Supplementary Data

Efficient synthesis of oligonucleotides carrying amino lipid groups at the 3'-end for RNA interference studies

Santiago Grijalvo,[†] Sandra M. OCampo,[†] José C. Perales[‡] and Ramón Eritja[*†]

[†] *Institute for Research in Biomedicine (IRB Barcelona); Networking Center on Bioengineering , Biomaterials and Biomedicine (CIBER-BBN) and Institute for Advanced Chemistry of Catalonia (IQAC), Spanish Research Council (CSIC); Cluster Building, Baldiri Reixac 10, E-08028 Barcelona, Spain,* [‡] *Biophysics Unit, Department of Physiological Sciences II, Universitat de Barcelona; Feixa Llarga s/n, E-08907 L'Hospitalet del Llobregat, Barcelona, Spain*

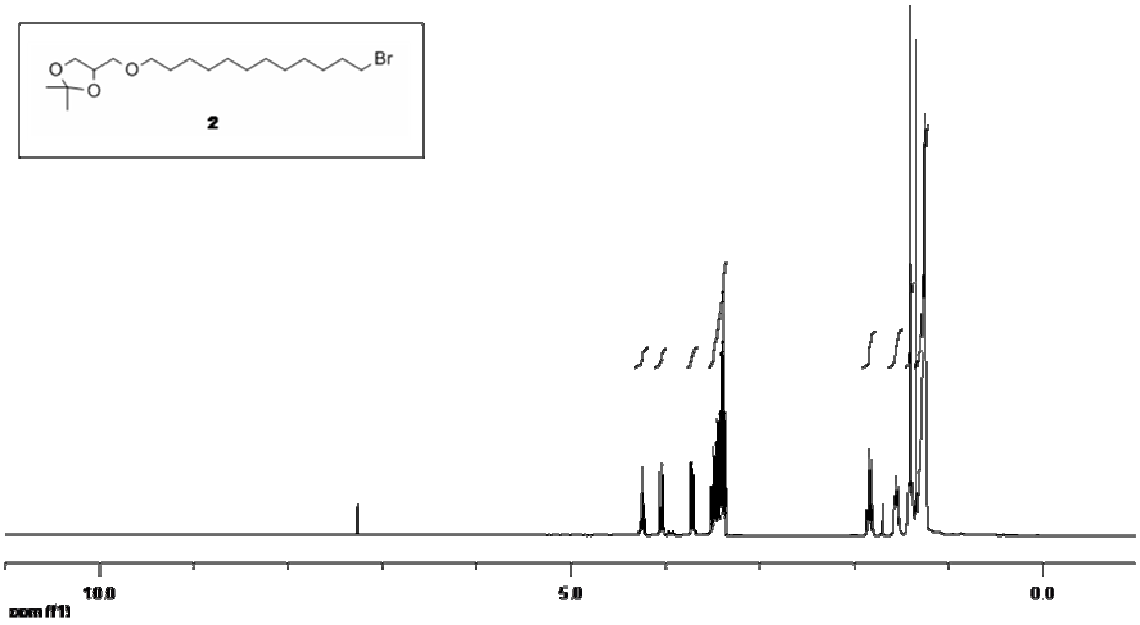
INDEX

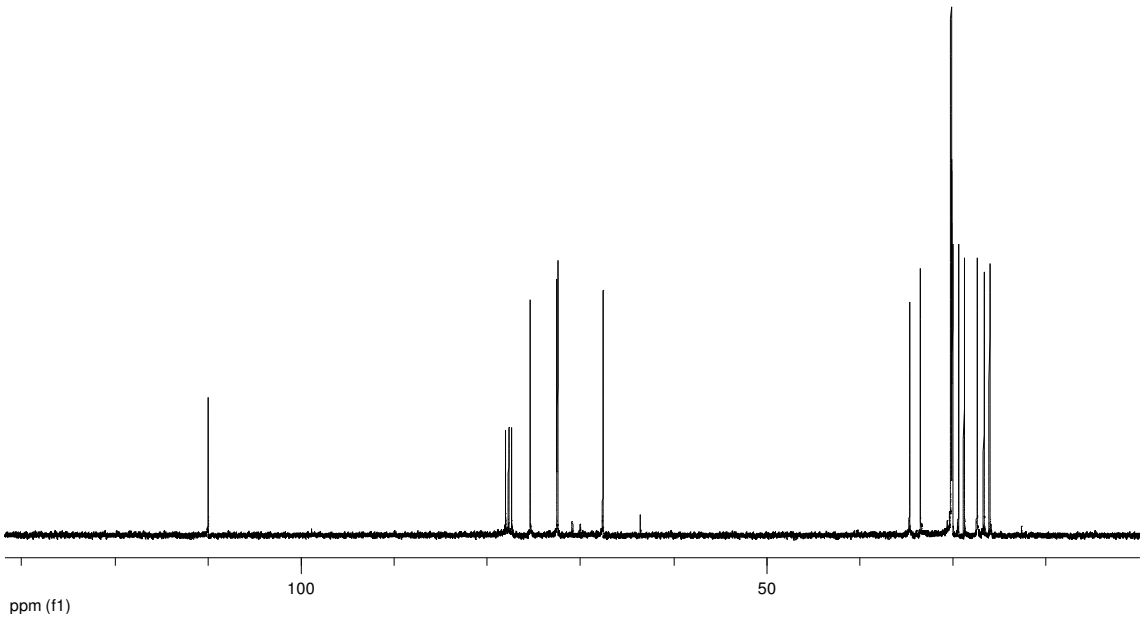
Characterization new compounds S-3

Oligodeoxyribonucleotide synthesis. MALDI-TOF mass spectrometry and Melting Temperatures S-16

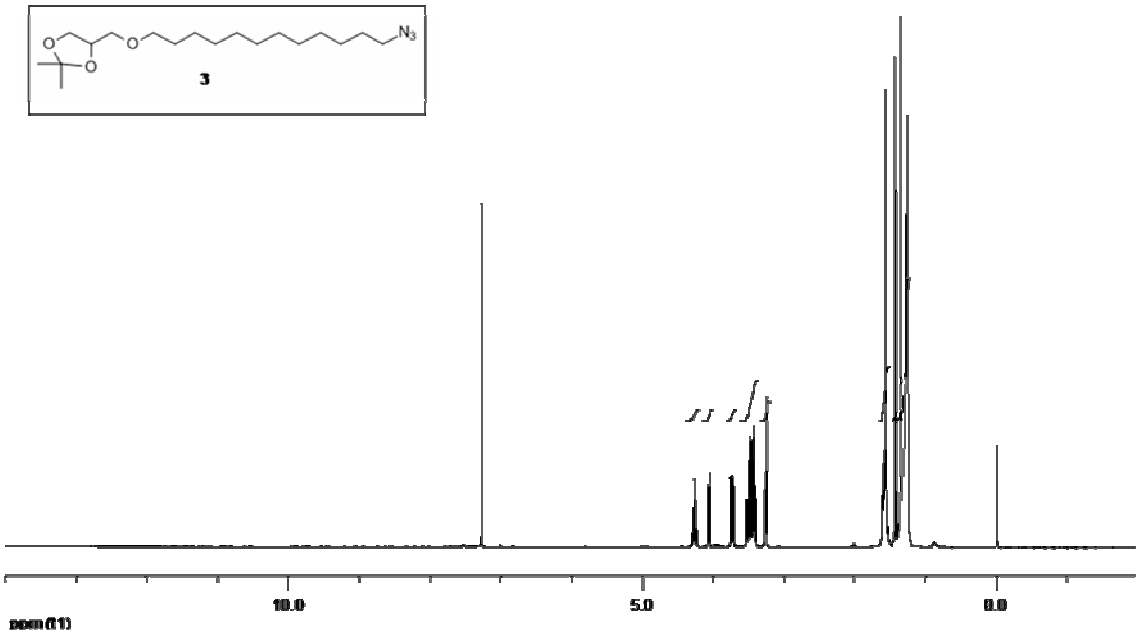
Oligoribonucleotide synthesis. MALDI-TOF mass spectrometry S-19

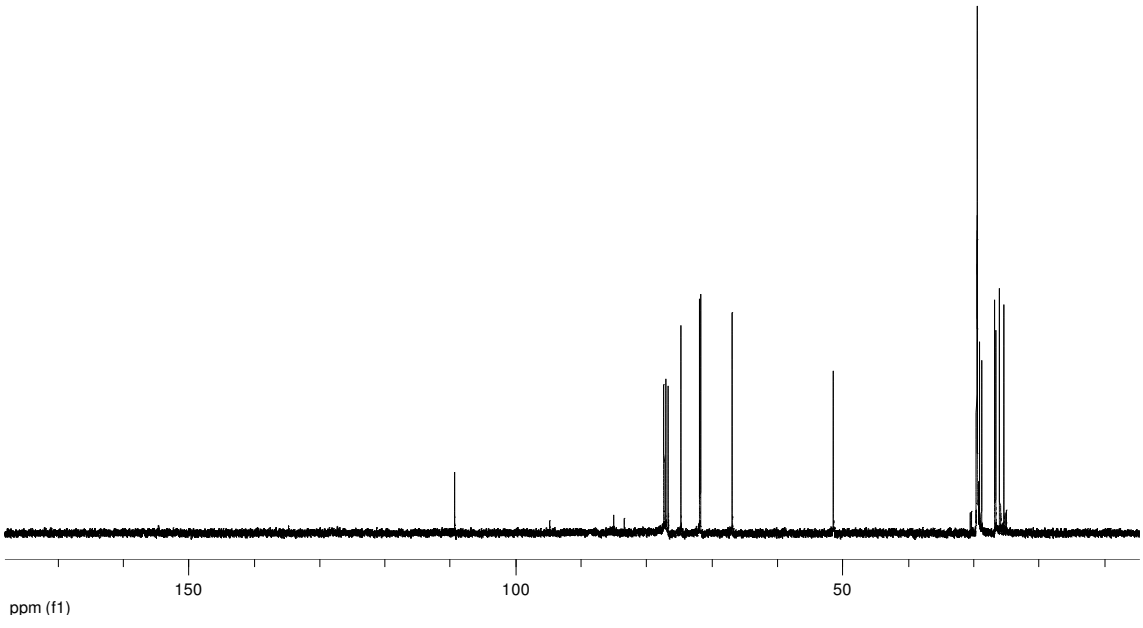
4-((12-bromododecyloxy)methyl)-2,2-dimethyl-1,3-dioxolane, 2



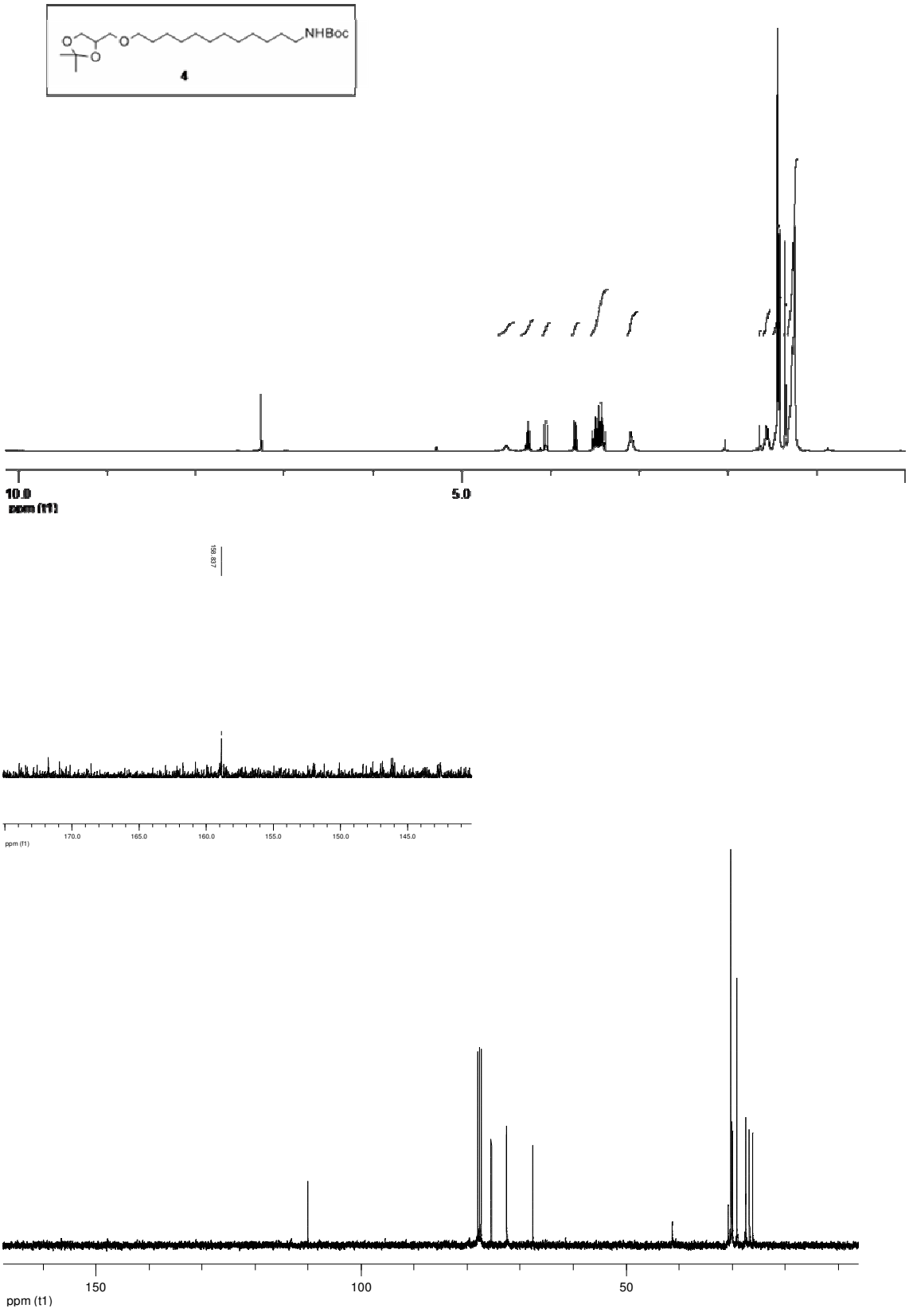


4-((12-azidododecyloxy)methyl)-2,2-dimethyl-1,3-dioxolane, 3

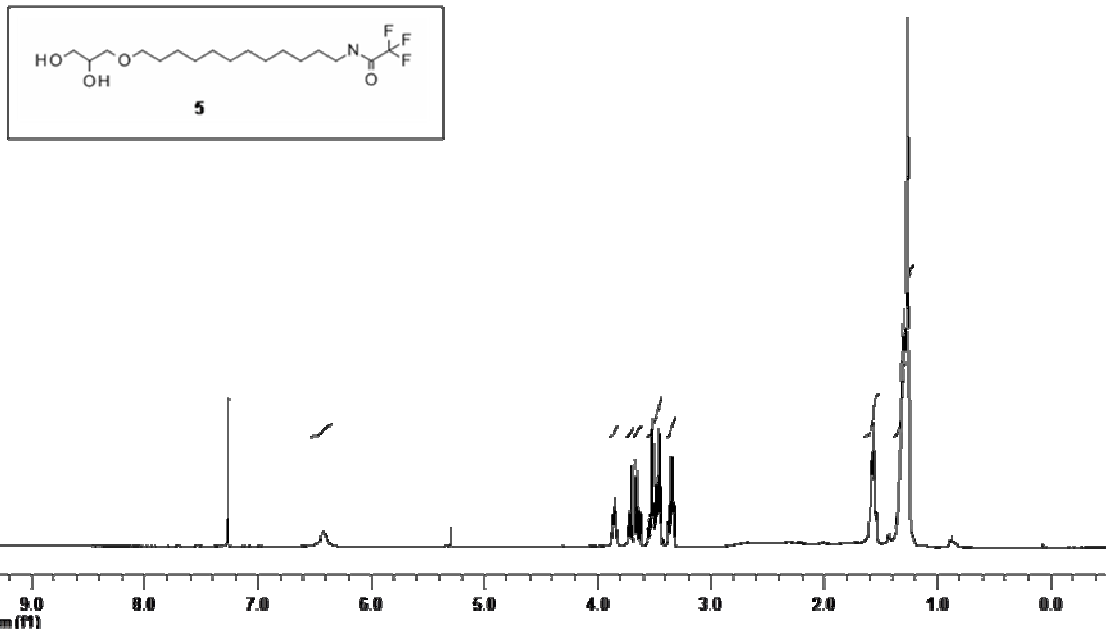


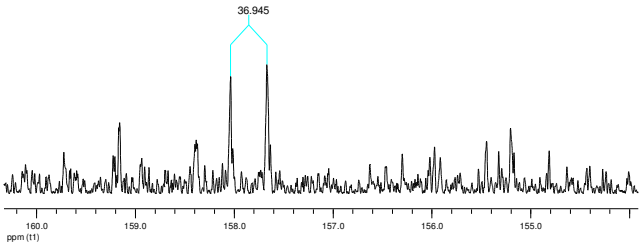


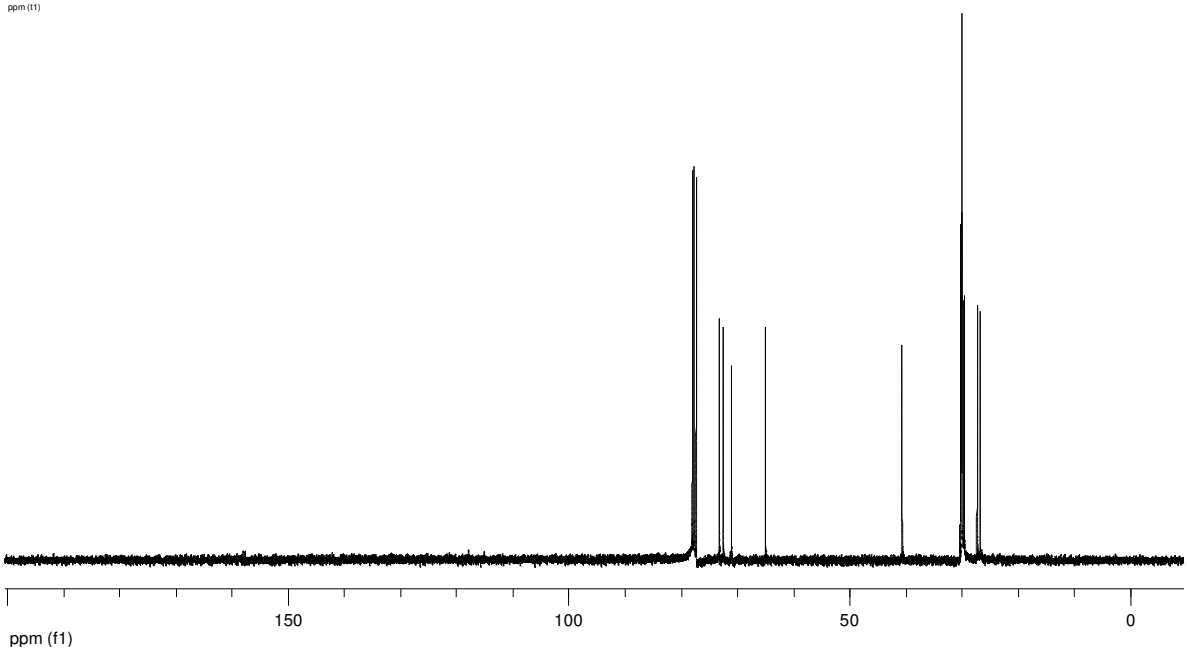
***tert*-butyl 12-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)dodecylcarbamate, 4**

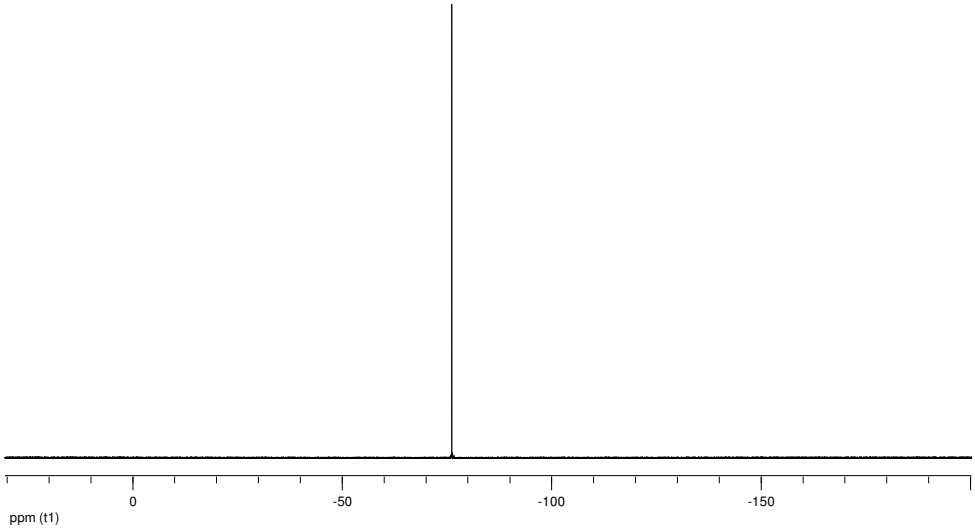


***N*-(12-(2,3-dihydroxypropoxy)dodecyl)-2,2,2-trifluoroacetamide, 5**

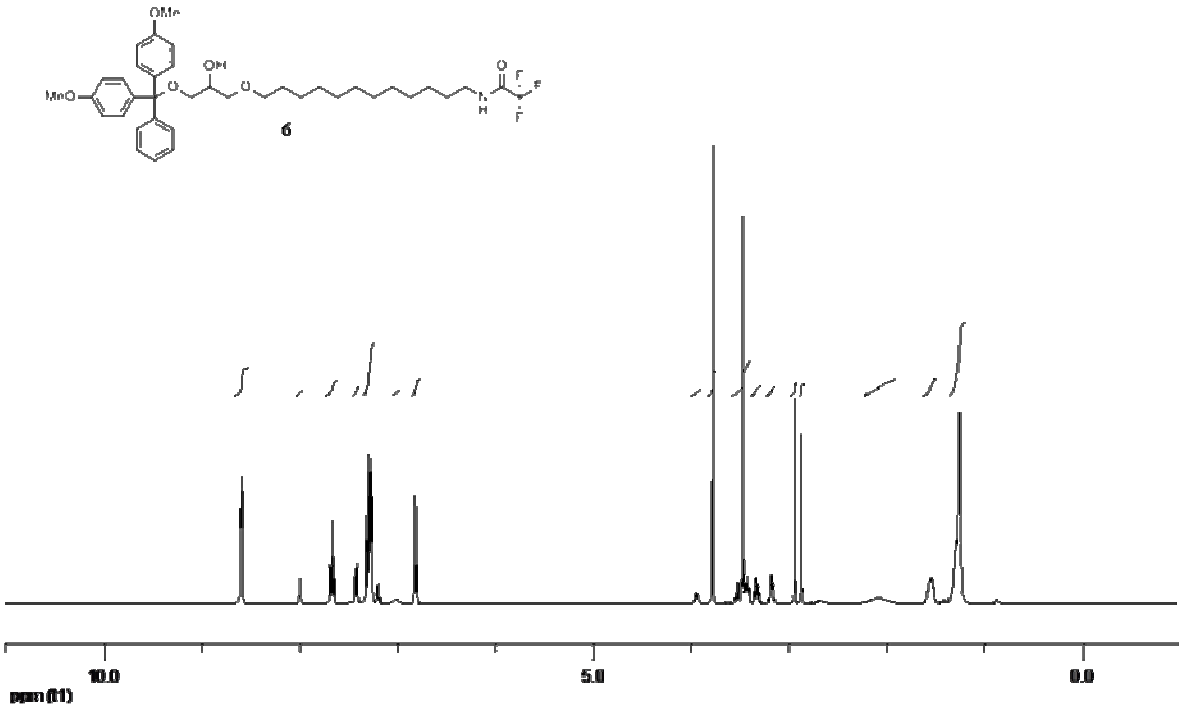


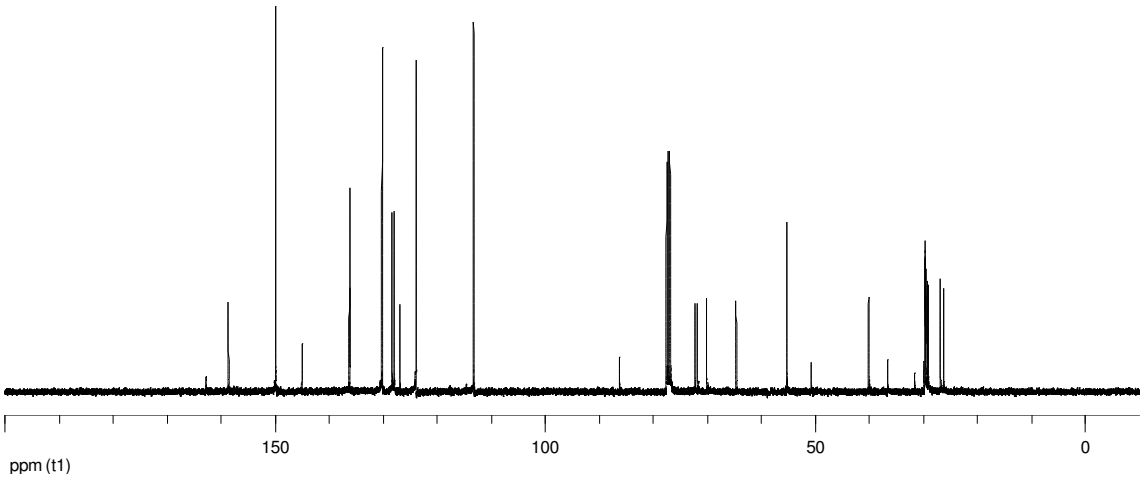




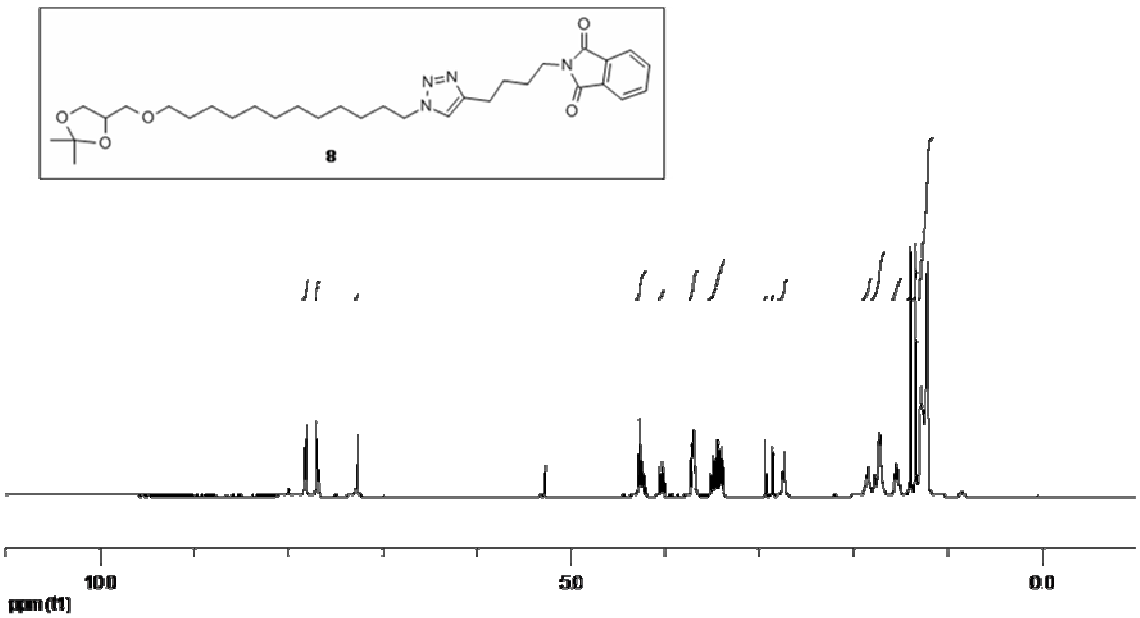

0
-50
-100
-150
ppm (t1)

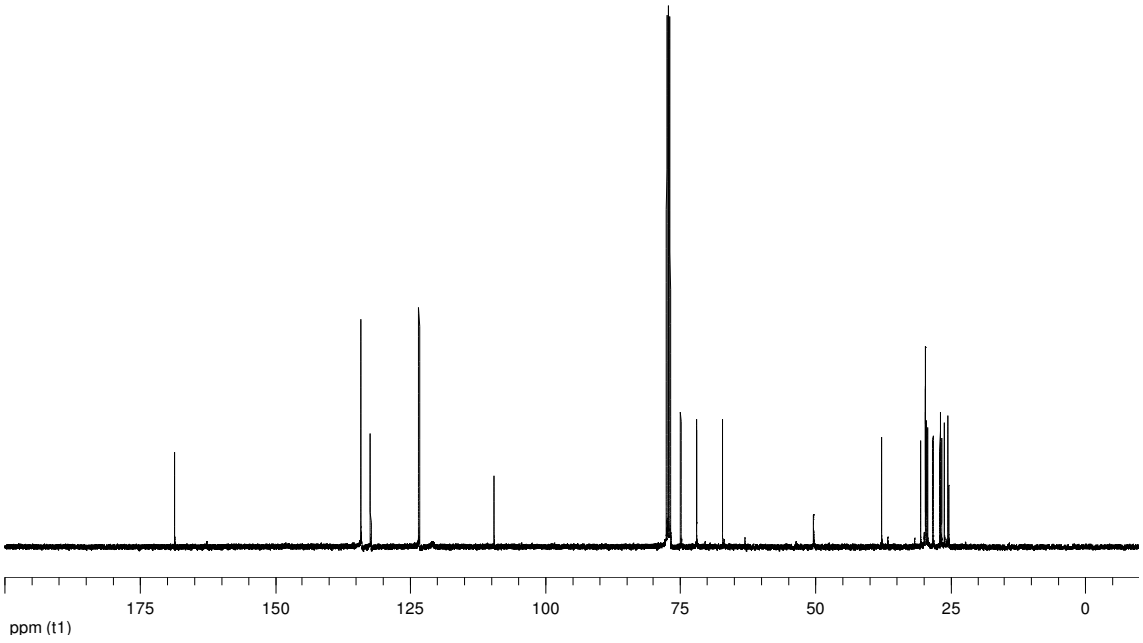
***N*-(12-(3-(bis(4-methoxyphenyl)(phenyl)methoxy)-2-hydroxypropoxy)dodecyl)-2,2,2-trifluoroacetamide, 6**



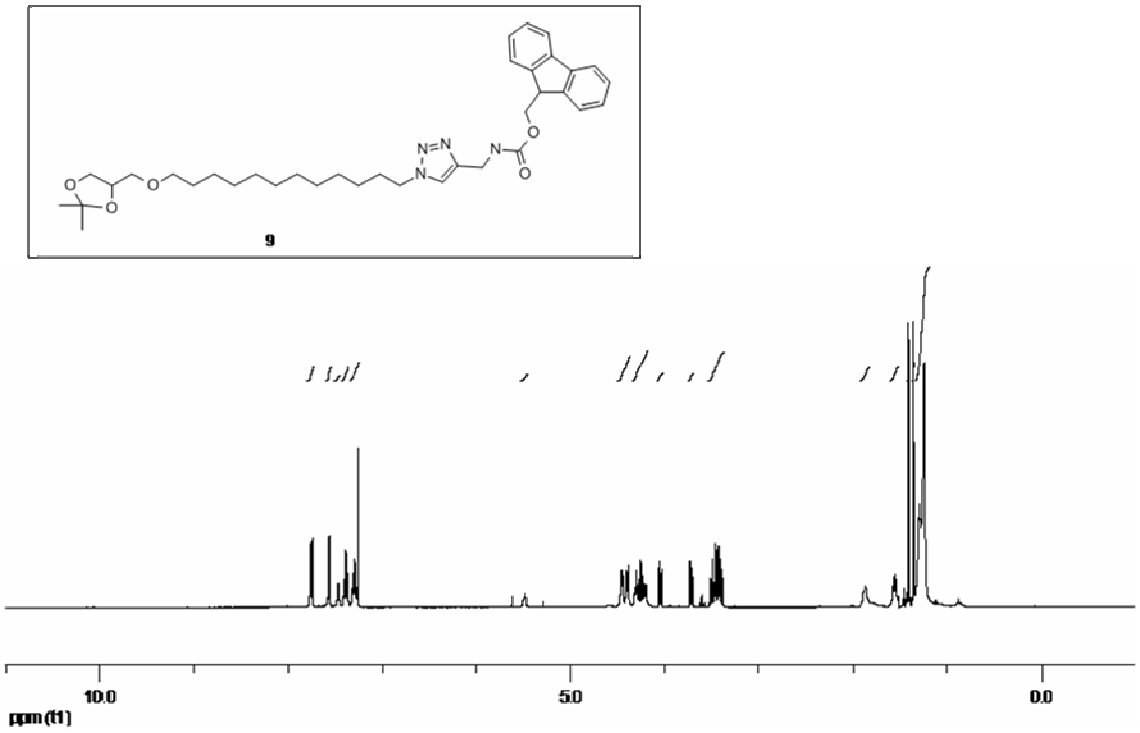


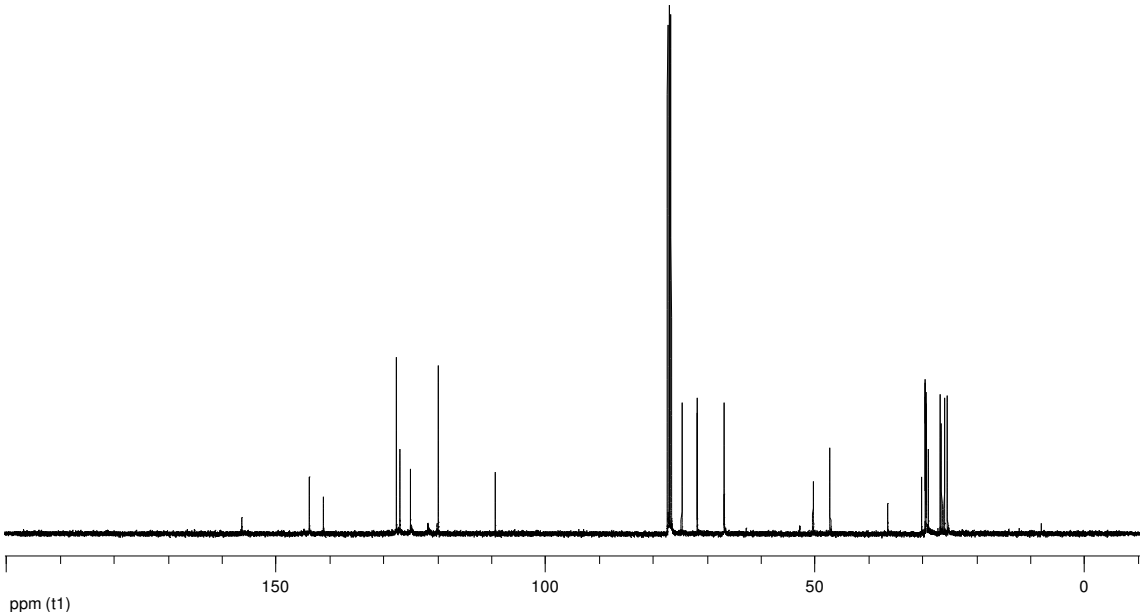
2-(4-(1-(12-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)dodecyl)-1*H*-1,2,3-triazol-4-yl)butyl)isoindoline-1,3-dione, 8



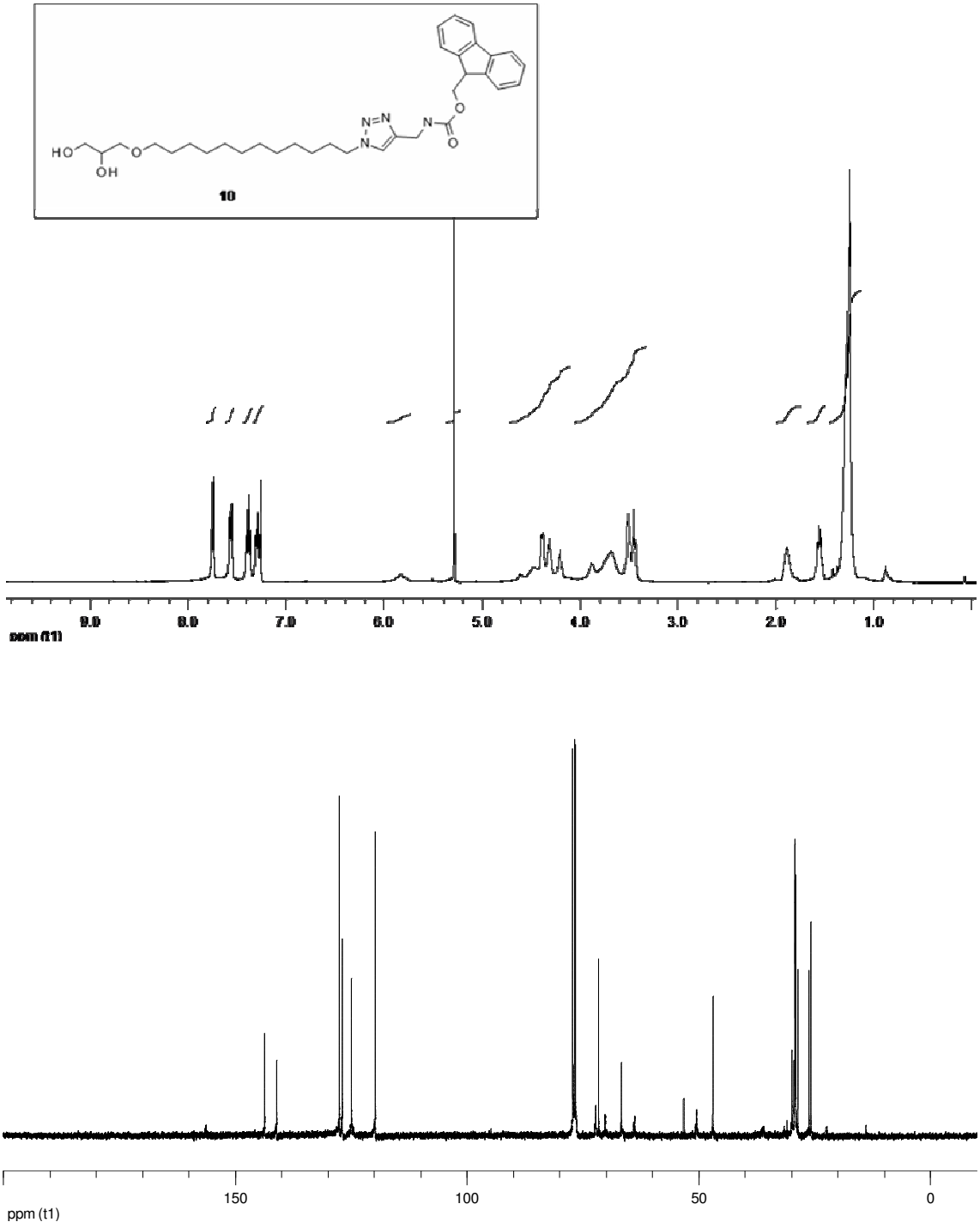


(9H-fluoren-9-yl)methyl-(1-(12-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)dodecyl)-1H-1,2,3-triazol-4-yl)methylcarbamate, 9

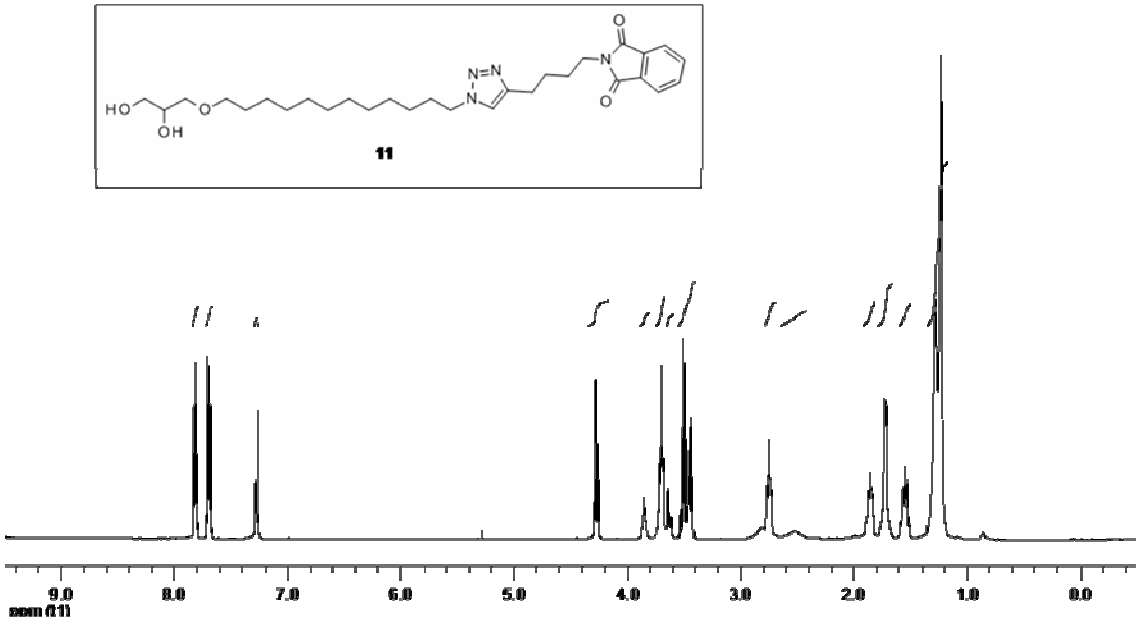


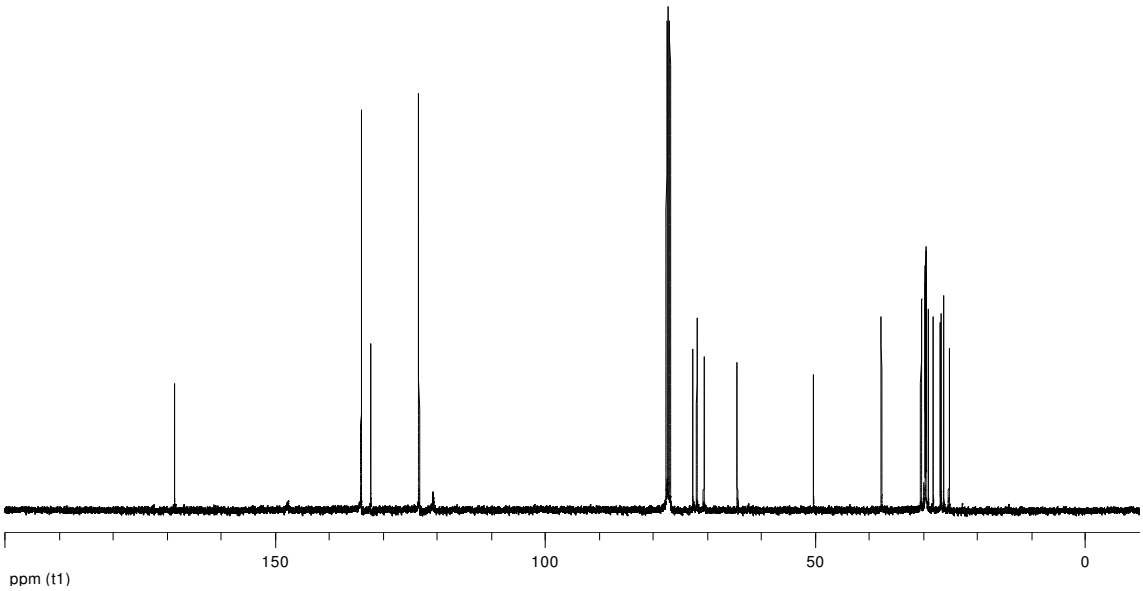


(9*H*-fluoren-9-yl)methyl-(1-(12-(2,3-dihydroxypropoxy)dodecyl)-1*H*-1,2,3-triazol-4-yl)methylcarbamate, 10

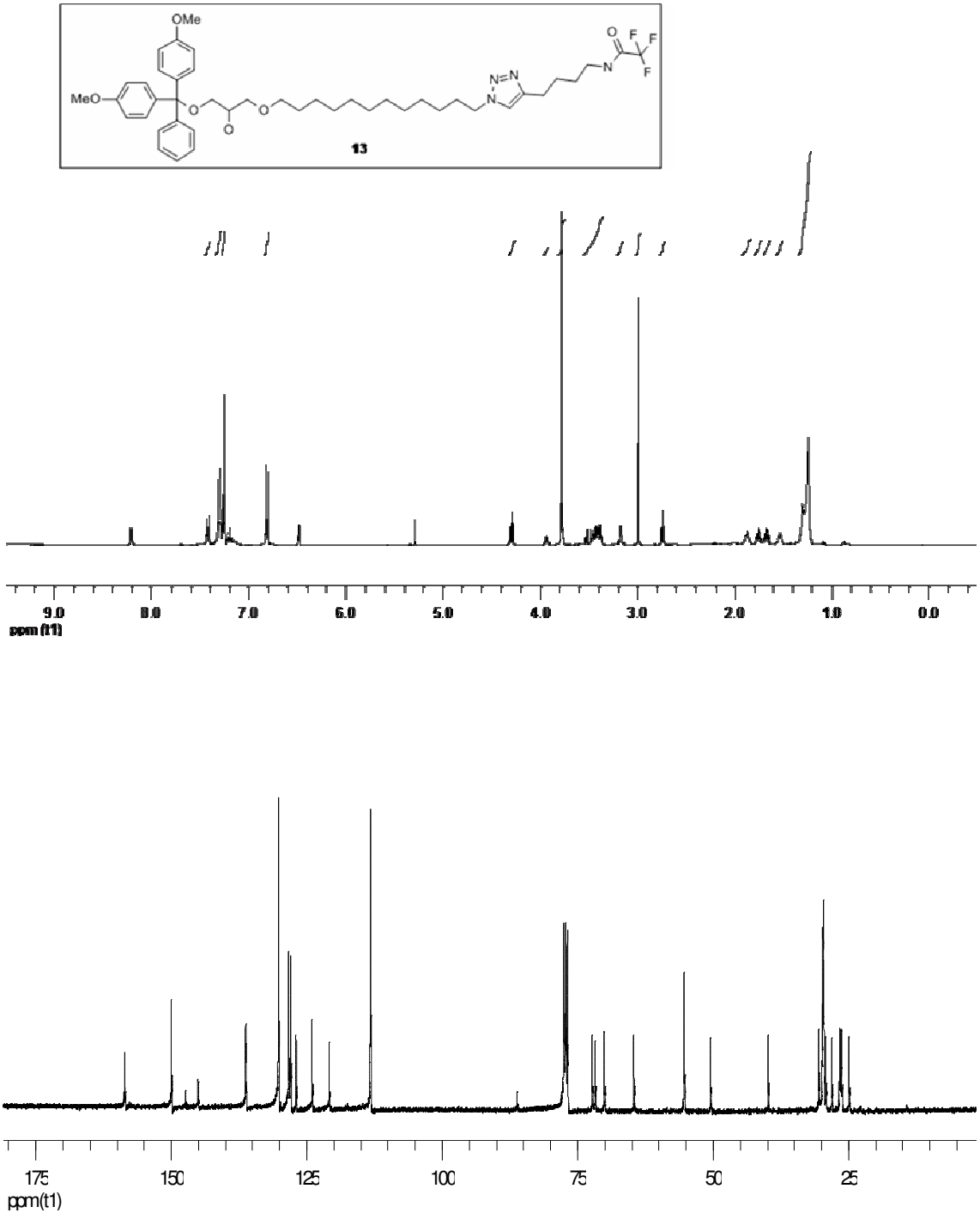


2-(4-(1-(12-(2,3-dihydroxypropoxy)dodecyl)-1*H*-1,2,3-triazol-4-yl)butyl)-isoindoline-1,3-dione, 11

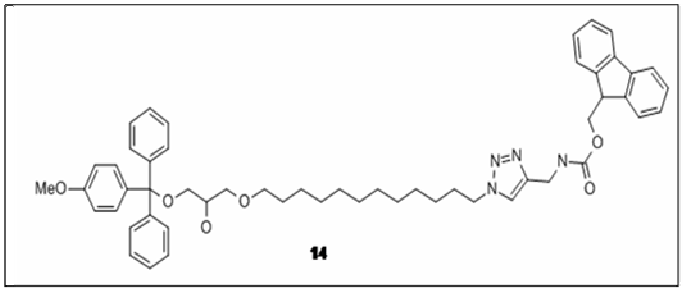


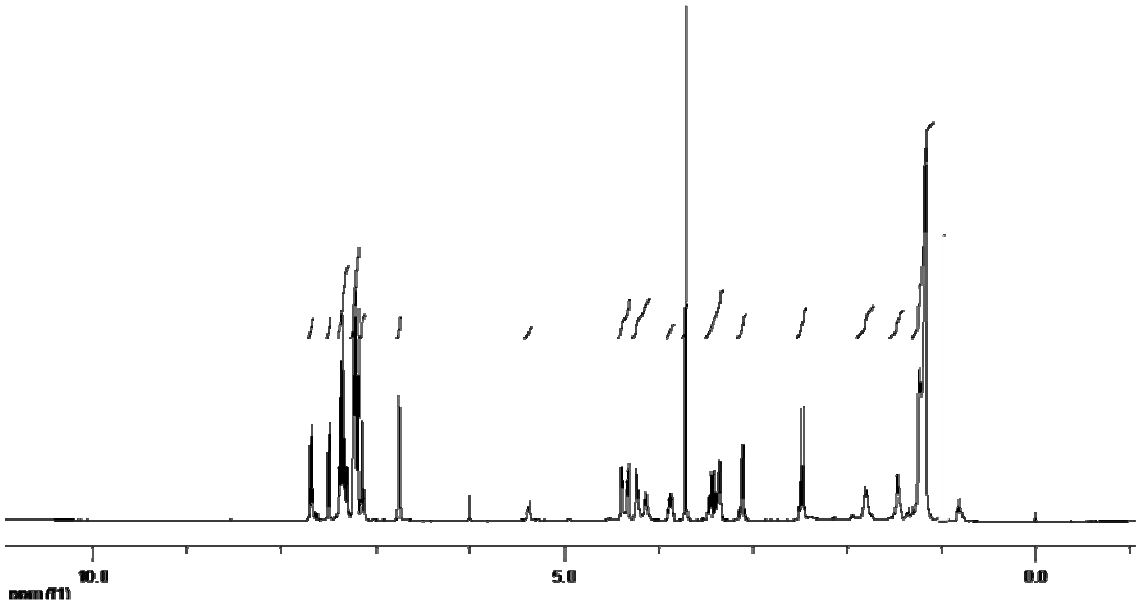


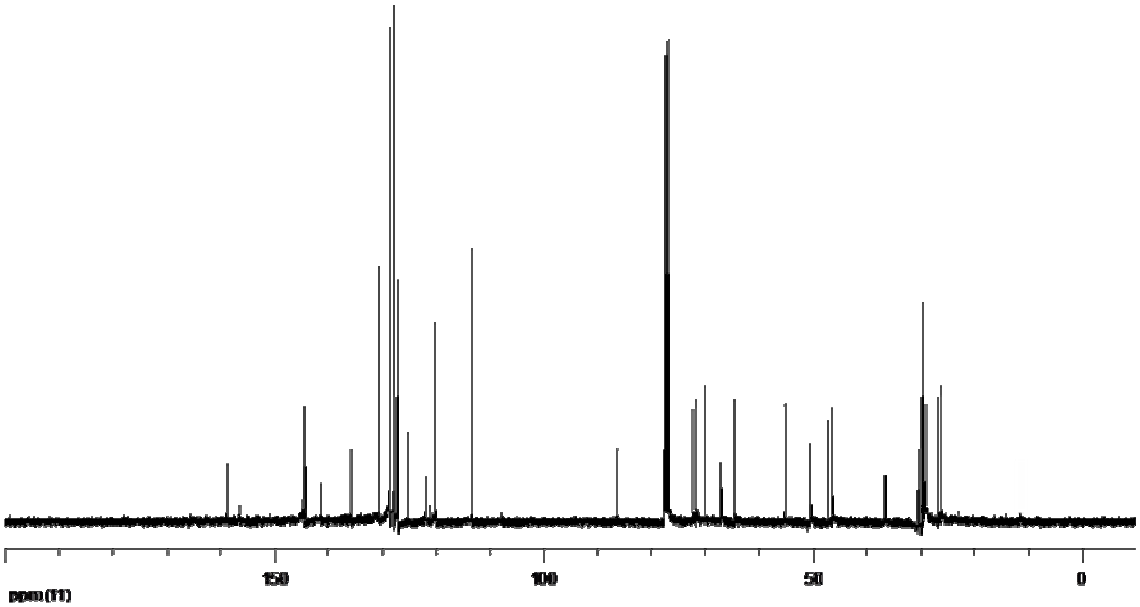
***N*-(4-(1-(12-(3-(bis(4-methoxyphenyl)(phenyl)methoxy)-2-hydroxypropoxy)dodecyl)-1*H*-1,2,3-triazol-4-yl)butyl)-2,2,2-trifluoroacetamide, 13**



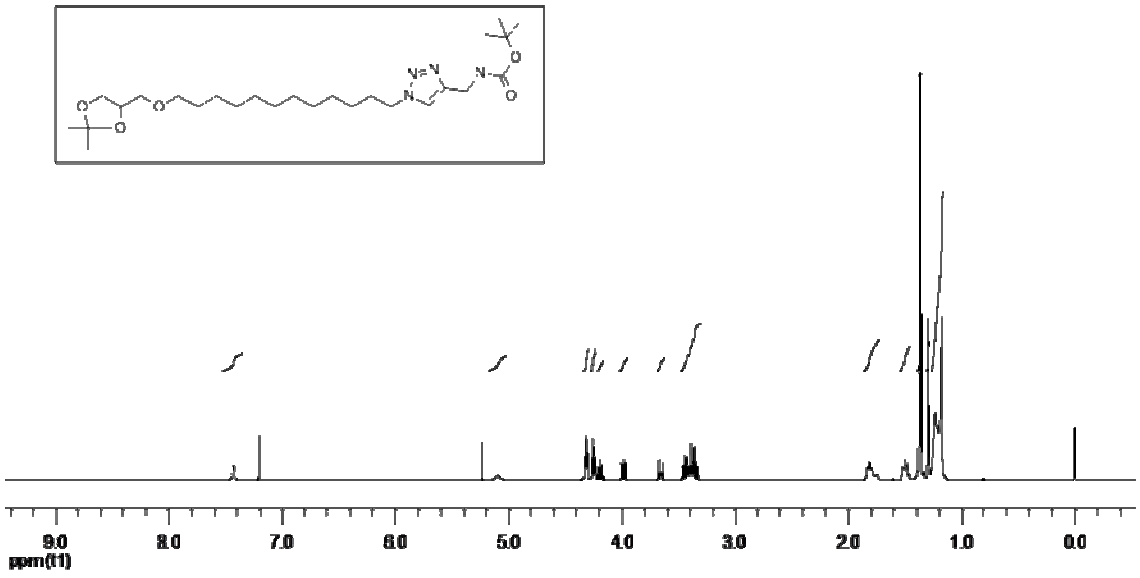
(9H-fluoren-9-yl)methyl-(1-(12-(2-hydroxy-3-((4-methoxyphenyl)-diphenylmethoxy)-propoxy)-dodecyl)-1H-1,2,3-triazol-4-yl)methylcarbamate, 14

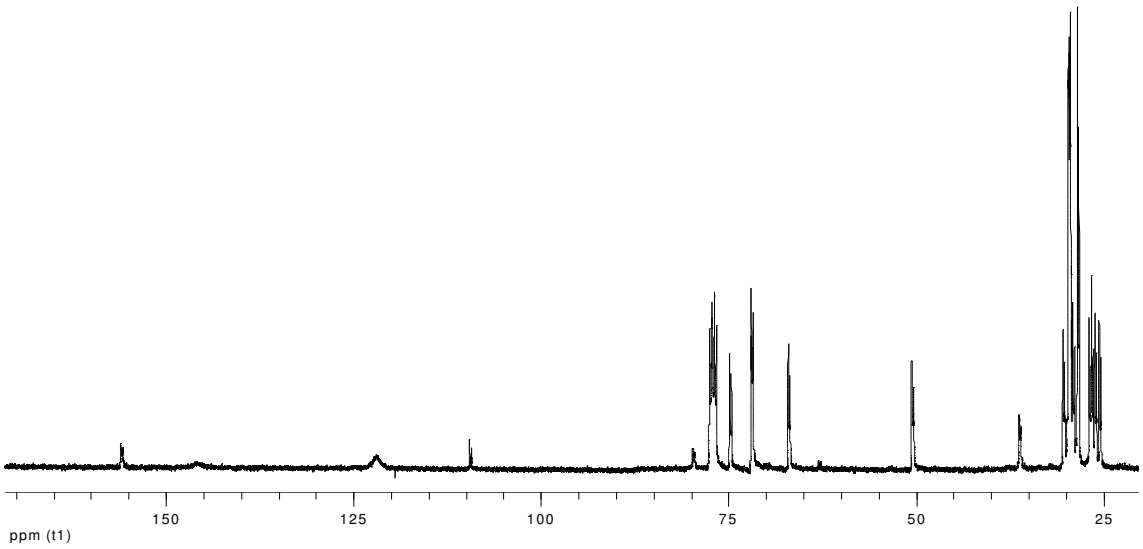






***tert*-butyl-(1-(12-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)dodecyl)-1*H*-1,2,3-triazol-4-yl)methylcarbamate.** Yield: 69%; IR (film) 2929, 2861, 1713, 1500, 1458, 1369, 1250, 1169, 1050, 752 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.43 (s, 1H), 5.09 (broad s, 1H), 4.32 (d, *J* = 5.9 Hz; 2H), 4.24 (t, *J* = 7.2 Hz; 2H), 4.19 (m, 1H), 3.98 (dd, *J* = 14.6 Hz, 8.2 Hz; 1H), 3.65 (dd, *J* = 14.6 Hz, 8.2 Hz; 1H), 3.44 (m, 2H), 3.38 (m, 2H), 1.81 (m, 2H), 1.49 (m, 2H), 1.37 (s, 9H), 1.35 (s, 3H), 1.29 (s, 3H), 1.21 (m, 16H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 156.0, 122.4, 109.5, 79.5, 74.9, 72.1, 72.0, 67.1, 50.6, 36.4, 36.3, 30.4, 29.7, 29.7, 29.7, 29.6, 29.6, 29.5, 29.1, 28.5, 26.9, 26.6, 26.2, 25.6;); HR ESI MS: *m/z* calcd for $C_{26}H_{48}N_4O_5$ (M^++1), 497.3697; found, ; *m/z* 497.3698; calcd for $C_{26}H_{48}N_4O_5$ Na (M^++23), 519.3516; found, *m/z* 519.3517.





Oligodeoxyribonucleotide synthesis

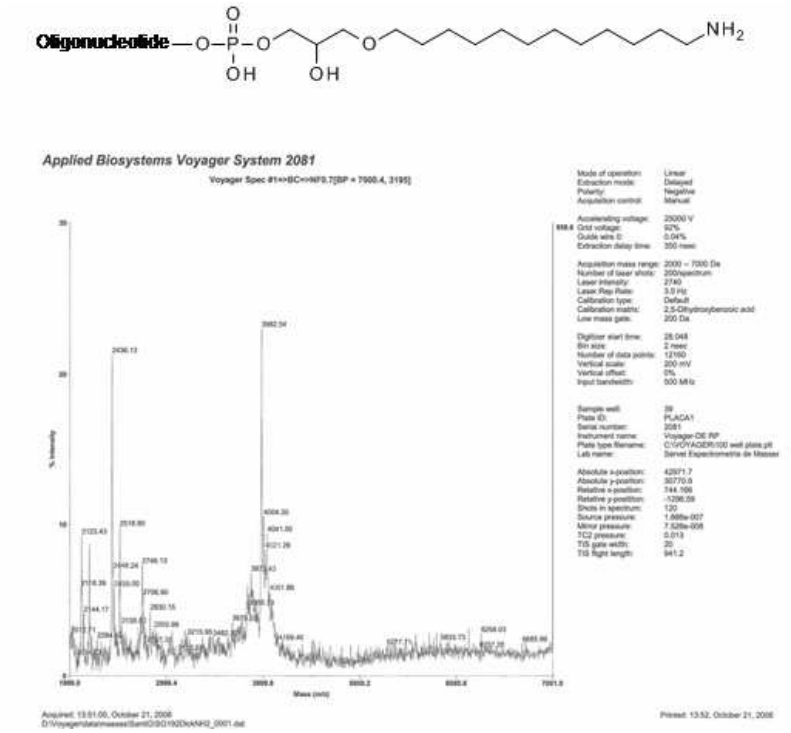
MALDI-TOF mass spectrometry and Melting Temperatures

TABLE S-1. Mass spectrometry and melting temperatutes (T_m) of lipid-modified *Dickerson-Drew* dodecamer derivatives.

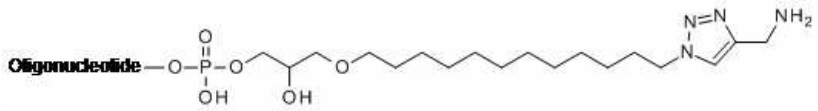
Oligonucleotide	Sequence 5' → 3'	MW calc	MW found	T_m (ºC)*
17	CGCGAATTCGCG-$C_{12}NH_2$	3983	3982.5	55
18	CGCGAATTCGCG-C_{12}-triazol-CH_2NH_2	4066	4065	58
19	CGCGAATTCGCG-C_{12}-triazol-$(CH_2)_4NH_2$	4106	4105	57
20	CGCGAATTCGCG-C_{12}NHC(NH)-NH_2	4027	4029	61
21	CGCGAATTCGCG-C_{12}-triazol-CH_2NHC(NH)-NH_2	4108	4109	56
22	CGCGAATTCGCG-C_{12}-triazol-$(CH_2)_4$NHC(NH)-NH_2	4150	4151	55

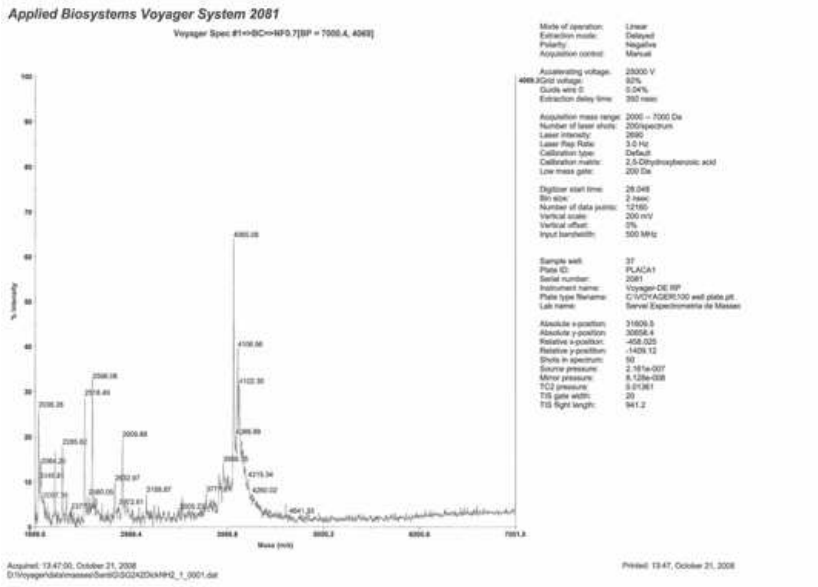
*conditions: sodium phosphate 10 mM, NaCl NaCl, pH 7.0, in these conditions unmodified *Dickerson-Drew* dodecamer melted at 49 ºC.

Aminolipid-Oligonucleotide conjugate, 17

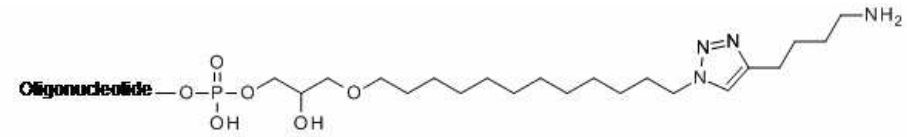


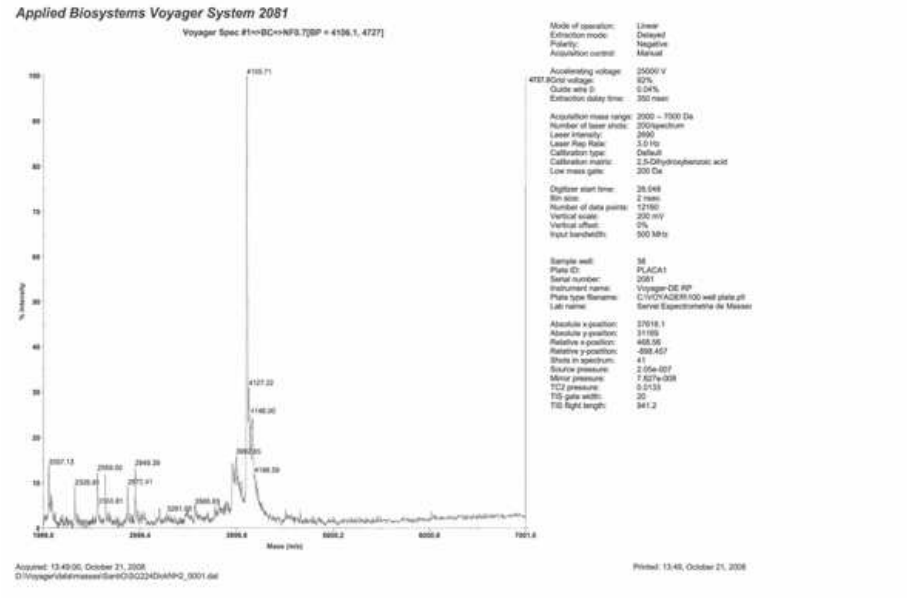
Aminolipid-Oligonucleotide conjugate, 18



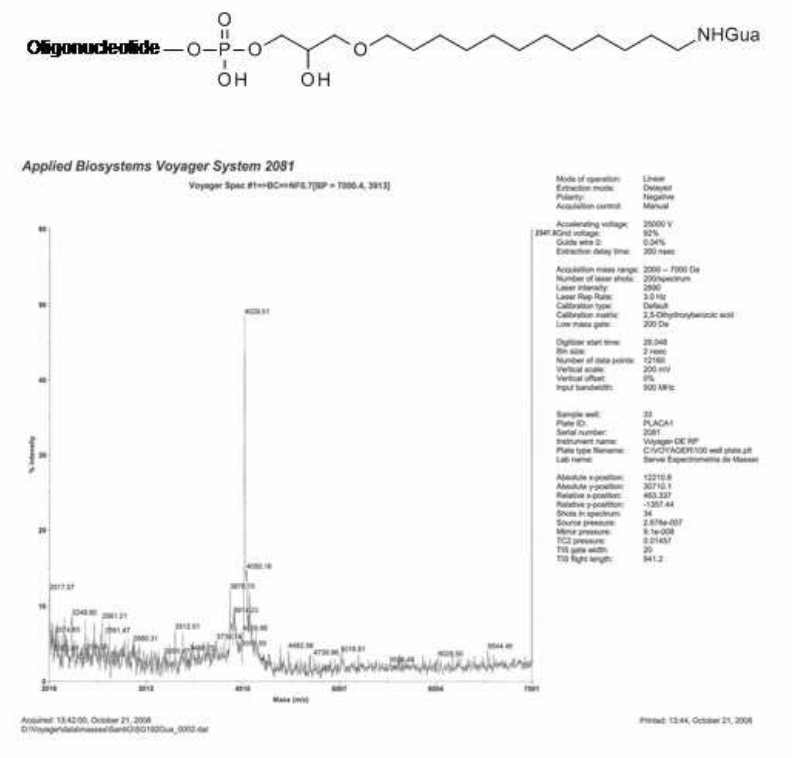


Aminolipid-Oligonucleotide conjugate, 19

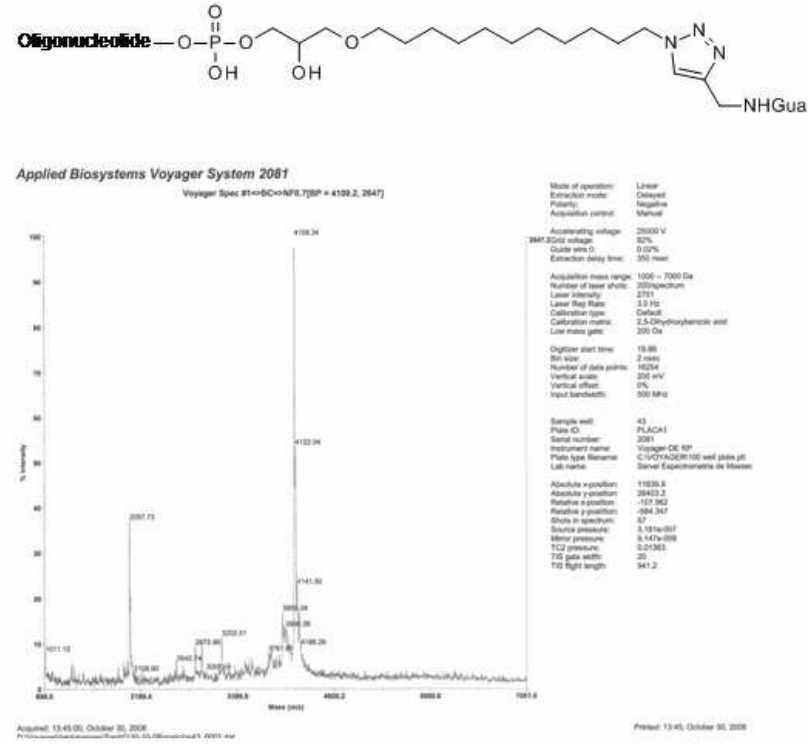




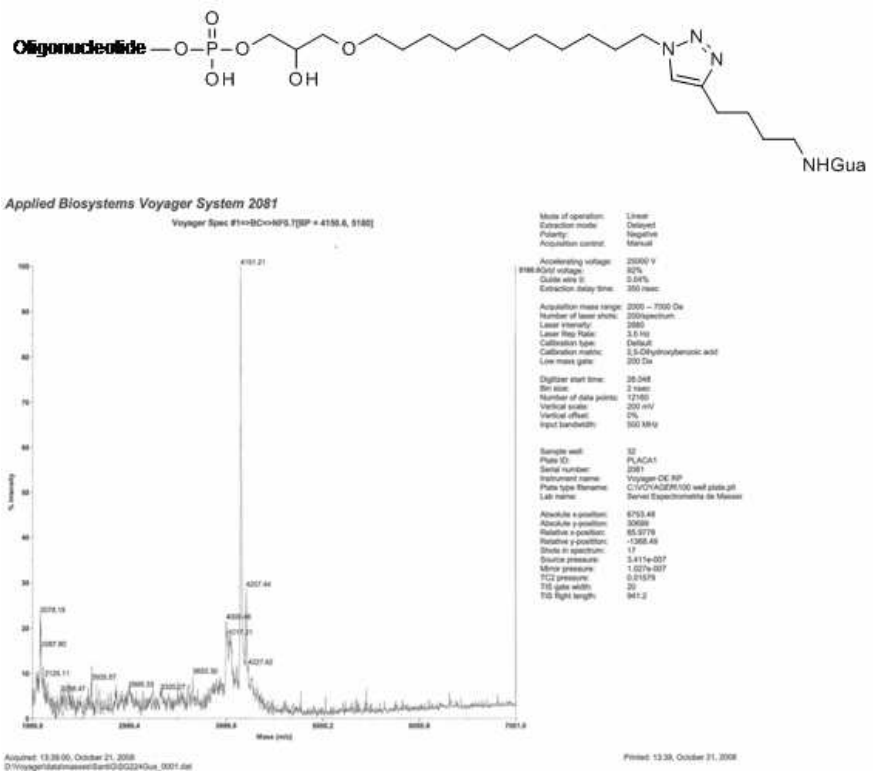
Aminolipid-Oligonucleotide conjugate, 20



Aminolipid-Oligonucleotide conjugate, 21



Aminolipid-Oligonucleotide conjugate, 22



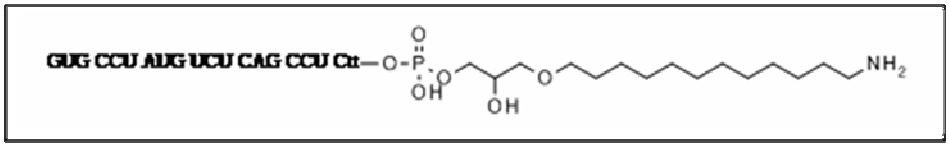
Oligoribonucleotide synthesis.

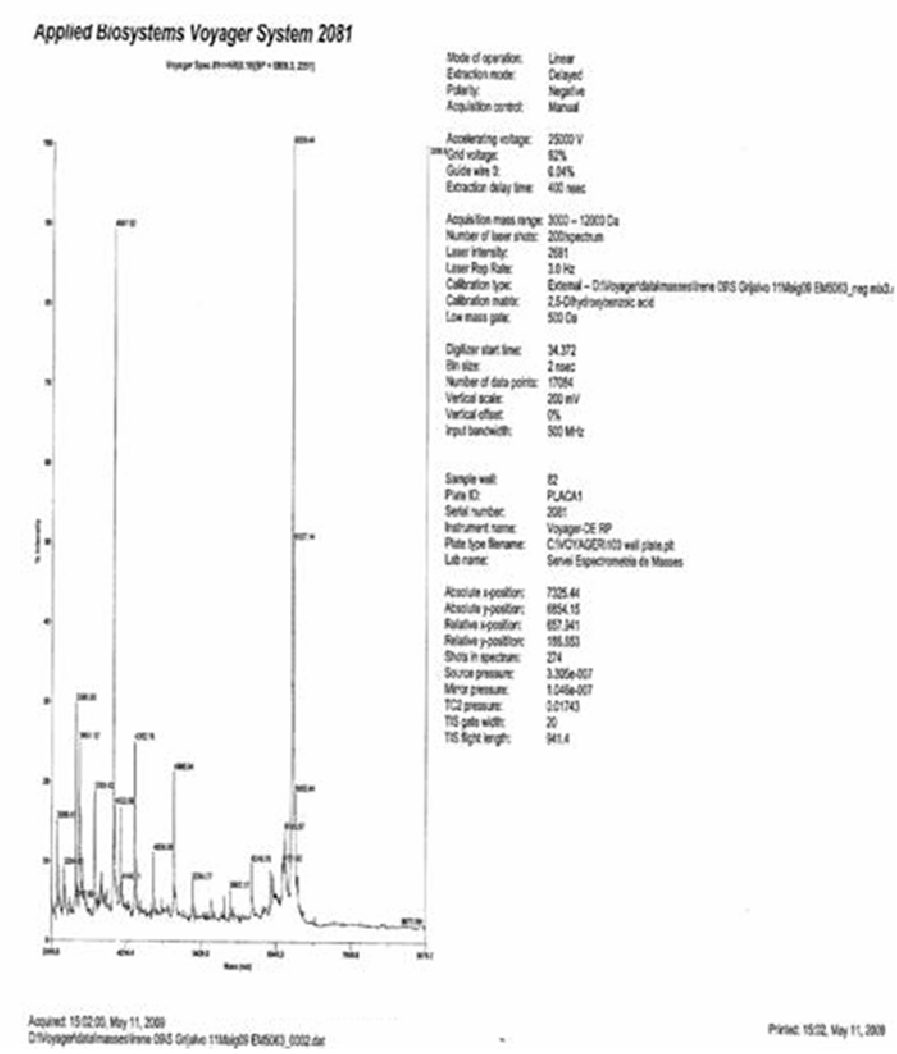
MALDI-TOF mass spectrometry

Table S-2

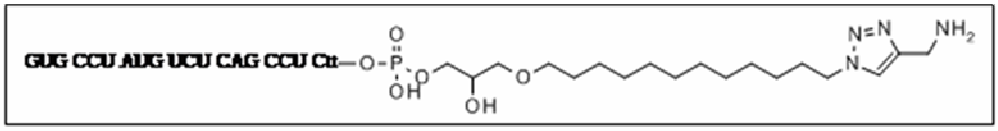
Lipid-RNA	MW (calcd)	MW (obs)
23	6896	6899
24	6977	6991 [M+Na^+]
25	7019	7054 [M+2Na^+]

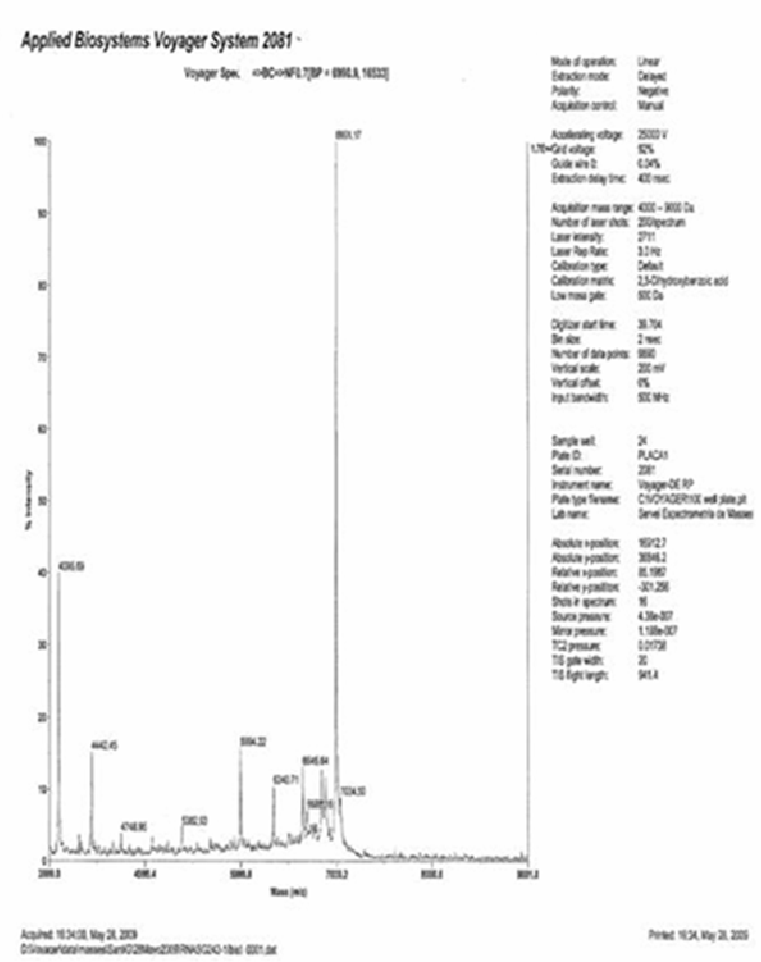
Lipid-RNA, 23


GUG CCU AUG UCU CAG CCU Ctt
O
P
OH
O
OH
O
NH2

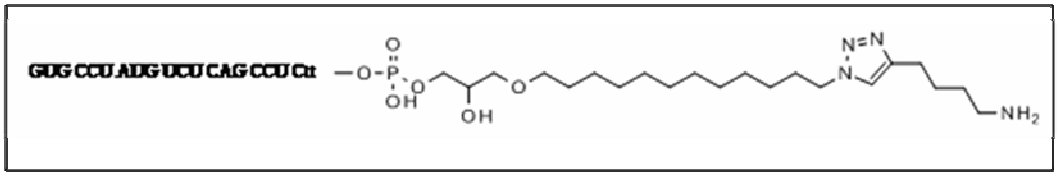

Applied Biosystems Voyager System 2081
Mode of operation: Linear
Extraction mode: Delayed
Polarity: Negative
Acquisition control: Manual
Accelerating voltage: 25000 V
Grid voltage: 92%
Guide wire 3: 0.04%
Extraction delay time: 400 nsec
Acquisition mass range: 3000 – 12000 Da
Number of laser shots: 200/spectrum
Laser intensity: 2681
Laser Rep Rate: 3.0 Hz
Calibration type: External – D:\Voyager\data\masses\Irene 09\S Grijalvo 11Maig09 EM5063_reg mix2.
Calibration matrix: 2,5-Dihydroxybenzoic acid
Low mass gate: 500 Da
Digitizer start time: 34.372
Bin size: 2 nsec
Number of data points: 17084
Vertical scale: 200 mV
Vertical offset: 0%
Input bandwidth: 500 MHz
Sample well: B2
Plate ID: PLACA1
Serial number: 2081
Instrument name: Voyager-DE RP
Plate type filename: C:\VOYAGER\b03 well plate.plt
Lab name: Servei Espectrometria de Masses
Absolute x-position: 7325.44
Absolute y-position: 6854.15
Relative x-position: 657.341
Relative y-position: 186.053
Shots in spectrum: 274
Source pressure: 3.305e-007
Mirror pressure: 1.046e-007
TC2 pressure: 0.01743
TIS gate width: 20
TIS flight length: 941.4
Acquired: 15:02:00, May 11, 2009
D:\Voyager\data\masses\Irene 09\S Grijalvo 11Maig09 EM5063_0002.dat
Printed: 15:02, May 11, 2009

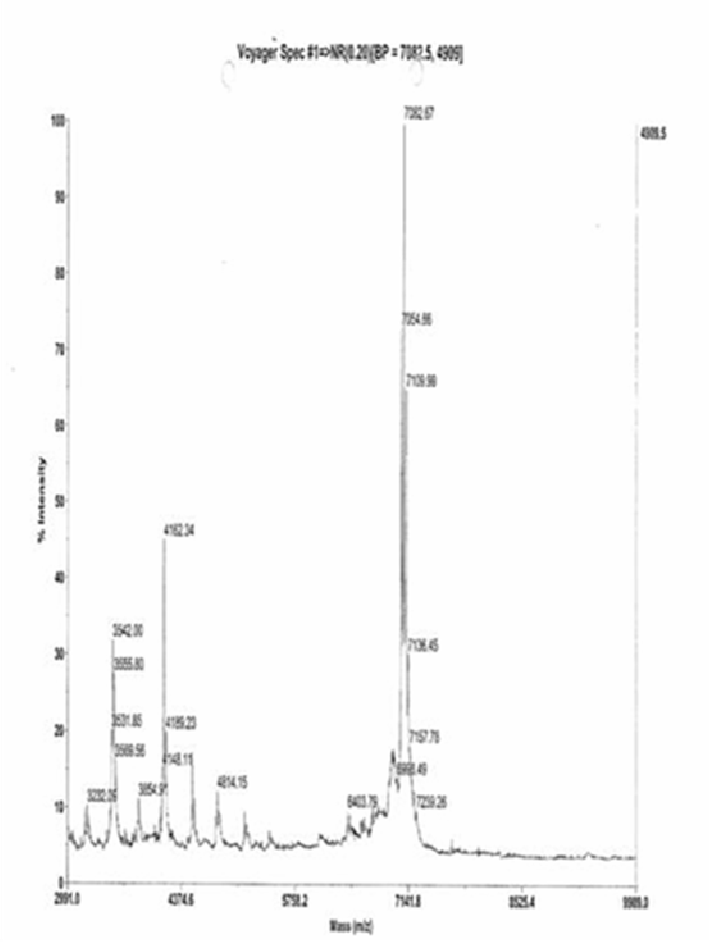
Lipid-RNA, 24


GUG CCU AUG UCU CAG CCU Ct
O
P
OH
O
OH
N=N
NH2
N


Applied Biosystems Voyager System 2081
Voyager Spec
Mode of operation: Linear
Extraction mode: Delayed
Polarity: Negative
Acquisition control: Manual
Accelerating voltage: 25000 V
Grid voltage: 92%
Guide wire 0: 0.04%
Extraction delay time: 400 nsec
Acquisition mass range: 4000 – 9000 Da
Number of laser shots: 250/spectrum
Laser intensity: 2711
Laser Rep Rate: 3.0 Hz
Calibration type: Default
Calibration matrix: 2,5-Dihydroxybenzoic acid
Low mass gate: 600 Da
Digitizer start time: 38.704
Bin size: 2 nsec
Number of data points: 8000
Vertical scale: 200 mV
Vertical offset: 0%
Input bandwidth: 500 MHz
Sample well: 2K
Plate ID: PLACA1
Serial number: 2081
Instrument name: Voyager-DE RP
Lab name: Servei Espectrometria de Masses
Absolute x-position: 16012.7
Absolute y-position: 36846.2
Relative x-position: 85.1960
Relative y-position: -321.256
Shots in spectrum: 16
Source pressure: 4.38e-007
Mirror pressure: 1.108e-007
TC2 pressure: 0.01730
TG gate width: 20
TG flight length: 941.4
% Intensity
Mass (m/z)
Printed: 16:34, May 28, 2009

Lipid-RNA, 25


GUG CCU AUG UCU CAG CCU Ct
O
O-P
OH
O
OH
O
N=N
N
NH2


% Intensity
Mass [m/z]