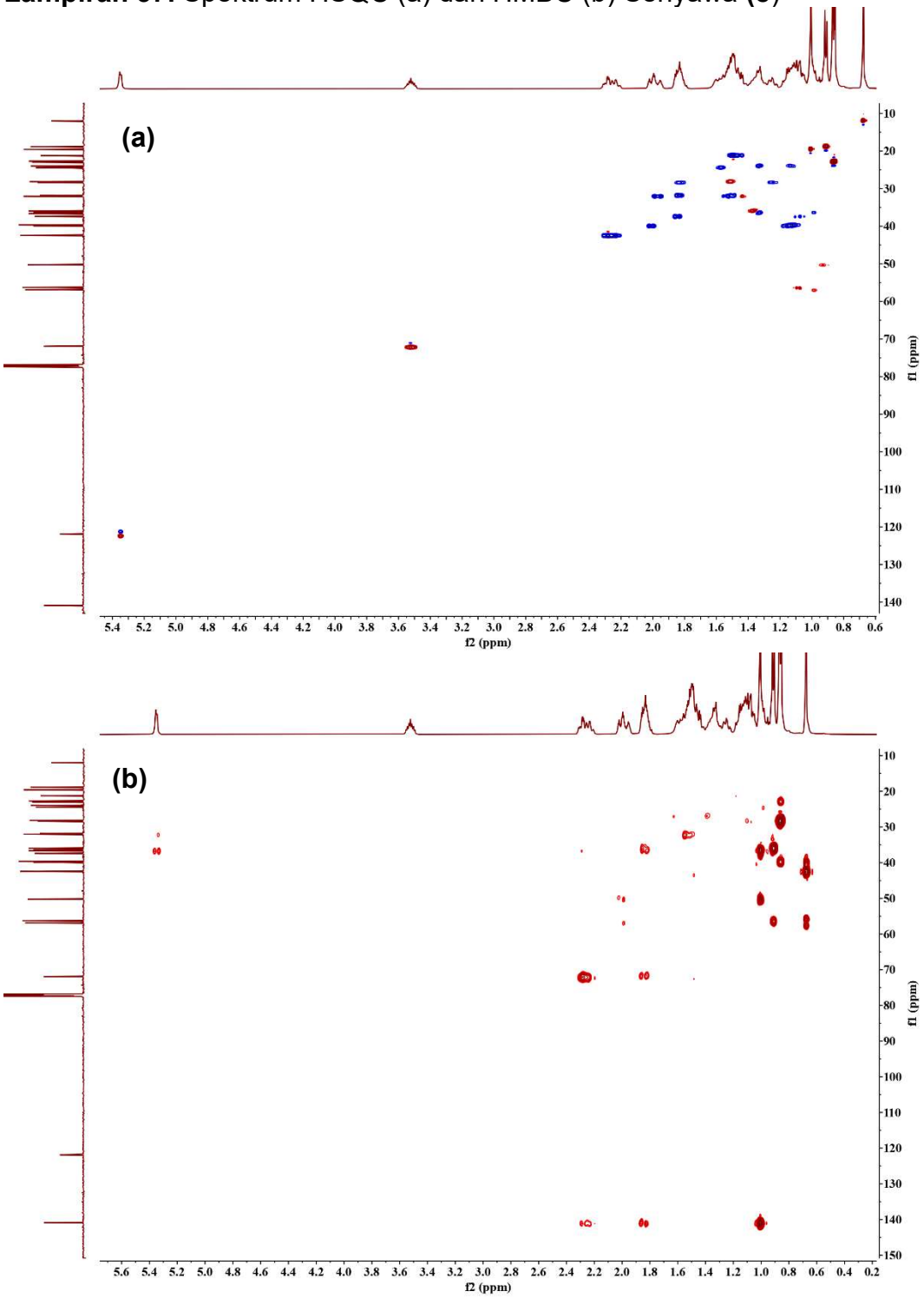
(a)



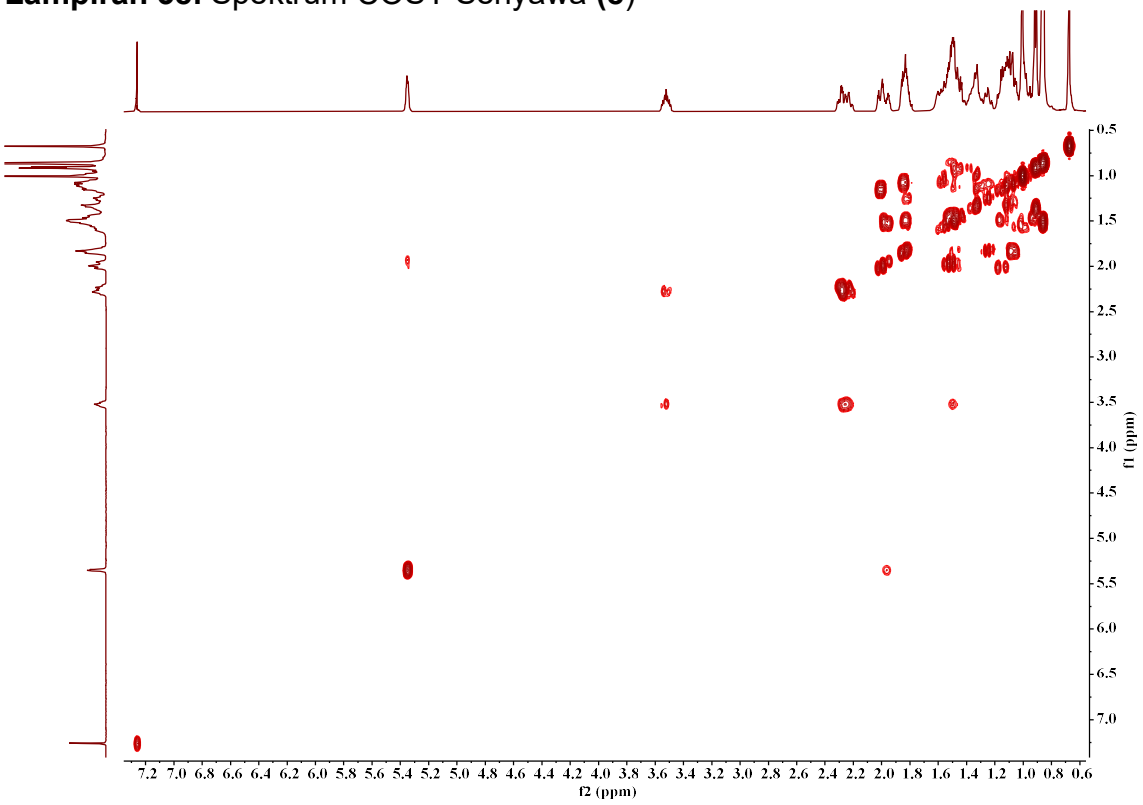
(b)



Lampiran 37. Spektrum HSQC (a) dan HMBC (b) Senyawa **(8)**



Lampiran 38. Spektrum COSY Senyawa **(8)**



Lampiran 39. Nama Senyawa berdasarkan Nomor

No	Nama Senyawa
1	3-hexadecyloxy-1,2-propanediol
2	Kolesterol
3	β-sitosteroltetrakosanoat
4	Stigmasterol
5	14-metil-stigmasterol
6	Asam behenat
7	Bis-floridoside
8	4a,6a-dimetil-7-(6metilheptan-2-il)-1,2,3,4,4b,5,6,7,8,9,10,10b,11-tetradecahydrochryse-2-ol
9	2,6-Dibromophenol
10	2,4-Dibromophenol
11	Tribromophenol
12	2-Bromophenol
13	4-Bromophenol
14	Stigmasterol
15	β-Stigmasterol
16	Brassicasterol
17	Campesterol
18	β-Cryptoxantin
19	8-Hydroxyeicosatetraenoic acid (8-HETE)
20	Dihydroxyeicosatetraenoic acid (7,8-di-HETE)
21	3-(2-ethyl-6-((3*Z*,7*Z*)-1,2,5,6- tetrahydroazocin-5-yl) hexyl)morpholin-6-one
22	2*H*-chromen, 2-acetoxy-2-(5-acetoxy-4-methyl-2-oxotetrahydro-2*H*-pyran-4-yl) ethyl4-(3-methoxy-2 (methoxymethyl)-7-methyl-3, 4,4a,7,8,8a-hexahydro-2*H*-chromen-4-yloxy)-5-methylheptanoate
23	5-(7-(5-ethyl-3,4-dimethoxycyclooctyl) benzofuran-6-yl)-7-methyl-3,4,7,8-tetrahydro-2H-oxocin-2-one
24	2-(3-ethyl-9-(2-methoxyethoxy)-1-oxo-2,3,4,9-tetrahydro-1H-xanthen-2-yl) ethyl-5-hydroxy-9-methoxy-7,8-dimethyl-8-(5-methylfuran-2-yl) nona-3,6-dienoate
25	1-β-D-glucosyl :Gracilarioside
26	Gracilamide A
27	Gracilamide B
28	Acyclic diterpenoid 3,7,11,15 tetramethyl-3-hexadec-en-1-ol
29	Phytol
30	13-[[2-(hexyloxy)-2,5,5,8a-tetramethyldecahydro-1-naphthalenyl] (methoxy)methyl]benzenol
31	1-butoxy-4,4,11b,11c-tetramethyl-decahydrobenzo [kl]xanthen-10-ol
32	3-(hept-3^6-enyloxy)-decahydro-4,6a,12a,12b-tetramethyl-1*H*-benzo[α]xanthene-4,10,12-triol

33	3-(hydroxymethyl)-7-(methoxymethyl)-3,11-dimethyl-9-oxo-spiro[5.5]undec-4-en-10-methylbutanoate
34	4-ethoxy-11,11-dimethyl-7-methylene-8-(propionyloxy)spiro[5.5]undec-2-en-10^4,10^6-dihydroxytetrahydro-2H-pyran-10-carboxylate
35	methyl-16(13→14)-abeo-7-labdene-(12-oxo) carboxylate
36	Salicornolide A
37	Salicornolide B
38	Salicornolide C
39	4'-[10'-[7-hydroxy-2,8-dimethyl-6-(pentyloxy)-2H-chromen-2-yl]ethyl]-30,40-dimethyl-cyclohexanone
40	3'-[10'-(8-hydroxy-5-methoxy-2,6,7-trimethyl-2*H*-chromen-2-yl)ethyl]-30-methyl-20-methylene cyclohexyl butyrate
41	14-(15-methyltetrahydrofuranyl)-(18-(23-(hydroxy-23-methylheptanyl)-10-methylhexahydro-2*H*-furo[3,2-b]pyranyl))-2,2,6-trimethyltetrahydro-2H-pyran-3- butyrate
42	3-methoxy-2,6-dimethyl-3,4-dihydro-2*H*-pyranyl-(6-(7-(10-methyloctahydropyrano[3,2-b]pyranyl)-14-((19-methyl-23-methyleneoxepan)-(15-pentene-18-ol))
43	22-Dehydrocholesterol
44	Kolesterol
45	Stigmasterol
46	Kolesterol oleate
47	Oleic acid
48	Stigmast-5-en-3β-ol (β-Sitosterol)
49	Epicatechin
50	Catechin
51	R=H Cymopol
52	R=CH_3 Cymopol monomethyl ether
53	R=H Cyclocymopol
54	R=CH_3 Monomethyl ether
55	Cymopolone
56	Isocymopolone
57	Cymopochromenol
58	3,6,7-Trihydroxycoumarin
59	6,7-dihydroxycoumarin-3-sulfate
60	7-hydroxycoumarin-3,6-disulfate (dasycladin B)
61	5,8'-di-(6(6'),7(7')-tetrahydroxy-3-sulfoxy-3'-sulfoxycoumarin (dasycladin A)
62	4-(sufooxy)benzoic acid
63	4-(sufooxy)phenylacetic acid

64	(PBQ2)
65	(PBQ1)
66	(2R,4S,4aS,6R,8aS)-2-bromo-4,8a-dimethyl-6-(prop-1-en-2-yl)octahydronaphthalen-4a(2H)-ol
67	(3R,4R,4aS,6R,8aS)-3-bromo-4,8a-dimethyl-6-(prop-1-en-2-yl) octahydronaphthalen-4a(2H)-ol
68	(1R,4R,4aR,7R,8aS)-4-bromo-1,4a-dimethyl-7-(prop-1-en-2-yl) decahydronaphthalen-1-ol