Supplementary Materials

Spectroscopic and chromatographic data

Chemo-enzymatic Protocol for the Synthesis of Enantiopure β-Blocker (*S*)-Bisoprolol

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1. NMR-Spectra

4-((2-Isopropoxyethoxy)methyl)phenol (2)

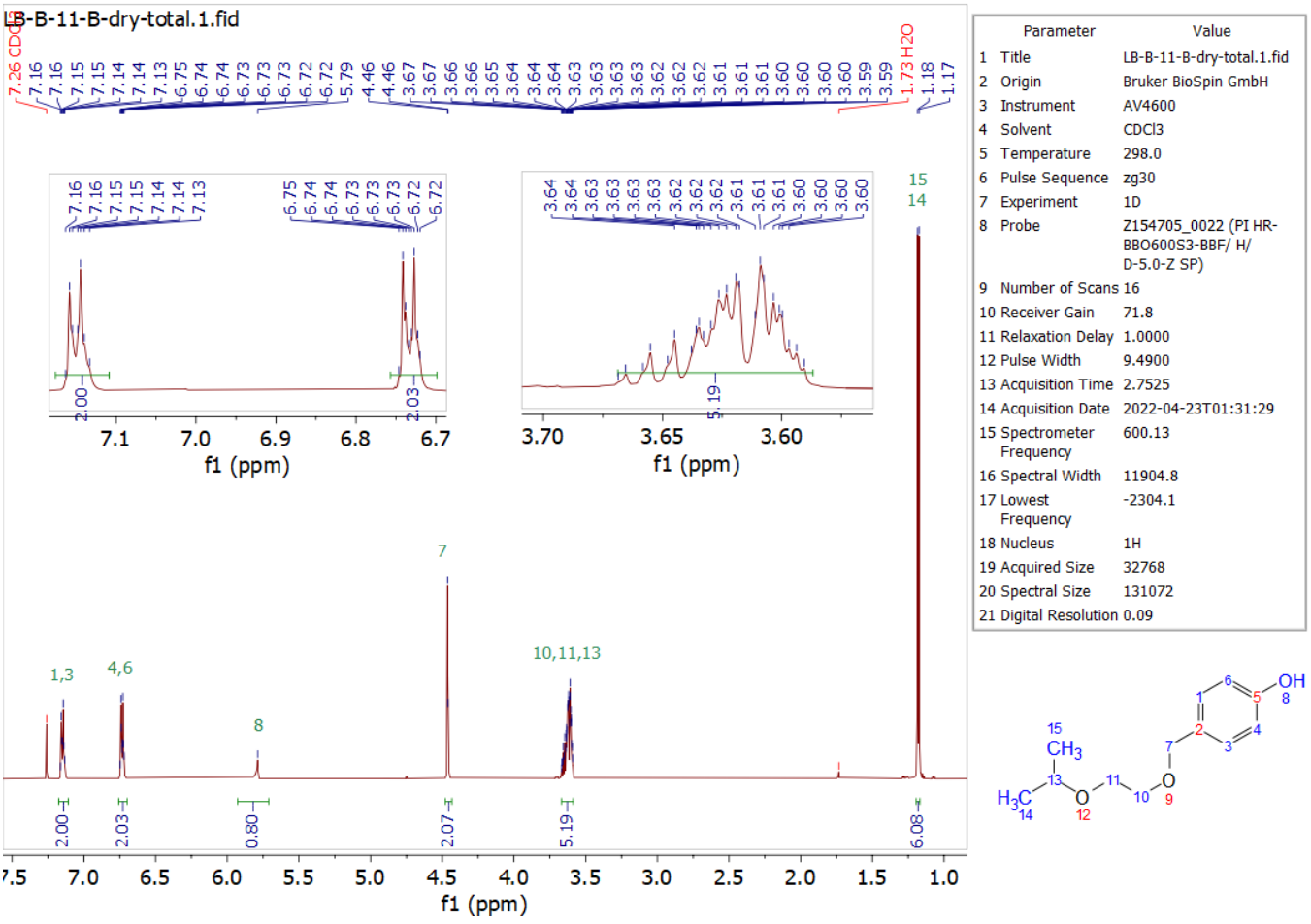


Figure S1. ^{1}H-NMR spectrum (600 MHz, $CDCl_3$) of 4-((2-isopropoxyethoxy)methyl)phenol (**2**).

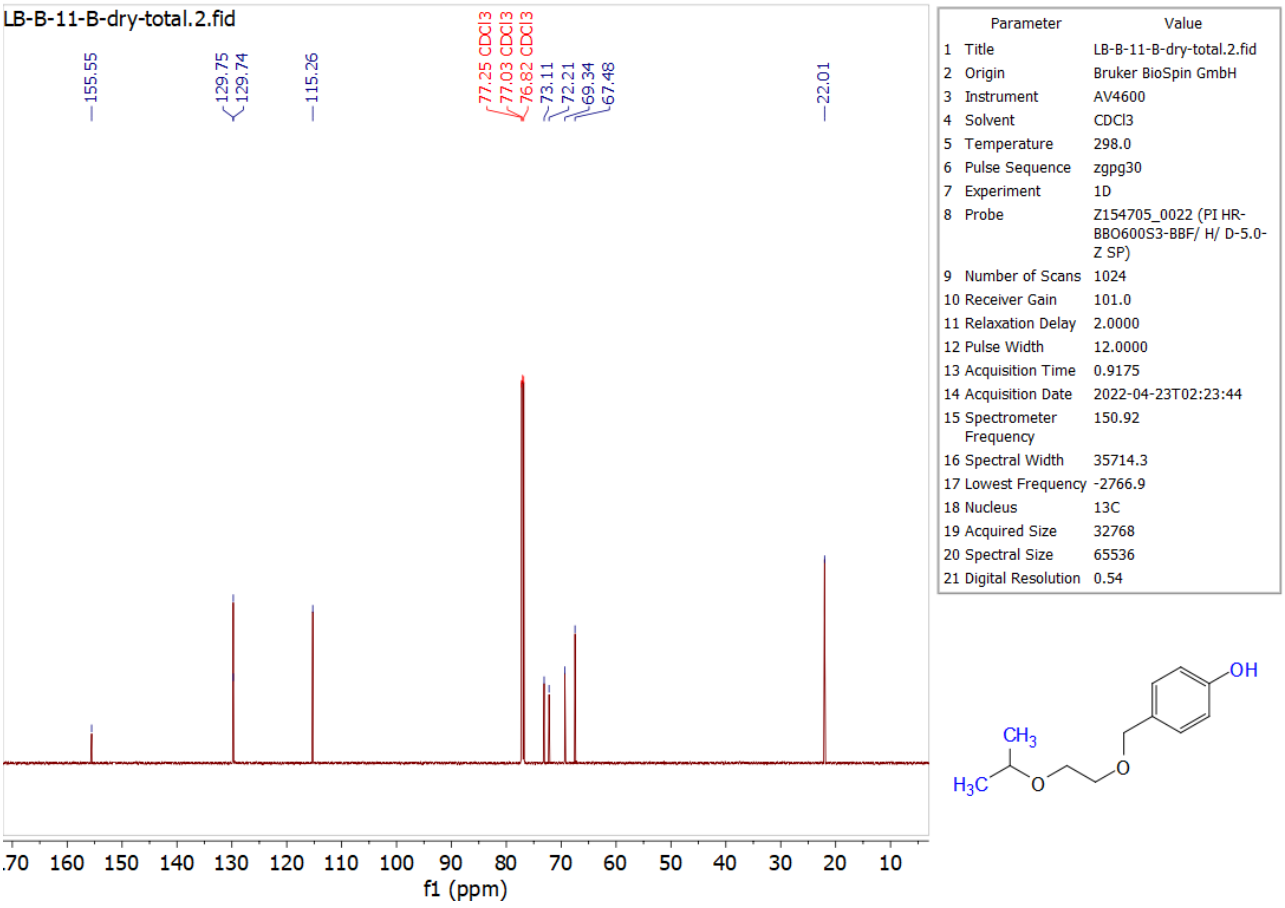


Figure S2. ^{13}C-NMR spectrum (151 MHz, $CDCl_3$) of 4-((2-isopropoxyethoxy)methyl)phenol (**2**).

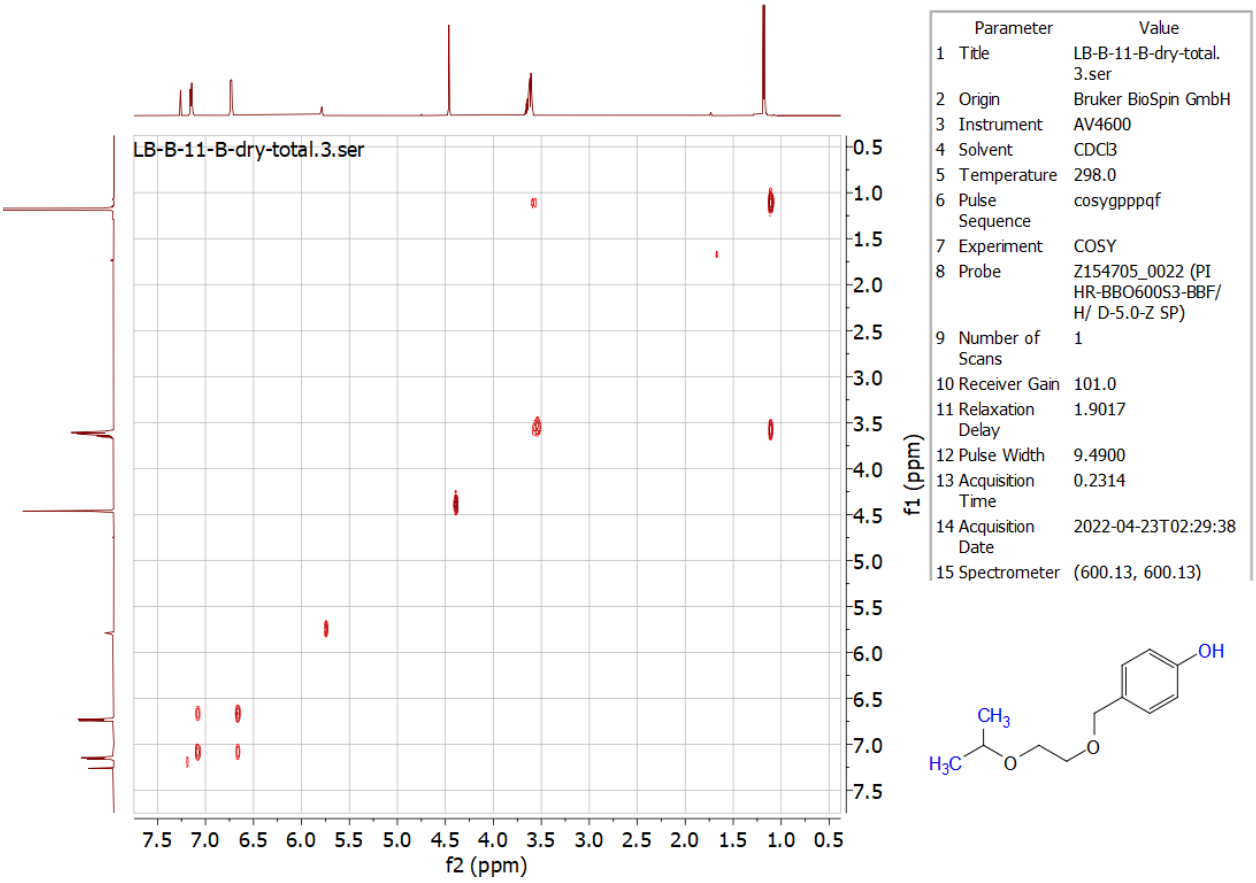


Figure S3. H,H-COSY-NMR spectrum (600 MHz, $CDCl_3$) of 4-((2-isopropoxyethoxy)methyl)phenol (**2**).

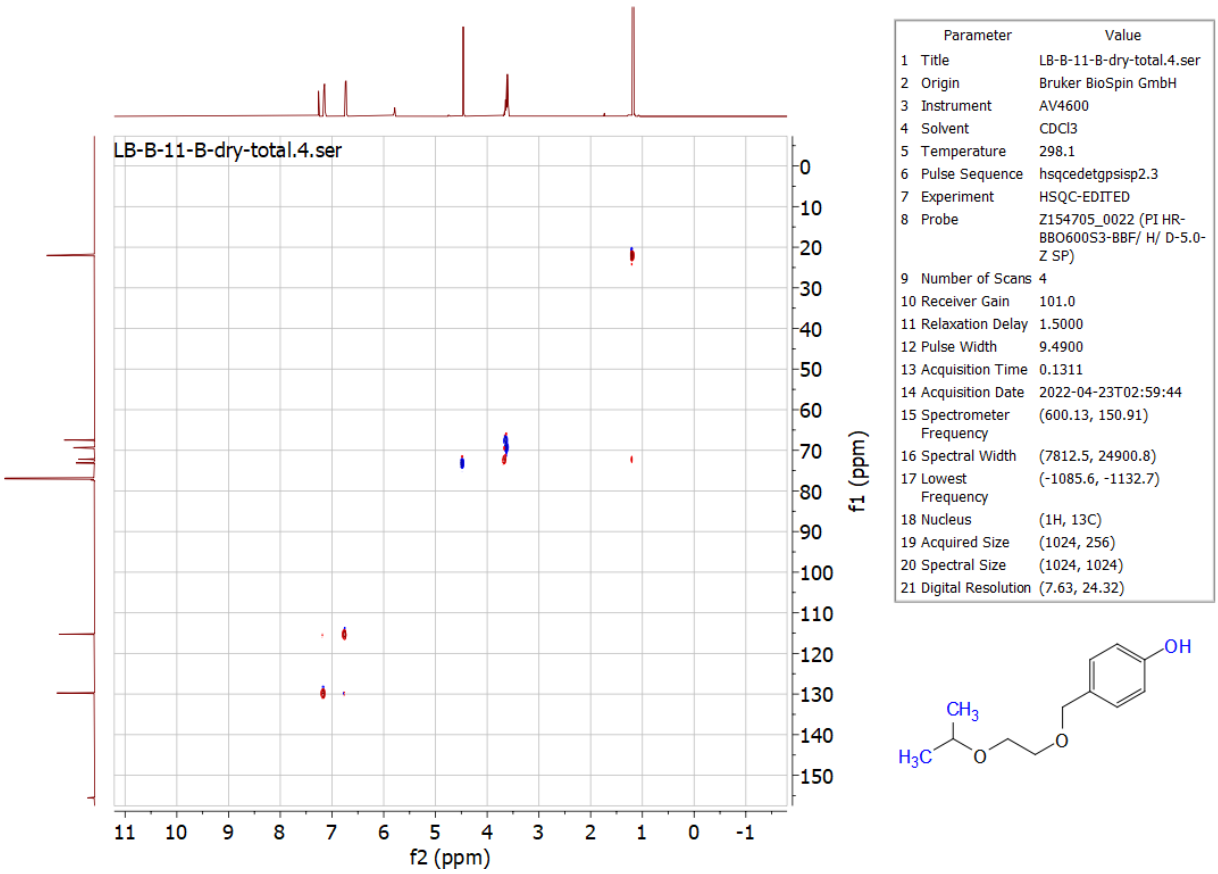


Figure S4. HSQC-NMR spectrum (600 MHz, $CDCl_3$) of 4-((2-isopropoxyethoxy)methyl)phenol (**2**).

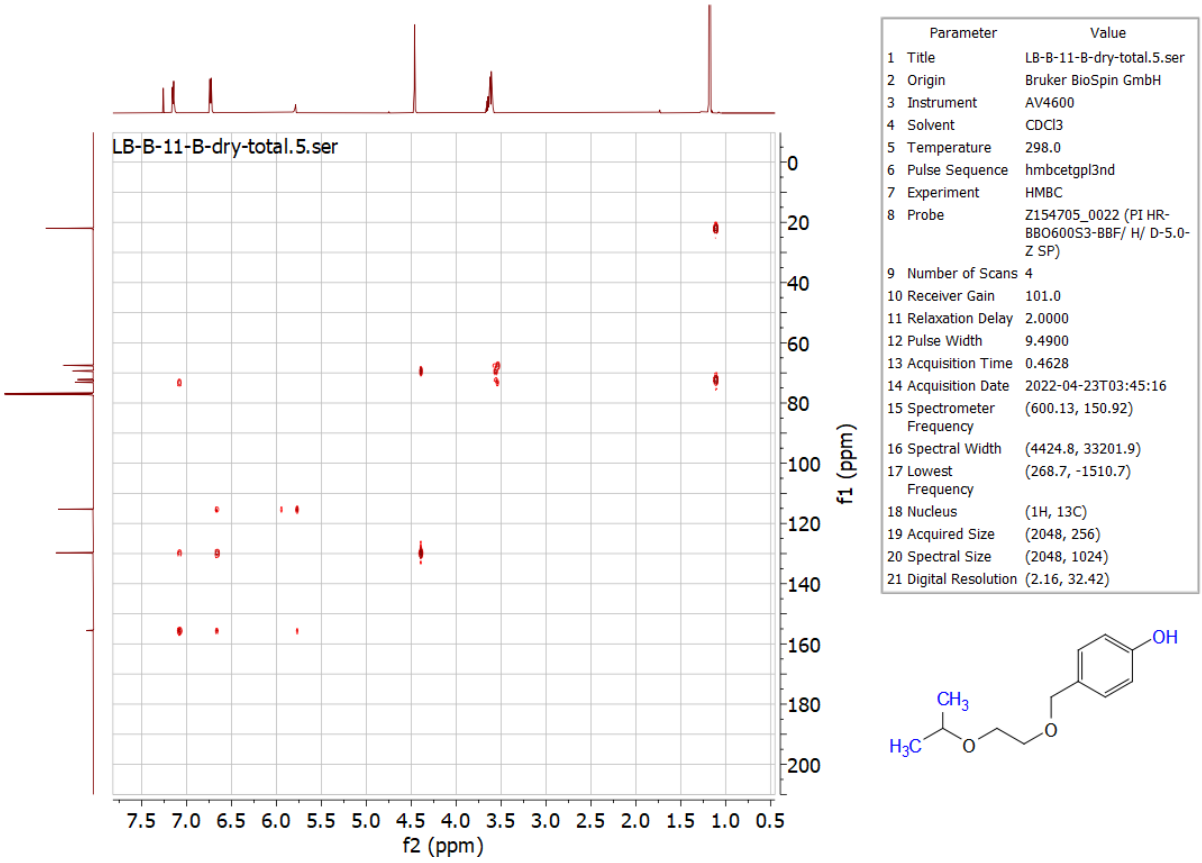
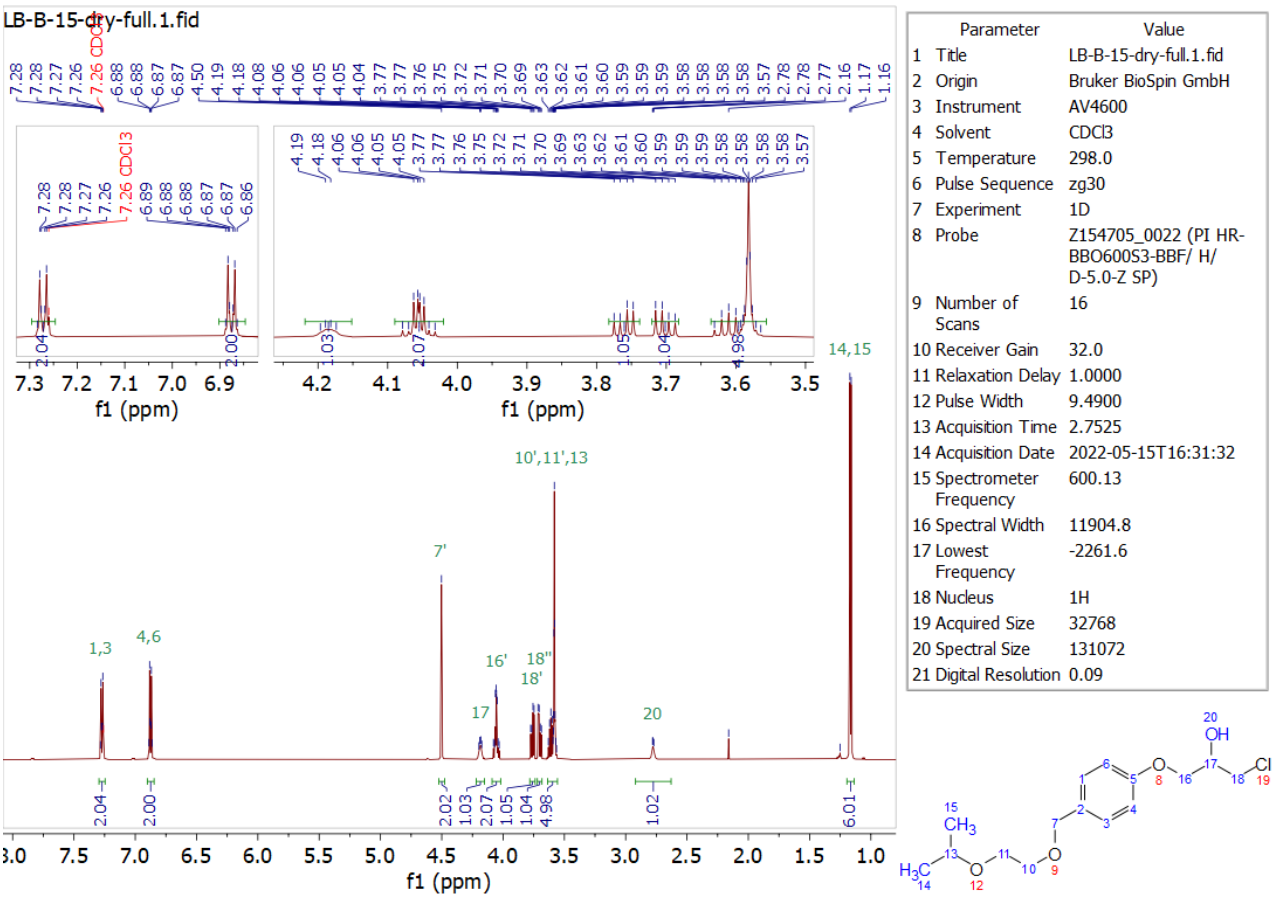


Figure S5. HMBC-NMR spectrum (600 MHz, $CDCl_3$) of 4-((2-isopropoxyethoxy)methyl)phenol (2).

1-Chloro-3-(4-((2-isopropoxyethoxy)methyl)phenoxy)propan-2-ol (4)



	Parameter	Value
1	Title	LB-B-15-dry-full.1.fid
2	Origin	Bruker BioSpin GmbH
3	Instrument	AV4600
4	Solvent	CDCl3
5	Temperature	298.0
6	Pulse Sequence	zg30
7	Experiment	1D
8	Probe	Z154705_0022 (PI HR-BBO600S3-BBF/ H/ D-5.0-Z SP)
9	Number of Scans	16
10	Receiver Gain	32.0
11	Relaxation Delay	1.0000
12	Pulse Width	9.4900
13	Acquisition Time	2.7525
14	Acquisition Date	2022-05-15T16:31:32
15	Spectrometer Frequency	600.13
16	Spectral Width	11904.8
17	Lowest Frequency	-2261.6
18	Nucleus	1H
19	Acquired Size	32768
20	Spectral Size	131072
21	Digital Resolution	0.09

Figure S6. ^{1}H-NMR spectrum (600 MHz, $CDCl_3$) of 1-chloro-3-(4-((2-isopropoxyethoxy)methyl)phenoxy)propan-2-ol (**4**).

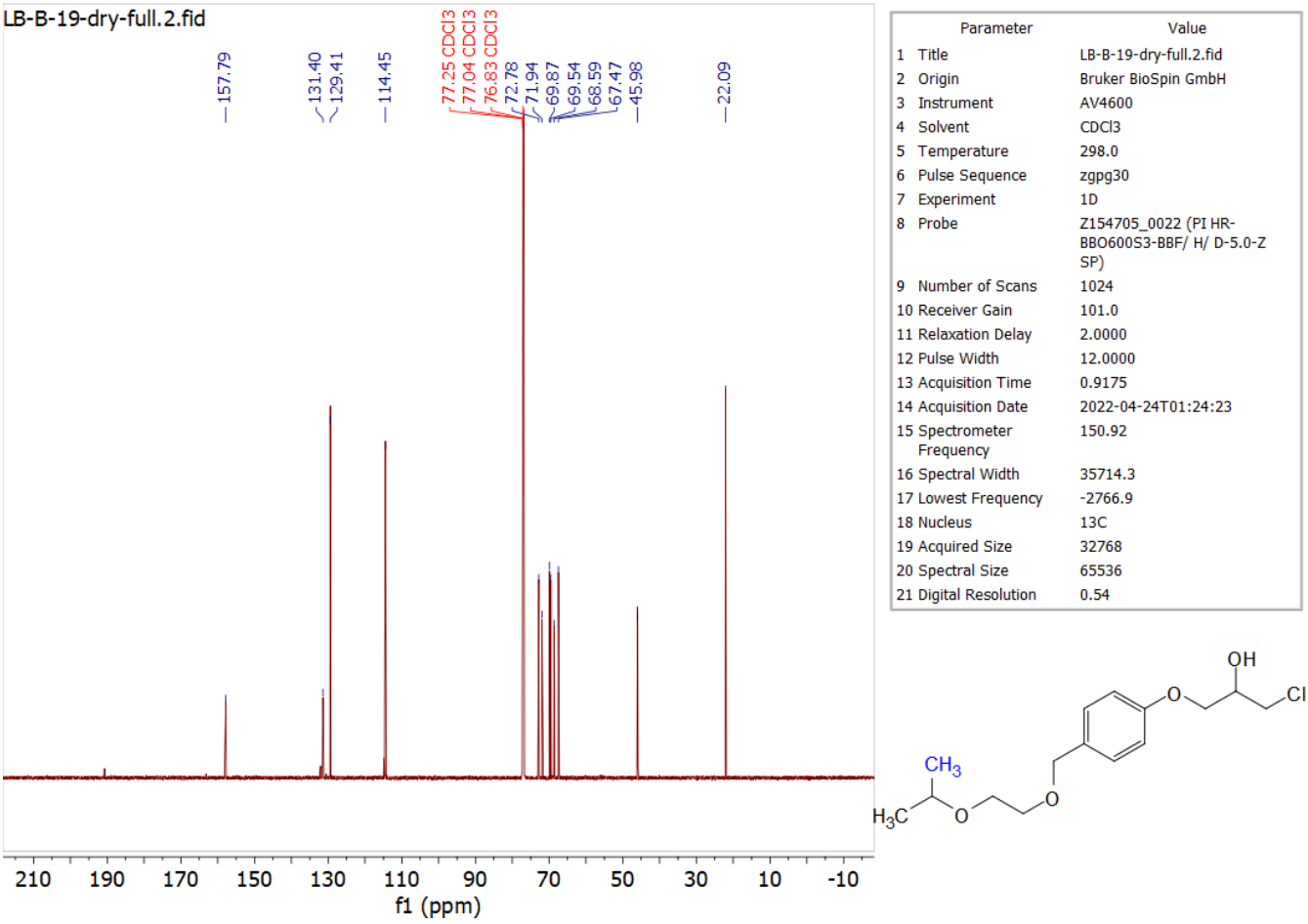


Figure S7. ^{13}C-NMR spectrum (151 MHz, $CDCl_3$) of 1-chloro-3-(4-((2-isopropoxyethoxy)methyl)phenoxy)propan-2-ol (**4**).

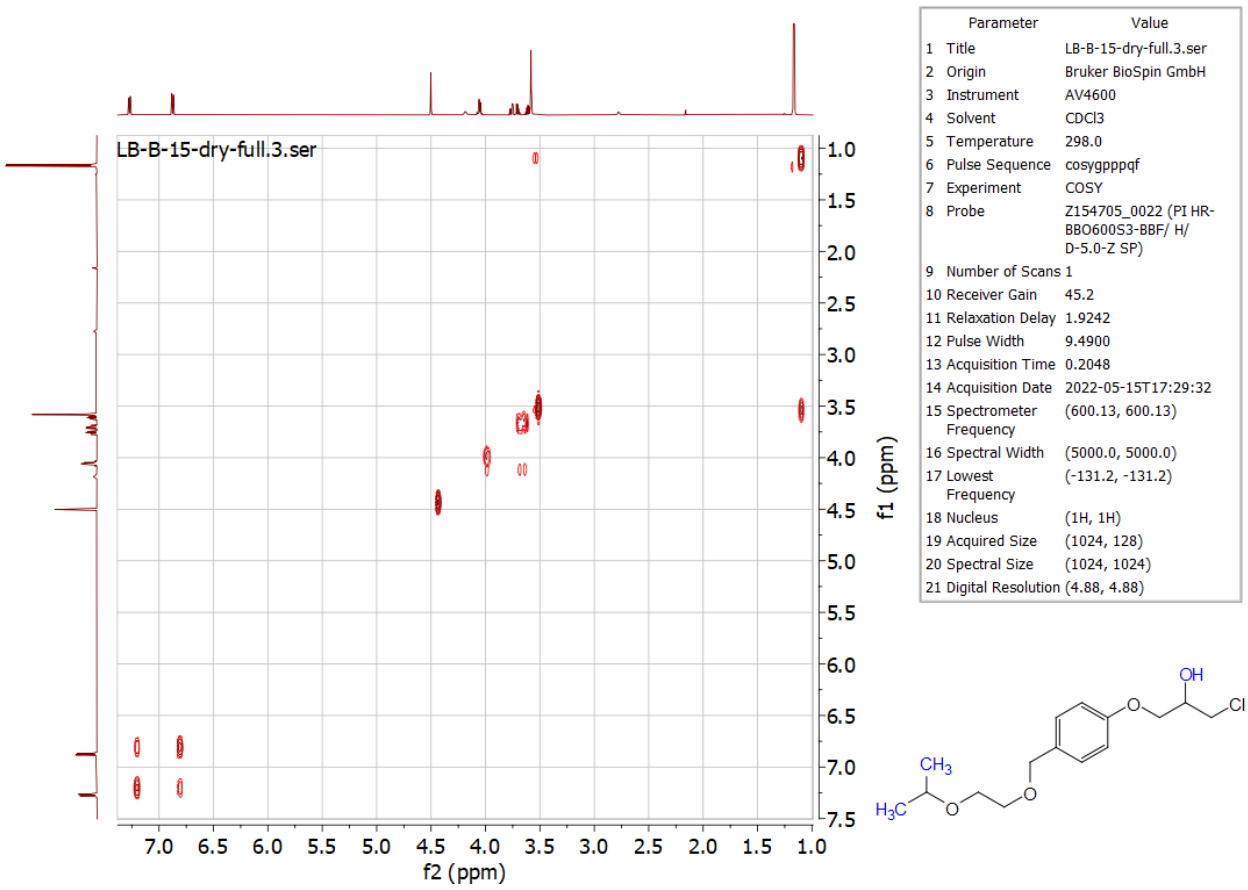


Figure S8. H,H-COSY-NMR spectrum (600 MHz, $CDCl_3$) of 1-chloro-3-(4-((2-isopropoxyethoxy)methyl)phenoxy)propan-2-ol (**4**).

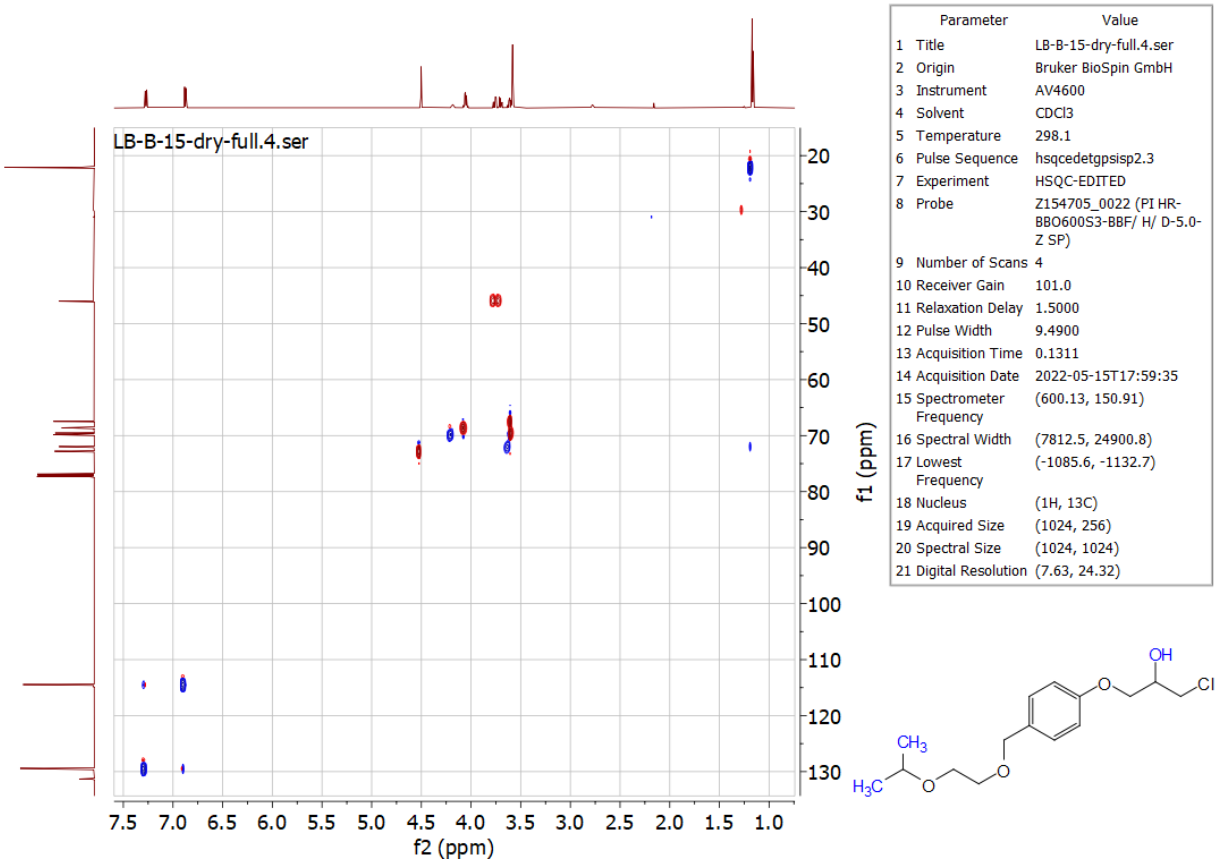


Figure S9. HSQC-NMR spectrum (600 MHz, $CDCl_3$) of 1-chloro-3-(4-((2-isopropoxyethoxy)methyl)phenoxy)propan-2-ol (**4**).

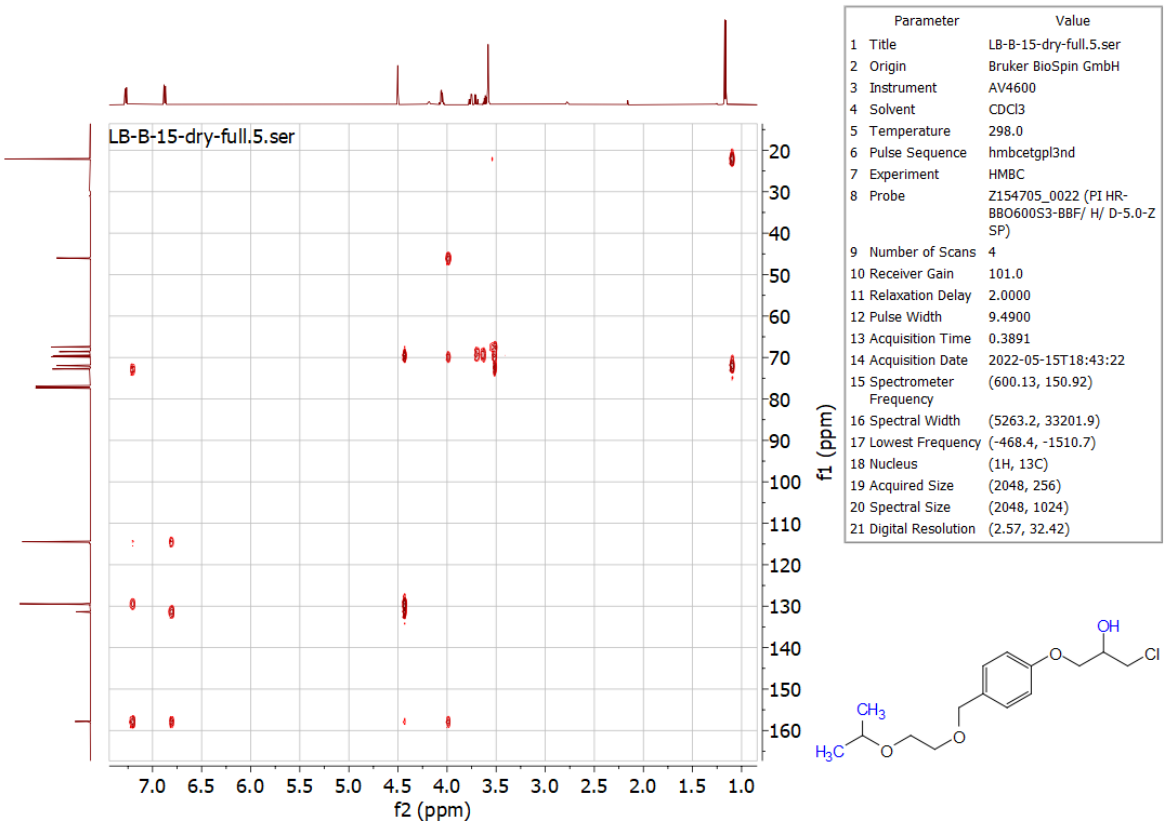
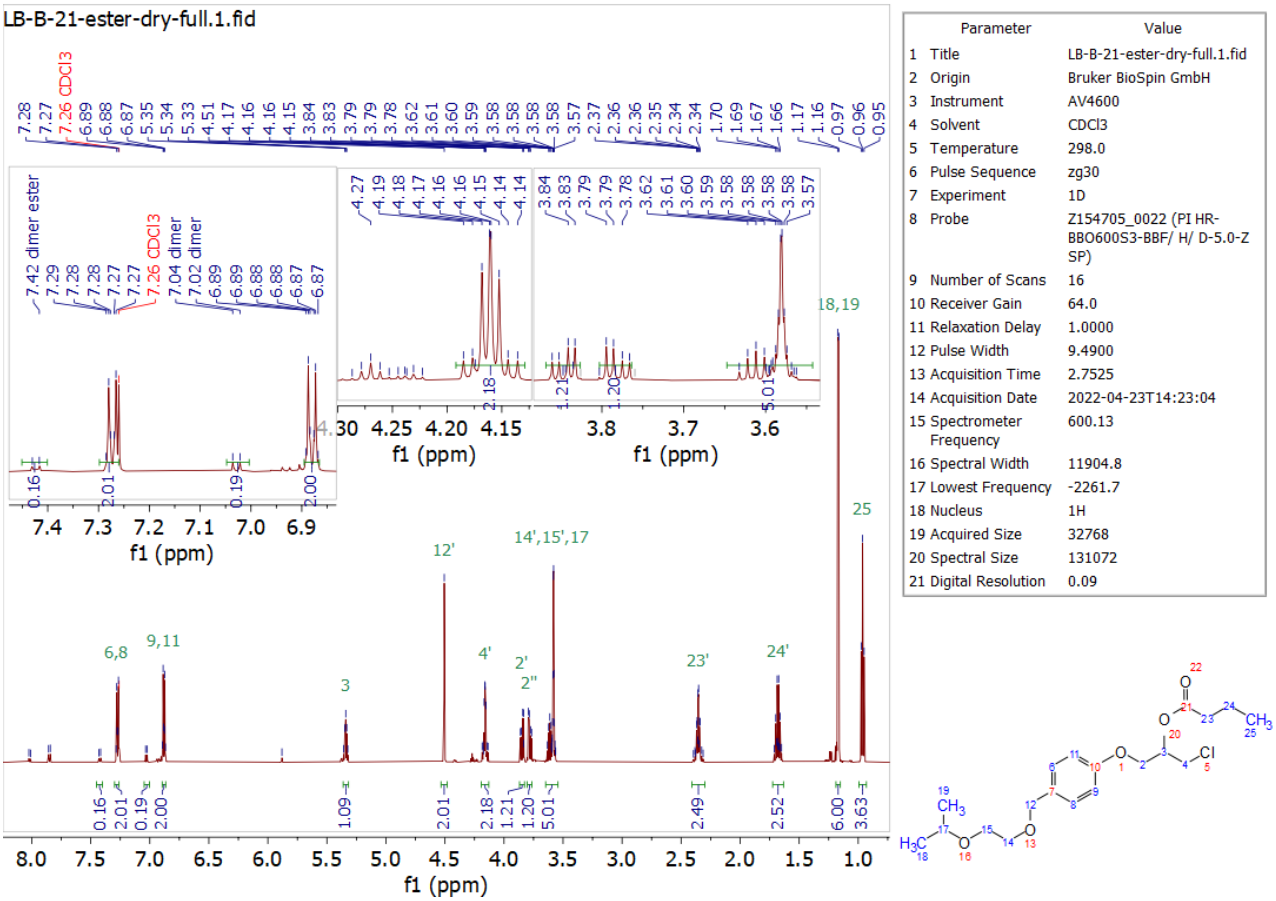


Figure S10. HMBC-NMR spectrum (600 MHz, $CDCl_3$) of 1-chloro-3-(4-((2-isopropoxyethoxy)methyl)phenoxy)propan-2-ol (**4**).

1-Chloro-3-(4-((2-isopropoxyethoxy)methyl)phenoxy)propan-2-yl butanoate (5)



	Parameter	Value
1	Title	LB-B-21-ester-dry-full.1.fid
2	Origin	Bruker BioSpin GmbH
3	Instrument	AV4600
4	Solvent	CDCl3
5	Temperature	298.0
6	Pulse Sequence	zg30
7	Experiment	1D
8	Probe	Z154705_0022 (PI HR-BBO600S3-BBF/ H/ D-5.0-Z SP)
9	Number of Scans	16
10	Receiver Gain	64.0
11	Relaxation Delay	1.0000
12	Pulse Width	9.4900
13	Acquisition Time	2.7525
14	Acquisition Date	2022-04-23T14:23:04
15	Spectrometer Frequency	600.13
16	Spectral Width	11904.8
17	Lowest Frequency	-2261.7
18	Nucleus	1H
19	Acquired Size	32768
20	Spectral Size	131072
21	Digital Resolution	0.09

Figure S11. ^{1}H-NMR spectrum (600 MHz, $CDCl_3$) of 1-chloro-3-(4-((2-isopropoxyethoxy)methyl)phenoxy)propan-2-yl butanoate (**5**).

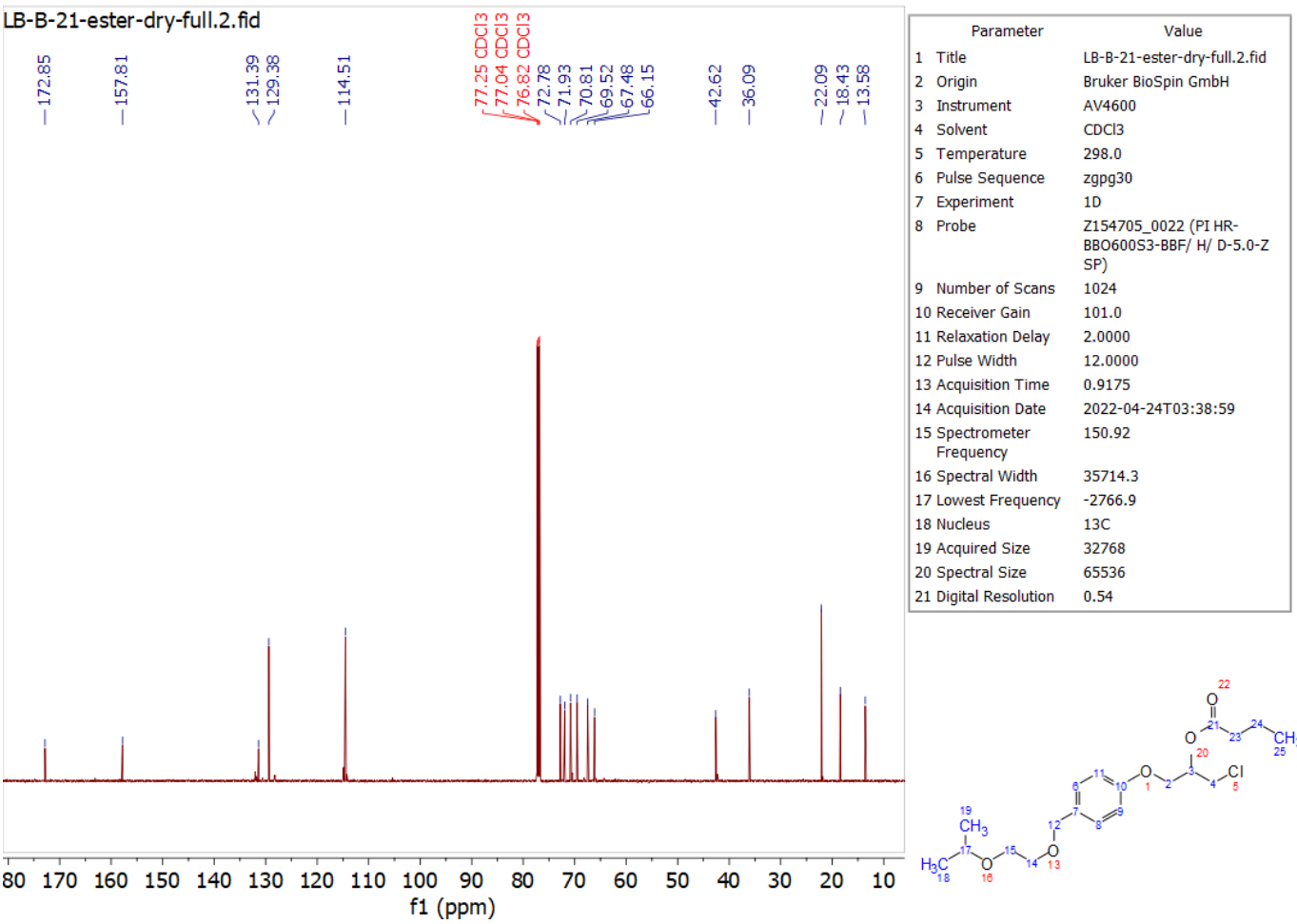


Figure S12. ^{13}C-NMR spectrum (151 MHz, $CDCl_3$) of 1-chloro-3-(4-((2-isopropoxyethoxy)methyl)phenoxy)propan-2-yl butanoate (**5**).

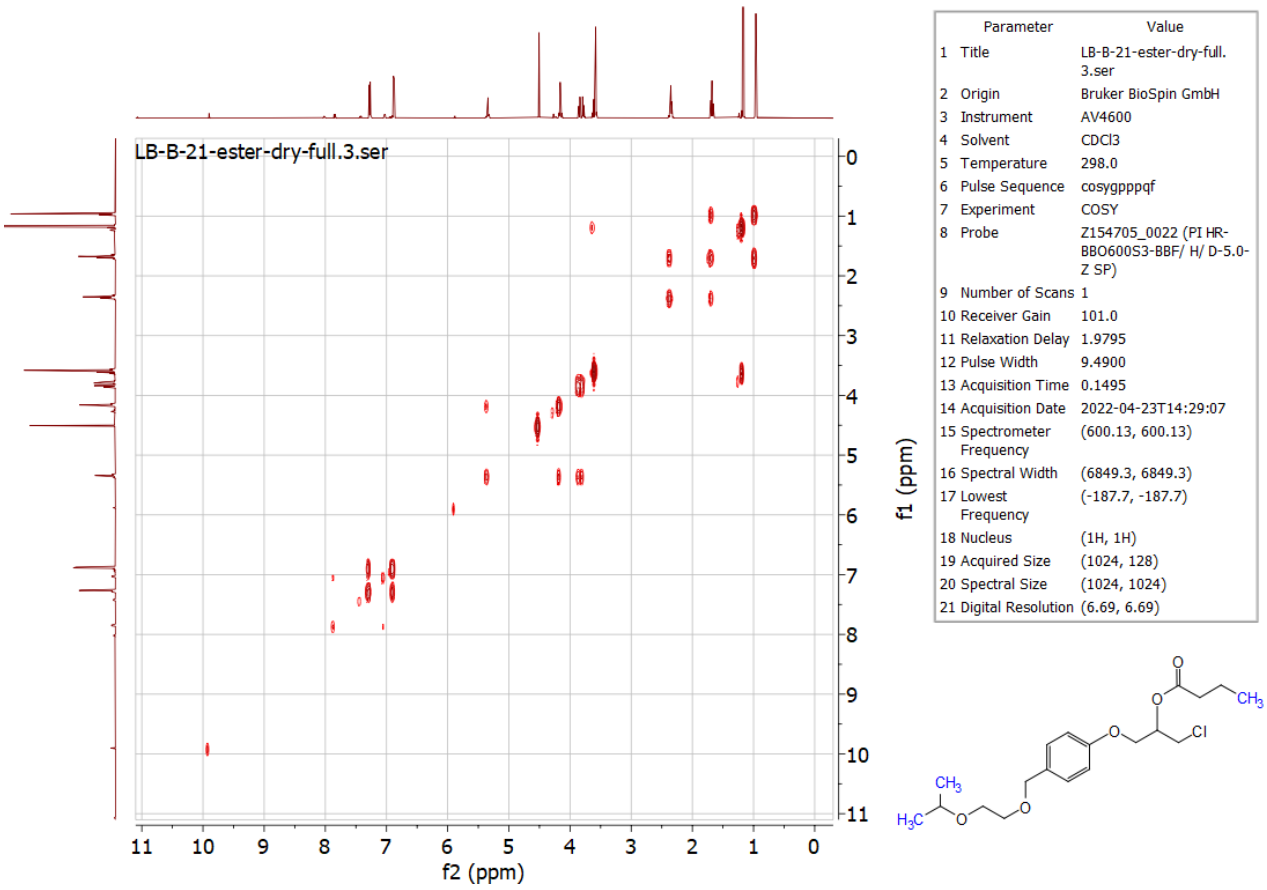


Figure S13. H,H-COSY-NMR spectrum (600 MHz, $CDCl_3$) of 1-chloro-3-(4-((2-isopropoxyethoxy)methyl)phenoxy)propan-2-yl butyrate (**5**).

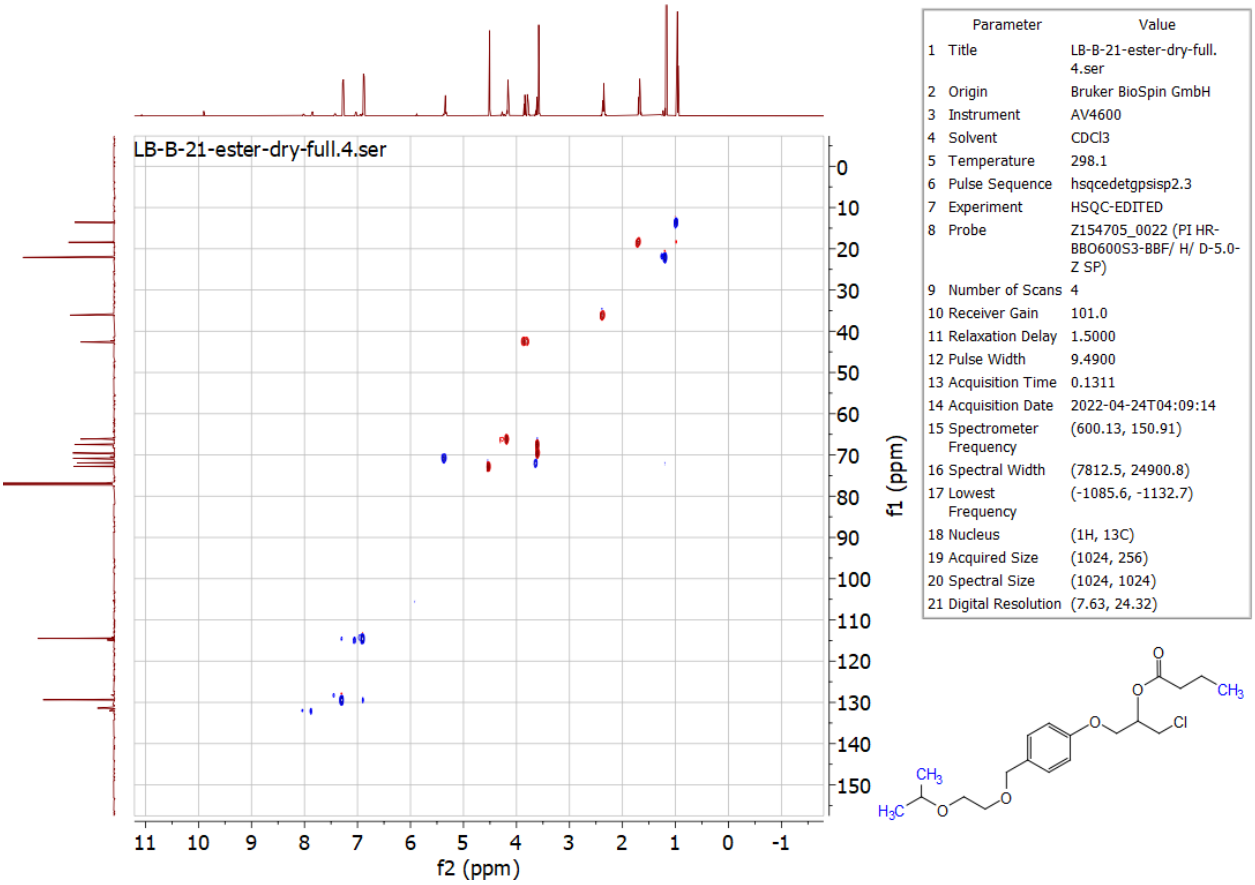


Figure S14. HSQC-NMR spectrum (600 MHz, $CDCl_3$) of 1-chloro-3-(4-((2-isopropoxyethoxy)methyl)phenoxy)propan-2-yl butyrate (**5**).

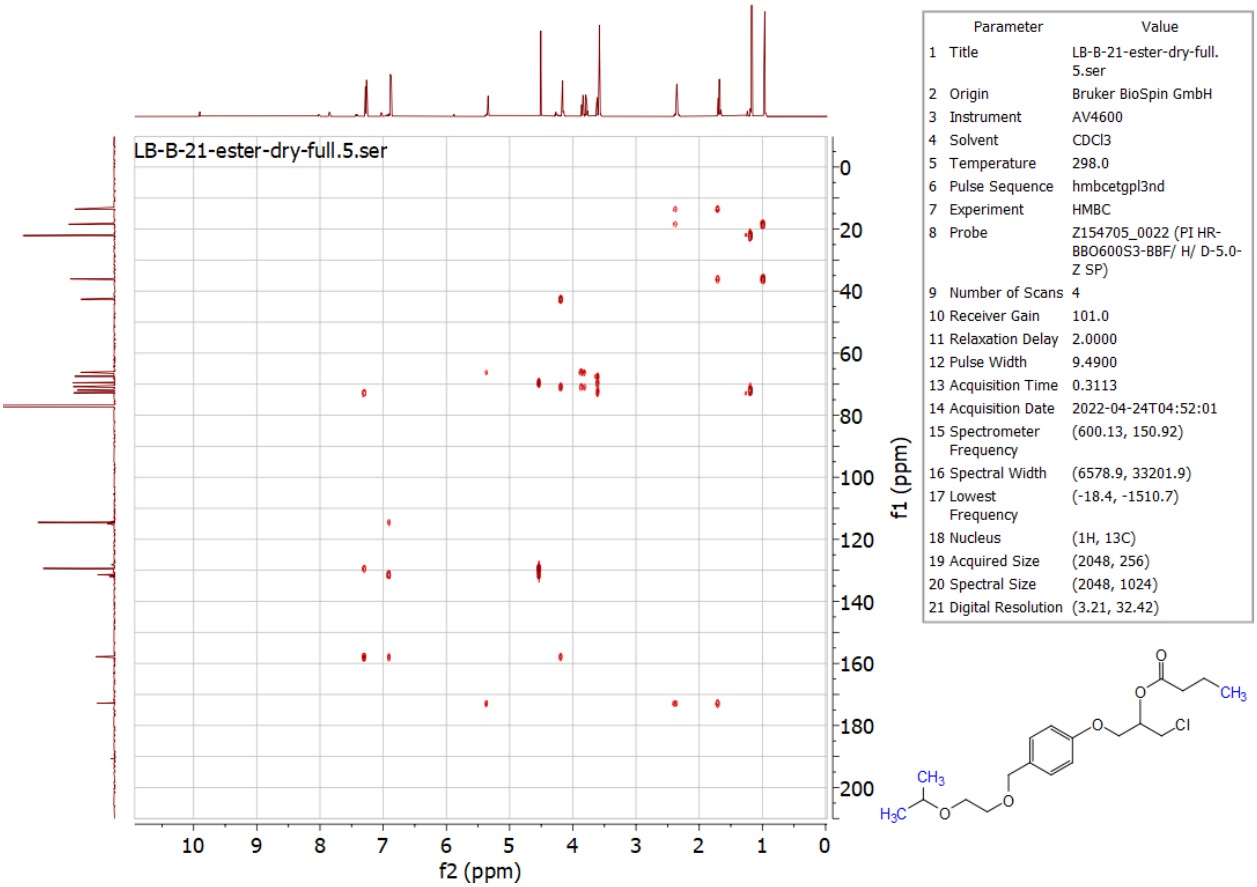
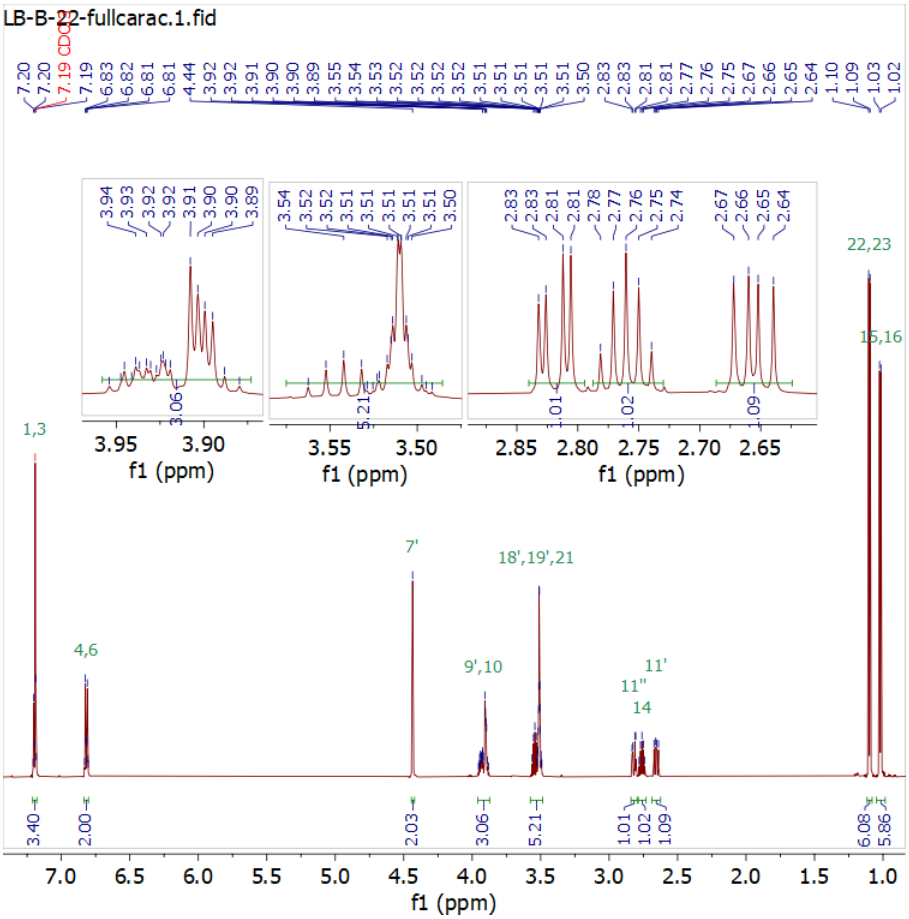


Figure S15. HMBC-NMR spectrum (600 MHz, $CDCl_3$) of 1-chloro-3-(4-((2-isopropoxyethoxy)methyl)phenoxy)propan-2-yl butyrate (**5**).

Bisoprolol (6)



	Parameter	Value
1	Title	LB-B-22-fullcarac.1.fid
2	Origin	Bruker BioSpin GmbH
3	Instrument	AV4600
4	Solvent	CDCl3
5	Temperature	298.0
6	Pulse Sequence	zg30
7	Experiment	1D
8	Probe	Z154705_0022 (PI HR-BBO600S3-BBF/ H/ D-5.0-Z SP)
9	Number of Scans	16
10	Receiver Gain	101.0
11	Relaxation Delay	1.0000
12	Pulse Width	9.4900
13	Acquisition Time	2.7525
14	Acquisition Date	2022-04-01T15:46:53
15	Spectrometer Frequency	600.13
16	Spectral Width	11904.8
17	Lowest Frequency	-2303.4
18	Nucleus	1H
19	Acquired Size	32768
20	Spectral Size	131072
21	Digital Resolution	0.09

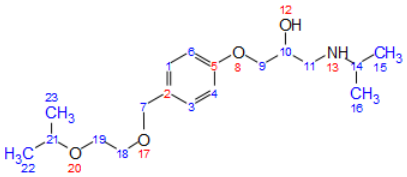


Figure S16. ^{1}H-NMR spectrum (600 MHz, $CDCl_3$) of bisoprolol (**6**).

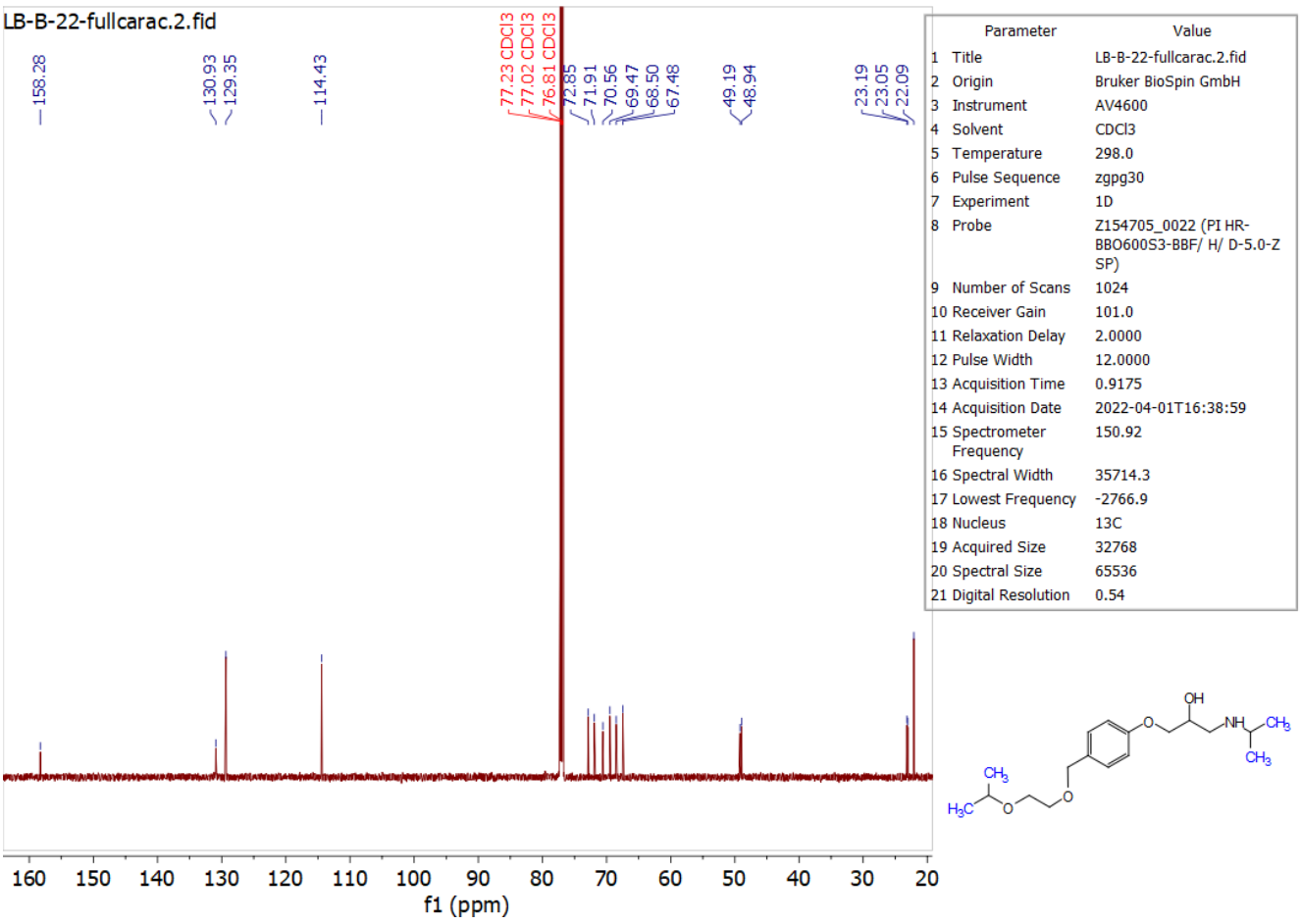


Figure S17. ^{13}C-NMR spectrum (151 MHz, $CDCl_3$) of bisoprolol (**6**).

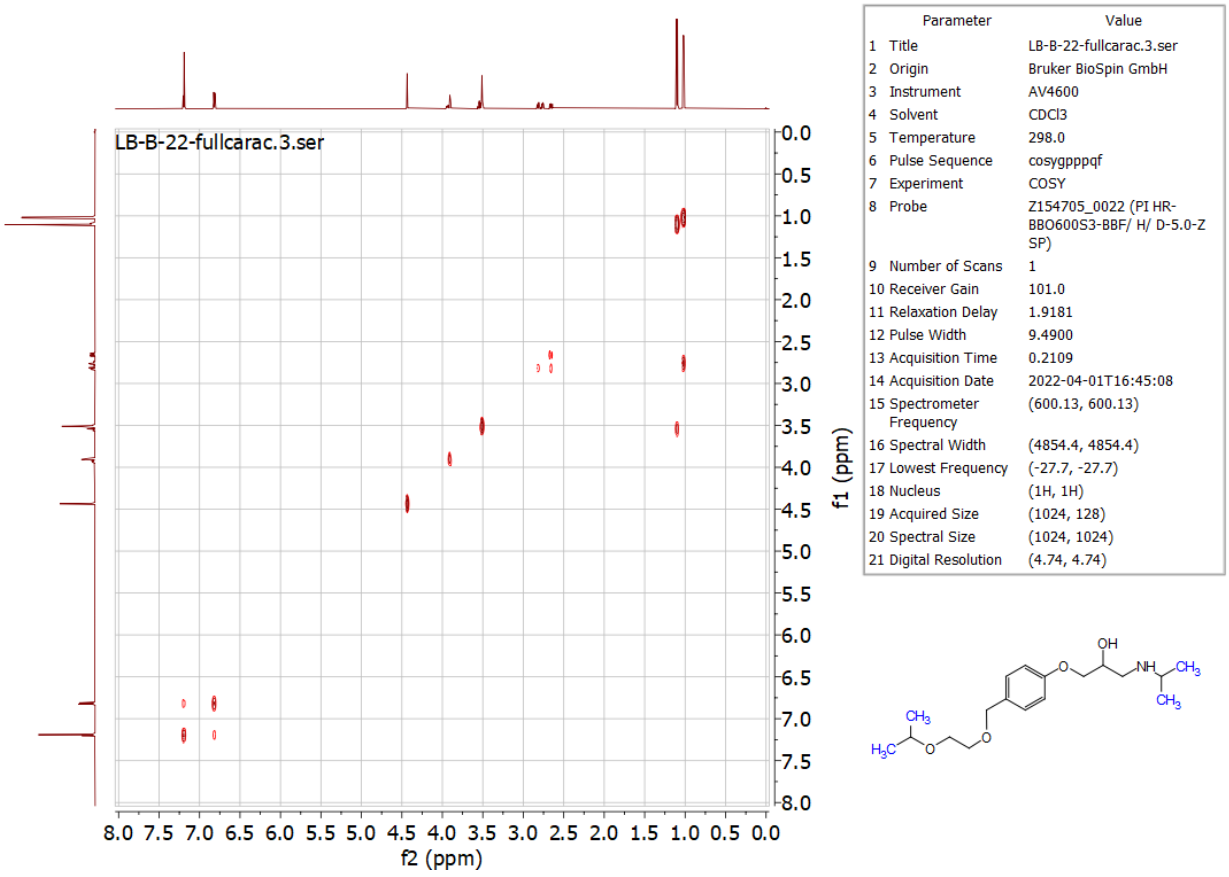
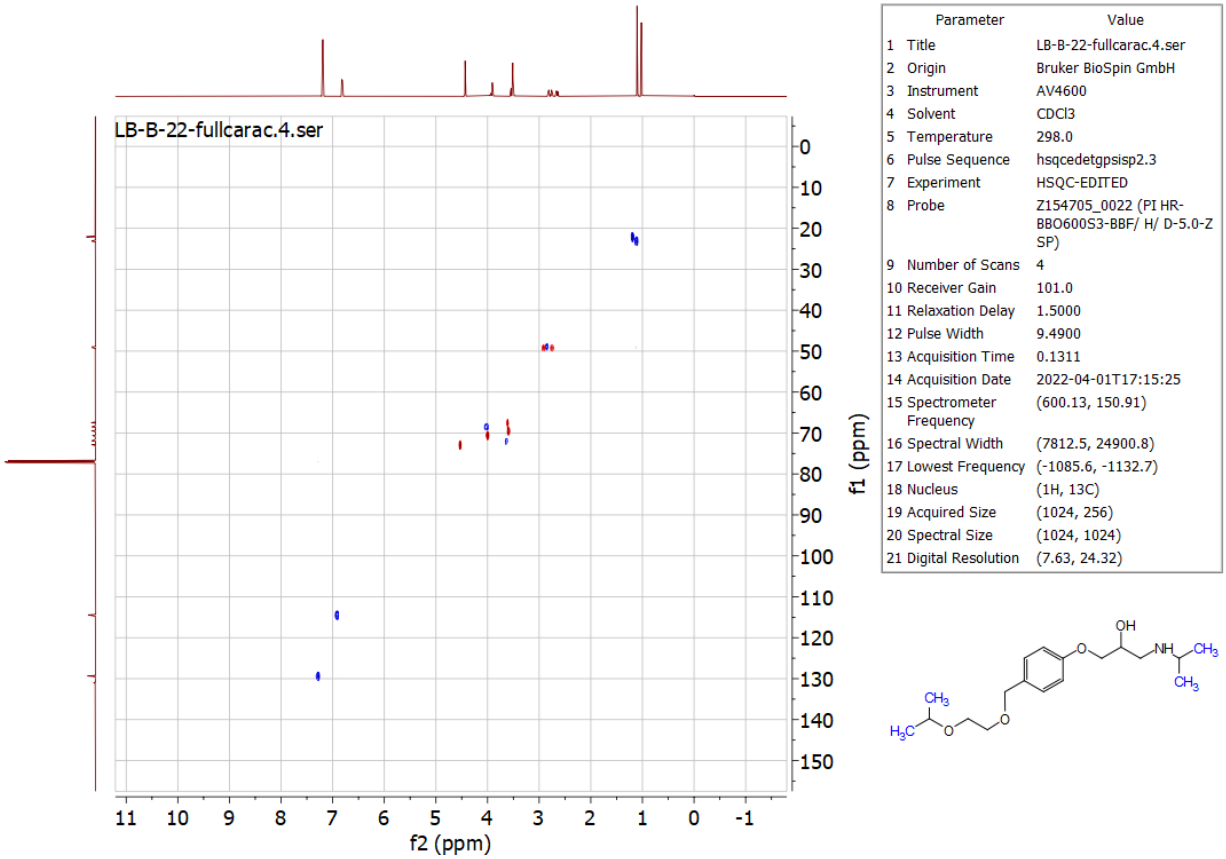
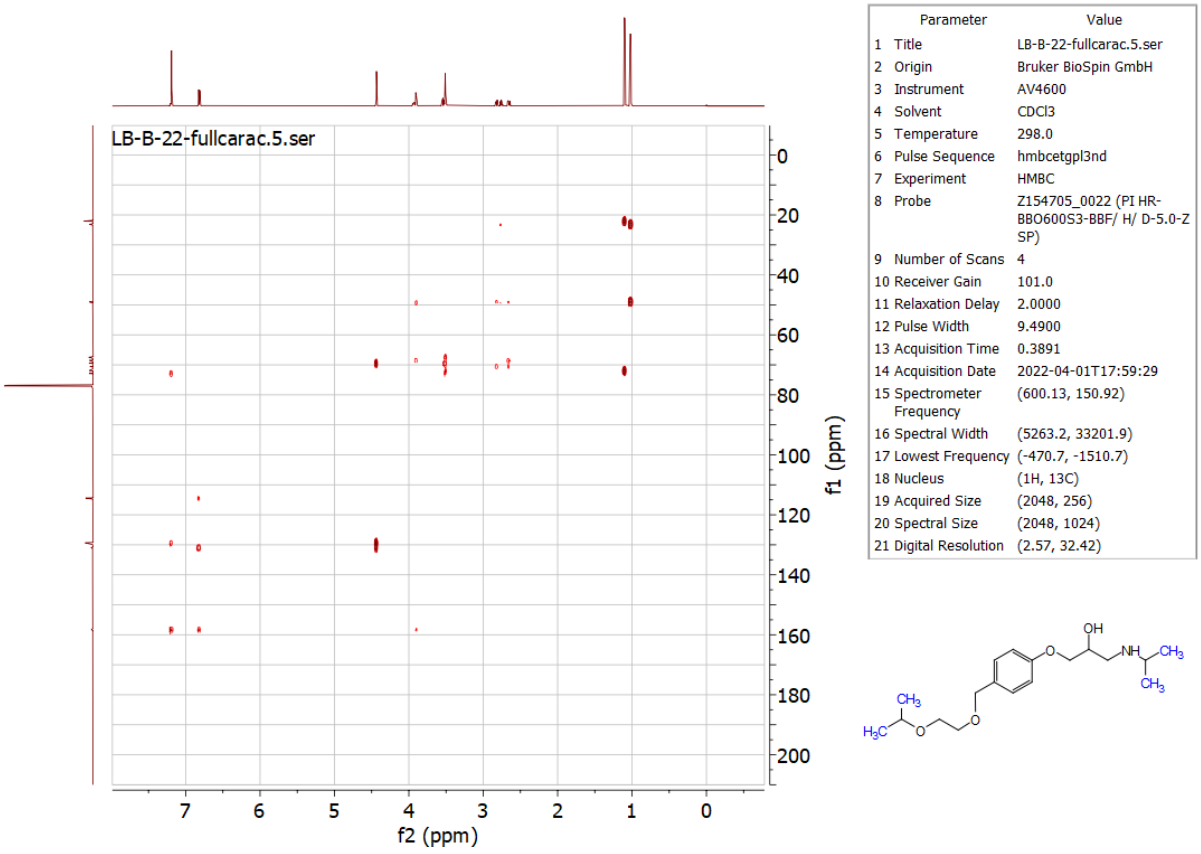


Figure S18. H,H-COSY-NMR spectrum (600 MHz, $CDCl_3$) of bisoprolol (**6**).



	Parameter	Value
1	Title	LB-B-22-fullcarac.4.ser
2	Origin	Bruker BioSpin GmbH
3	Instrument	AV4600
4	Solvent	CDCl3
5	Temperature	298.0
6	Pulse Sequence	hsqcedetgpsisp2.3
7	Experiment	HSQC-EDITED
8	Probe	Z154705_0022 (PI HR-BBO600S3-BBF/ H/ D-5.0-Z SP)
9	Number of Scans	4
10	Receiver Gain	101.0
11	Relaxation Delay	1.5000
12	Pulse Width	9.4900
13	Acquisition Time	0.1311
14	Acquisition Date	2022-04-01T17:15:25
15	Spectrometer Frequency	(600.13, 150.91)
16	Spectral Width	(7812.5, 24900.8)
17	Lowest Frequency	(-1085.6, -1132.7)
18	Nucleus	(1H, 13C)
19	Acquired Size	(1024, 256)
20	Spectral Size	(1024, 1024)
21	Digital Resolution	(7.63, 24.32)

Figure S19. HSQC-NMR spectrum (600 MHz, $CDCl_3$) of bisoprolol (**6**).



	Parameter	Value
1	Title	LB-B-22-fullcarac.5.ser
2	Origin	Bruker BioSpin GmbH
3	Instrument	AV4600
4	Solvent	CDCl3
5	Temperature	298.0
6	Pulse Sequence	hmbcetgpl3nd
7	Experiment	HMBC
8	Probe	Z154705_0022 (PI HR-BBO600S3-BBF/ H/ D-5.0-Z SP)
9	Number of Scans	4
10	Receiver Gain	101.0
11	Relaxation Delay	2.0000
12	Pulse Width	9.4900
13	Acquisition Time	0.3891
14	Acquisition Date	2022-04-01T17:59:29
15	Spectrometer Frequency	(600.13, 150.92)
16	Spectral Width	(5263.2, 33201.9)
17	Lowest Frequency	(-470.7, -1510.7)
18	Nucleus	(1H, 13C)
19	Acquired Size	(2048, 256)
20	Spectral Size	(2048, 1024)
21	Digital Resolution	(2.57, 32.42)

Figure S20. HMBC-NMR spectrum (600 MHz, $CDCl_3$) of bisoprolol (**6**).

Bisoprolol hemifumarate (7)

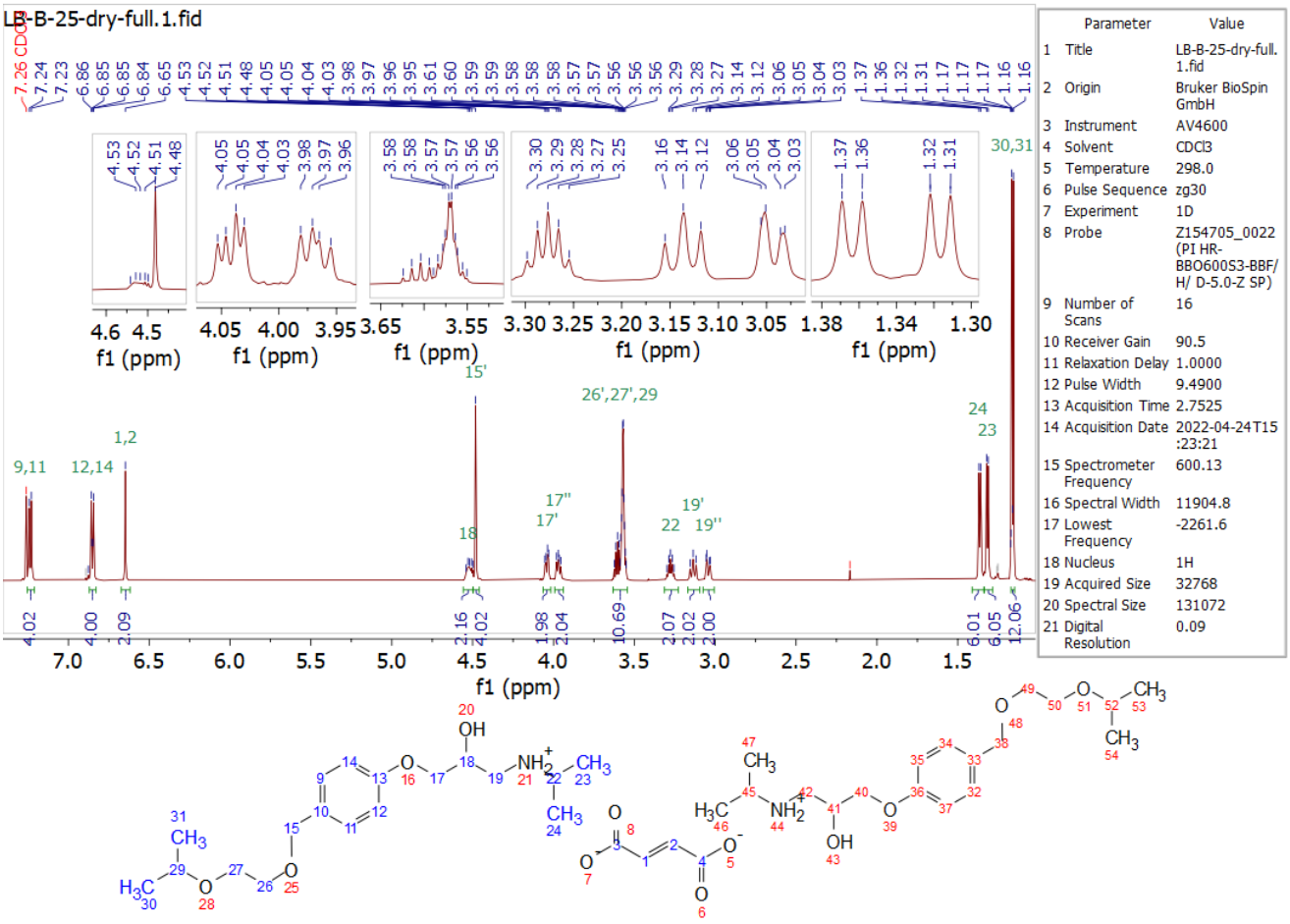


Figure S21. ^{1}H-NMR spectrum (600 MHz, $CDCl_3$) of bisoprolol hemifumarate (**7**).

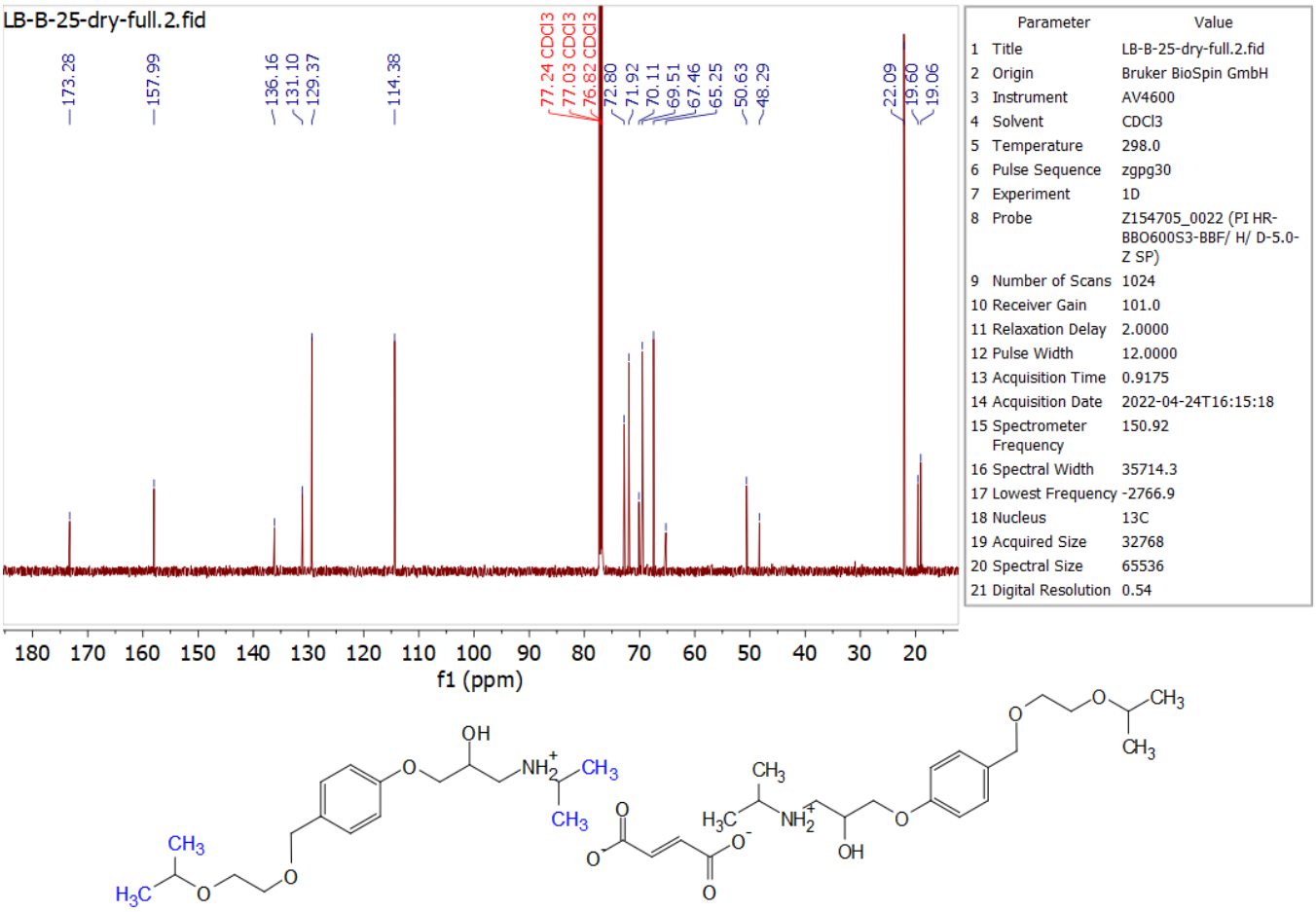


Figure S22. ^{13}C-NMR spectrum (151 MHz, $CDCl_3$) of bisoprolol hemifumarate (**7**).

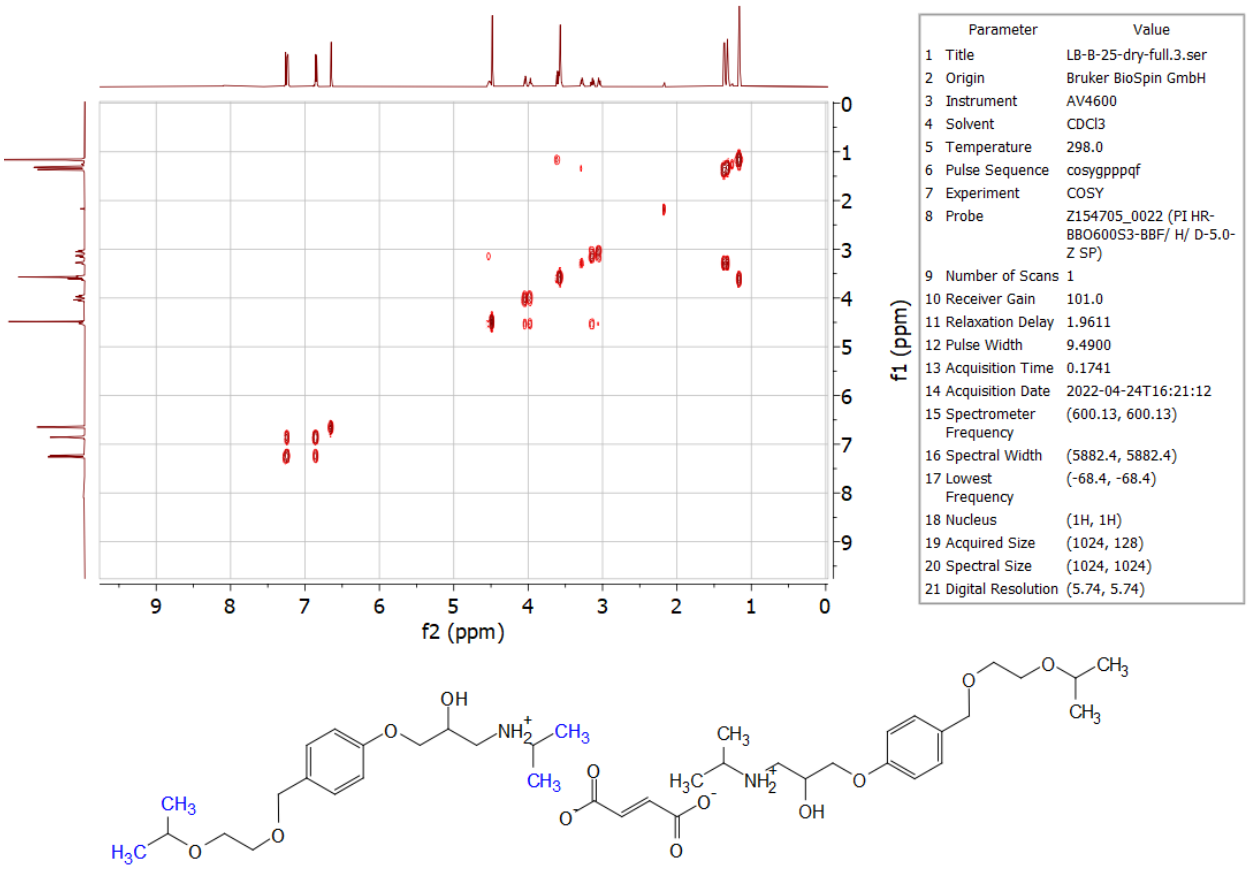


Figure S23. H,H-COSY-NMR spectrum (600 MHz, $CDCl_3$) of bisoprolol hemifumarate (**7**).

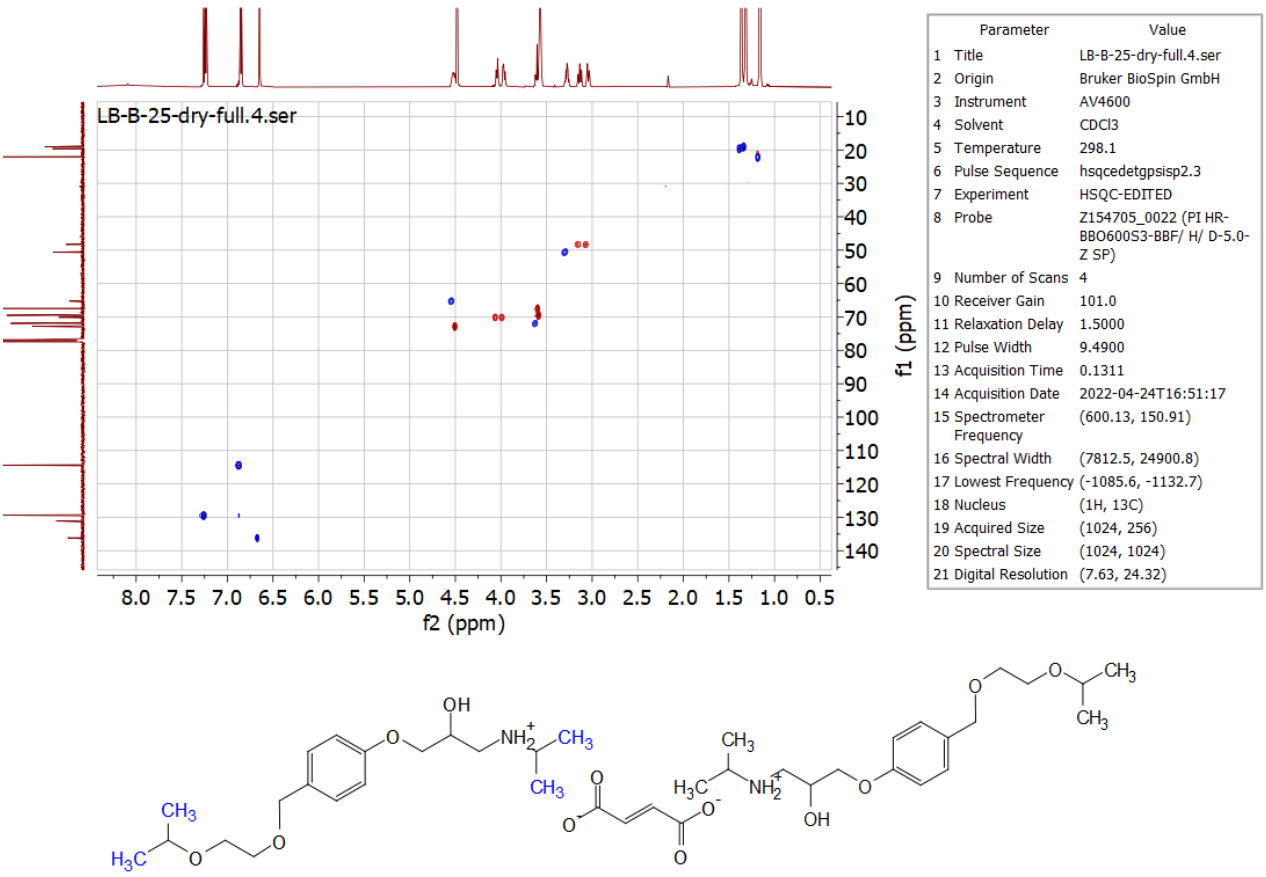


Figure S24. HSQC-NMR spectrum (600 MHz, $CDCl_3$) of bisoprolol hemifumarate (7).

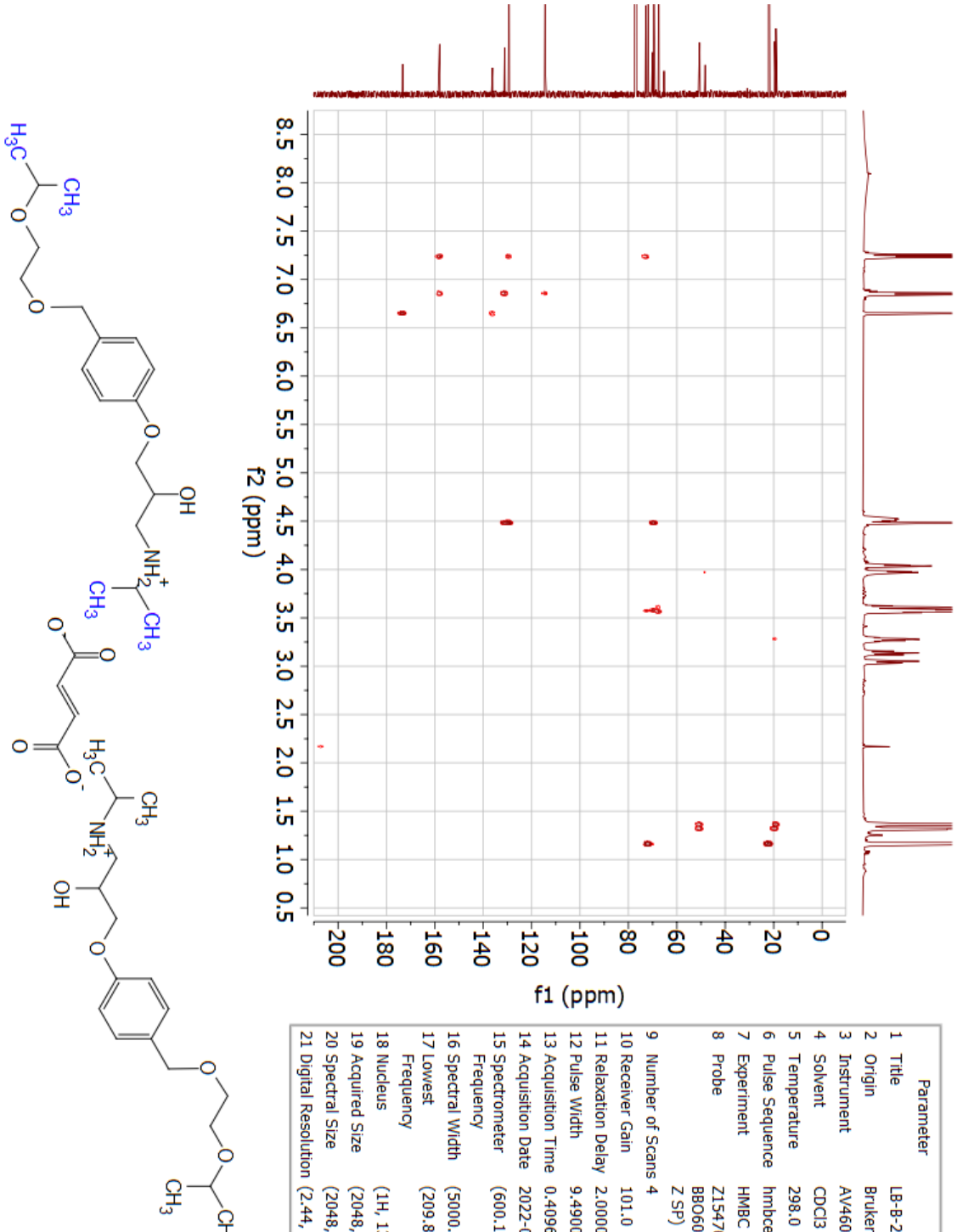


Figure S25. HMBC-NMR spectrum (600 MHz, $CDCl_3$) of bisoprolol hemifumarate (**7**).

2. HPLC chromatograms.

Separation of enantiomers of **4, 5** and **7** on Chiralcel OD-H column

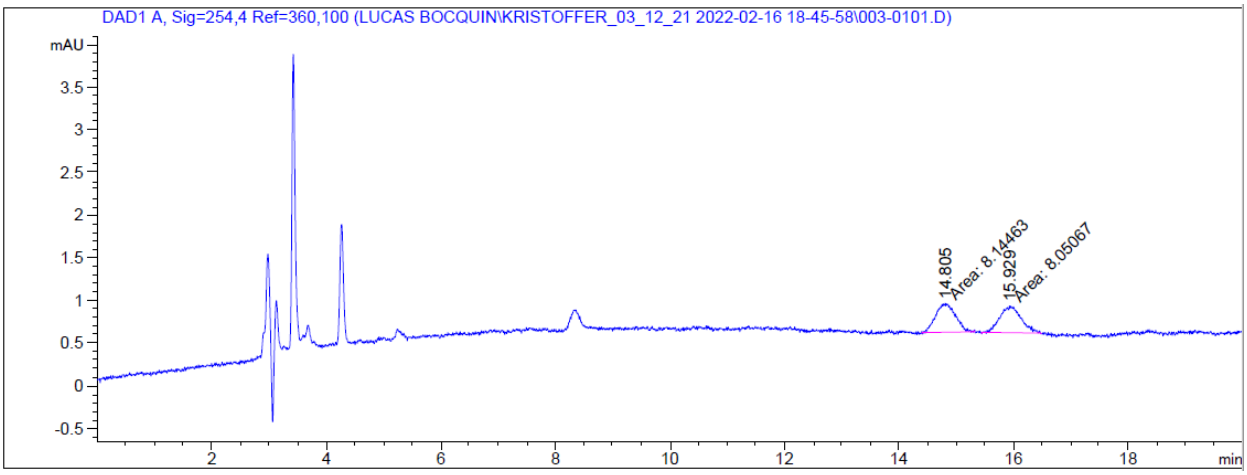


Figure S26. Chiral HPLC chromatogram of chlorohydrin **4**. The analysis was performed on a Chiralcel OD-H column with hexane and 2-propanol (90:10) as eluent and 1 mL/min flow, and with a detection wavelength of 280.8 nm. The retention times are $\boldsymbol{t_R}$((*R*)-**4**) = 14.81 min and $\boldsymbol{t_R}$((*S*)-**4**) = 15.93 min. $\boldsymbol{R_S}$((*S*)/(*R*)-**4**) = 1.63. The column impurities do not affect the separation of the enantiomers.

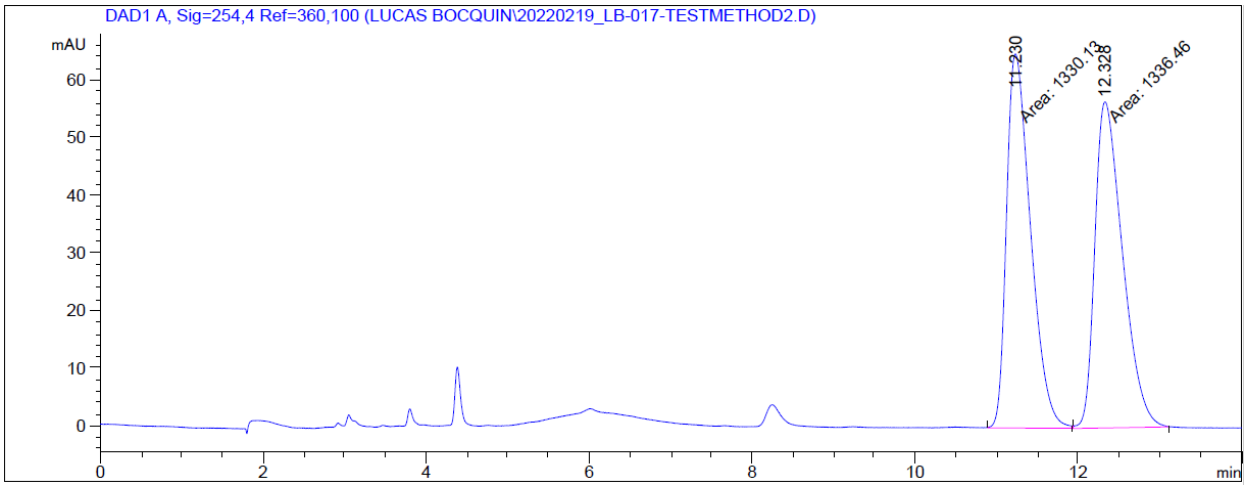


Figure S27. Chiral HPLC chromatogram of ester **5**. The analysis was performed on a Chiralcel OD-H column with hexane and 2-propanol (96:4) as eluent and 1 mL/min flow, and with a detection wavelength of 280.8 nm. The retention times obtained are $\boldsymbol{t_R}$((*R*)-**5**) = 11.23 min

and t_R((*S*)-**5**) = 12.33 min. R_S((*S*)/(*R*)-**5**) = 1.89. The column impurities do not affect the separation of the enantiomers.

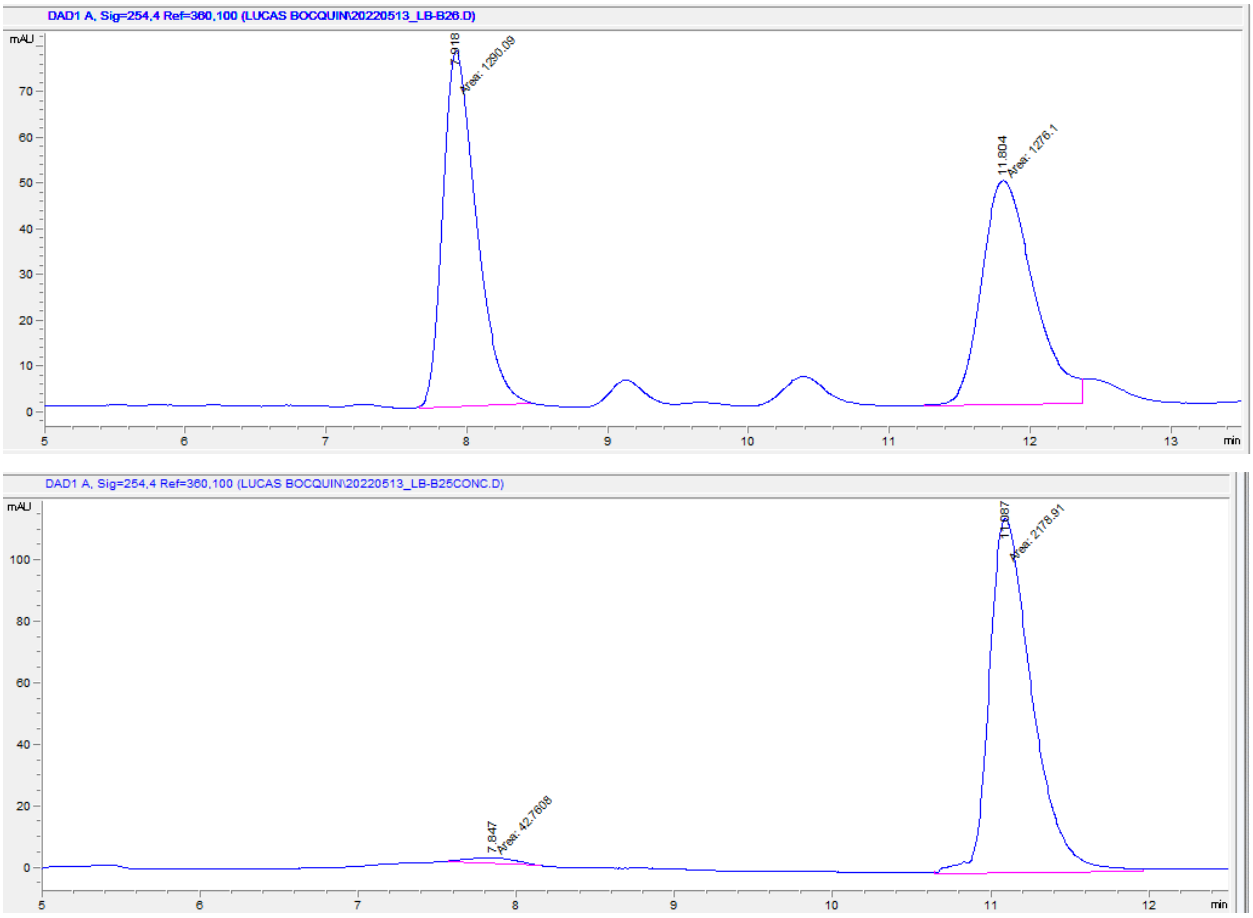


Figure S28. Chiral HPLC chromatogram of racemic bisoprolol hemifumarate (**7**) (upper chromatogram) and enantiopure (*S*)-**7** (*ee* =96%) (bottom chromatogram). The analysis was performed on a Chiralcel OD-H column with hexane (90%) and 2-propanol containing 2% diethanolamine (10%) as eluent and 1 mL/min flow, and with a detection wavelength of 254.4 nm. The retention times are $\mathbf{t_R}$((*R*)-**7**) = 7.84 min and $\mathbf{t_R}$((*S*)-**7**) = 11.08 min (lower chromatogram). The resolution factor in the upper chromatogram is $\mathbf{R_S}$((*S*)/(*R*)-**7**) = 7.02.