

Supplementary material

Synthesis, gene transfection efficiency, and serum compatibility of β -sitosterol-based cationic lipids with different headgroups

Chopaka Thongbamrer, ^{a,b} Boon-ek Yingyongnarongkul, ^b Uthai Sakee, ^a Wang Nguitragee ^{c,d}
and Widchaya Radchatawedchakoon ^{*a}

^a Creative Chemistry and Innovation Research Unit, Department of Chemistry and Center of Excellence for Innovation in Chemistry (PERCH-CIC), Faculty of Science, Mahasarakham University, Kantharawichai, Maha Sarakham, 44150 Thailand

^b Department of Chemistry and Center of Excellence for Innovation in Chemistry (PERCH-CIC), Faculty of Science, Ramkhamhaeng University, Bangkapi, Bangkok, 10240 Thailand

^c Department of Molecular Tropical Medicine and Genetics, Faculty of Tropical Medicine, Mahidol University, Bangkok, 10400 Thailand

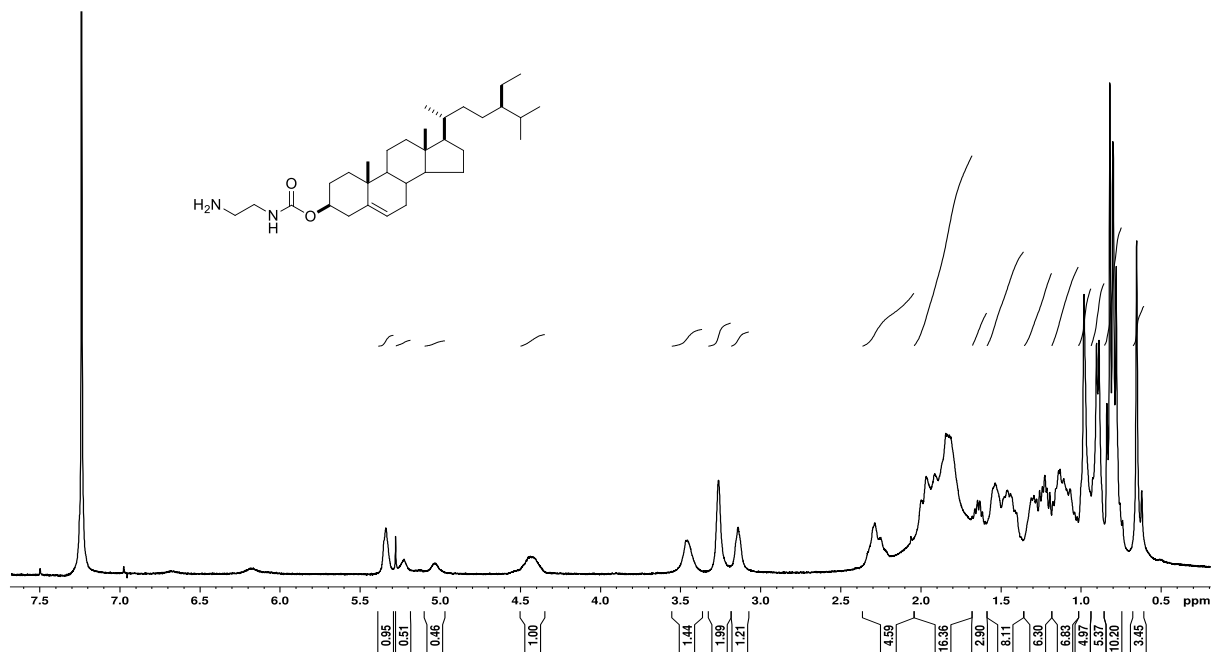
^d Mahidol Vivax Research Unit, Faculty of Tropical Medicine, Mahidol University, Bangkok, 10400 Thailand

* Corresponding author. Tel.: +6643 754 246; fax: +6643 754 246.

E-mail addresses: widchaya.r@msu.ac.th; widchaya@hotmail.com

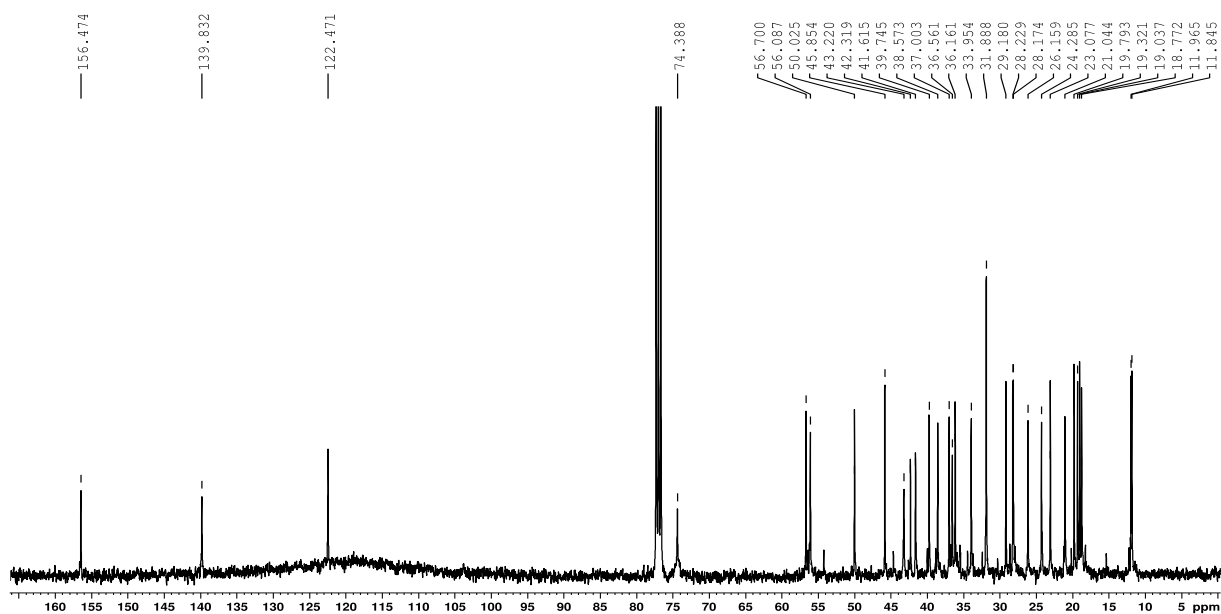
^1H , ^{13}C NMR and HR-ESI-MS Spectra of All Synthesized Compounds

WR-NMR178 Chopaka CT(1)-58 (7.8 mg, CDCl_3)



^1H NMR Spectrum (400 MHz, CDCl_3) of 3β-[N-(2-Aminoethyl)carbamoyl]β-sitosterol (1A)

^{13}C WR-NMR178 Chopaka CT(1)-58 (7.8 mg, CDCl_3)



^{13}C NMR Spectrum (100 MHz, CDCl_3) of 3β-[N-(2-Aminoethyl)carbamoyl]β-sitosterol (1A)

Mass Spectrum SmartFormula Report

Analysis Info

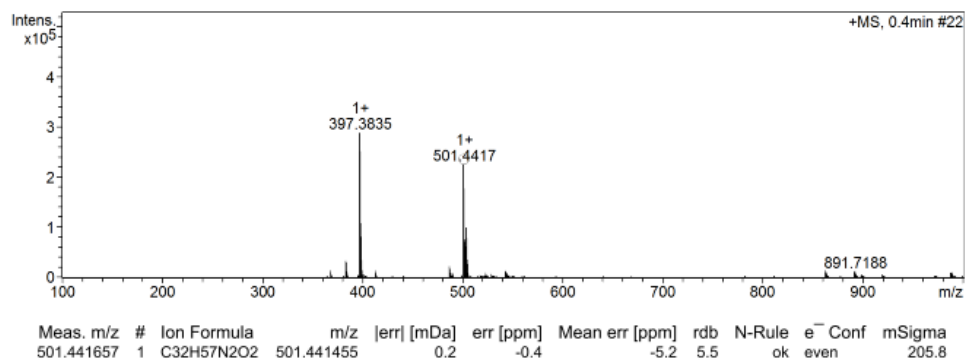
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Acquisition Date 6/29/2016 3:32:56 PM

Operator RU
Instrument micrOTOF 8213750.10411

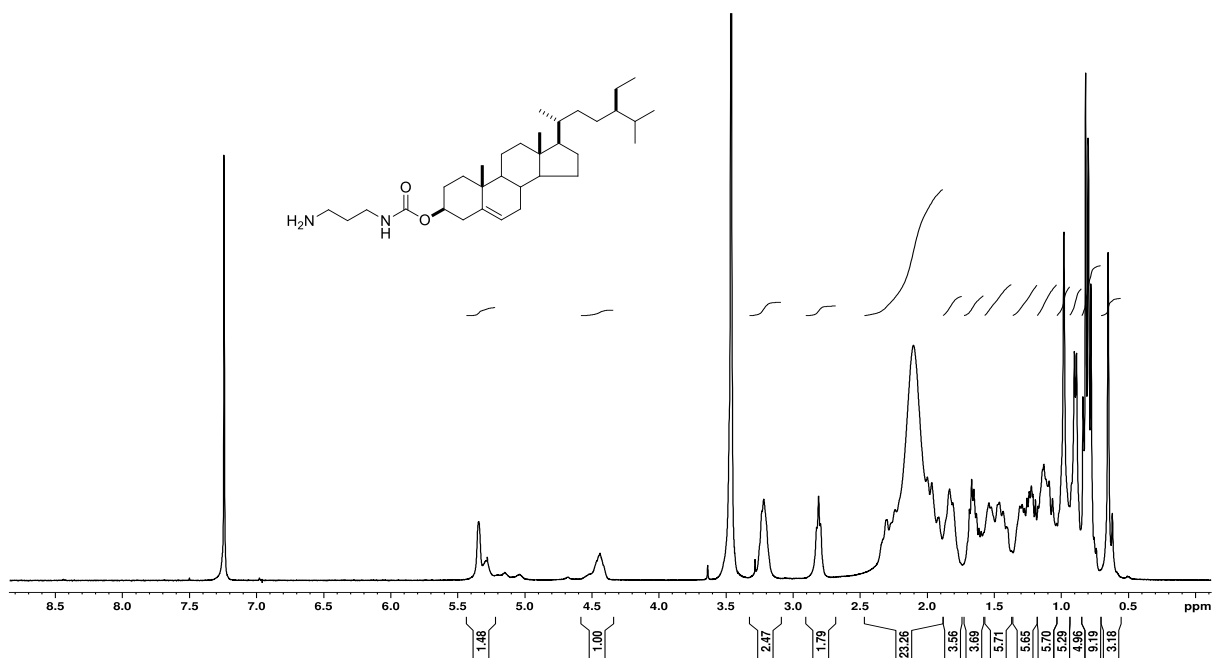
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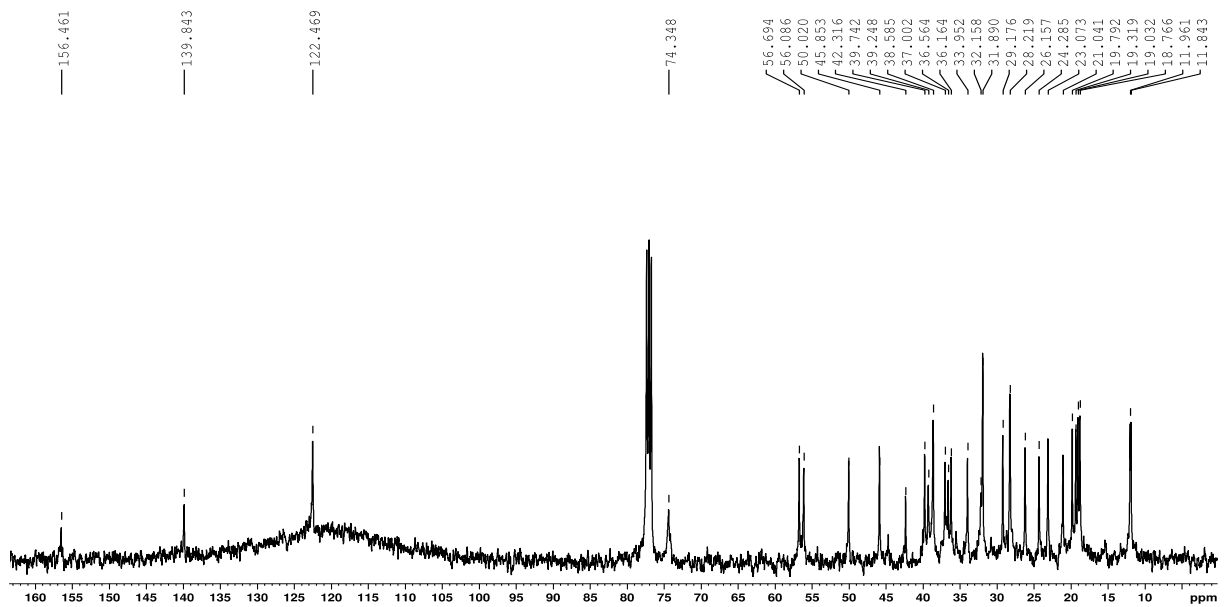
HR-ESI-MS Spectrum of 3 β -[N-(2-Aminoethyl)carbamoyl] β -sitosterol (**1A**)

WR-NMR179 Chopaka CT(1)-59 (8.6 mg, CDCl₃)



¹H NMR Spectrum (400 MHz, CDCl₃) of 3β-[N-(3-Aminopropyl)carbamoyl]β-sitosterol (**5A**)

¹³C WR-NMR179 Chopaka CT(1)-59 (8.6 mg, CDCl₃)



¹³C NMR Spectrum (100 MHz, CDCl₃) of 3β-[N-(3-Aminopropyl)carbamoyl]β-sitosterol (**5A**)

Mass Spectrum SmartFormula Report

Analysis Info

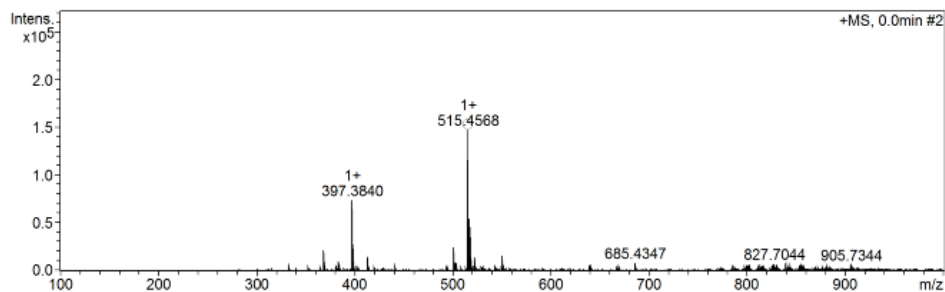
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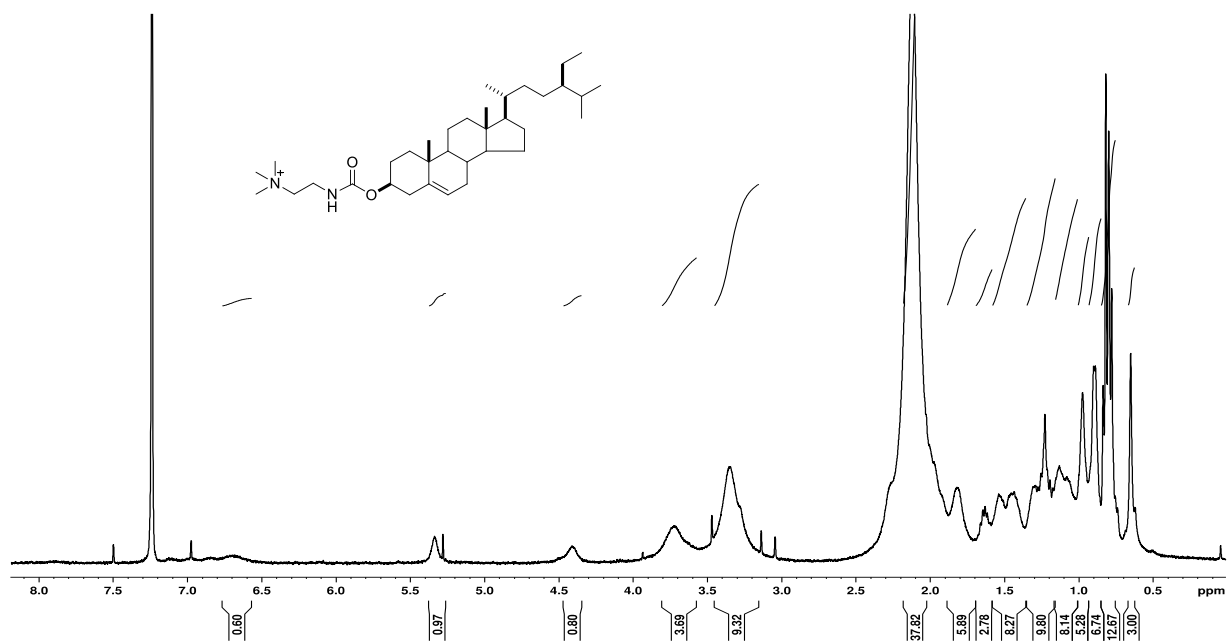
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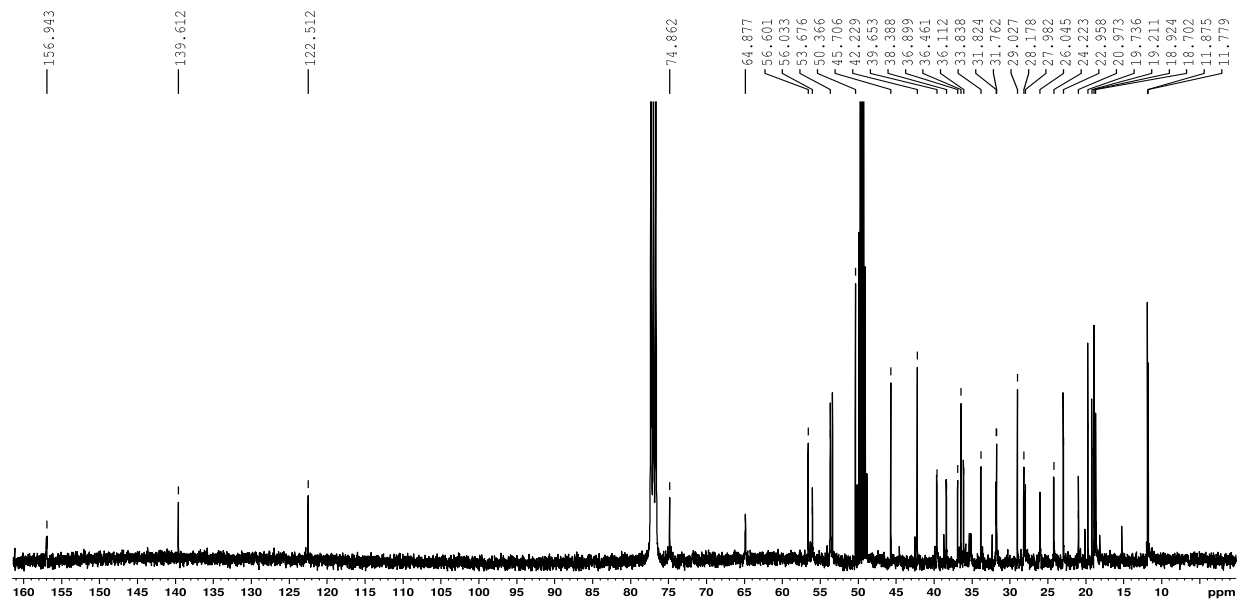
HR-ESI-MS Spectrum of 3 β -[N-(3-Aminopropyl)carbamoyl] β -sitosterol (**5A**)

WR-NMR184 Chopaka CT(1)-64 (8.4 mg, CDCl₃ + CD₃OD)



¹H NMR Spectrum (400 MHz, CDCl₃) of 3β-[N-(2-(*N,N,N*-Trimethyl)aminoethyl)carbamoyl]β-sitosterol (**2A**)

¹³C WR-NMR184 Chopaka CT(1)-64 (8.4 mg, CDCl₃ + CD₃OD)



¹³C NMR Spectrum (100 MHz, CDCl₃) of 3β-[N-(2-(*N,N,N*-Trimethyl)aminoethyl)carbamoyl]β-sitosterol (**2A**)

Mass Spectrum SmartFormula Report

Analysis Info

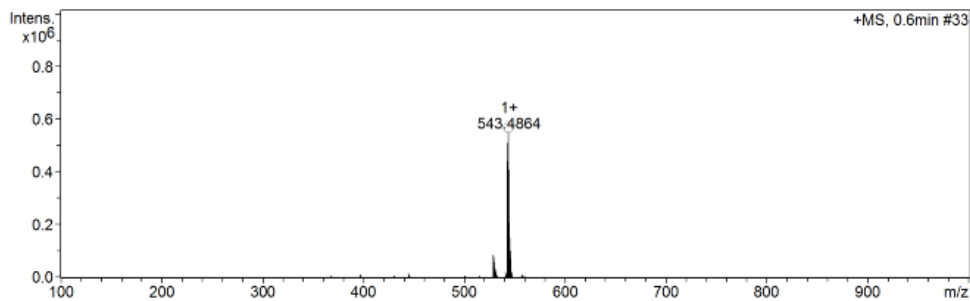
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Operator RU
Instrument micrOTOF 8213750.10411

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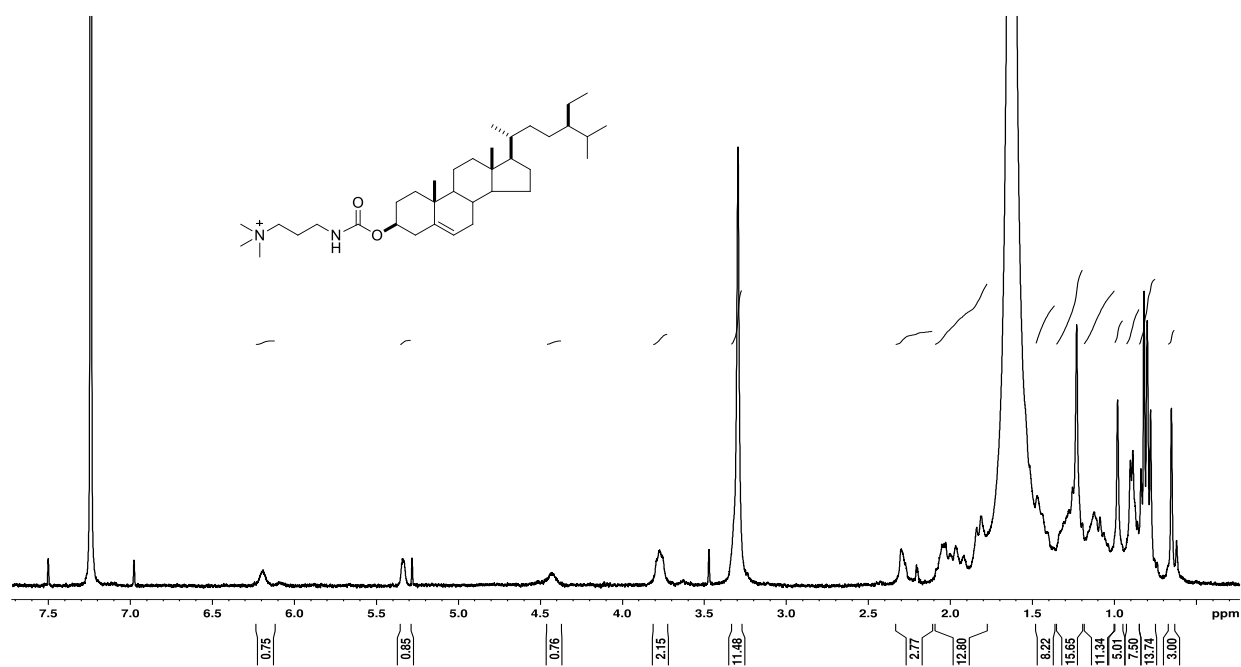
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Meas. m/z	#	Ion Formula	m/z	err [mDa]	err [ppm]	Mean err [ppm]	rdb	N-Rule	e ⁻ Conf	mSigma
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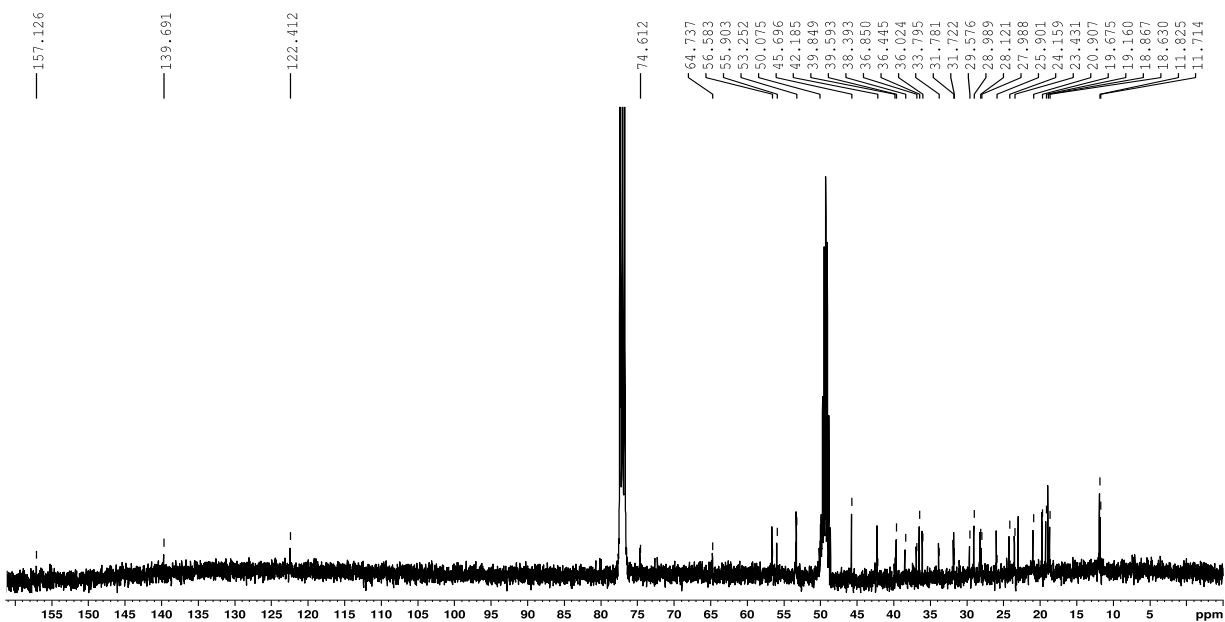
HR-ESI-MS Spectrum of 3 β -[*N*-(2-(*N*', *N*', *N*'-Trimethyl)aminoethyl)carbonyl] β -sitosterol (**2A**)

WR-NMR185 Chopaka CT(1)-65 (9.2 mg, CDCl₃ + CD₃OD)



¹H NMR Spectrum (400 MHz, CDCl₃) of 3β-[N-(3-(N', N', N'-Trimethyl)aminopropyl)carbamoyl]β-sitosterol (**6A**)

¹³C WR-NMR185 Chopaka CT(1)-65 (9.2 mg, CDCl₃ + CD₃OD)



¹³C NMR Spectrum (100 MHz, CDCl₃) of 3β-[N-(3-(N', N', N'-Trimethyl)aminopropyl)carbamoyl]β-sitosterol (**6A**)

Mass Spectrum SmartFormula Report

Analysis Info

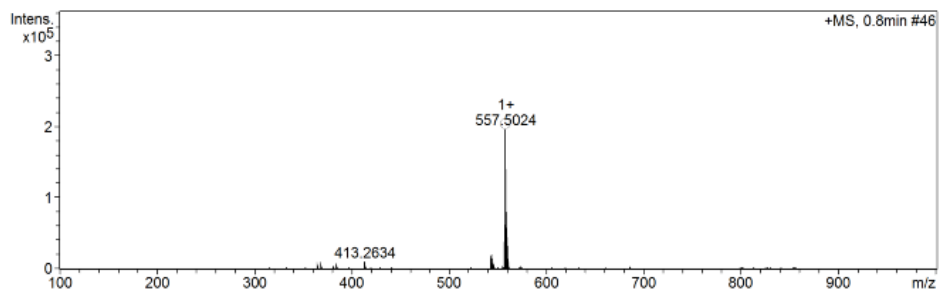
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Sample Name CT (1)-65
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Operator RU
Instrument micrOTOF 8213750.10411

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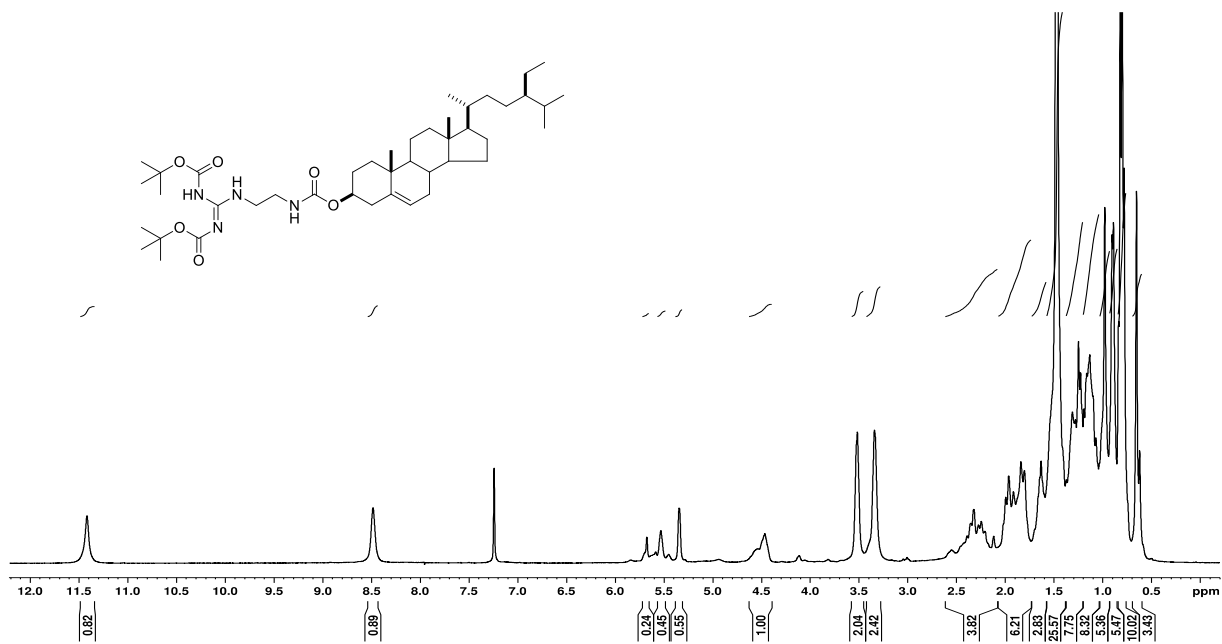
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Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



Meas. m/z	#	Ion Formula	m/z	err [mDa]	err [ppm]	Mean err [ppm]	rdb	N-Rule	e ⁻ Conf	mSigma
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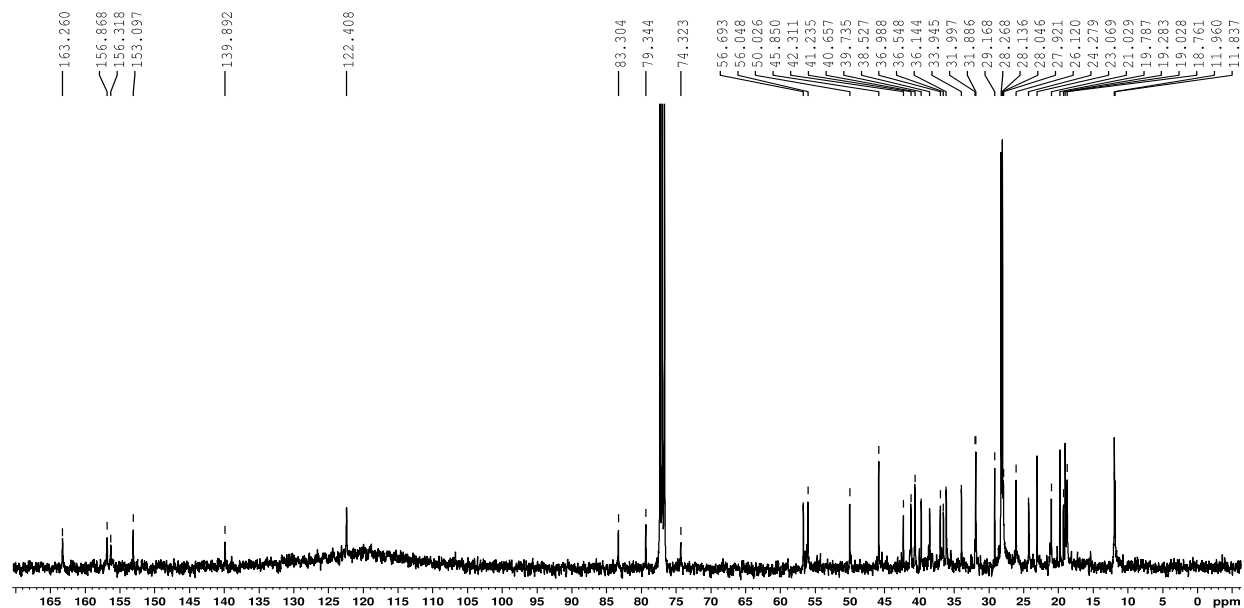
HR-ESI-MS Spectrum of 3 β -[N-(3-(N', N', N'-Trimethyl)aminopropyl)carbamoyl] β -sitosterol
(6A)

WR-NMR180 Chopaka CT(1)-60 (20 mg, CDCl₃)



¹H NMR Spectrum (400 MHz, CDCl₃) of 3β-[(2-((N',N''-Di(*tert*-butoxycarbonyl)guanidiny)ethyl) carbamoyl]β-sitosterol (**11**)

¹³C WR-NMR180 Chopaka CT(1)-60 (20.0 mg, CDCl₃)

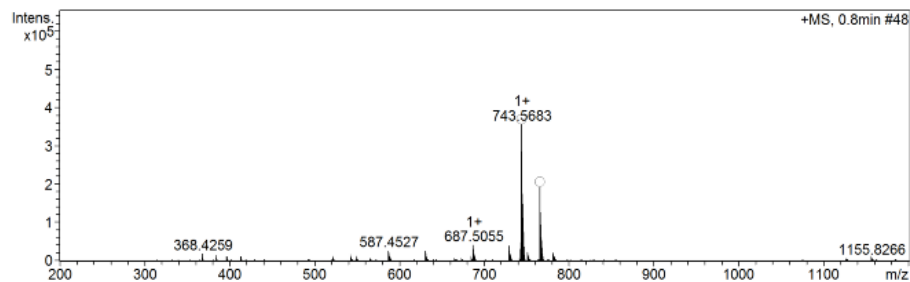


¹³C NMR Spectrum (100 MHz, CDCl₃) of 3β-[(2-((N',N''-Di(*tert*-butoxycarbonyl)guanidiny)ethyl) carbamoyl]β-sitosterol (**11**)

Mass Spectrum SmartFormula Report

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Sample Name	CT (1)-60	Instrument micrOTOF 8213750.10411
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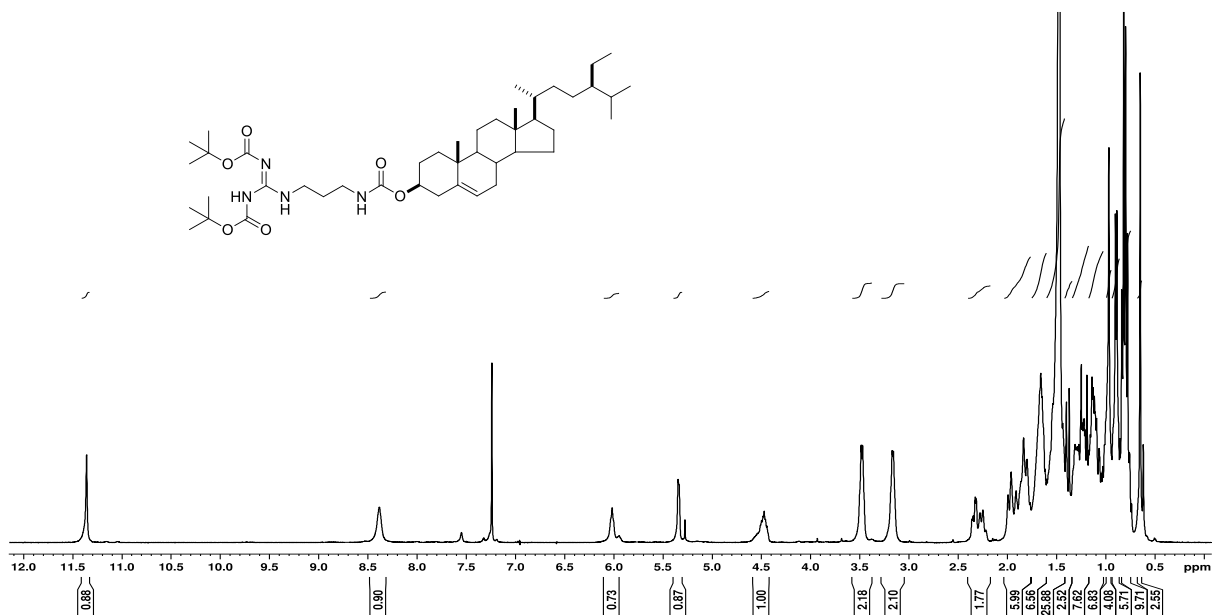
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765.548899	1	C43H74N4NaO6	765.550057	1.2	-1.5	0.4	8.5	ok	even	75.0

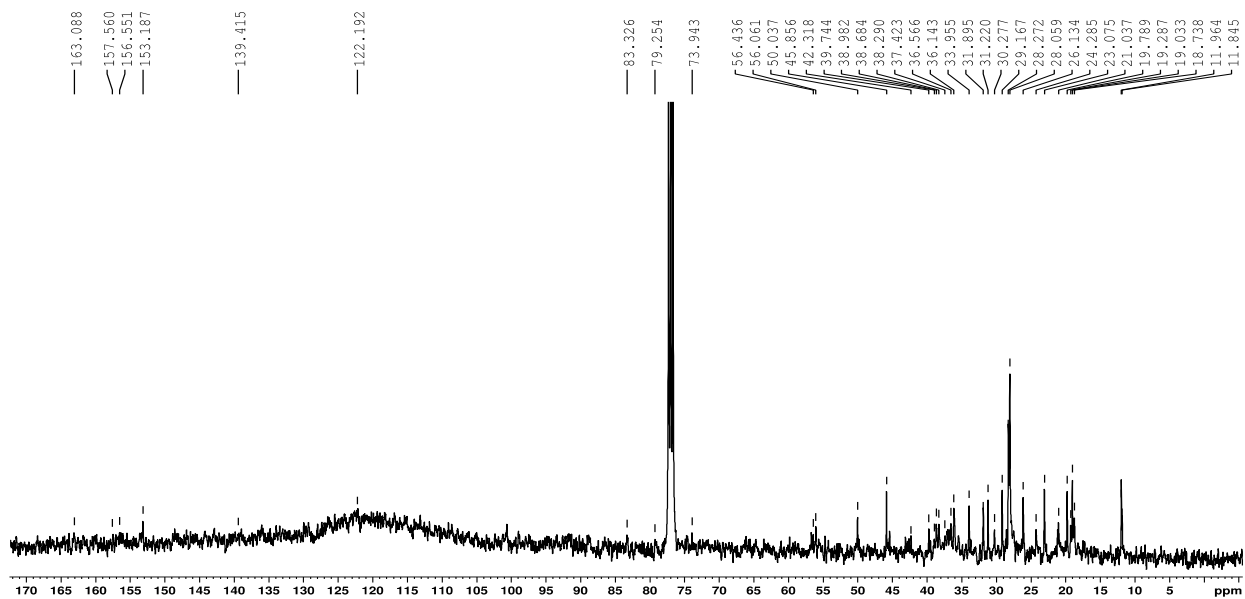
HR-ESI-MS Spectrum of 3β-[(2-((*N'*, *N''*-Di(*tert*-butoxycarbonyl)guanidiny)ethyl) carbamoly]β-sitosterol (**11**)

WR-NMR181 Chopaka CT(1)-61 (12.5 mg, CDCl₃)



¹H NMR Spectrum (400 MHz, CDCl₃) of 3β-[(3-((N',N''-Di(tert-butoxycarbonyl))guanidiny)propyl)carbamoyl]β-sitosterol (**12**)

¹³C WR-NMR181 Chopaka CT(1)-61 (12.5 mg, CDCl₃)



¹³C NMR Spectrum (100 MHz, CDCl₃) of 3β-[(3-((N',N''-Di(tert-butoxycarbonyl))guanidiny)propyl)carbamoyl]β-sitosterol (**12**)

Mass Spectrum SmartFormula Report

Analysis Info

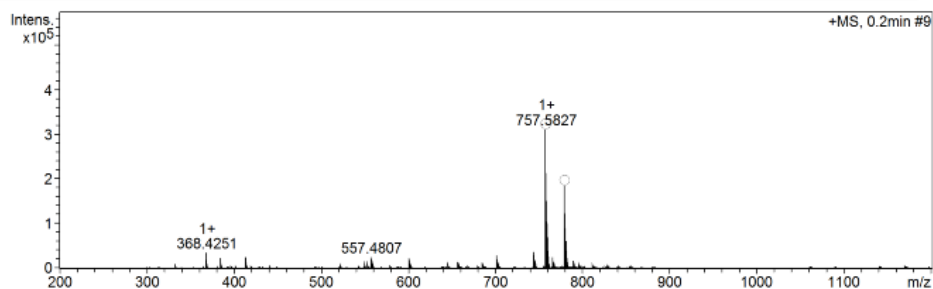
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Operator RU
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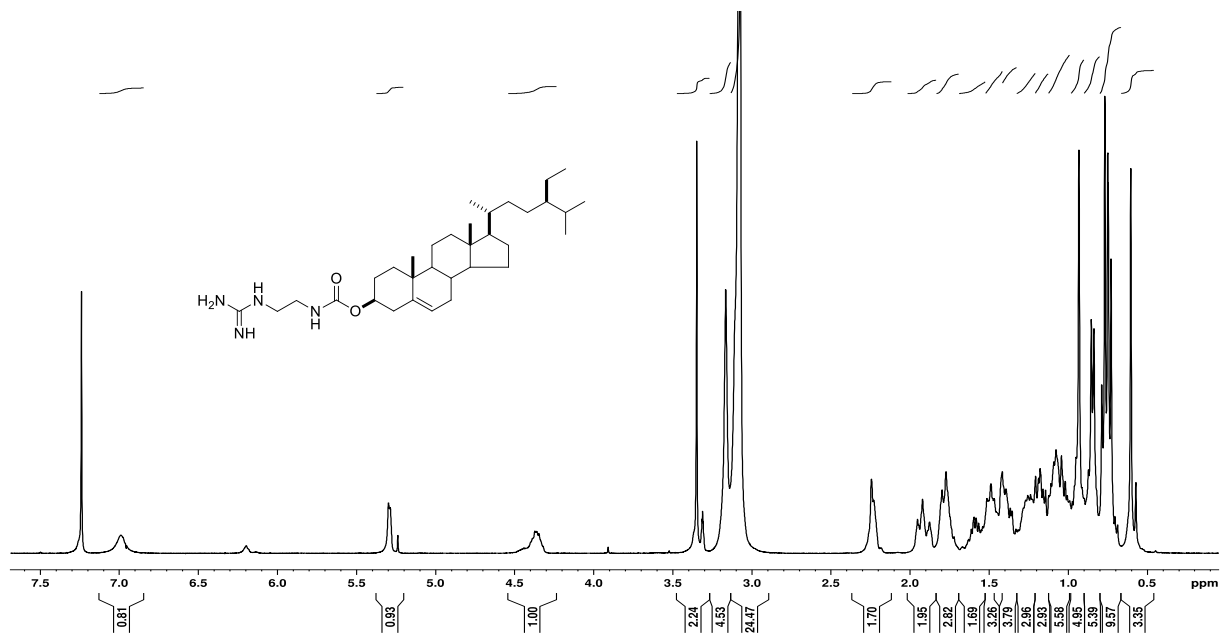
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Meas. m/z	#	Ion Formula	m/z	[err] [mDa]	err [ppm]	Mean err [ppm]	rdb	N-Rule	e ⁻ Conf	mSigma
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779.564673	1	C44H76N4NaO6	779.565707	1.0	-1.3	-0.6	8.5	ok	even	95.2

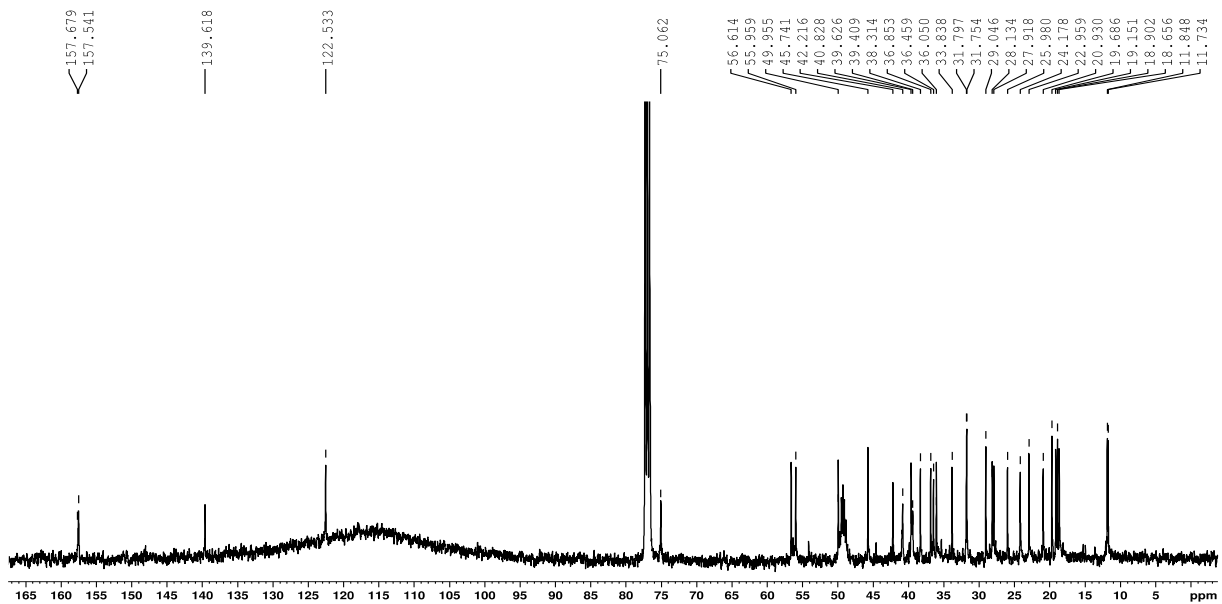
HR-ESI-MS Spectrum of 3 β -[(3-((N',N''-Di(*tert*-butoxycarbonyl))guanidiny)propyl)carbamoyl]
 β -sitosterol (**12**)

WR-NMR186 Chopaka CT(1)-66 (9.1 mg, CDCl₃ + CD₃OD 3 drops)



¹H NMR Spectrum (400 MHz, CDCl₃) of 3β-[(2-(Guanidiny)ethyl)carbamoyl]β-sitosterol (**3A**)

¹³C WR-NMR186 Chopaka CT(1)-66 (9.1 mg, CDCl₃ + 3 drops of CD₃OD)



¹³C NMR Spectrum (100 MHz, CDCl₃) of 3β-[(2-(Guanidiny)ethyl)carbamoyl]β-sitosterol (**3A**)

Mass Spectrum SmartFormula Report

Analysis Info

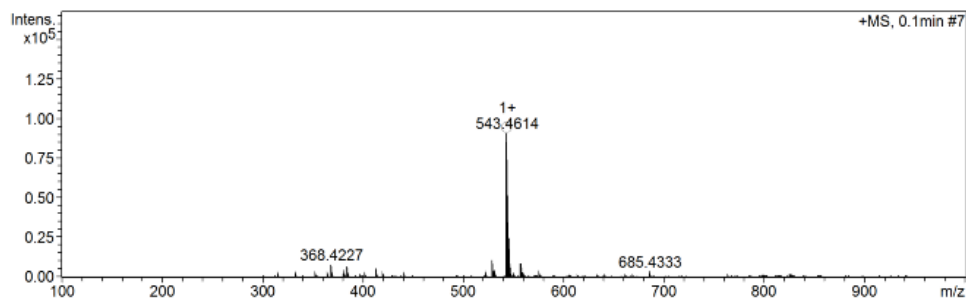
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Operator RU
Instrument micrOTOF 8213750.10411

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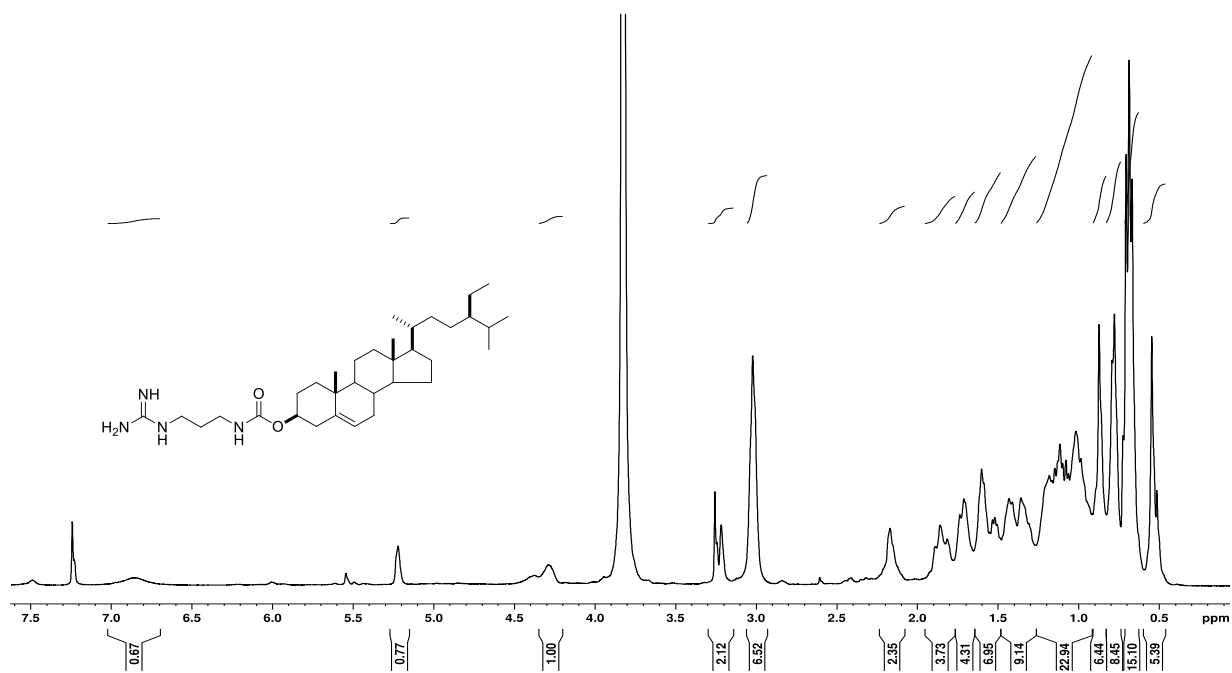
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Meas. m/z	#	Ion Formula	m/z	err [mDa]	err [ppm]	Mean err [ppm]	rdb	N-Rule	e ⁻ Conf	mSigma
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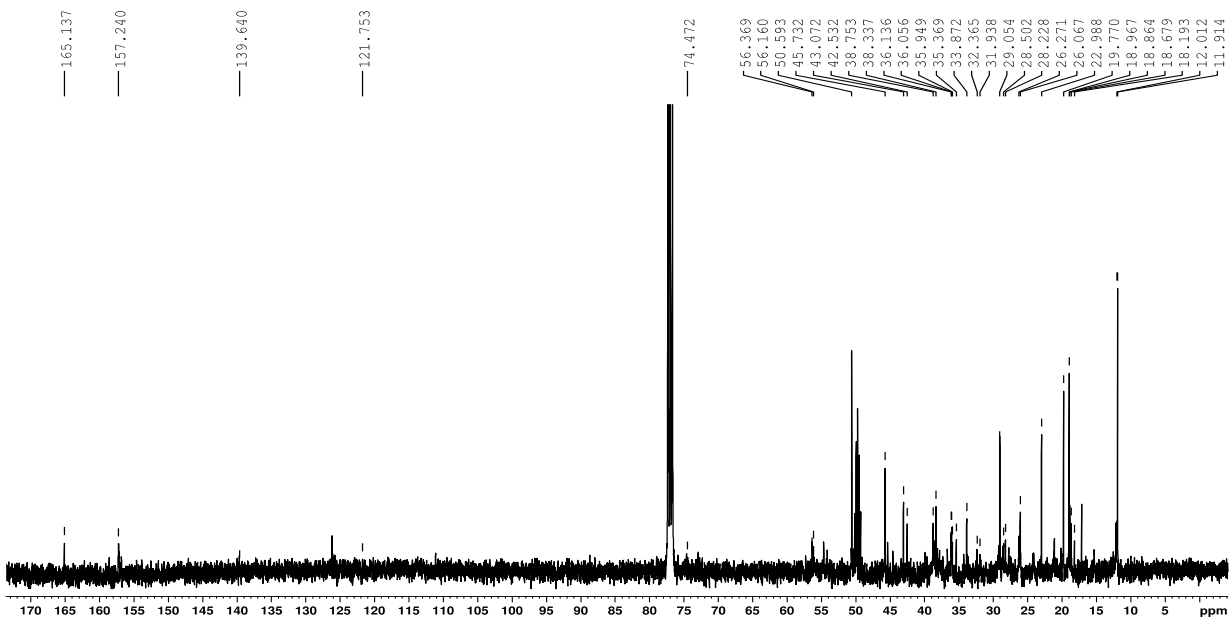
HR-ESI-MS Spectrum of 3β-[(2-(Guanidiny)ethyl)carbamoyl]β-sitosterol (**3A**)

WR-NMR218 Chopaka CT(1)-67 (20.6 mg, CDCl₃ + 10 drops of CD₃OD)



¹H NMR Spectrum (400 MHz, CDCl₃) of 3β-[(3-(Guanidiny)propyl)carbamoyl]β-sitosterol (**7A**)

13C WR-NMR128 Chopaka CT(1)-67 (20.6 mg, CDCl₃ + 10 drops of CD₃OD)



¹³C NMR Spectrum (100 MHz, CDCl₃) of 3β-[(3-(Guanidiny)propyl)carbamoyl]β-sitosterol (**7A**)

Mass Spectrum SmartFormula Report

Analysis Info

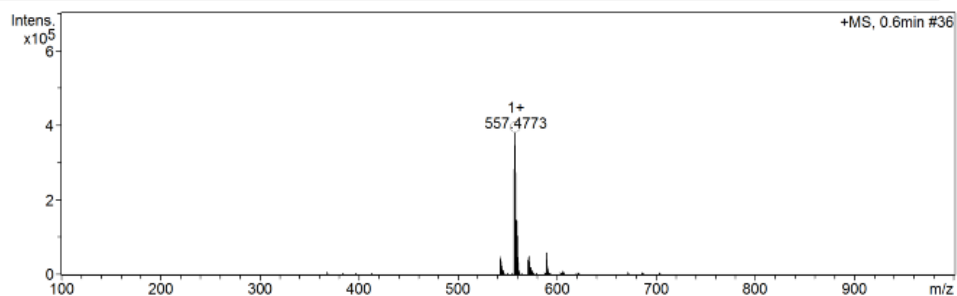
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Operator RU
Instrument micrOTOF 8213750.10411

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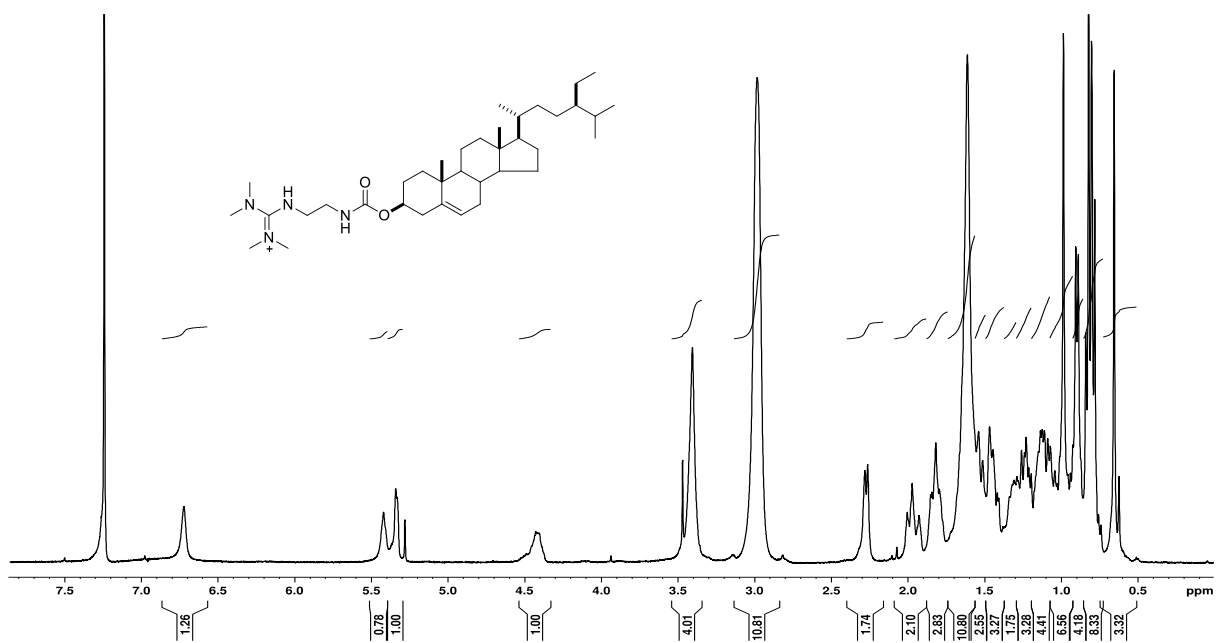
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Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



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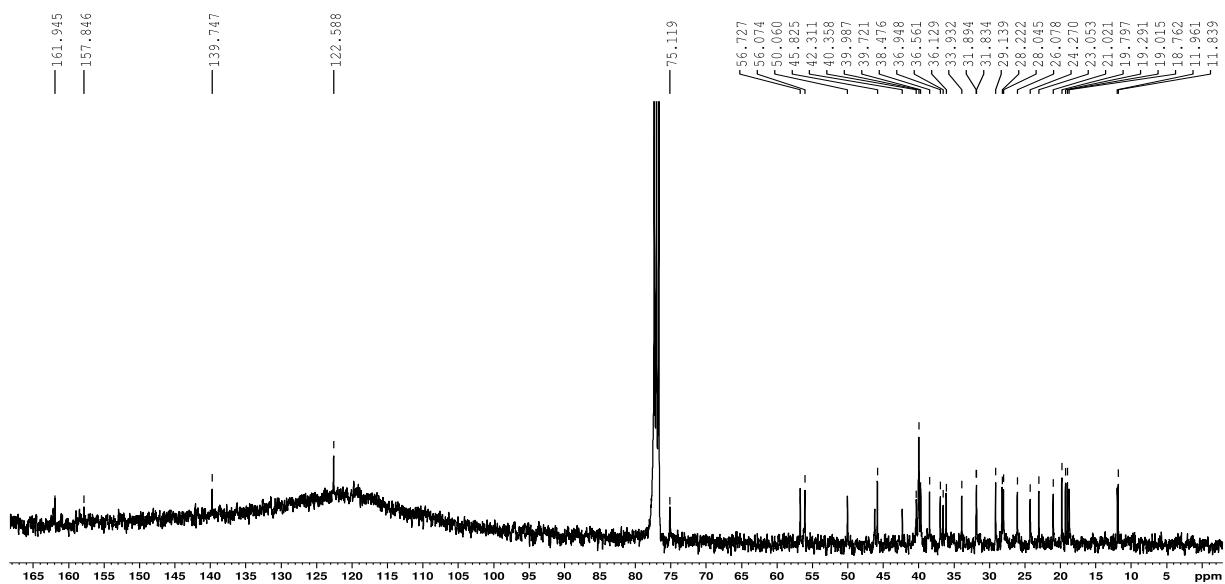
HR-ESI-MS Spectrum of 3 β -[(3-(Guanidinyl)propyl)carbamoyl] β -sitosterol (**7A**)

WR-NMR182 Chopaka CT(1)-62 (10 mg, CDCl₃)



¹H NMR Spectrum (400 MHz, CDCl₃) of 3β-[(2-((*N'*, *N'*, *N''*, *N''*-Tetramethyl)guanidiny) ethyl)carbamoyl]β-sitosterol (**4A**)

13C WR-NMR182 Chopaka CT(1)-62 (10 mg, CDCl₃)



¹³C NMR Spectrum (100 MHz, CDCl₃) of 3β-[(2-((*N'*, *N'*, *N''*, *N''*-Tetramethyl)guanidiny) ethyl)carbamoyl]β-sitosterol (**4A**)

Mass Spectrum SmartFormula Report

Analysis Info

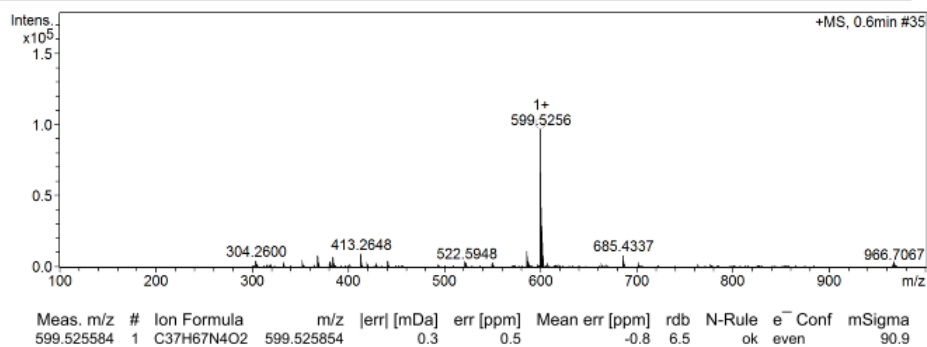
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Operator RU
Instrument micrOTOF 8213750.10411

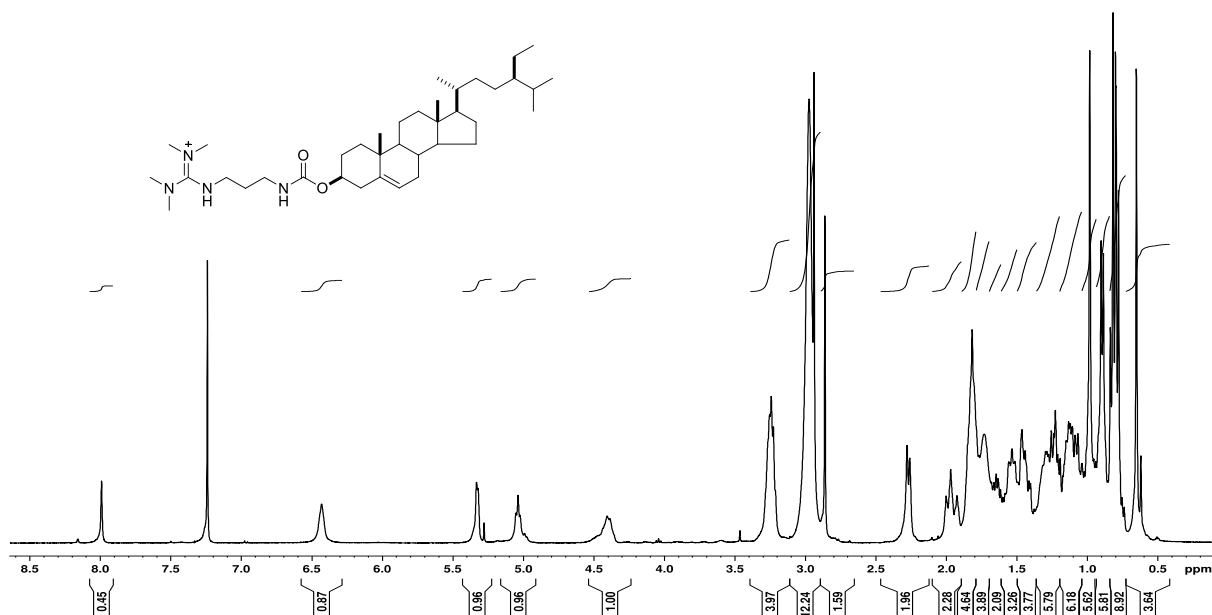
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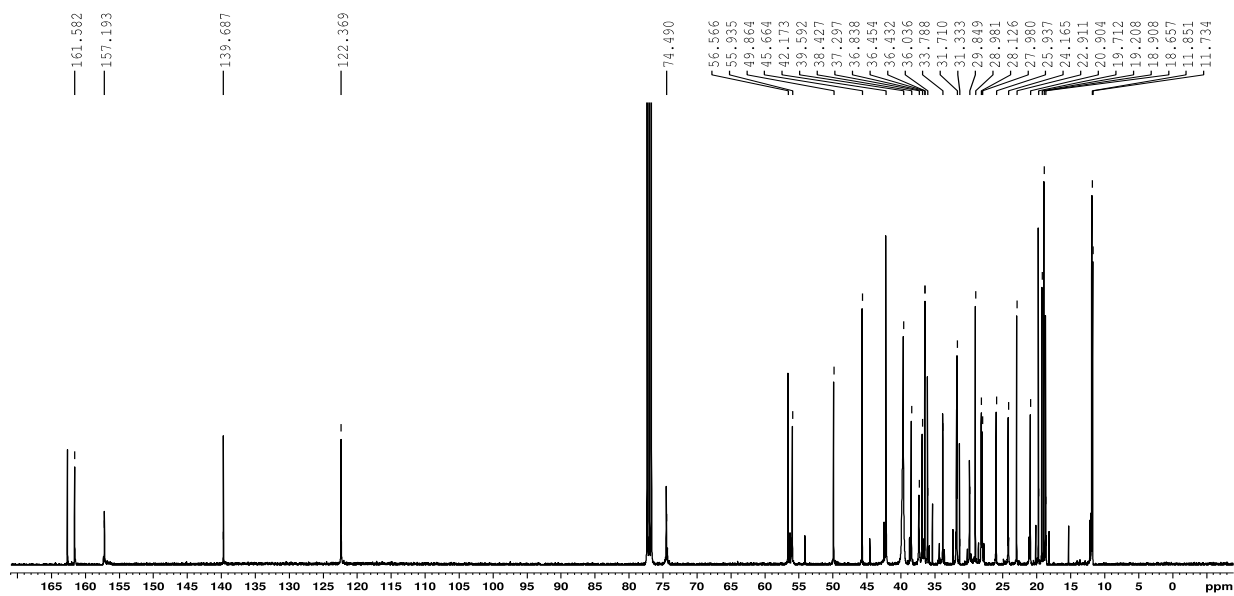
HR-ESI-MS Spectrum of 3β -[(2-((*N'*, *N'*, *N''*, *N''*-Tetramethyl)guanidinyl) ethyl)carbamoyl] β -sitosterol (**4A**)

WR-NMR183 Chopaka CT(1)-63 (9.8 mg, CDCl₃)



¹H NMR Spectrum (400 MHz, CDCl₃) of 3β-[(3-((N', N', N'', N''-Tetramethyl)guanidiny)propyl)carbamoyl]β-sitosterol (**8A**)

¹³C WR-NMR183 Chopaka CT(1)-63 (9.8 mg, CDCl₃)



¹³C NMR Spectrum (100 MHz, CDCl₃) of 3β-[(3-((N', N', N'', N''-Tetramethyl)guanidiny)propyl)carbamoyl]β-sitosterol (**8A**)

Mass Spectrum SmartFormula Report

Analysis Info

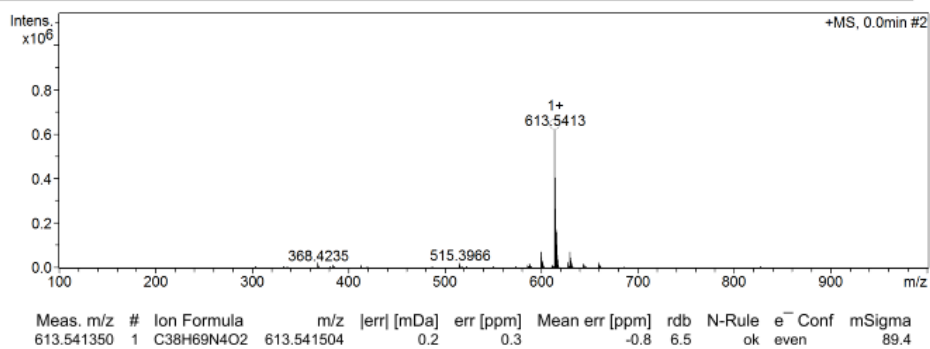
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Sample Name CT (1)-63
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Operator RU
Instrument micrOTOF 8213750.10411

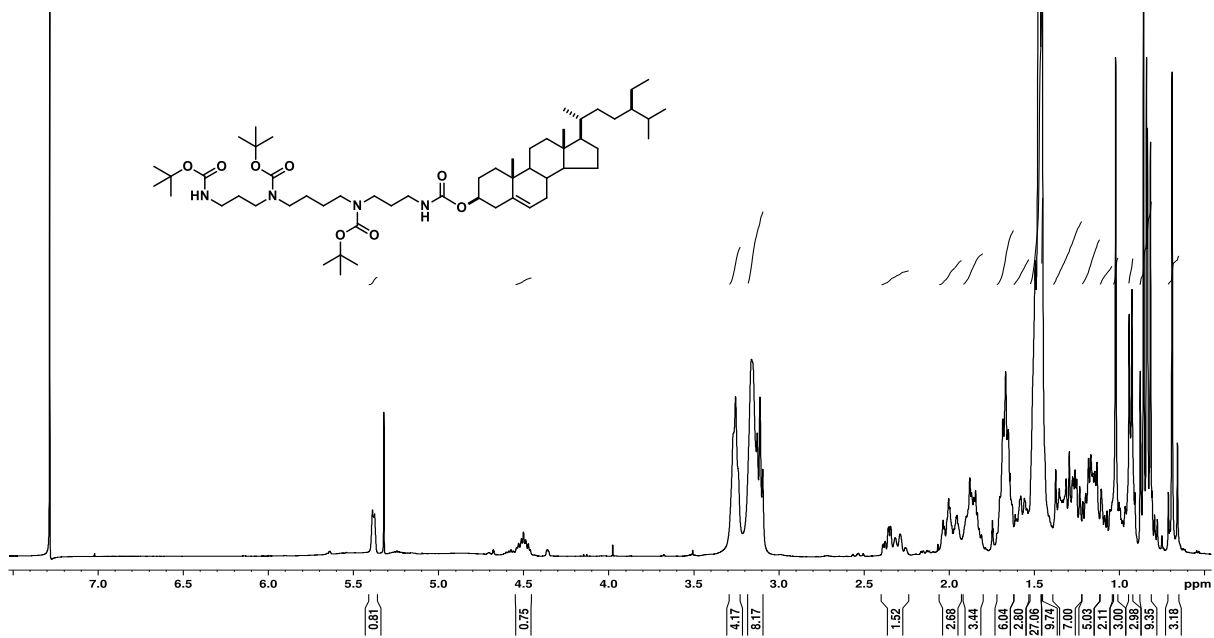
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Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste

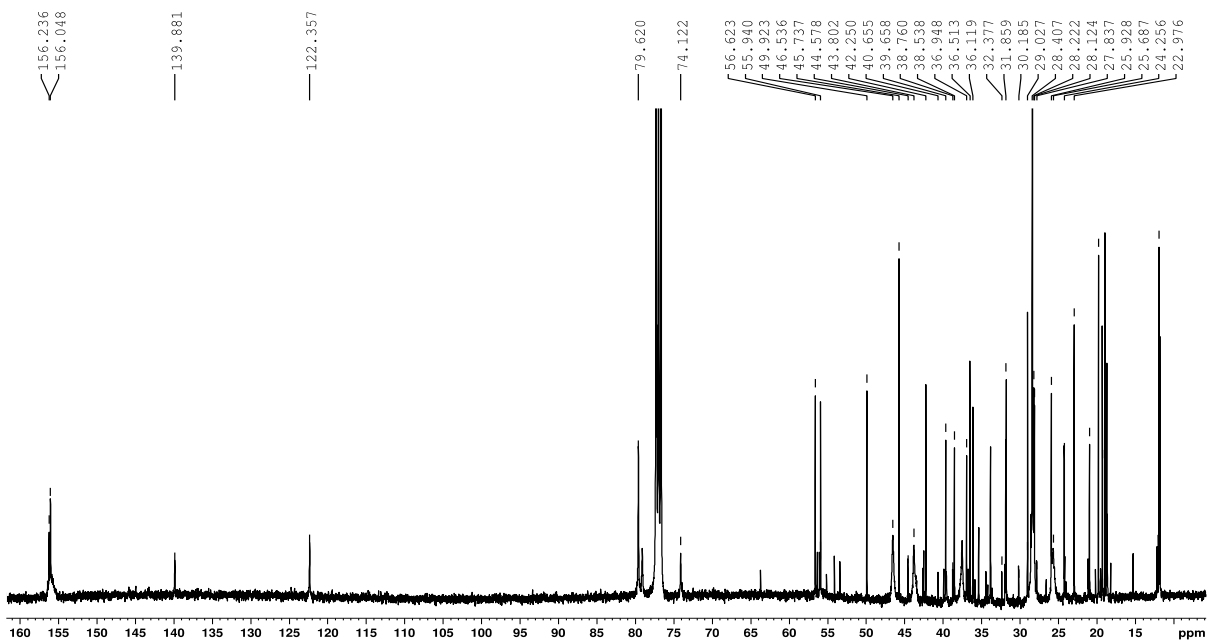


HR-ESI-MS Spectrum of 3 β -[(3-((*N'*, *N'*, *N''*, *N''*-Tetramethyl)guanidiny) propyl)carbamoyl] β -sitosterol (**8A**)

WR-NMR225 Chopaka CT(1)-93 (50.7 mg, CDC13)



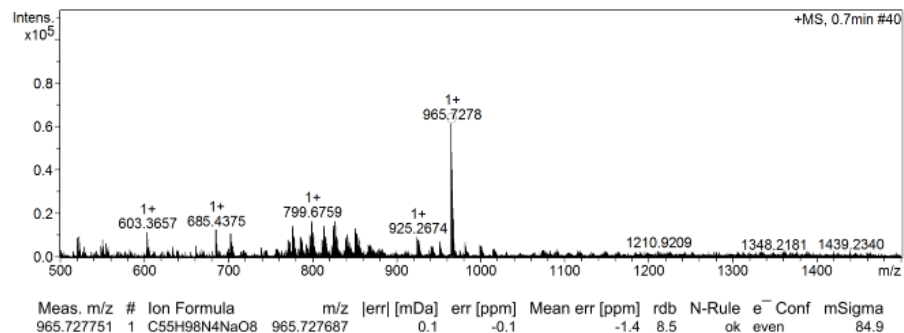
WR-NMR225 Chopaka CT(1)-93 (50.7 mg, CDC13)



Mass Spectrum SmartFormula Report

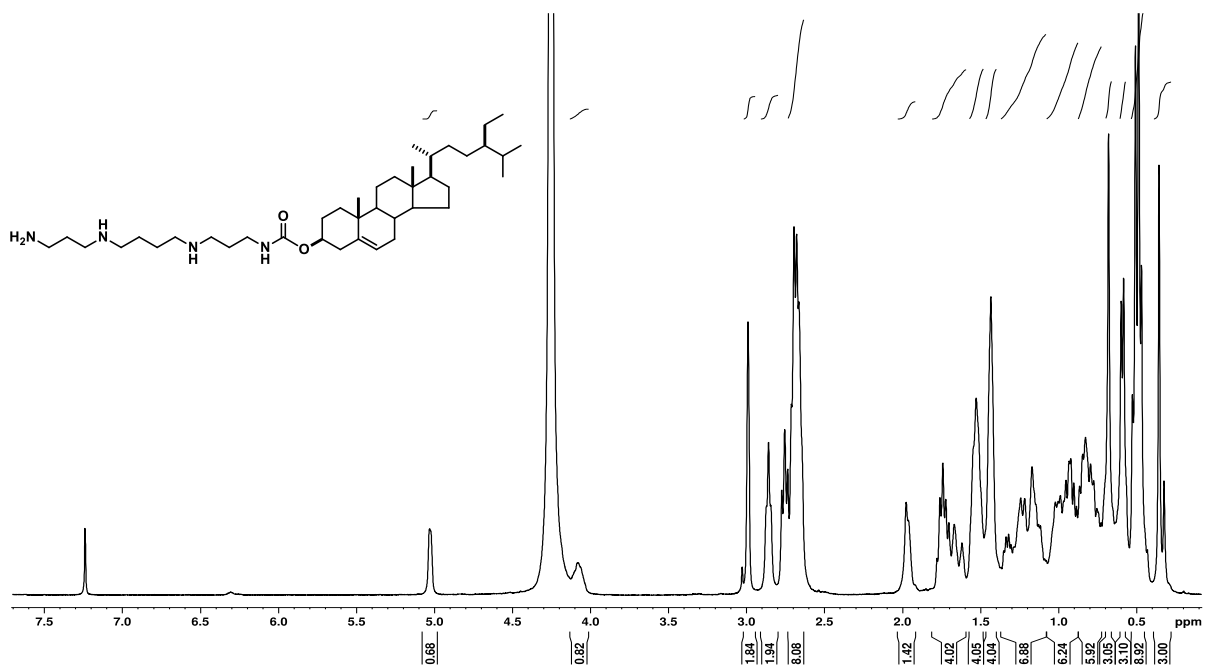
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Method tune_wide2.m Operator RU
Sample Name CT(1) -93 Instrument micrOTOF 8213750.10411
Comment

Acquisition Parameter
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Scan Begin 50 m/z Set Capillary 4500 V Set Dry Gas 4.0 l/min
Scan End 3000 m/z Set End Plate Offset -500 V Set Divert Valve Waste



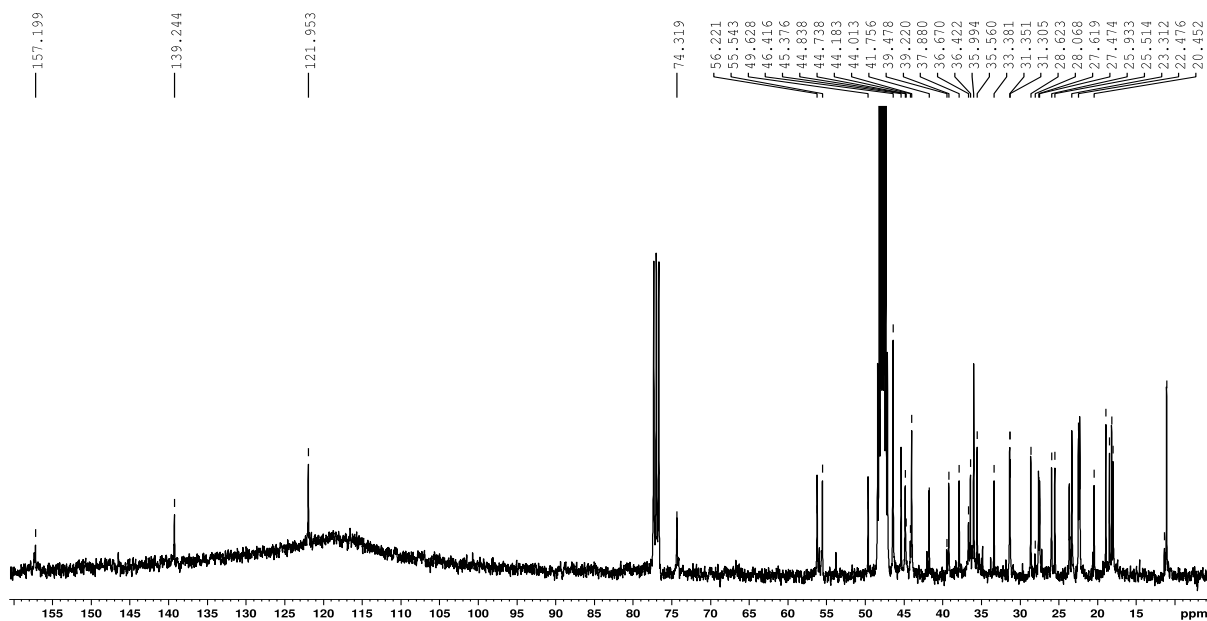
HR-ESI-MS Spectrum of 3β -[(N^5 , N^{10} , N^{14} -Tri(*tert*-butyloxycarbonyl-(N^1 -sperminyl)))
carbonyloxy] β -sitosterol (**13**)

WR-NMR224 Chopaka CT(1)-93-2F1 (24.9 mg, CDCl₃ + CD₃OD)



¹H NMR Spectrum (400 MHz, CDCl₃) of 3β-[(N¹-Sperminyl)carbonyloxy]β-sitosterol (1B)

WR-NMR224 Chopaka (24.9 mg, CDCl₃ + CD₃OD)



¹³C NMR Spectrum (100 MHz, CDCl₃) of 3β-[(N¹-Sperminyl)carbonyloxy]β-sitosterol (1B)

Mass Spectrum SmartFormula Report

Analysis Info

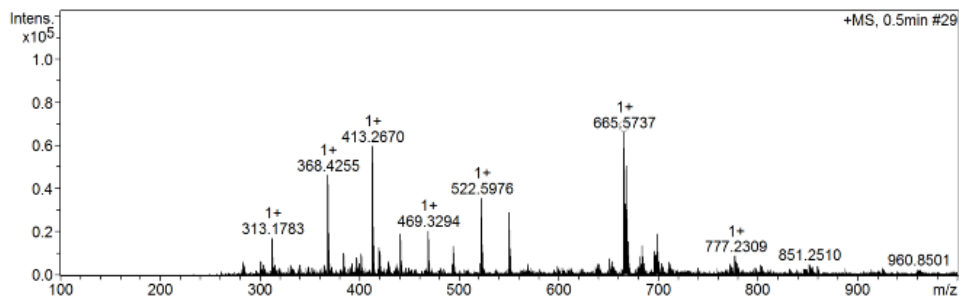
Analysis Name D:\Data\Boon-ek\ESI\BY-HRMS 236 (pos).d
 Method tune_wide1.m
 Sample Name CT(1)-93-2F1
 Comment

Acquisition Date 11/23/2016 4:18:18 PM

Operator RU
 Instrument micrOTOF 8213750.10411

Acquisition Parameter

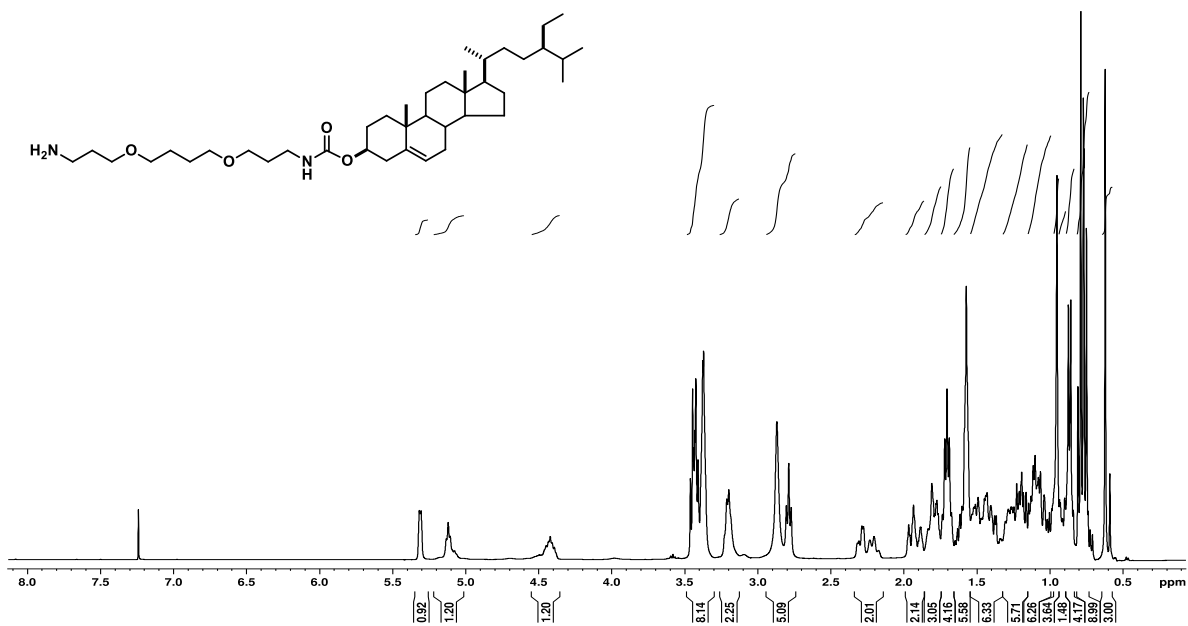
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.5 Bar
Focus	Not active			Set Dry Heater	180 °C
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



Meas. m/z # Ion Formula	m/z	err [ppm]	Mean rdb err [ppm]	N-Rule e ⁻ Conf	mSigm	Std I a	Std Mean m/z	Std VarNo m	Std m/z Diff	Std Comb Dev	
665.573727 1 C40H74N4NaO2	665.570398	5.0	-12.7	5.5	ok even	381.6	379.9	9.7	170.2	6.5	891.3

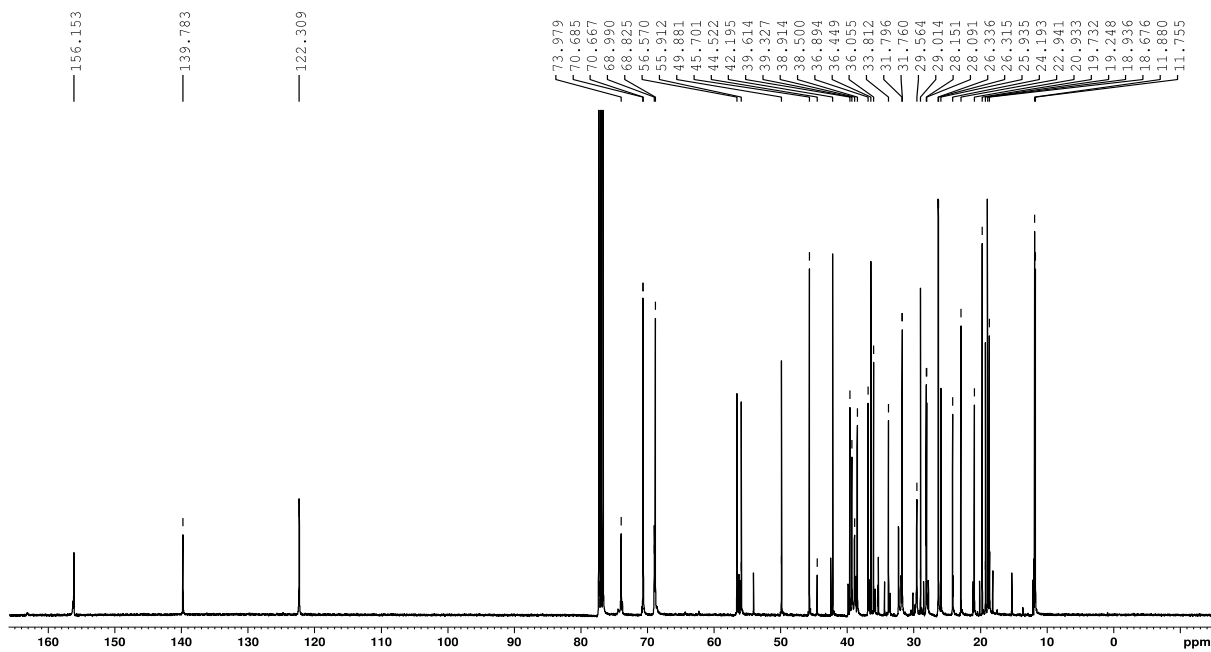
HR-ESI-MS Spectrum of 3β-[(N¹-Sperminyl)carbonyloxy]β-sitosterol (**1B**)

1R-NMR251 Chopaka CT(1)-100 (20.3 mg, CDCl₃)



^1H NMR Spectrum (400 MHz, CDCl_3) of 3β -[N^1 -(1,4-Bis(3-aminopropoxy)butyl)carbonyloxy]
 β -sitosterol (**2B**)

1R-NMR251 Chopaka CT(1)-100 (20.3 mg, CDCl₃)



^{13}C NMR Spectrum (100 MHz, CDCl_3) of 3β -[N^1 -(1,4-Bis(3-aminopropoxy)butyl)carbonyloxy]
 β -sitosterol (**2B**)

Mass Spectrum SmartFormula Report

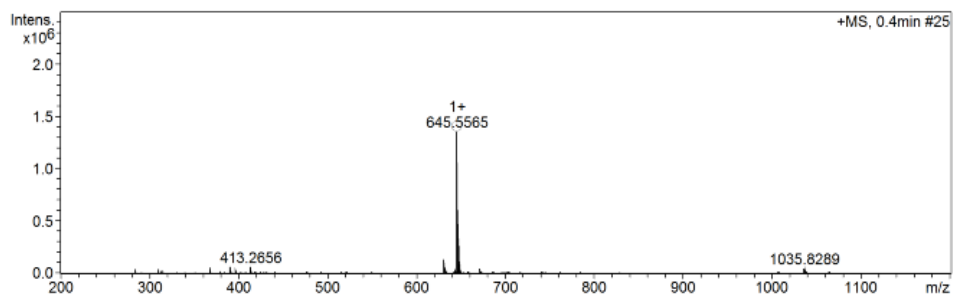
Analysis Info

Analysis Name D:\Data\Boon-ek\ESI\BY-HRMS 356 (pos).d
Method tune_wide 4 19-6-60.m
Sample Name CT(1)-100
Comment

Acquisition Date 7/20/2017 4:14:03 PM
Operator RU
Instrument micrOTOF 8213750.10411

Acquisition Parameter

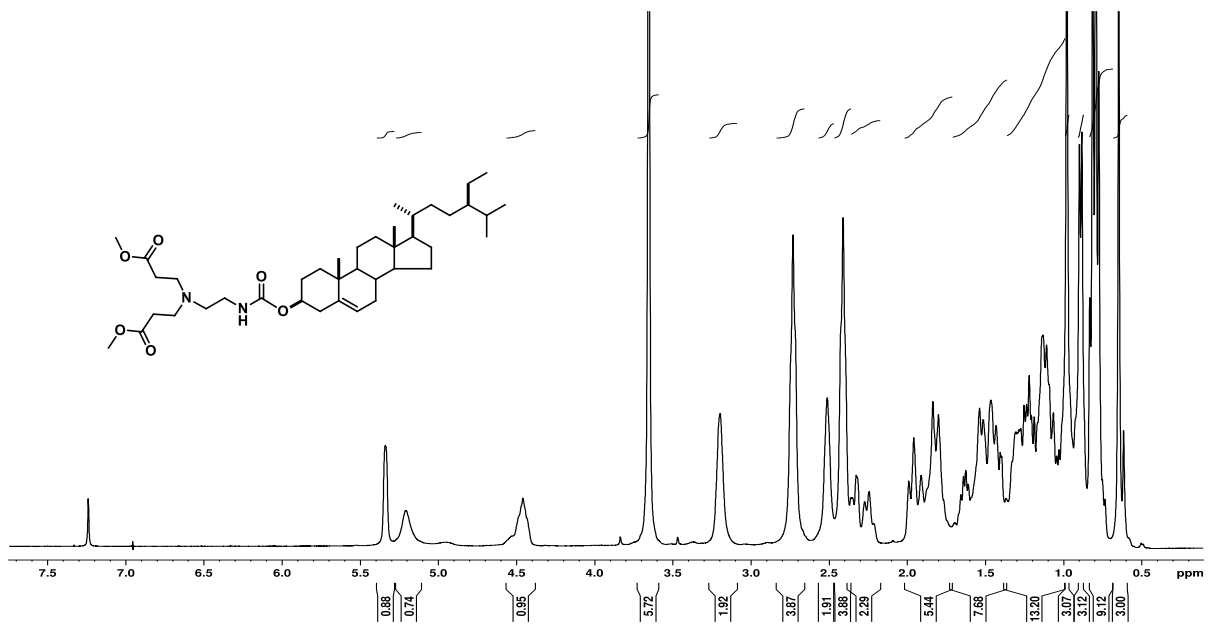
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.5 Bar
Focus	Not active			Set Dry Heater	180 °C
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



Meas. m/z	#	Ion Formula	m/z	[err] [mDa]	err [ppm]	Mean err [ppm]	rdb	N-Rule	e ⁻ Conf	mSigma
645.556454	1	C ₄₀ H ₇₃ N ₂ O ₄	645.556485	0.0	-0.0	-1.1	5.5	ok	even	77.7

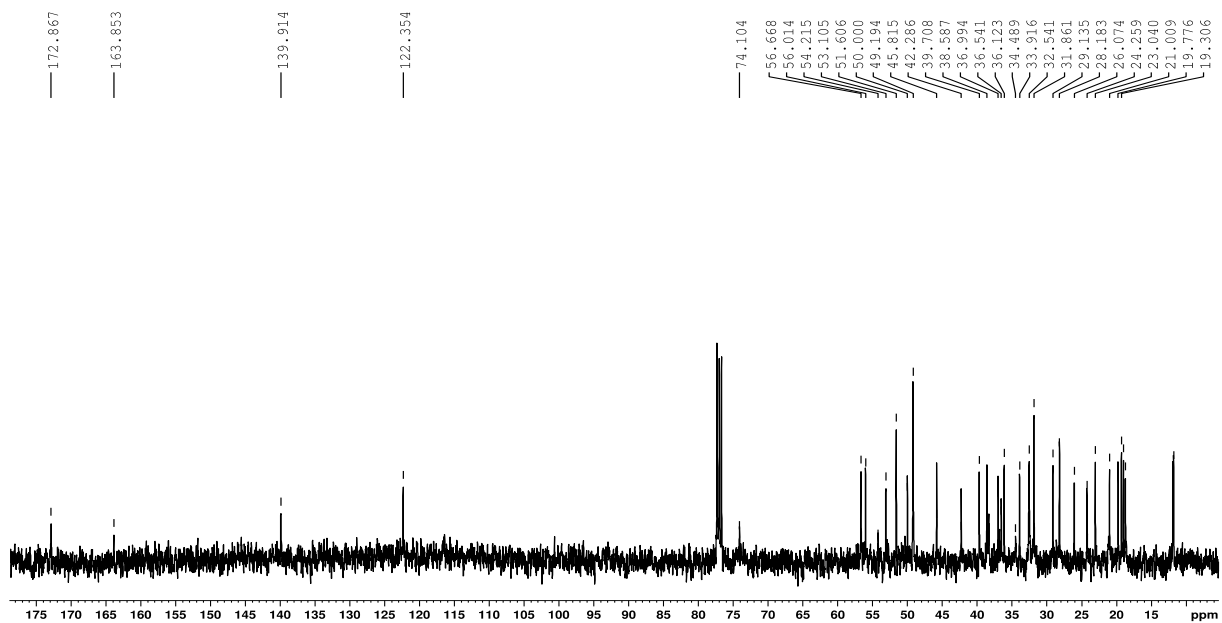
HR-ESI-MS Spectrum of 3β -[*N*¹-(1,4-Bis(3-aminopropoxy)butyl)carbonyloxy] β -sitosterol (**2B**)

WR-NMR226 Chopaka CT(1)-96 (42.2 mg, CDCl₃)



¹H NMR Spectrum (400 MHz, CDCl₃) of 3β-[N-(2-(N,N-Bis(methoxycarbonyl)ethyl)aminoethyl)carbamoyl]β-sitosterol (**14**)

WR-NMR226 Chopaka CT(1)-96 (42.2 mg, CDCl₃)



¹³C NMR Spectrum (400 MHz, CDCl₃) of 3β-[N-(2-(N,N-Bis(methoxycarbonyl)ethyl)aminoethyl)carbamoyl]β-sitosterol (**14**)

Mass Spectrum SmartFormula Report

Analysis Info

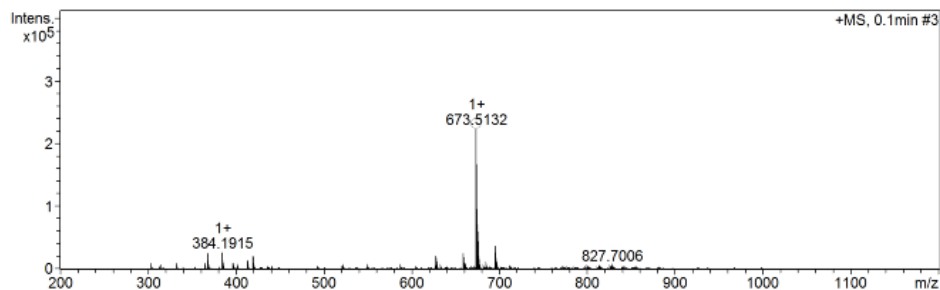
Analysis Name D:\Data\Boon-ek\ESI\BY-HRMS 210 (pos).d
Method tune_wide2.m
Sample Name CT(1) -96
Comment

Acquisition Date 7/1/2016 12:47:51 PM

Operator RU
Instrument micrOTOF 8213750.10411

Acquisition Parameter

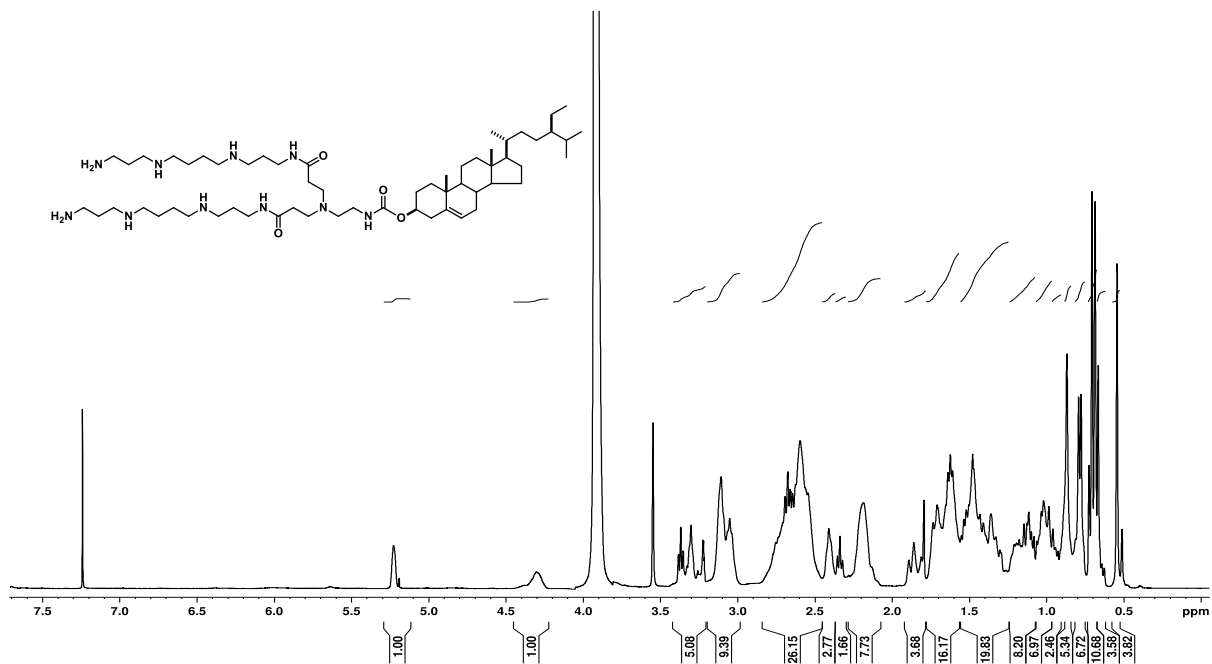
Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.5 Bar
Focus	Not active			Set Dry Heater	180 °C
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



Meas. m/z	#	Ion Formula	m/z	err [mDa]	err [ppm]	Mean err [ppm]	rdb	N-Rule	e ⁻ Conf	mSigma
673.513240	1	C40H69N2O6	673.515014	1.8	-2.6	1.0	7.5	ok	even	86.3

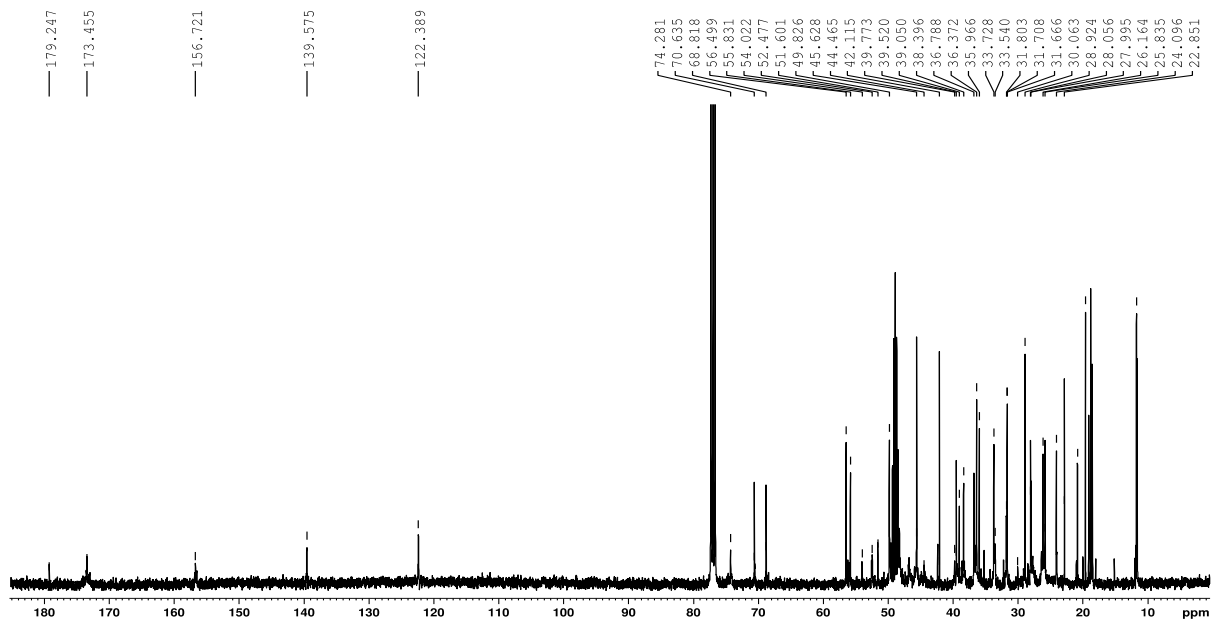
HR-ESI-MS Spectrum of 3 β -[*N*-(2-(*N,N*-Bis(methoxycarbonyl)ethyl)aminoethyl)carbamoyl] β -sitosterol (**14**)

WR-NMR285 Chopaka CT(1)-109/2 (20.5 mg, CDCl₃ + CD₃OD)



¹H NMR Spectrum (400 MHz, CDCl₃) of 3β[*N*-(2-(*N,N*-Bis(ethylcarbonyl(*N*¹-sperminyl))) aminoethyl)carbamoyl]β-sitosterol (**3B**)

WR-NMR285 Chopaka CT(1)-109/2 (20.5 mg, CDCl₃ + CD₃OD)



¹³C NMR Spectrum (100 MHz, CDCl₃) of 3β[*N*-(2-(*N,N*-Bis(ethylcarbonyl(*N*¹-sperminyl))) aminoethyl)carbamoyl]β-sitosterol (**3B**)

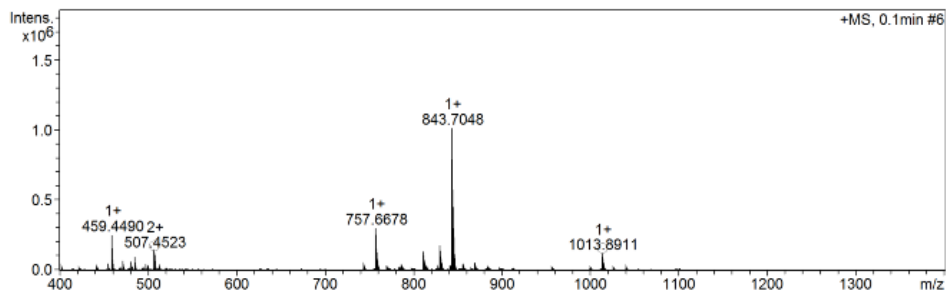
Mass Spectrum SmartFormula Report

Analysis Info

Analysis Name	D:\Data\Boon-ek\ESI\BY-HRMS 358 (pos).d	Acquisition Date	7/20/2017 4:18:03 PM
Method	tune_wide 4 19-6-60.m	Operator	RU
Sample Name	CT(1)-109/2	Instrument	micrOTOF 8213750.10411
Comment			

Acquisition Parameter

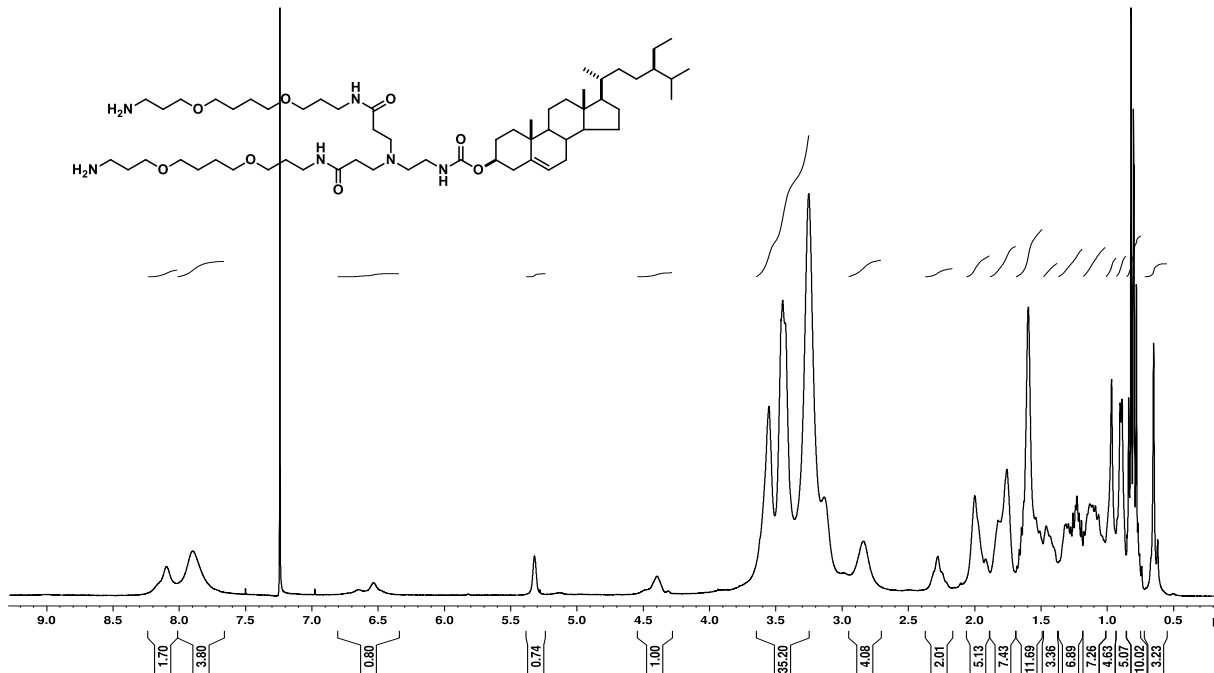
Source Type	ESI	Ion Polarity	Positive
Focus	Not active	Set Nebulizer	0.5 Bar
Scan Begin	50 m/z	Set Dry Heater	180 °C
Scan End	3000 m/z	Set Dry Gas	4.0 l/min
		Set End Plate Offset	-500 V
		Set Divert Valve	Waste



Meas. m/z	#	Ion Formula	m/z	err [mDa]	err [ppm]	Mean err [ppm]	rdb	N-Rule	e ⁻ Conf	mSigma
507.452307	1	C58H114N10O4	507.450678	1.6	-3.2	-3.2	7.0	ok	even	93.9
1013.891099	1	C58H113N10O4	1013.894079	3.0	2.9	3.1	7.5	ok	even	75.5

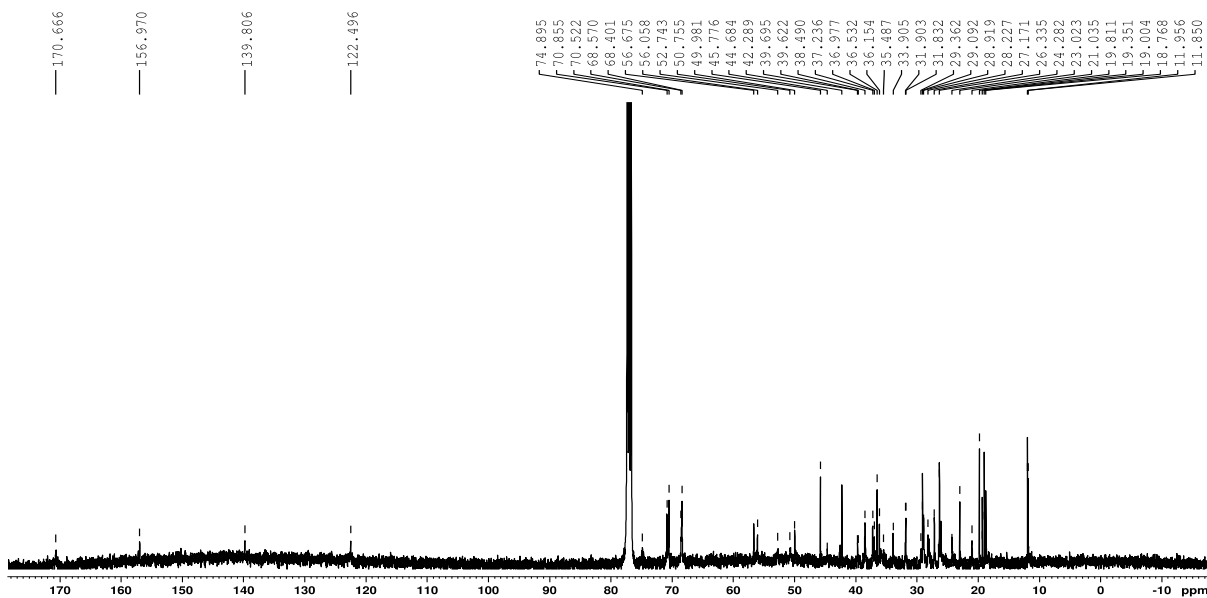
HR-ESI-MS Spectrum of 3β[*N*-(2-(*N,N*-Bis(ethylcarbonyl(*N*¹-sperminyl)))aminoethyl) carbamoyl]β-sitosterol (**3B**)

WR-NMR283 Chopaka CT(1)-107/2 (8 mg, CDC13)



¹H NMR Spectrum (400 MHz, CDCl₃) of 3β[*N*-(2-(*N,N*-Bis(ethylcarbonyl(*N*¹-1,4-bis(3-aminopropoxy)butyl))))aminoethyl) carbamoyl]β-sitosterol (**4B**)

WR-NMR283 Chopaka CT(1)-107/2 (8 mg, CDC13)



¹³C NMR Spectrum (100 MHz, CDCl₃) of 3β[*N*-(2-(*N,N*-Bis(ethylcarbonyl(*N*¹-1,4-bis(3-aminopropoxy)butyl))))aminoethyl) carbamoyl]β-sitosterol (**4B**)

Mass Spectrum SmartFormula Report

Analysis Info

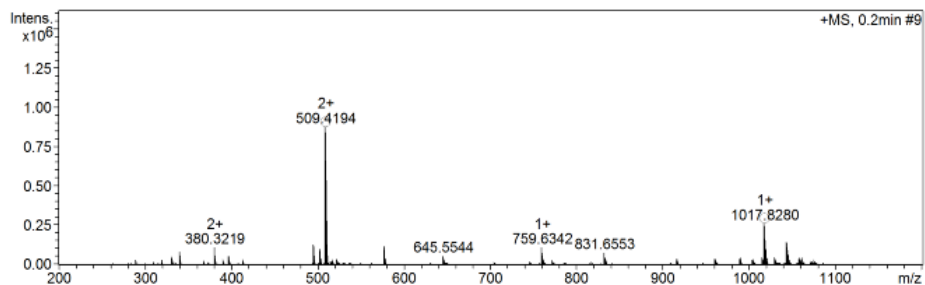
Analysis Name D:\Data\Boon-ek\ESI\BY-HRMS 357 (pos).d
 Method tune_wide 4 19-6-60.m
 Sample Name CT(1)-107/2
 Comment

Acquisition Date 7/20/2017 4:16:03 PM

Operator RU
 Instrument micrOTOF 8213750.10411

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.5 Bar
Focus	Not active			Set Dry Heater	180 °C
Scan Begin	50 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Waste



Meas. m/z	#	Ion Formula	m/z	err [mDa]	err [ppm]	Mean err [ppm]	rdb	N-Rule	e ⁻	Conf	mSigma
509.419393	1	C58H110N6O8	509.418709	0.7	-1.3	-1.8	7.0	ok	even		86.7
1017.828011	1	C58H109N6O8	1017.830141	2.1	-2.1	0.8	7.5	ok	even		78.0

HR-ESI-MS Spectrum of 3β [*N*-(2-(*N,N*-Bis(ethylcarbonyl(*N*¹-1,4-bis(3-aminopropoxy)butyl)))) aminoethyl)carbamoyl] β -sitosterol (**4B**)