

Supporting Information

3-Nitroatenolol: First Synthesis, Chiral Resolution and Enantiomers' Absolute Configuration

Rosa Sparaco,¹ Pierfrancesco Cinque,¹ Antonia Scognamiglio,¹ Angela Corvino,¹ Giuseppe Caliendo,¹ Ferdinando Fiorino,¹ Elisa Magli,² Elisa Perissutti,¹ Vincenzo Santagada,¹ Beatrice Severino,¹ Paolo Luciano,¹ Marcello Casertano,¹ Anna Aiello,¹ Gustavo Yuri Martins Viegas,^{3,4} Gilberto De Nucci,^{3,4,*} and Francesco Frecentese¹

¹ *Department of Pharmacy, University of Naples Federico II, Via D. Montesano 49, Naples, Italy*

² *Department of Public Health, University of Naples Federico II, Via Pansini 5, Naples, Italy*

³ *Department of Pharmacology, Faculty of Medical Sciences, State University of Campinas (UNICAMP), São Paulo, Campinas, Brazil*

⁴ *Department of Health Studies, Metropolitan University of Santos (UNIMES), Santos-SP, Brazil.*

Content:

Chiral analytical HPLC: stereochemical purity and LC/MS analyses for (+)-3-nitroatenol and (-)-3-nitroatenol.....	pages S2-S5
Mono and bidimensional NMR spectra of (±)-3-nitroatenol.....	pages S6-S7
LC-MS spectra of (±)-3-nitroatenol pre-SCX and post-SCX pretreatment.....	page S8
¹ H-NMR, COSY, HSQC, HMBC and HRMS spectra for bis-MPA derivatives of (+)-3-nitroatenol.....	pages S9-S14
¹ H-NMR, HMBC and HRMS spectra for bis-MPA derivatives of (-)-3-nitroatenol.....	pages S15-S18

Peak 1 - (+)-3-nitroatenolol

Item	Specification	Result
Appearance	Yellow powder	Compliant
Purity	LC-MS purity >95%	Compliant
Enantiomeric purity	HPLC > 95%	Compliant
Amount	23.7 mg	

Stereochemical purity

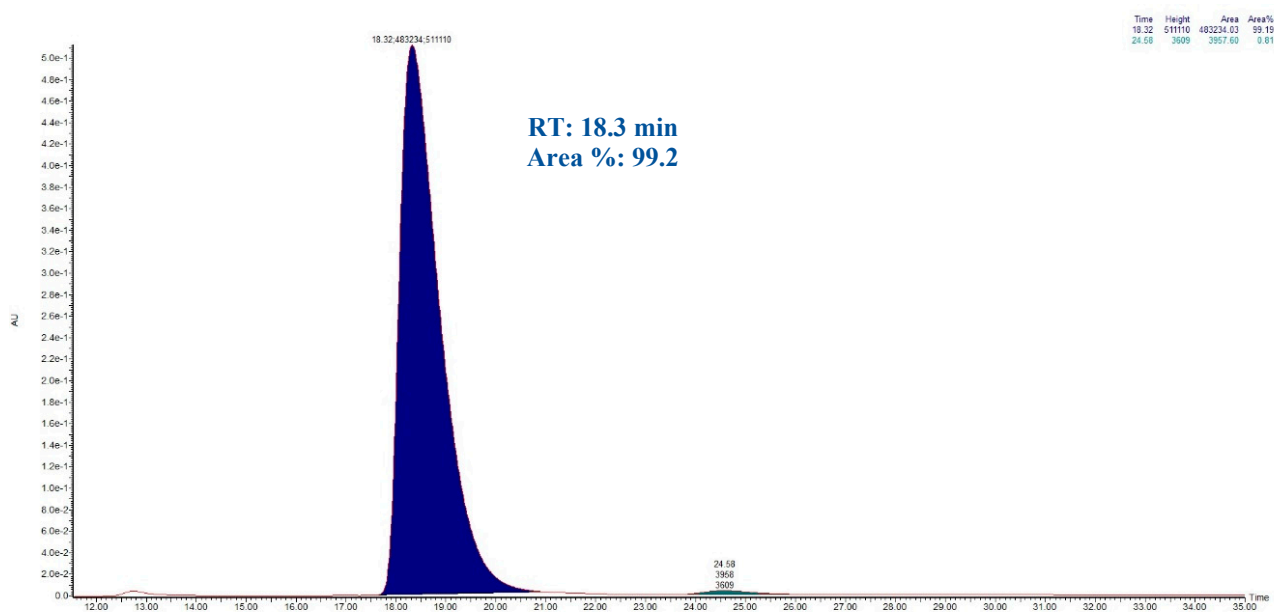
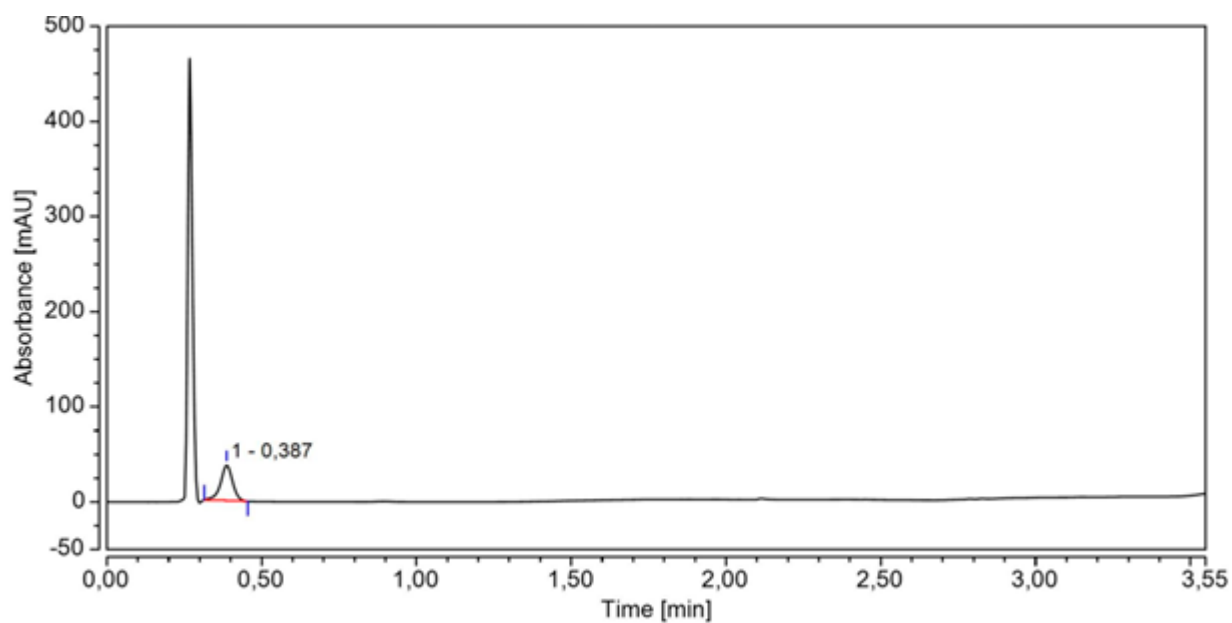


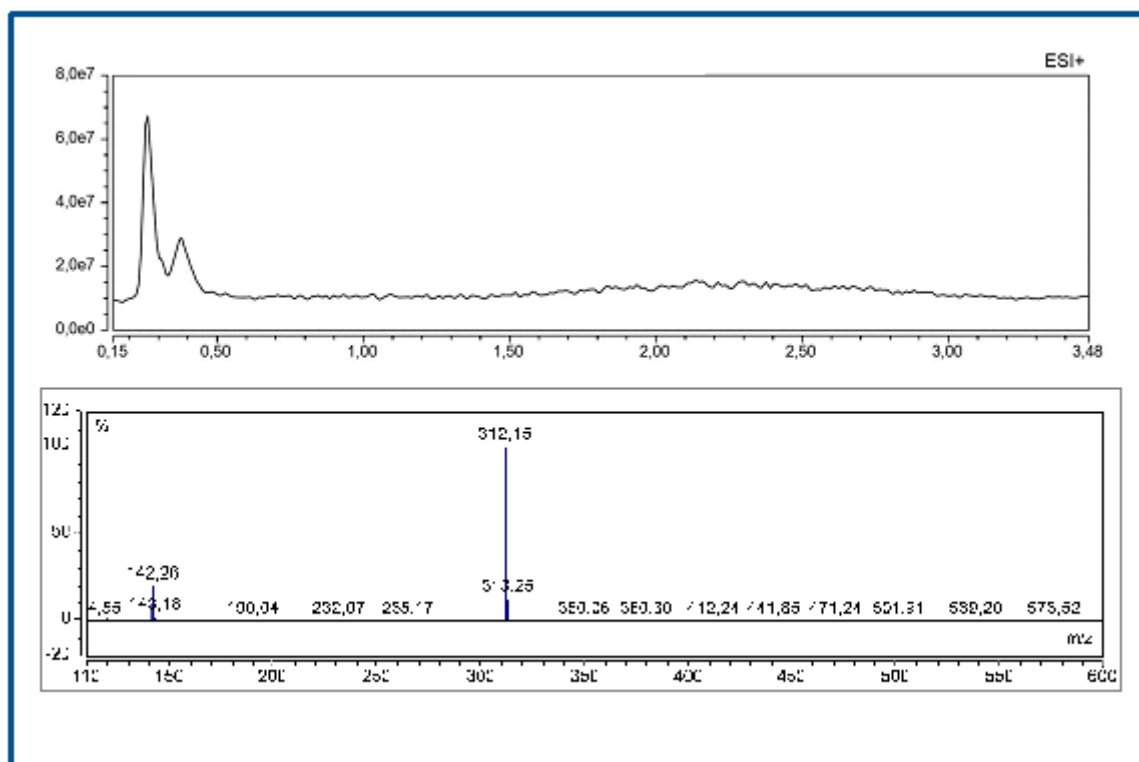
Figure S1 – chiral analytical HPLC: analysis of (+)-3-nitroatenolol

Sample:	NAT1 – Peak 1	Flow:	1 mL/min
Column:	5 AMY-Coat -5 µm Phenomenex	Wavelength:	254/280 nm
Dimension:	250 x 4,6 mm	Mobile Phase:	n-hexane-ethanol 80:20

LC-MS Analysis - Peak 1 - (+)-3-nitroatenolol



Peak Name	Retention Time min	Area mAU*min	Relative Area %
1	0.38	1.67	100



Peak 2 - (-)-3-nitroatenolol

Item	Specification	Result
Appearance	Yellow powder	Compliant
Purity	LC-MS purity >95%	Compliant
Enantiomeric purity	HPLC > 95%	Compliant
Amount	23.7 mg	

Stereochemical purity

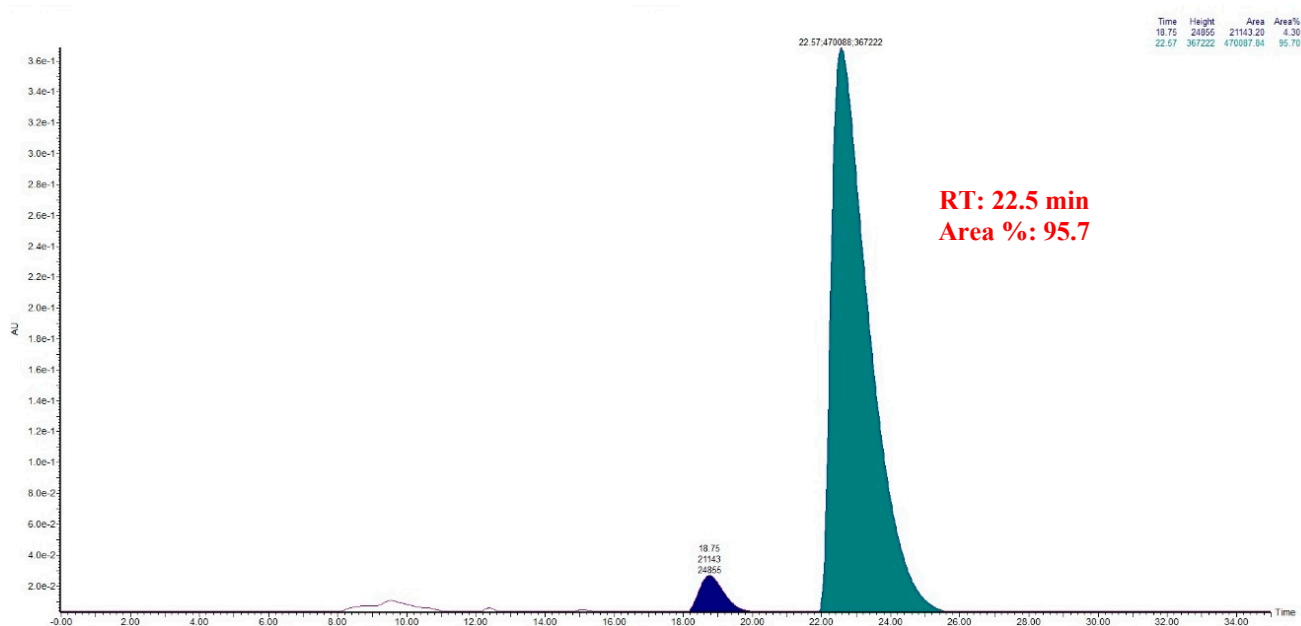
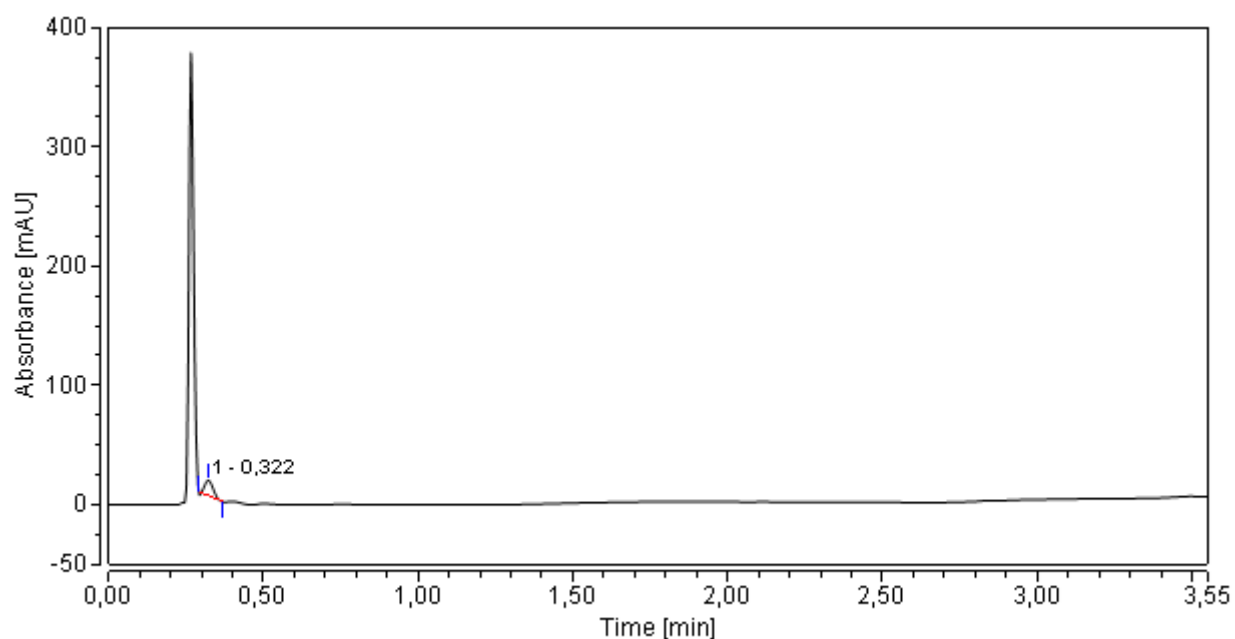


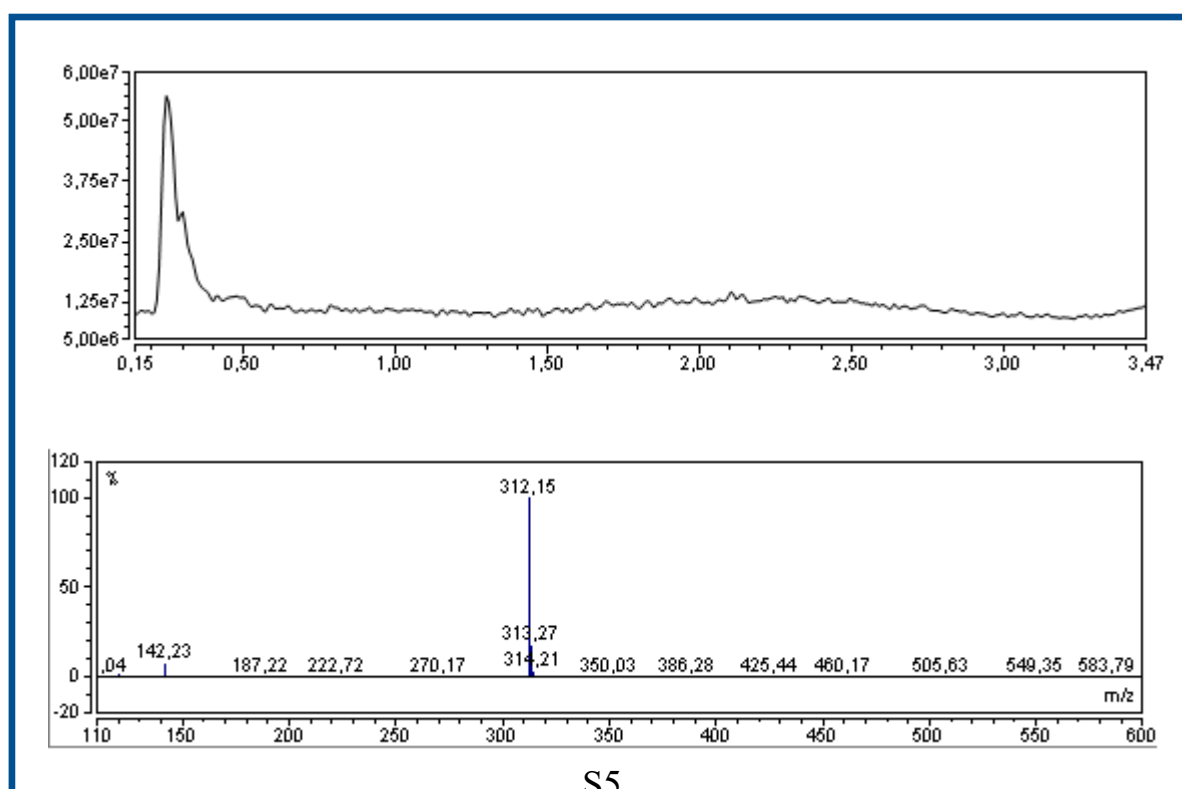
Figure S2 – chiral analytical HPLC: analysis of (-)-3-nitroatenolol.

Sample:	NAT1 – Peak 2	Flow:	1 mL/min
Column:	5 AMY-Coat -5 µm Phenomenex	Wavelength:	254/280 nm
Dimension:	250 x 4,6 mm	Mobile Phase:	n-hexane-ethanol 80:20

LC-MS Analysis



Peak Name	Retention Time min	Area mAU*min	Relative Area %
1	0.32	0.37	100



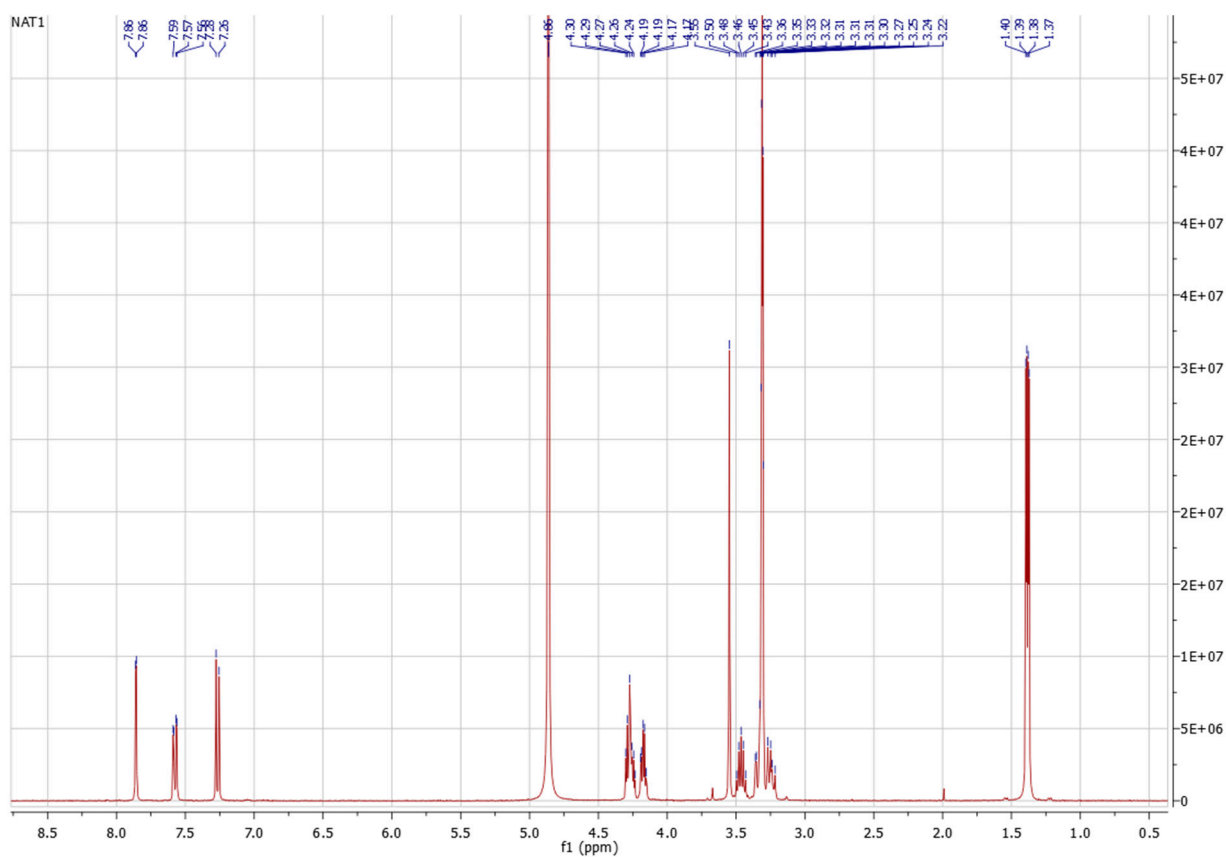


Figure S3 - ^1H -NMR of 3-nitroatenolol (CD_3OD)

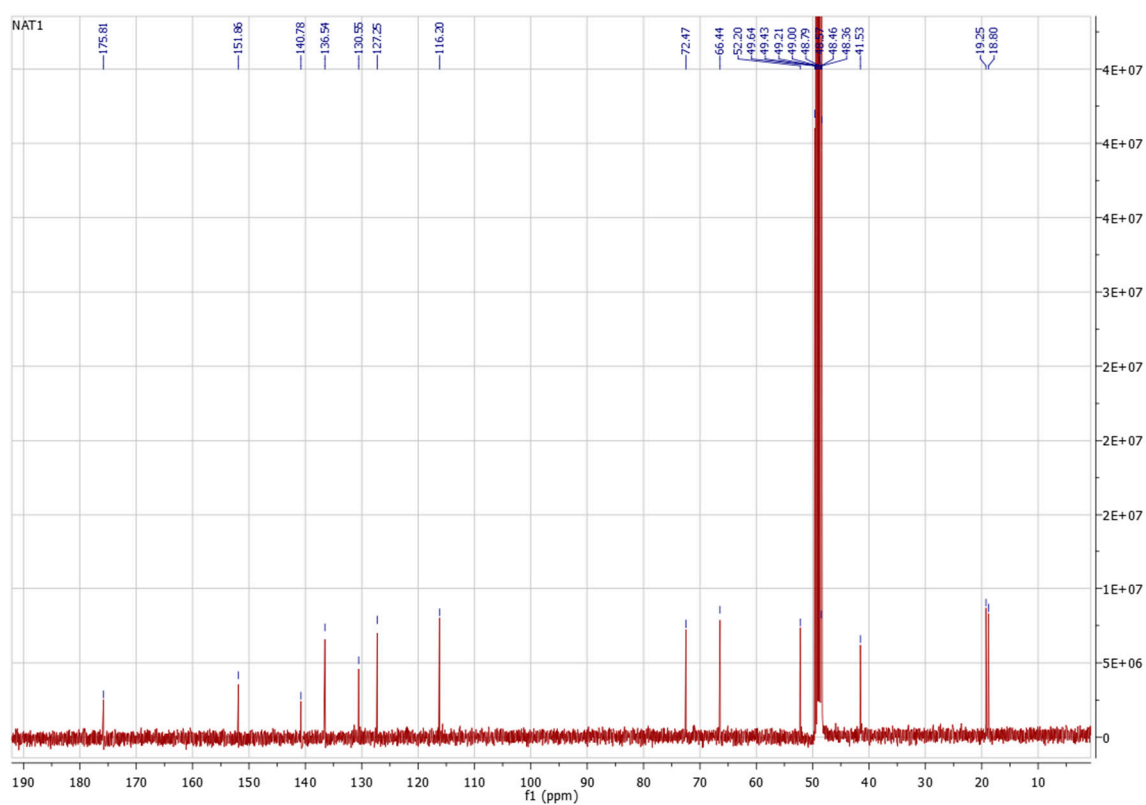


Figure S4 - ^{13}C -NMR of 3-nitroatenolol (CD_3OD)

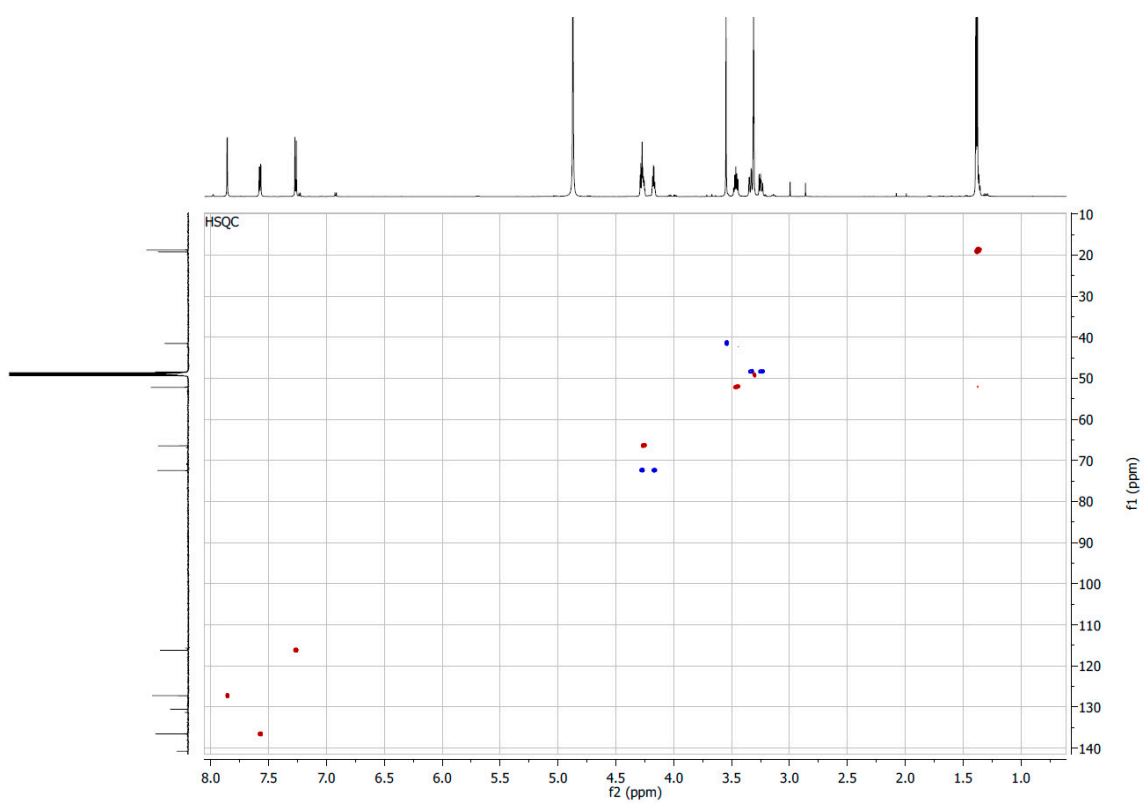


Figure S5 - HSQC of 3-nitroatenolol

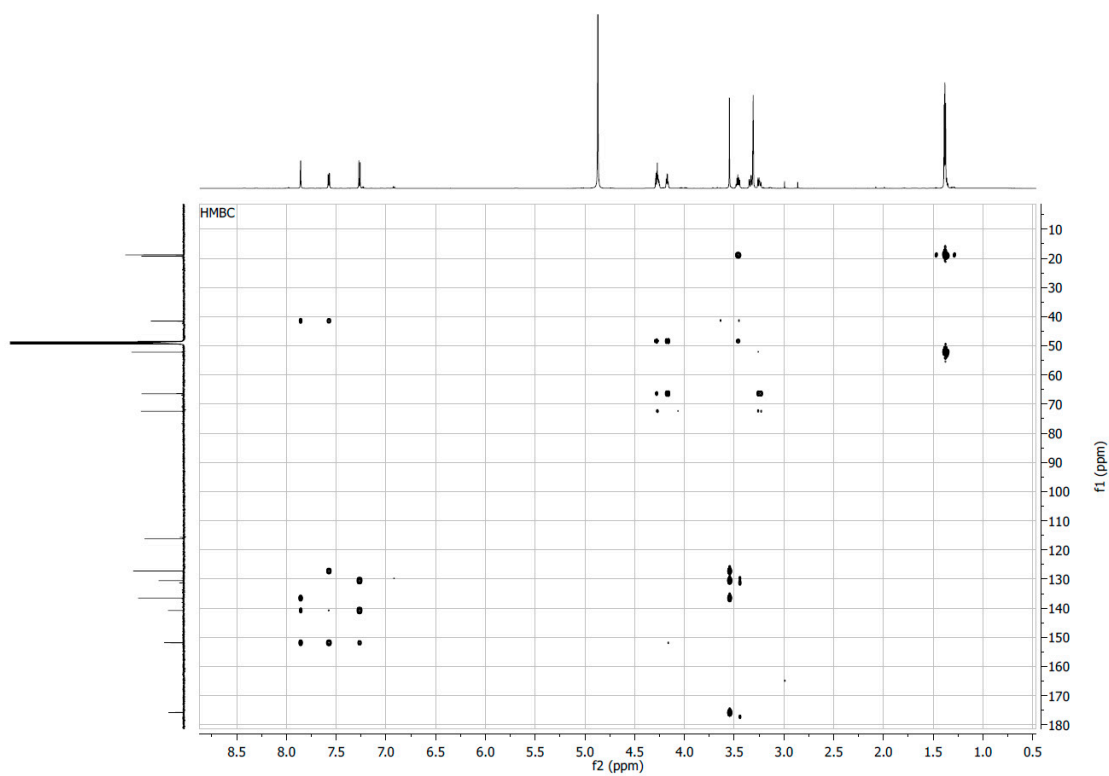


Figure S6 - HMBC of 3-nitroatenolol

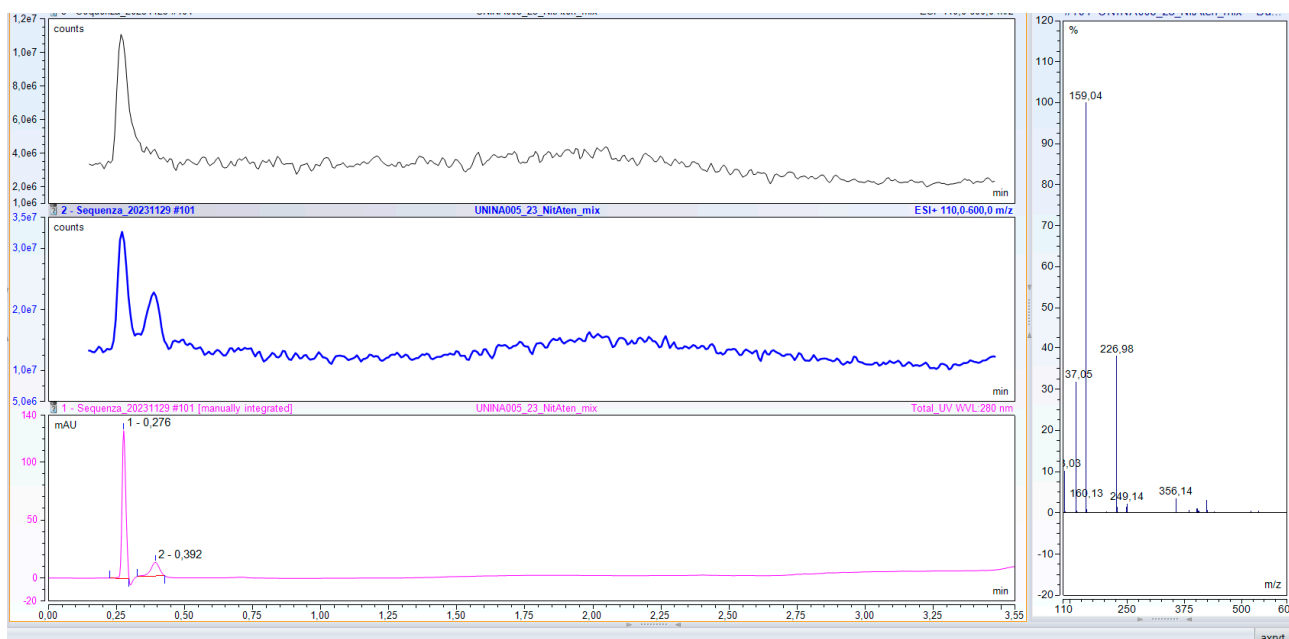


Figure S7 - LC-MS of (±)-3-nitroatenolol Pre-SCX. Mass spectrum in ESI⁺ (blue profile) matches with MW of compound. We detect in ESI⁻ (black profile) a peak corresponding to TFA (Dimer in negative mode: 226.9).

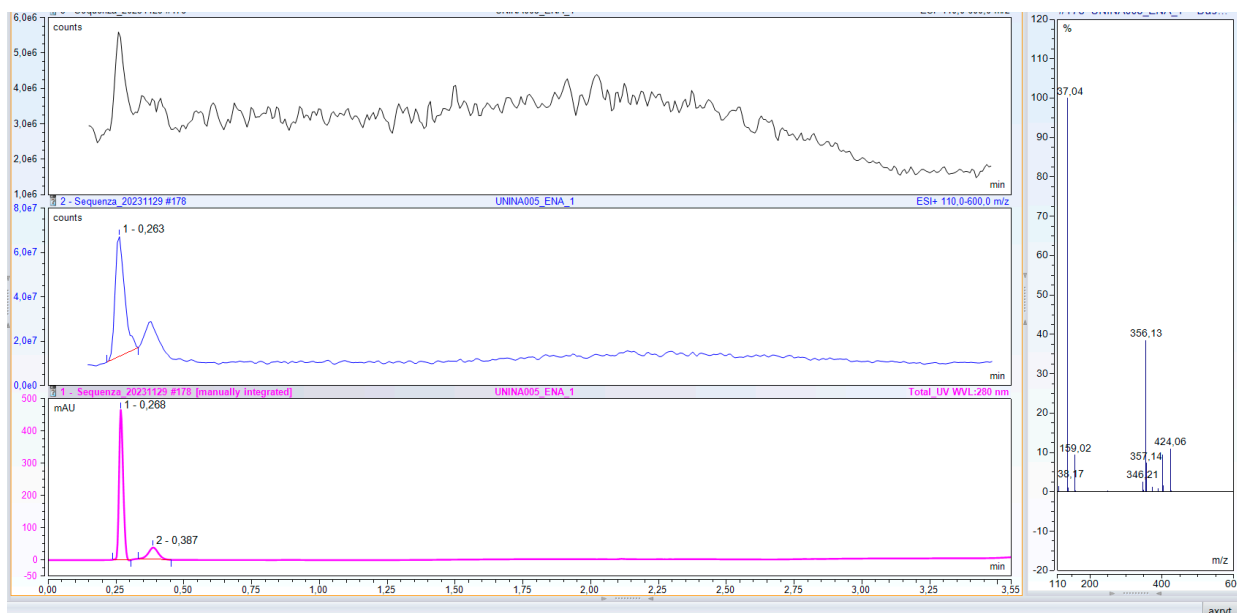


Figure S8 - LC-MS of (±)-3-nitroatenolol Post-SCX. In ESI⁻ (black profile) we observed only a peak corresponding to 3-nitroatenolol [M+HCOOH]⁻

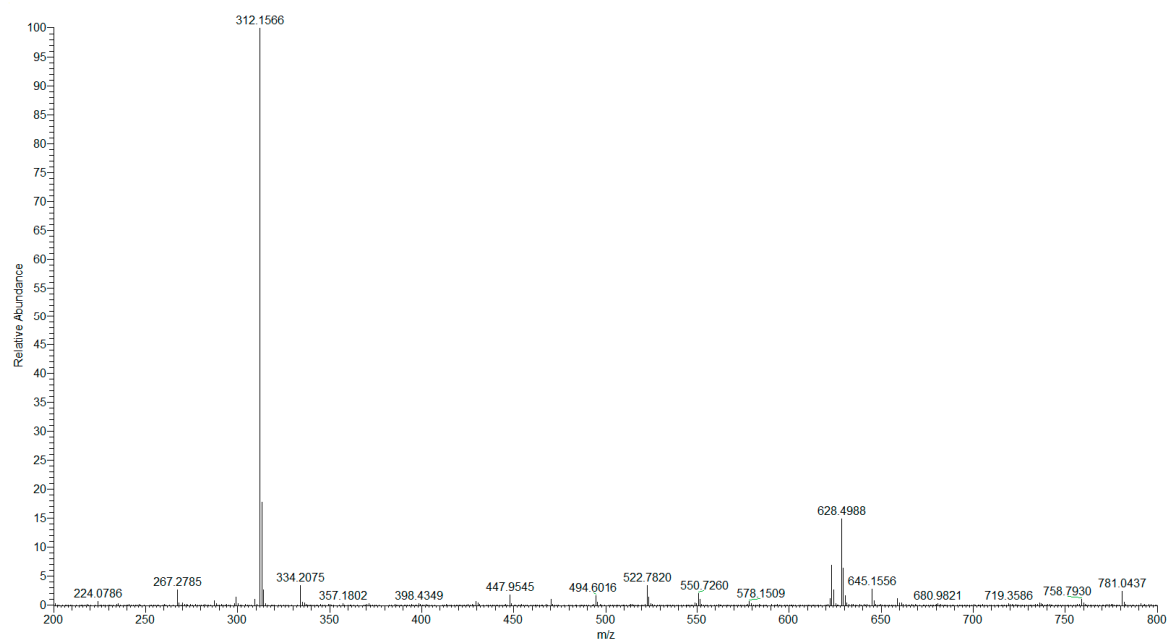


Figure S9 - HRESI-MS spectrum of (±)-3-nitroatenolol.

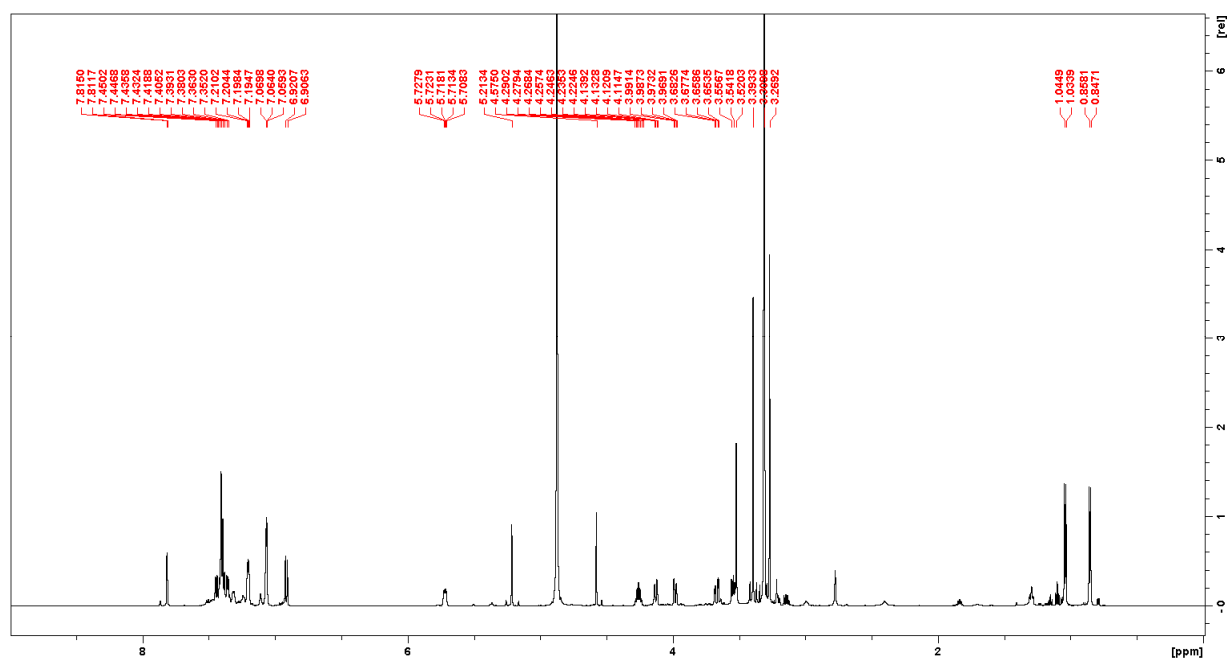


Figure S10 - ¹H NMR spectrum in CD₃OD of bis-(R)-MPA-(+)-3-nitroatenolol.

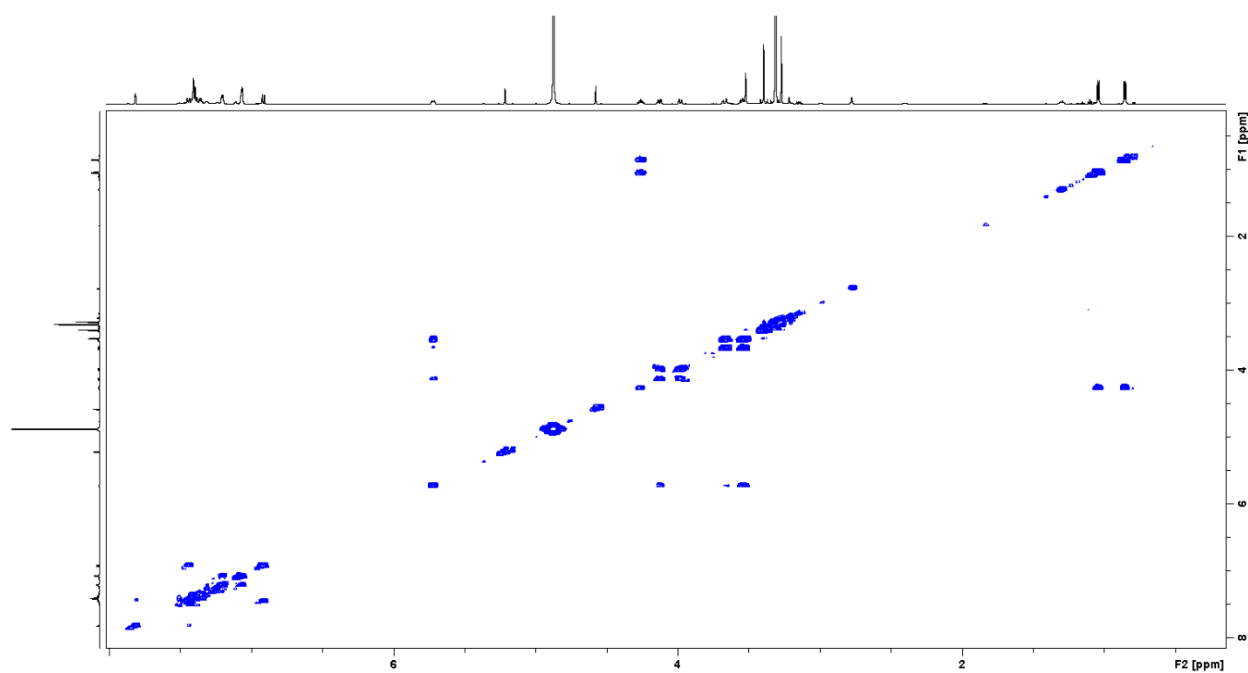


Figure S11 – COSY spectrum in CD₃OD of bis-(R)-MPA-(+)-3-nitroatenolol.

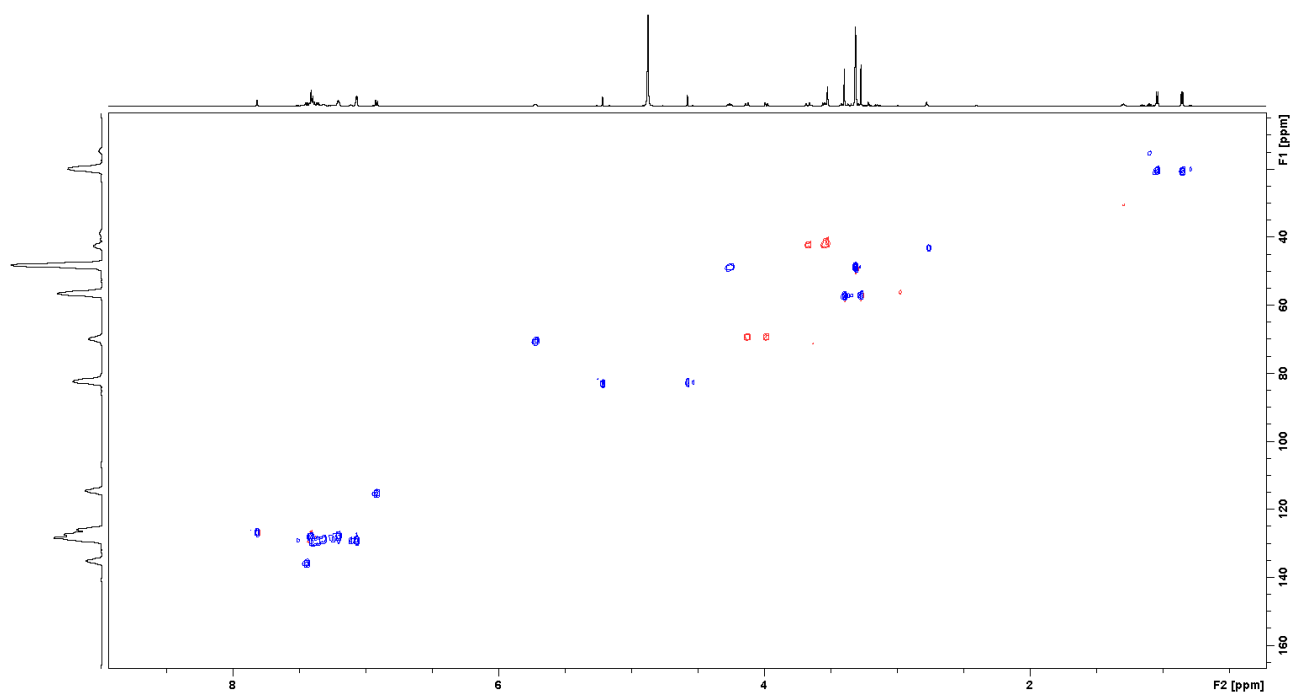


Figure S12 - HSQC spectrum in CD₃OD of bis-(R)-MPA-(+)-3-nitroatenolol.

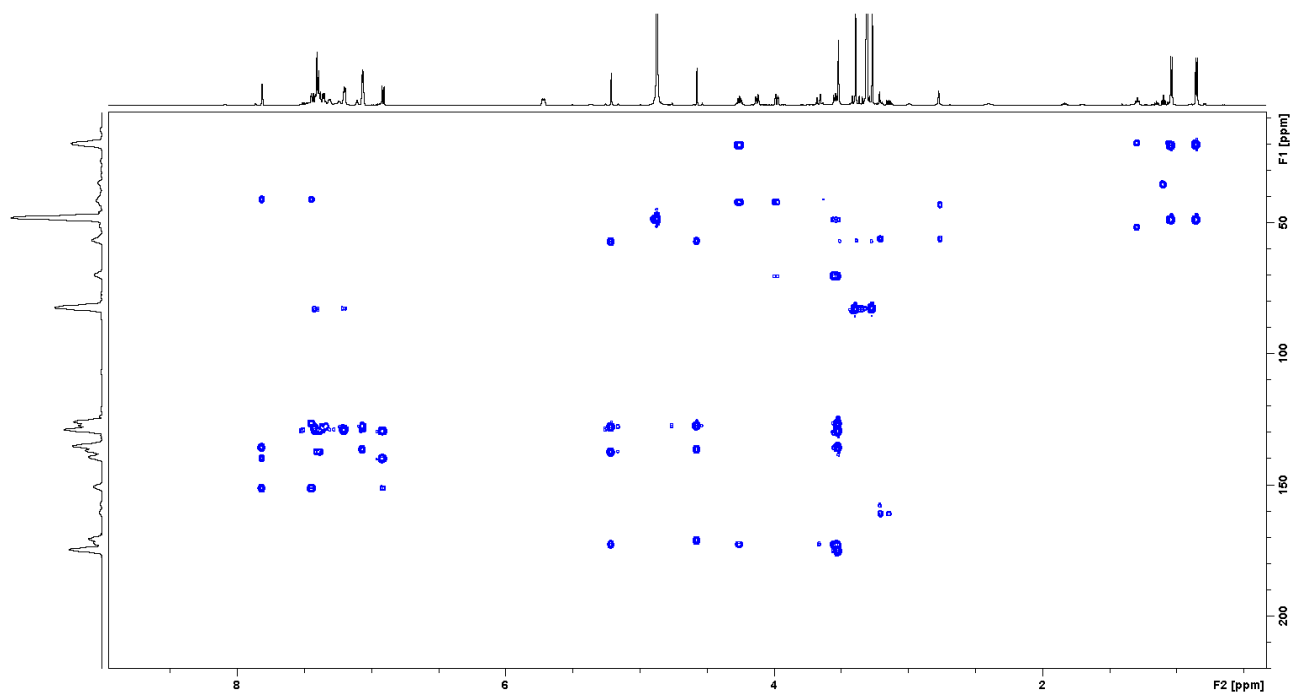


Figure S13- HMBC spectrum in CD₃OD of bis-(R)-MPA-(+)-3-nitroatenolol.

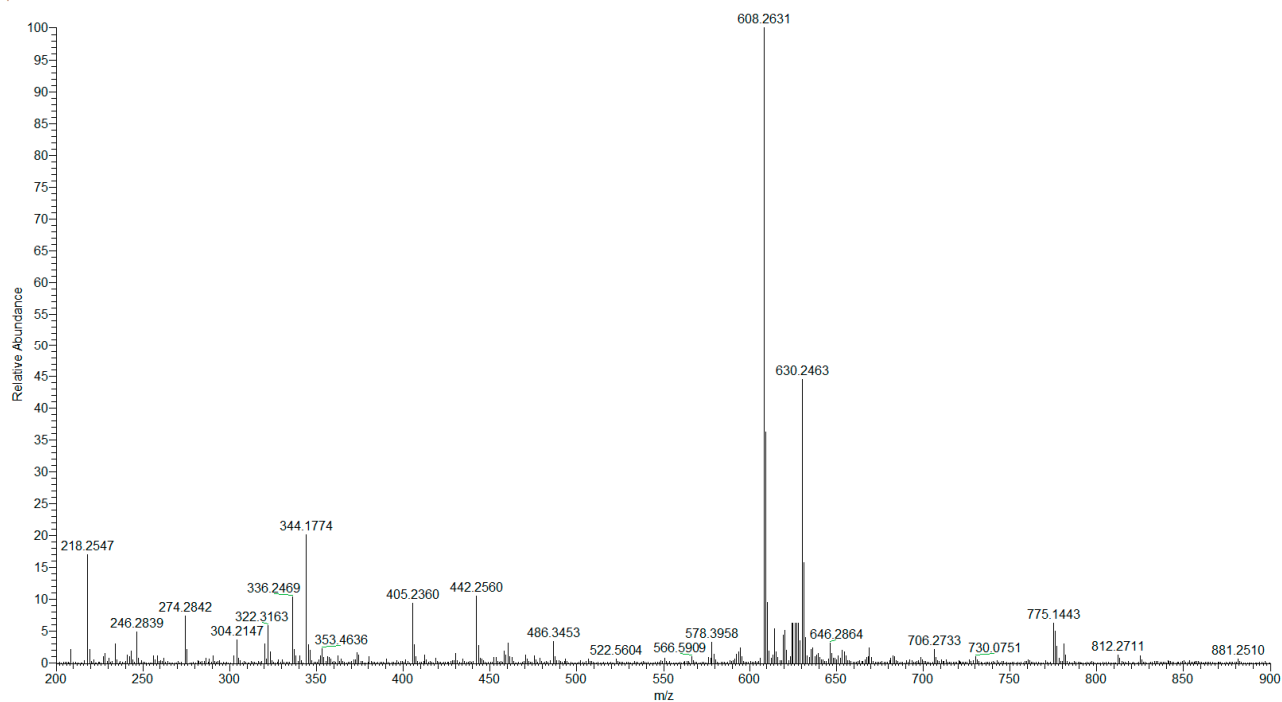


Figure S14 – HRESI-(+)-MS spectrum of bis-(R)-MPA-(+)-3-nitroatenolol.

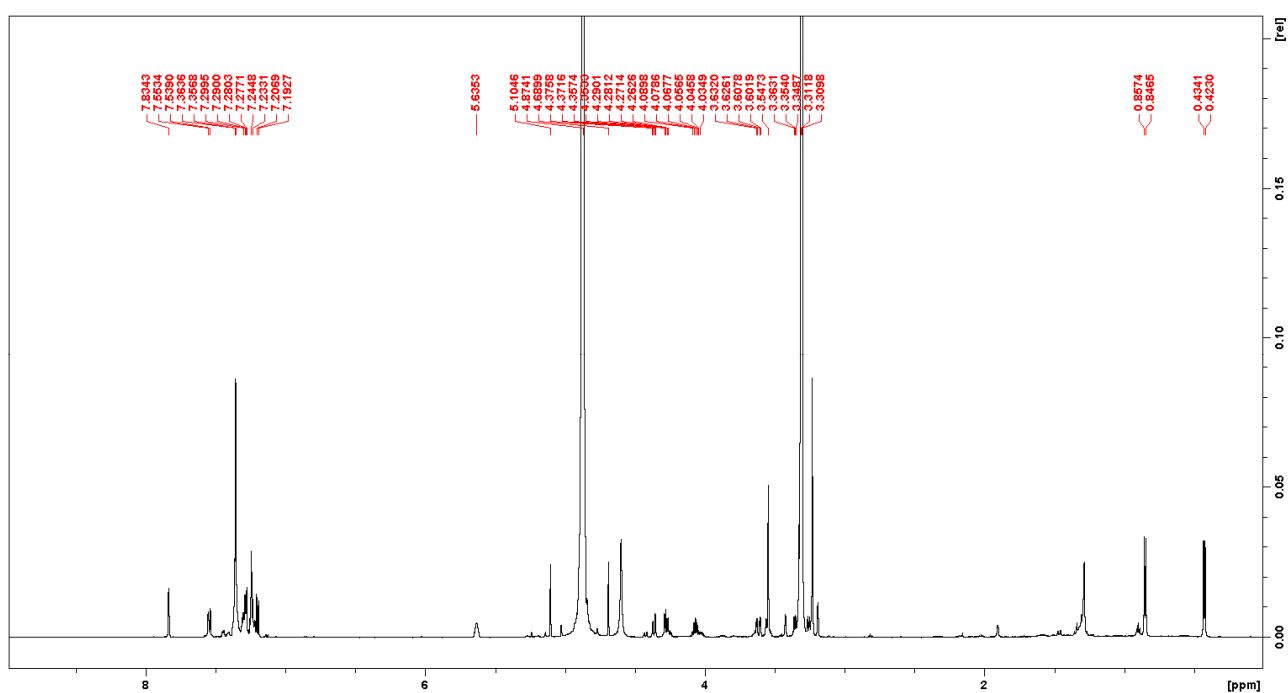


Figure S15- ¹H-NMR of bis-(S)-MPA-(+)-3-nitroatenolol.

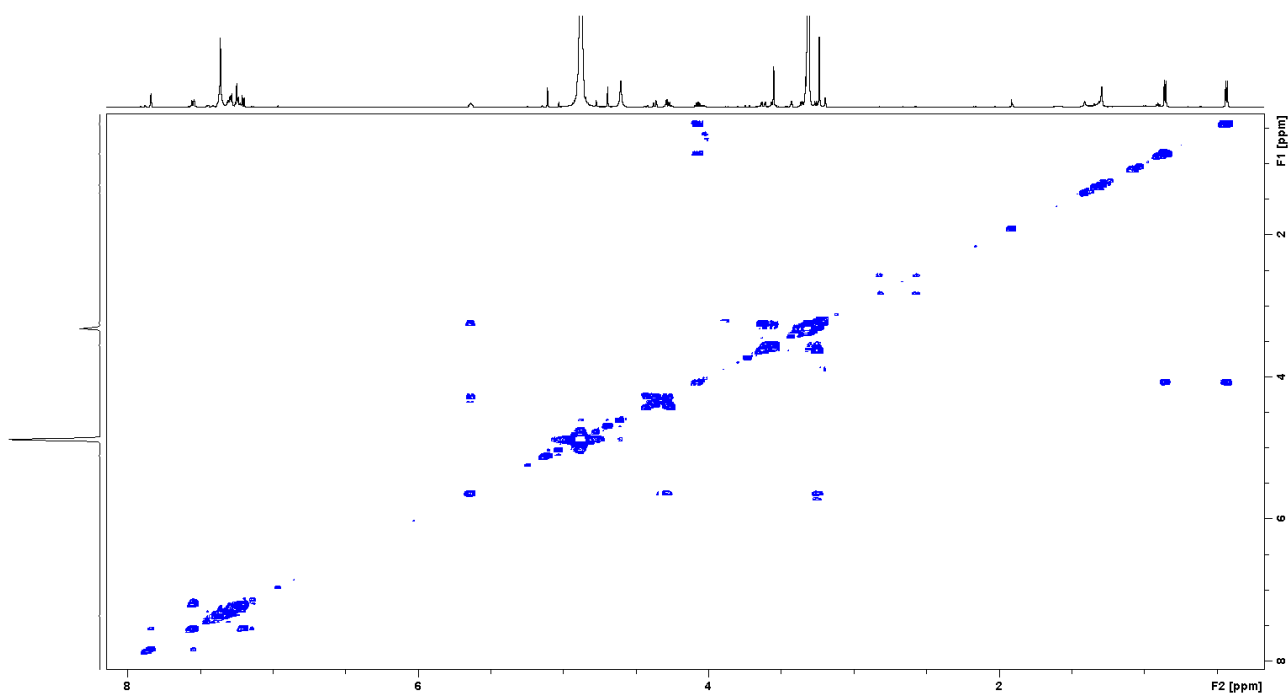


Figure S16 – COSY spectrum of bis-(S)-MPA-(+)-3-nitroatenolol.

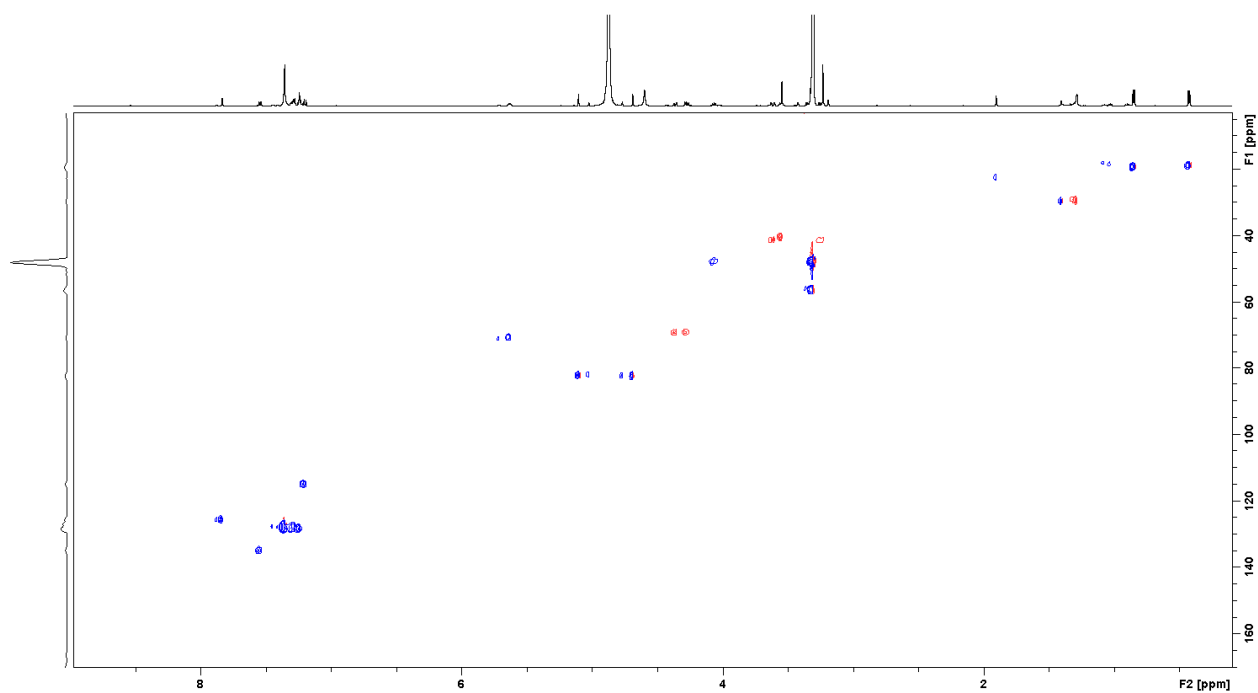


Figure S17 – HSQC spectrum of bis-(S)-MPA-(+)-3-nitroatenolol.

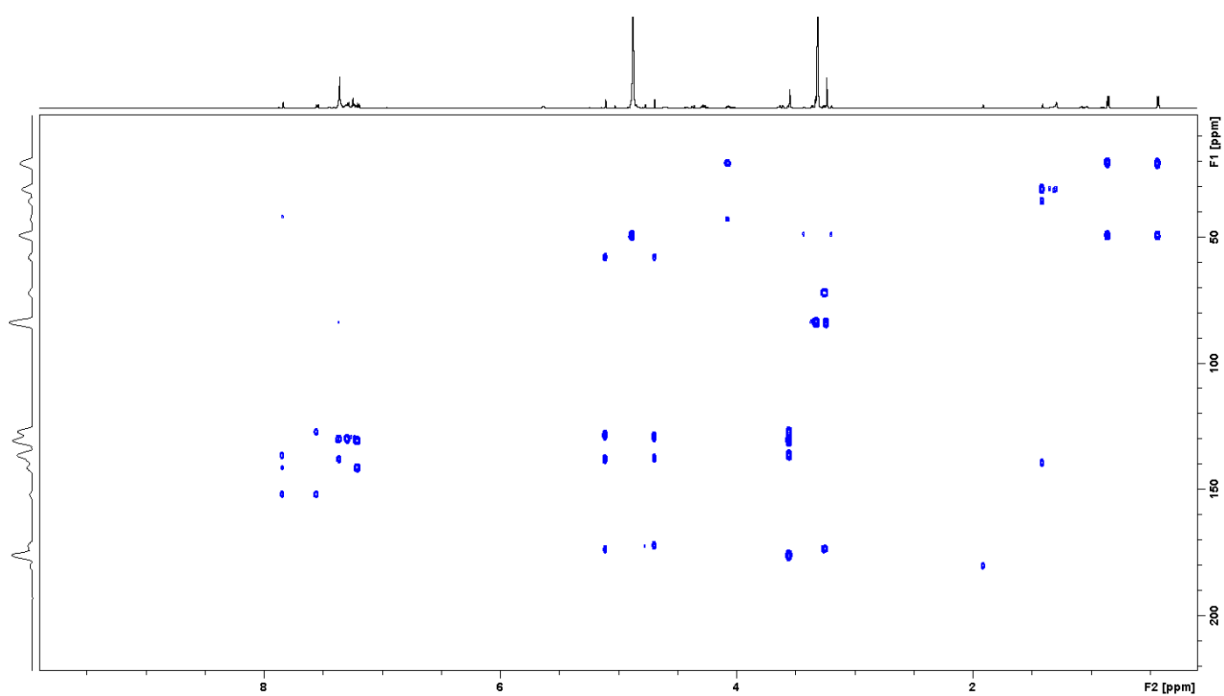


Figure S18 – HMBC spectrum of bis-(S)-MPA-(+)-3-nitroatenolol.

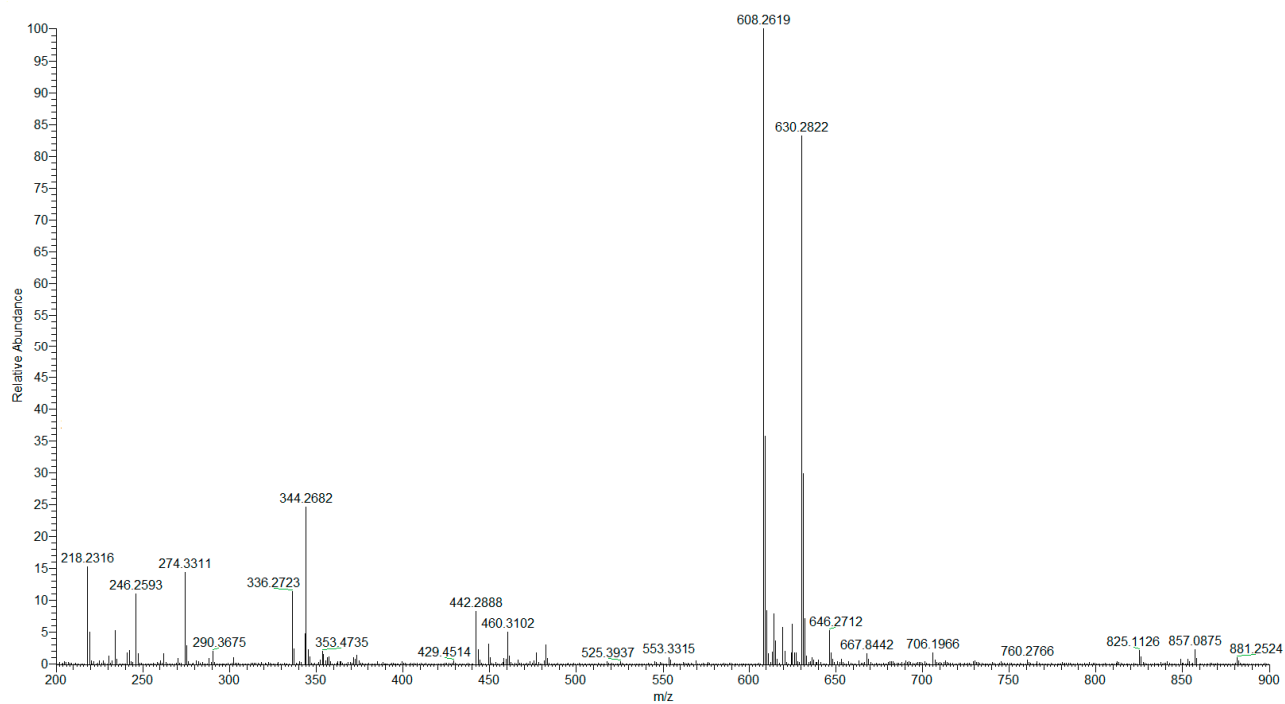


Figure S19 – HRESI-(+)-MS spectrum of bis-(S)-MPA-(+)-3-nitroatenolol.

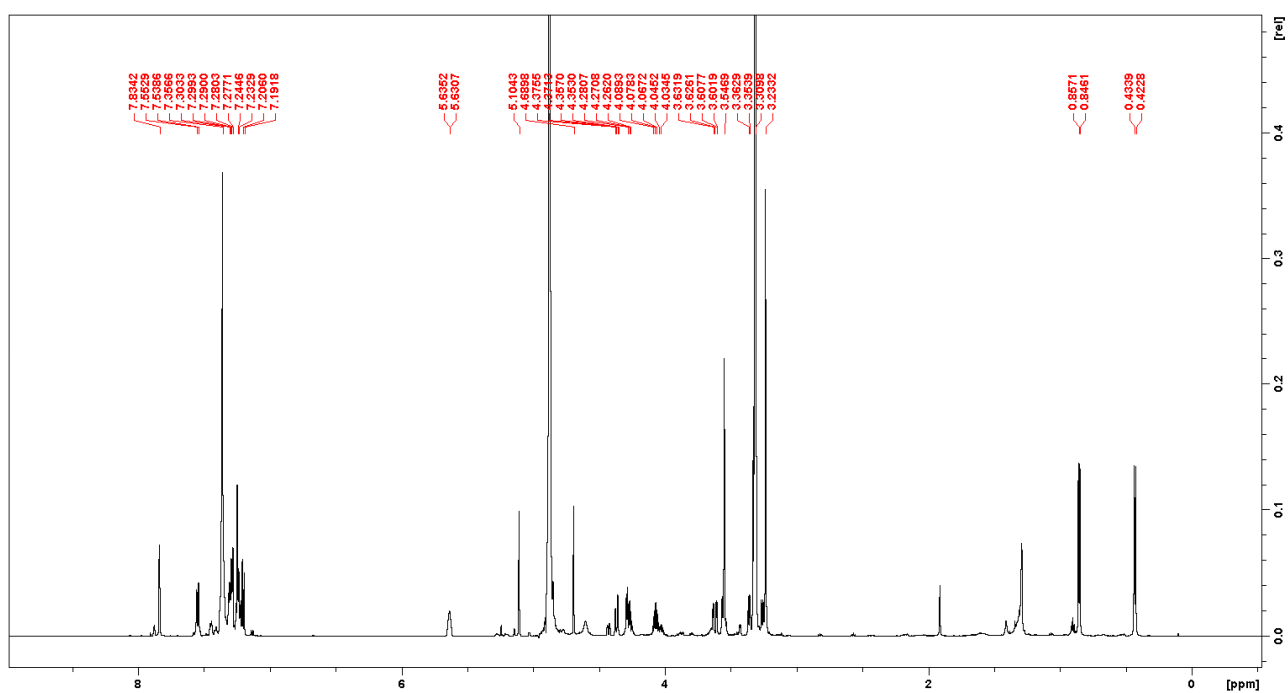


Figure S20 – NMR signals of bis-(R)-MPA derivative of (-)-3-nitroatenolol.

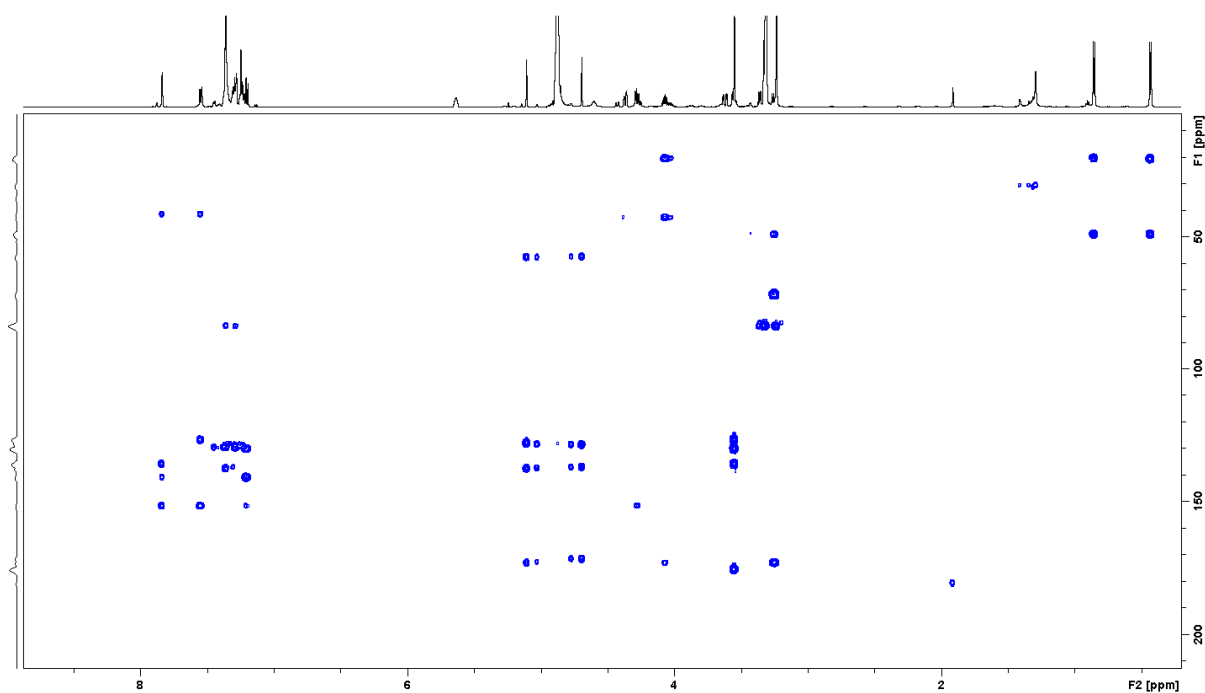


Figure S21 – HMBC spectrum of bis-(R)-MPA-(-)-3-nitroatenolol.

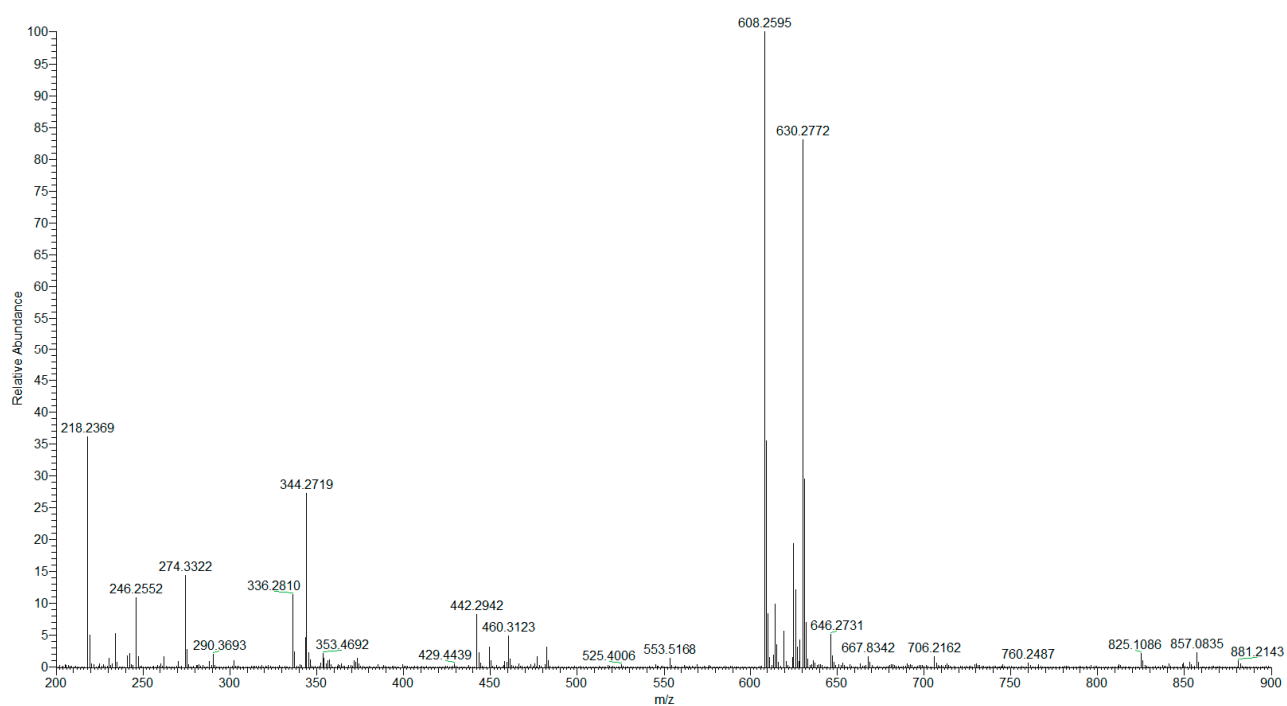


Figure S22 – HRESI-MS spectrum of bis-(R)-MPA-(-)-3-nitroatenolol.

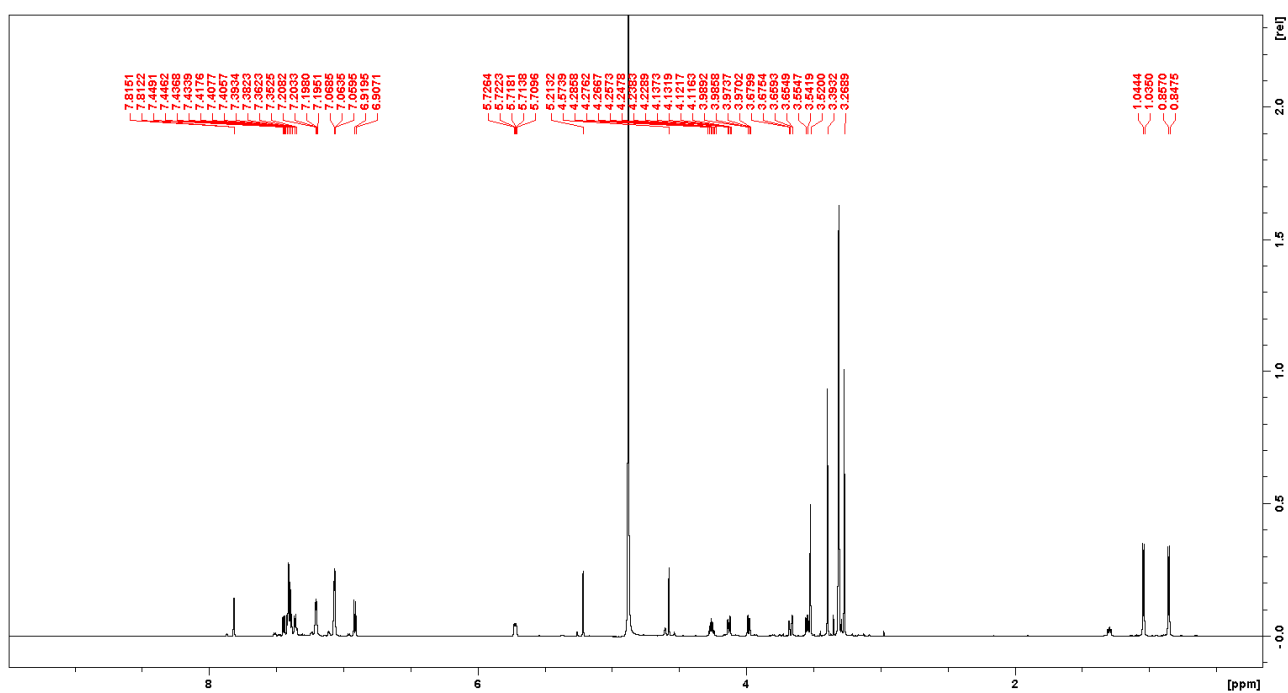


Figure S23 – NMR signals of bis-(S)-MPA derivative of (-)-3-nitroatenolol.

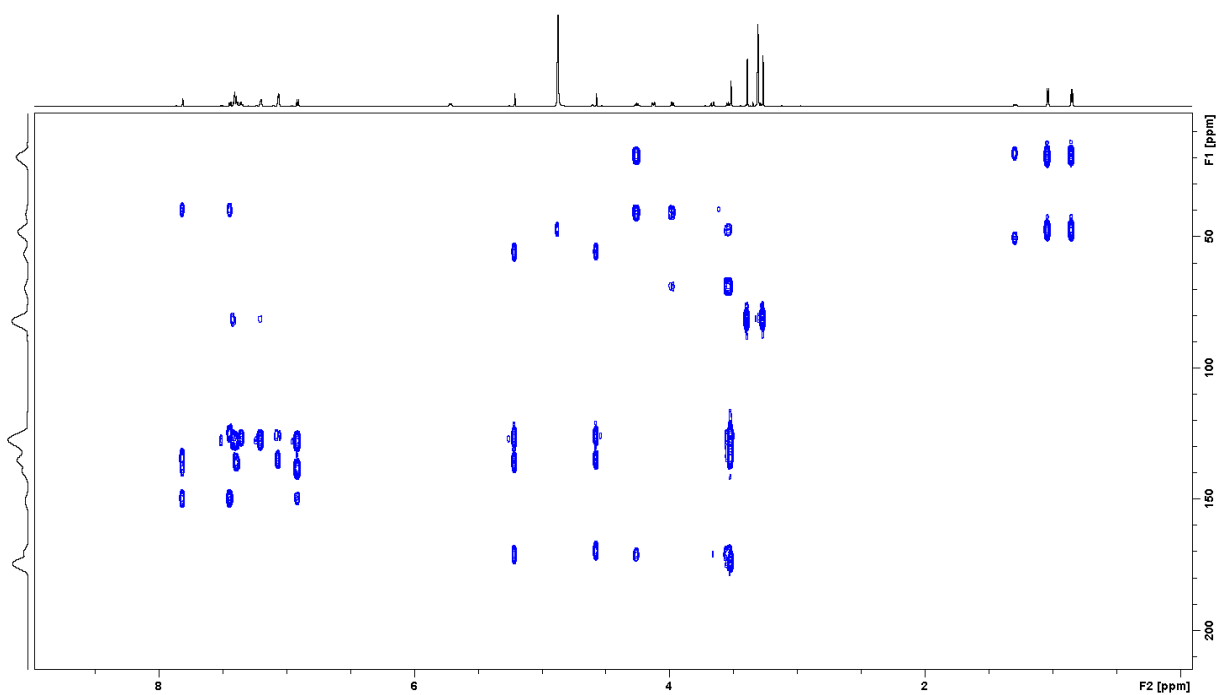


Figure S24 - HMBC of bis-(S)-MPA-(-)-3-nitroatenolol.

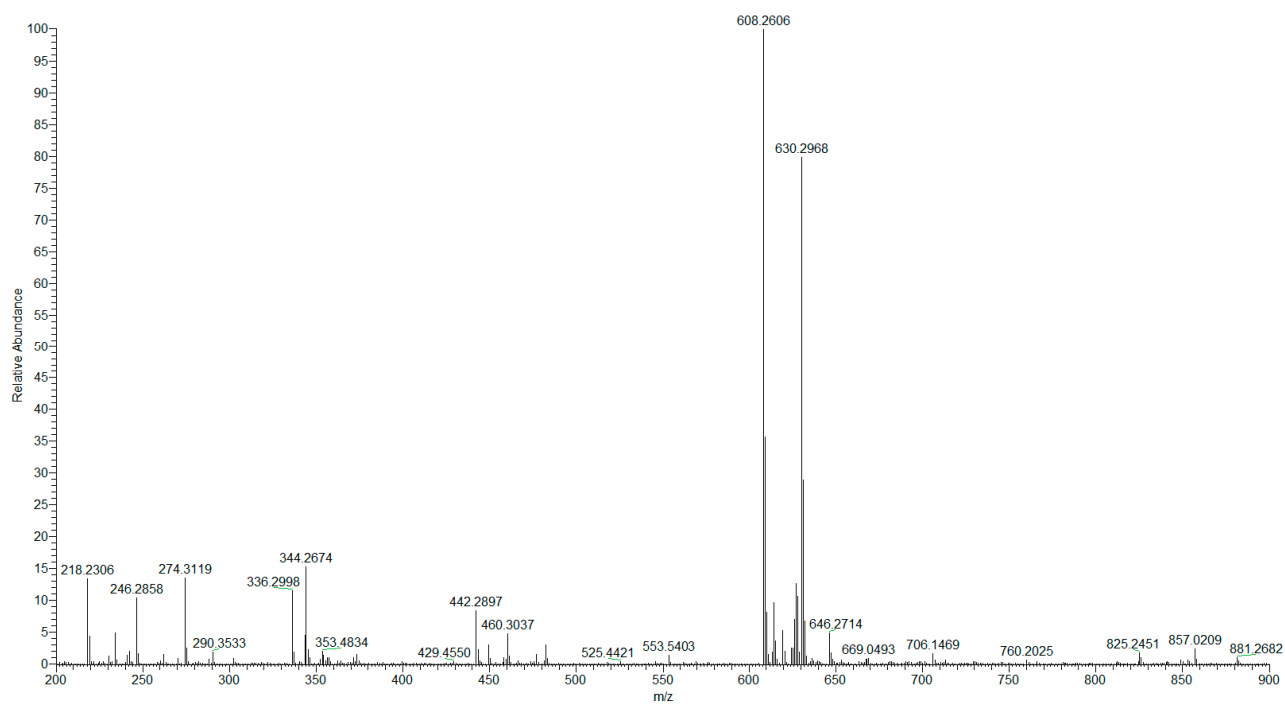


Figure S25 – HRESI-MS spectrum of bis-(S)-MPA-(-)-3-nitroatenolol.