

CHEMISTRY

A **European** Journal

Supporting Information

Genetic Code Expansion Facilitates Position-Selective Labeling of RNA for Biophysical Studies

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Genetic code expansion facilitates position-selective labeling of RNA for biophysical studies

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In vitro transcription and NMR of RNA

Figure S1: Denaturing polyacrylamide gel of 14mer and 14mer^X for analysis of the transcription efficiency

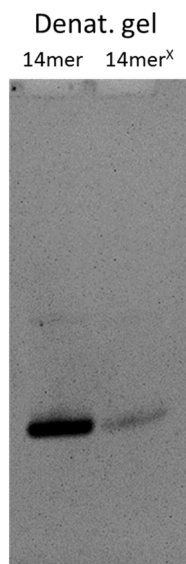


Table S1: Analysis of the relative yield of 14mer^x to 14mer based on the gel shown in Figure 1 done with ImageJ.

Lane	Band No.	Rel. Quant.	Band%
14mer	1	1	100
14merX	1	0,13010668	100

Figure S2: 1D ^1H Imino region of 14mer and 14mer^X at 278 K.

The partial assignment was has been transferred from Fürtig et al. [\[31\]](#)

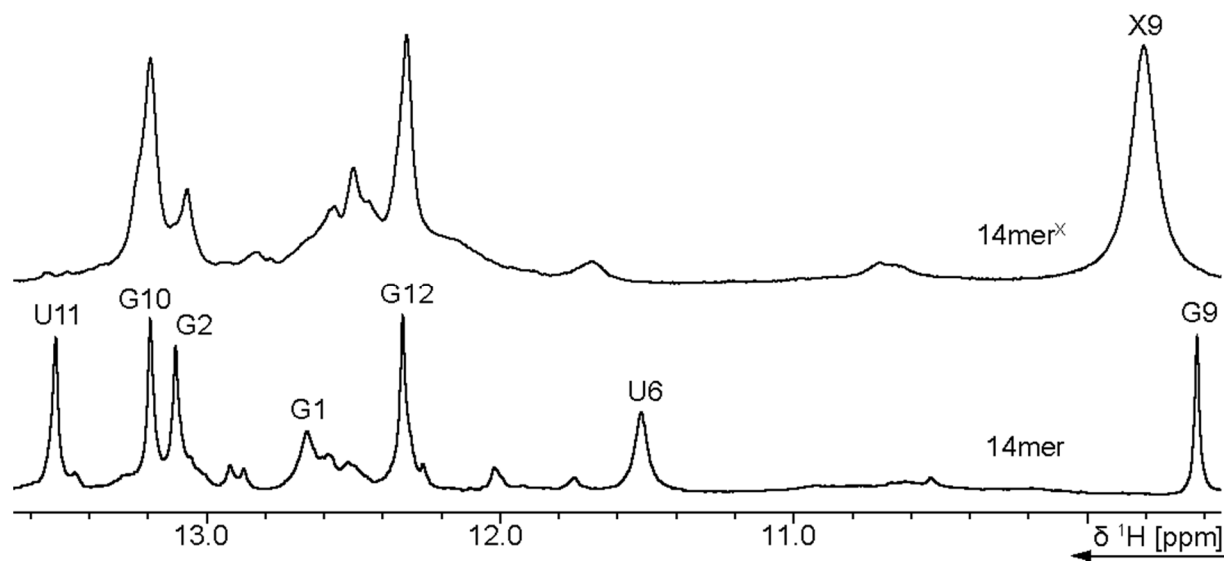


Figure S3: Optimization of XTP concentration for *in vitro* transcription of G79X.

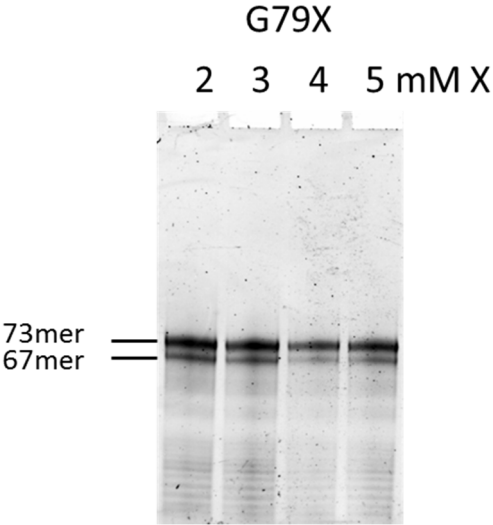


Table S2: Analysis of denaturing polyacrylamide gels (Figure 5B) to determine the absolute and relative transcription yield of G79X (73mer) and abortion product G79X abortion (67mer).

	Lane	Lane #	Band No.	Length	Rel. Quant.	Band %
	Gsw ⁷³	1	1	73mer	1	100
-XTP	G79X	2	1	73mer	0,17	21,62
	G79X abortion		2	67mer	0,62	78,38
+XTP	G79X	3	1	73mer	0,71	76,73
	G79X abortion		2	67mer	0,22	23,27

Figure 5B for reference:

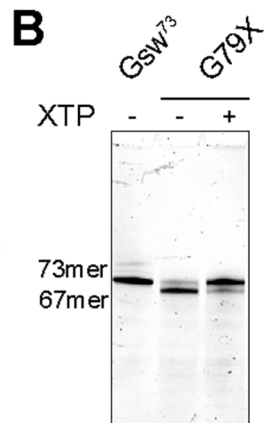


Figure S4: Optimization of 7-deazaxanthosine concentration for *in vitro* transcription of G79-7dX.

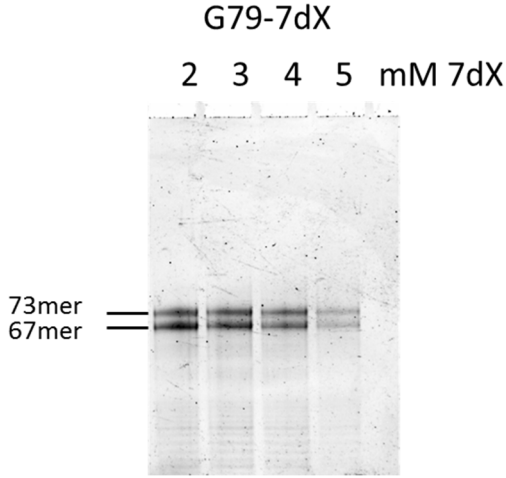
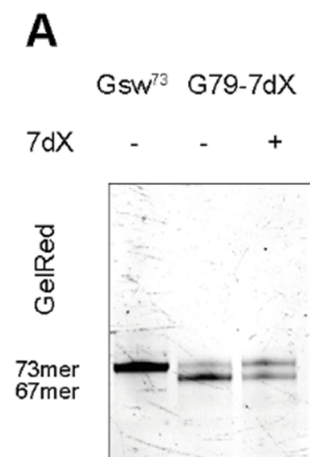


Table S3: Analysis of denaturing polyacrylamide gel (Figure 6A) to determine the absolute and relative transcription yield of G79-7dX (73mer) and abortion product G79-7dX abortion (67mer).

	Lane	Lane #	Band No.	Length	Rel. Quant.	Band %
	Gsw ⁷³	1	1	73mer	1	100
-7dXTP	G79-7dX	2	1	73mer	0,16	19,16
	G79-7dX abortion		2	68mer	0,67	80,84
+7dXTP	G79-7dX	3	1	73mer	0,35	45,3
	G79-7dX abortion		2	68mer	0,42	54,7

Figure 6A for reference:



Analytics of 3',5'-Bis-O-(*tert*-butyldimethylsilyl)thymidine (2)

Figure S5: ¹H-spectrum

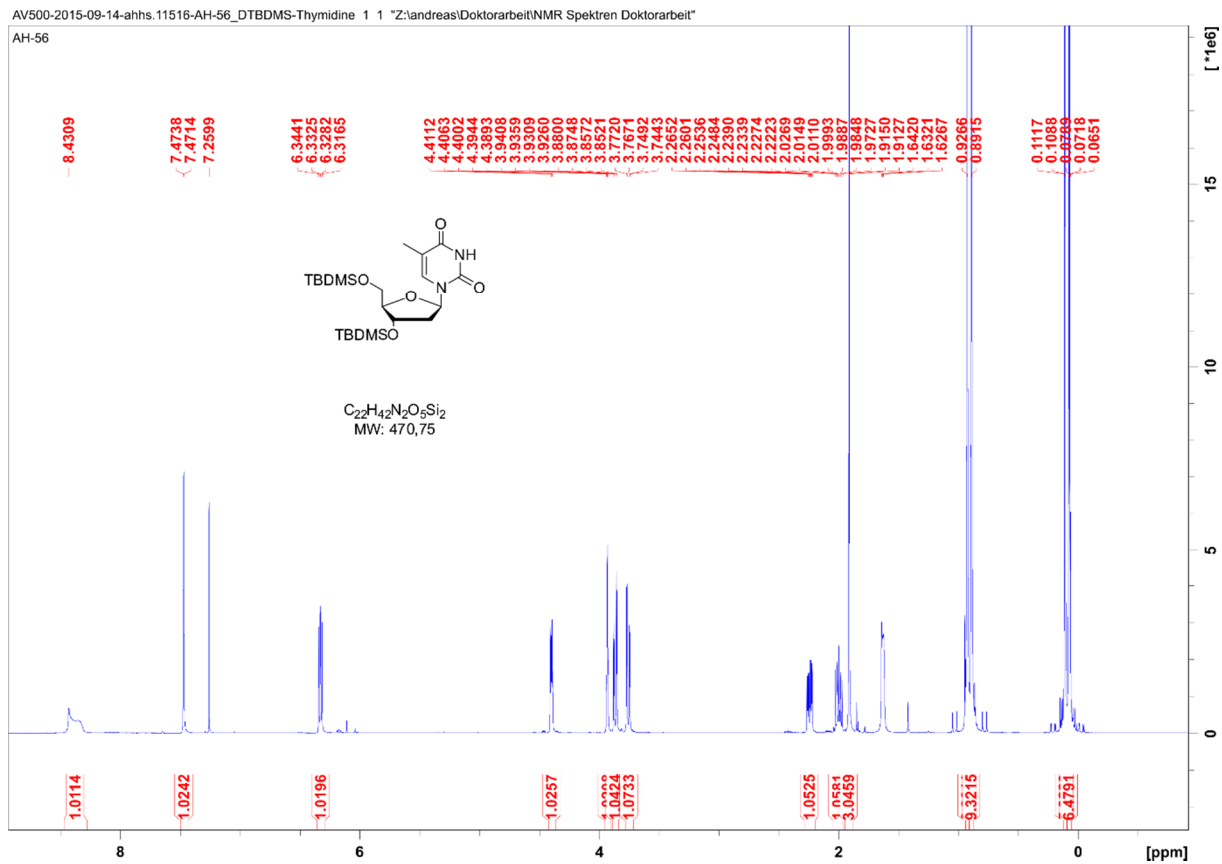


Figure S6: ¹³C-spectrum

AV500-2015-09-14-ahhs.11516-AH-56_DTBDMS-Thymidine 2 1 *Z:\andreas\Doktorarbeit\NMR Spektren Doktorarbeit\

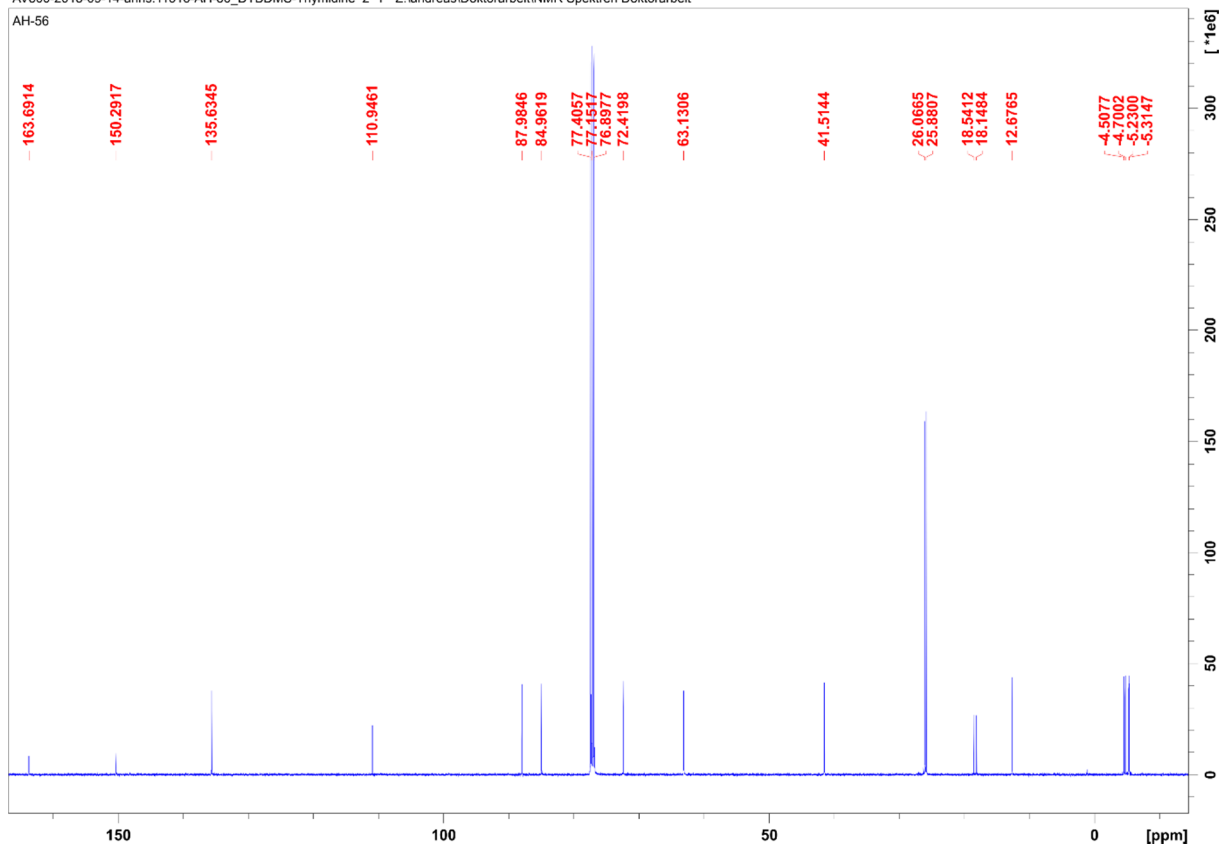
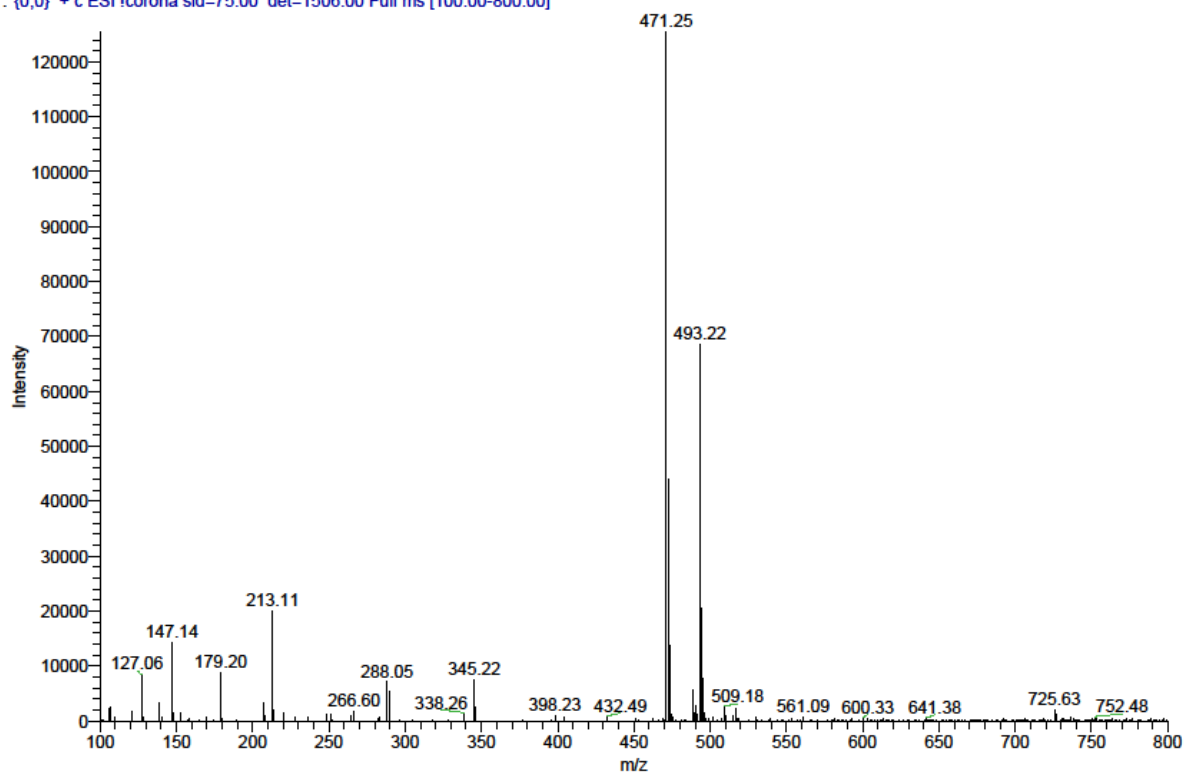


Figure S7: MALDI-spectrum

C:\Xcalibur\data\AH_10

10/20/2014 9:24:54 AM

AH_10 #32-54 RT: 0.54-0.93 AV: 23 SB: 27 0.02-0.47 NL: 1.25E5
T: {0,0} + c ESI Icorona sid=75.00 det=1506.00 Full ms [100.00-800.00]



Analytics of 1,4-anhydro-3,5-bis-*O*-(*tert*-butyldimethylsilyl)-2-deoxy-D-erythro-pent-1-enitol (3)

Figure S8: ¹H-spectrum

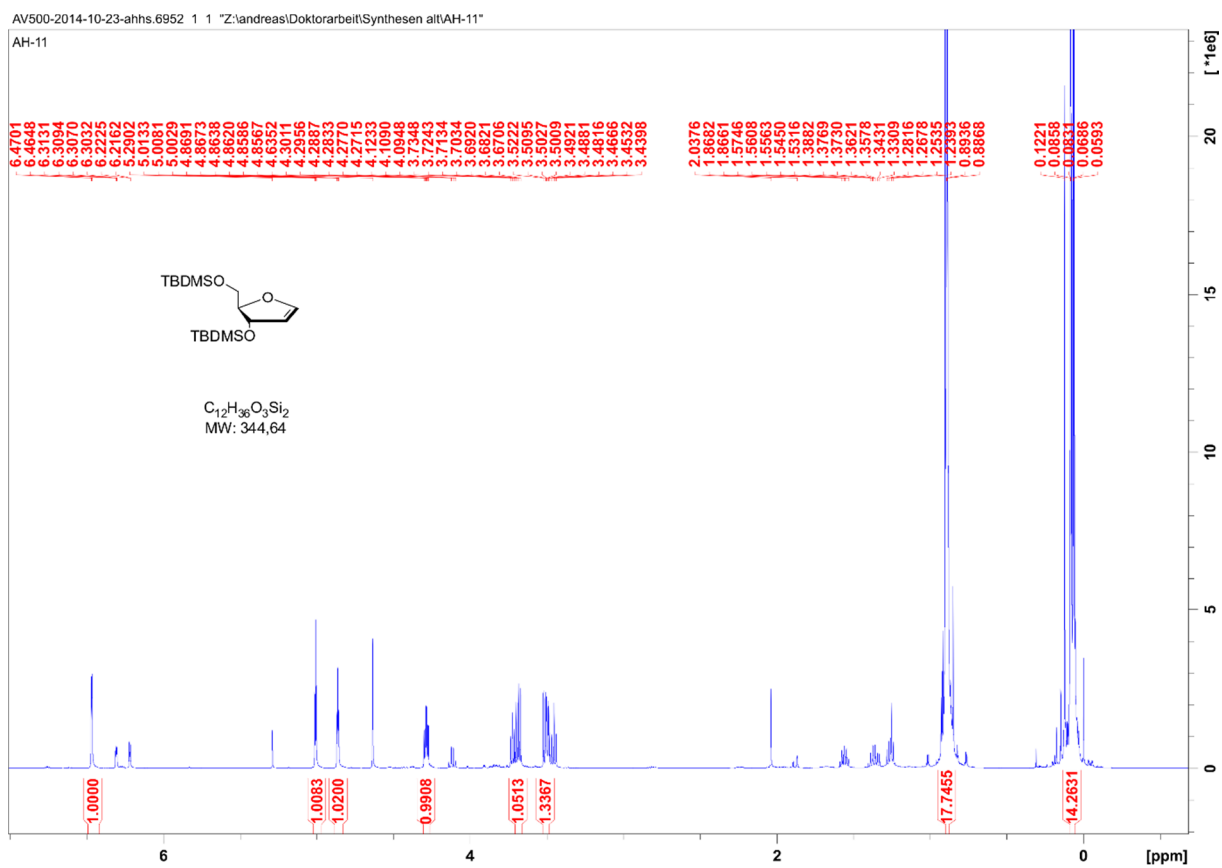


Figure S9: ¹³C-spectrum

AV500-2014-10-23-ahhs.6952 2 1 "Z:\andreas\Doktorarbeit\Synthesen alt\AH-11"

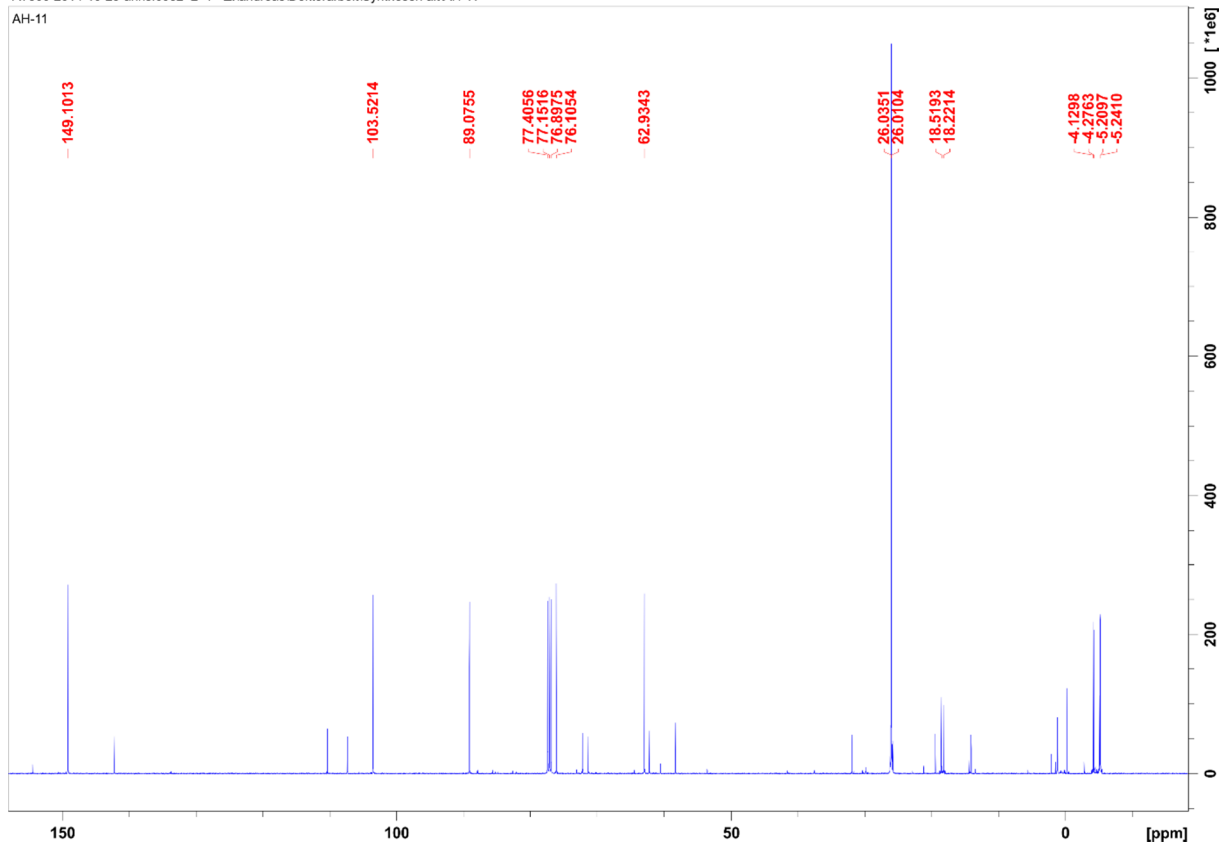
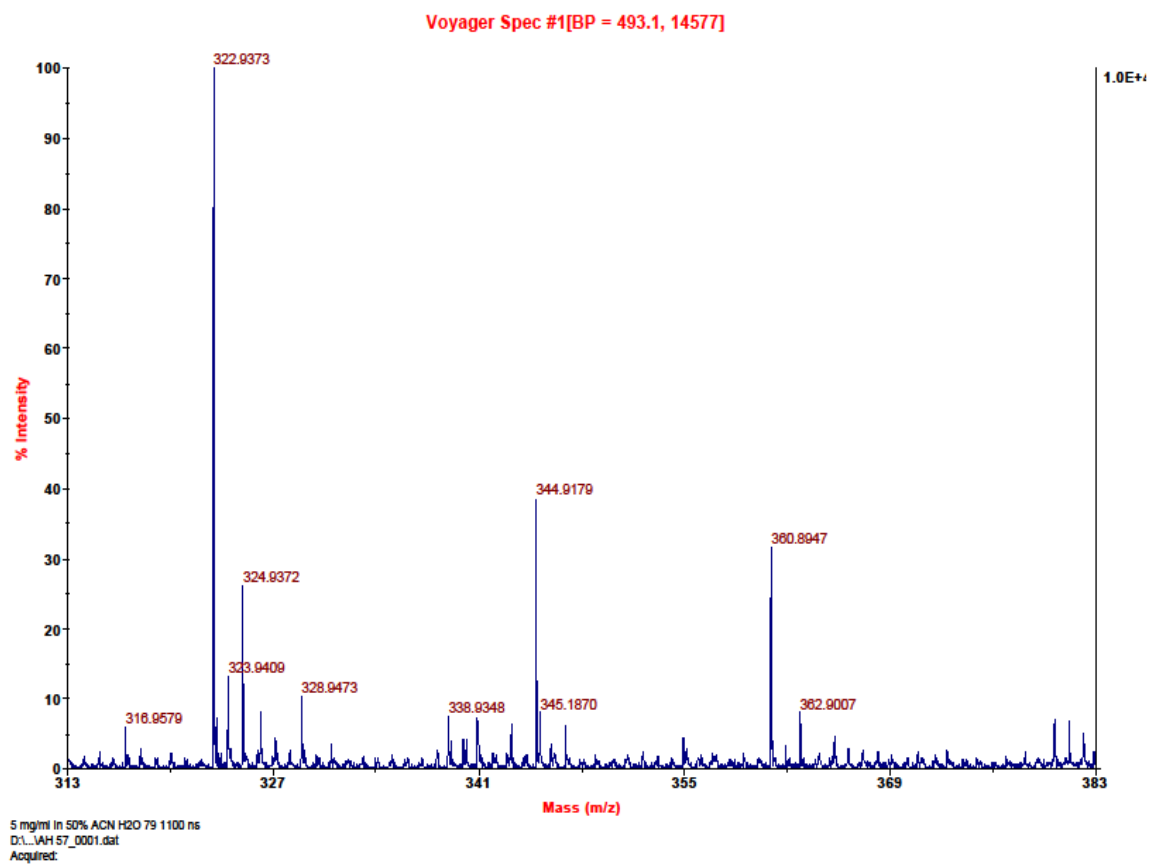


Figure S10: MALDI-spectrum



Analytics of 3-*O*-(*tert*-butyldimethylsilyl)-1,2-dideoxy-2,3-didehydro-D-ribofuranose (4)

Figure S11: ¹H-spectrum

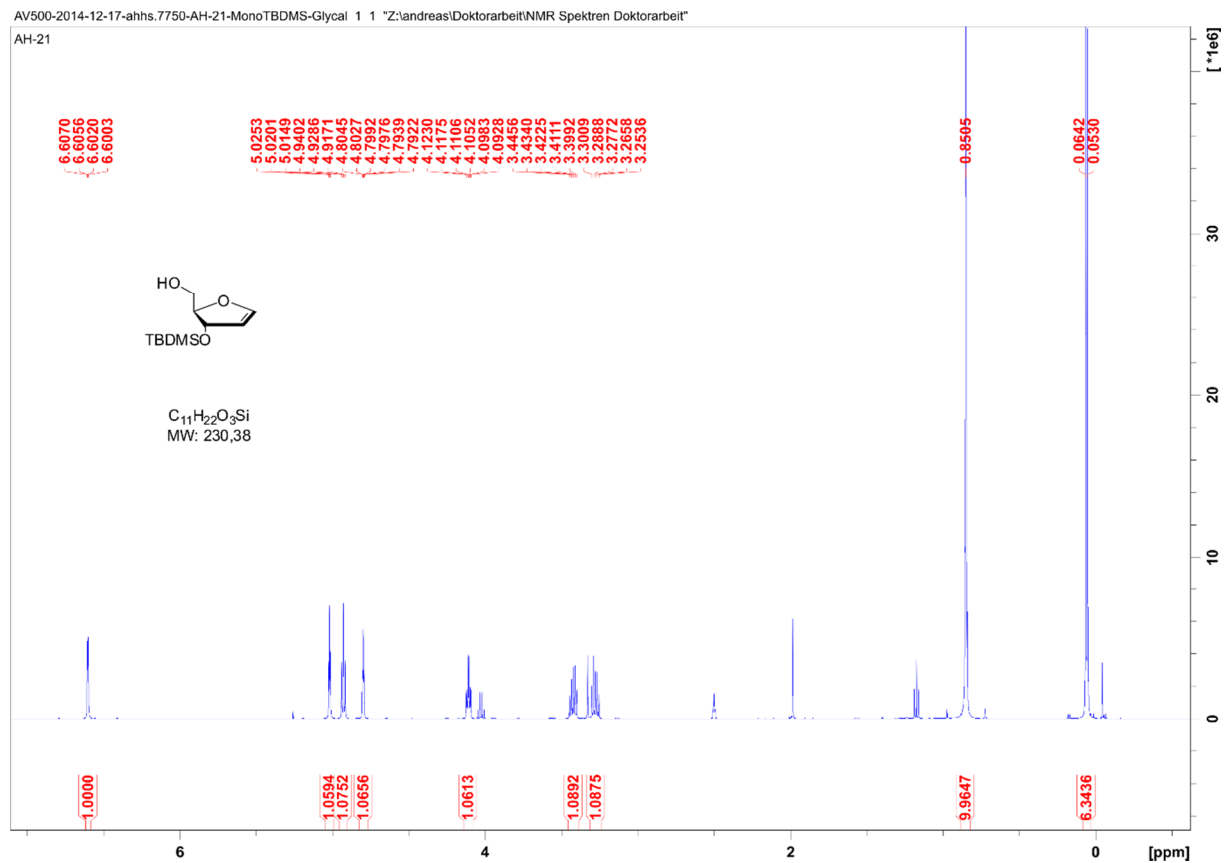


Figure S12: ¹³C-spectrum

AV500-2014-12-17-ahhs.7750-AH-21-MonoTBDMS-Glycal 2 1 *Z:landreasIDoktorarbeit\NMR Spektren Doktorarbeit*

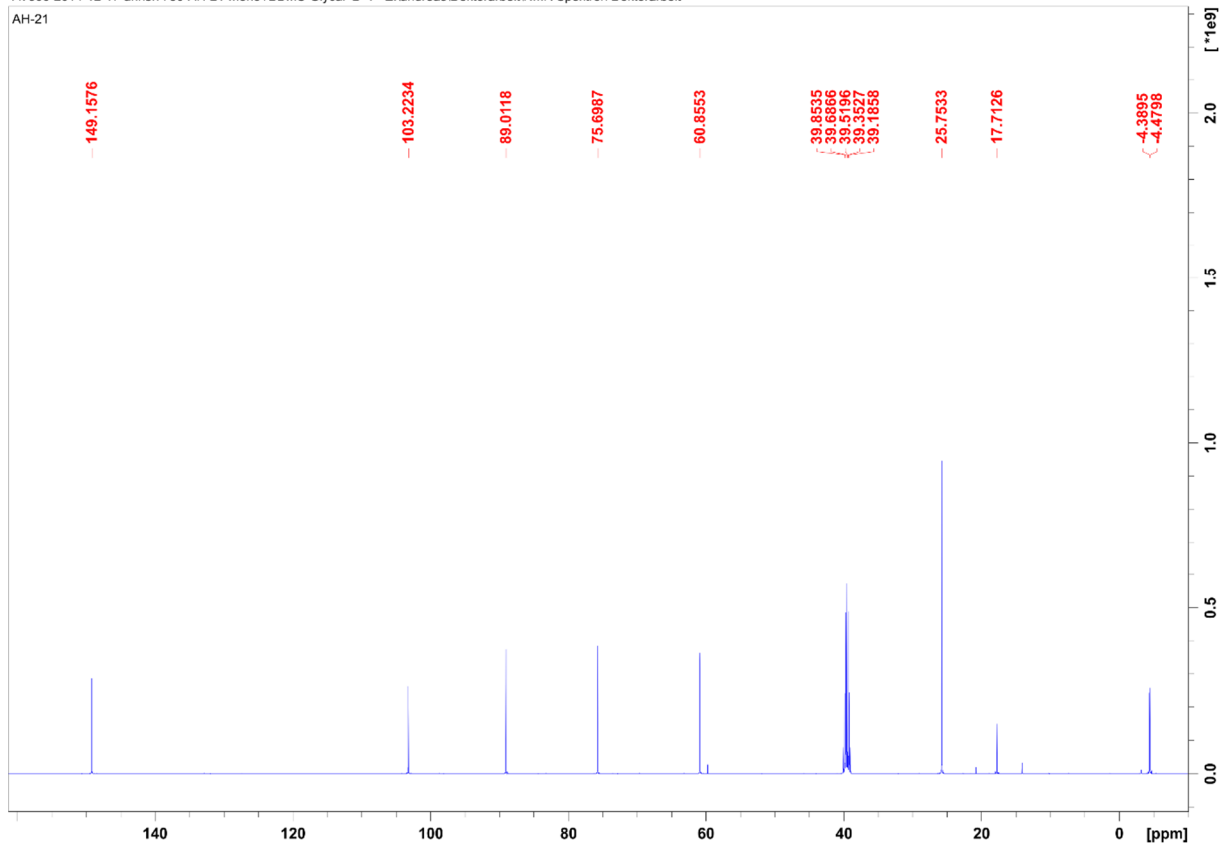
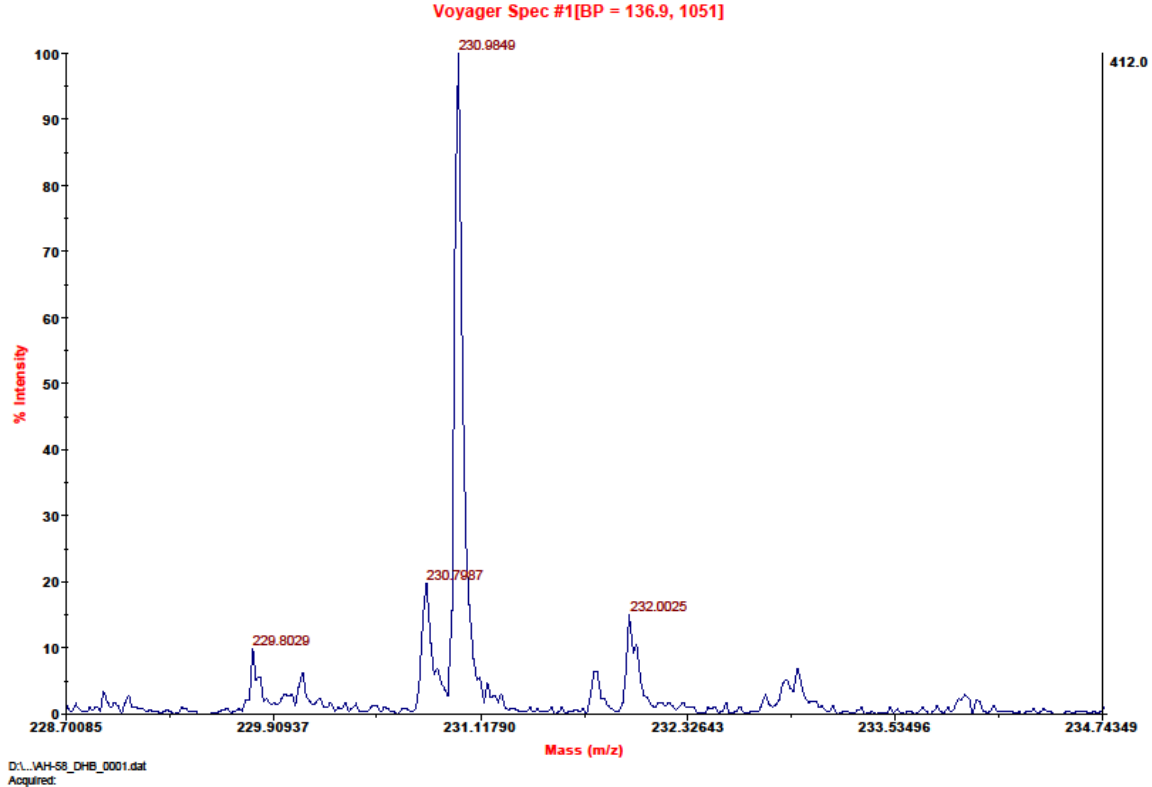


Figure S13: MALDI-spectrum



Analytics of 1β-(2,4-dichloropyrimidin-5-yl)-1,2,3-trideoxy-3-oxo-D-ribofuranose (6)

Figure S14: ¹H-spectrum

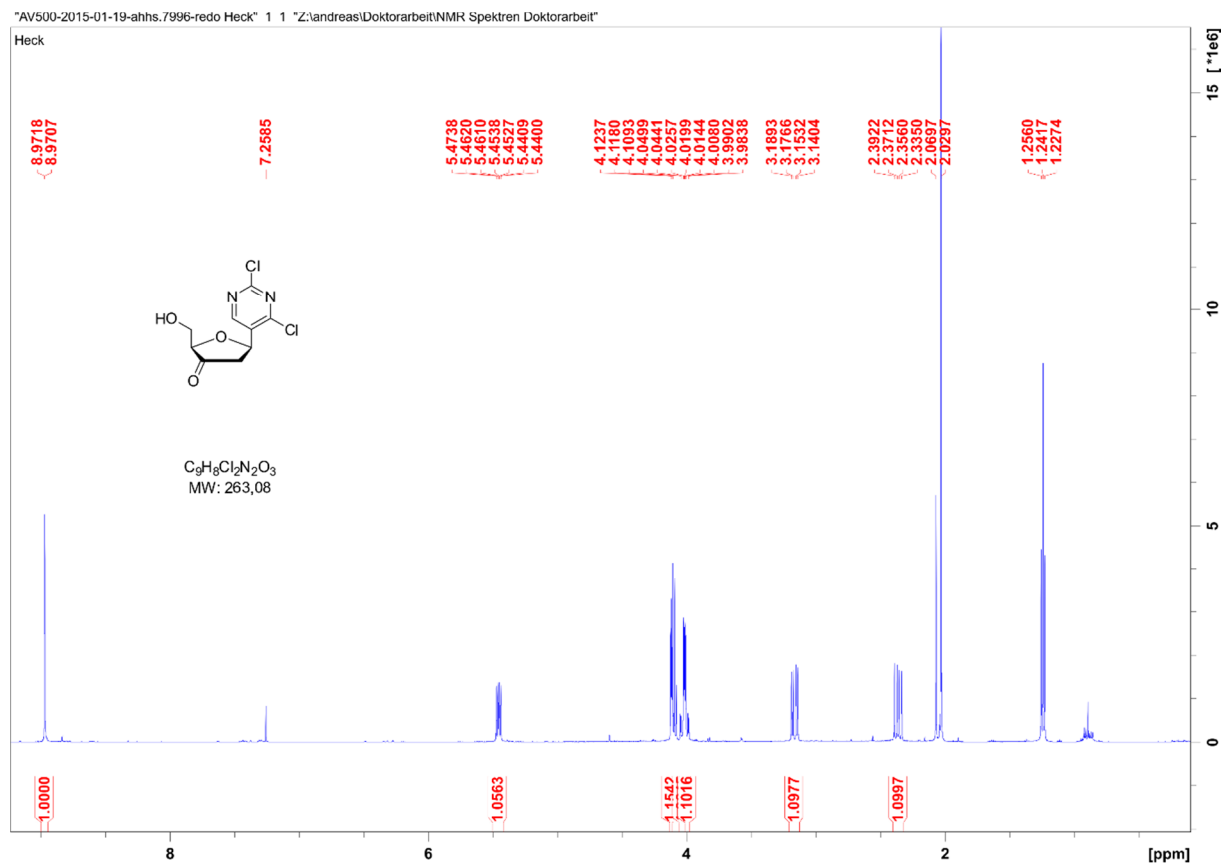


Figure S15: ^{13}C -spectrum

AV500-2015-10-05-ahhs.1752-AH-59 2 1 "Z:\andreas\Doktorarbeit\NMR Spektren Doktorarbeit"

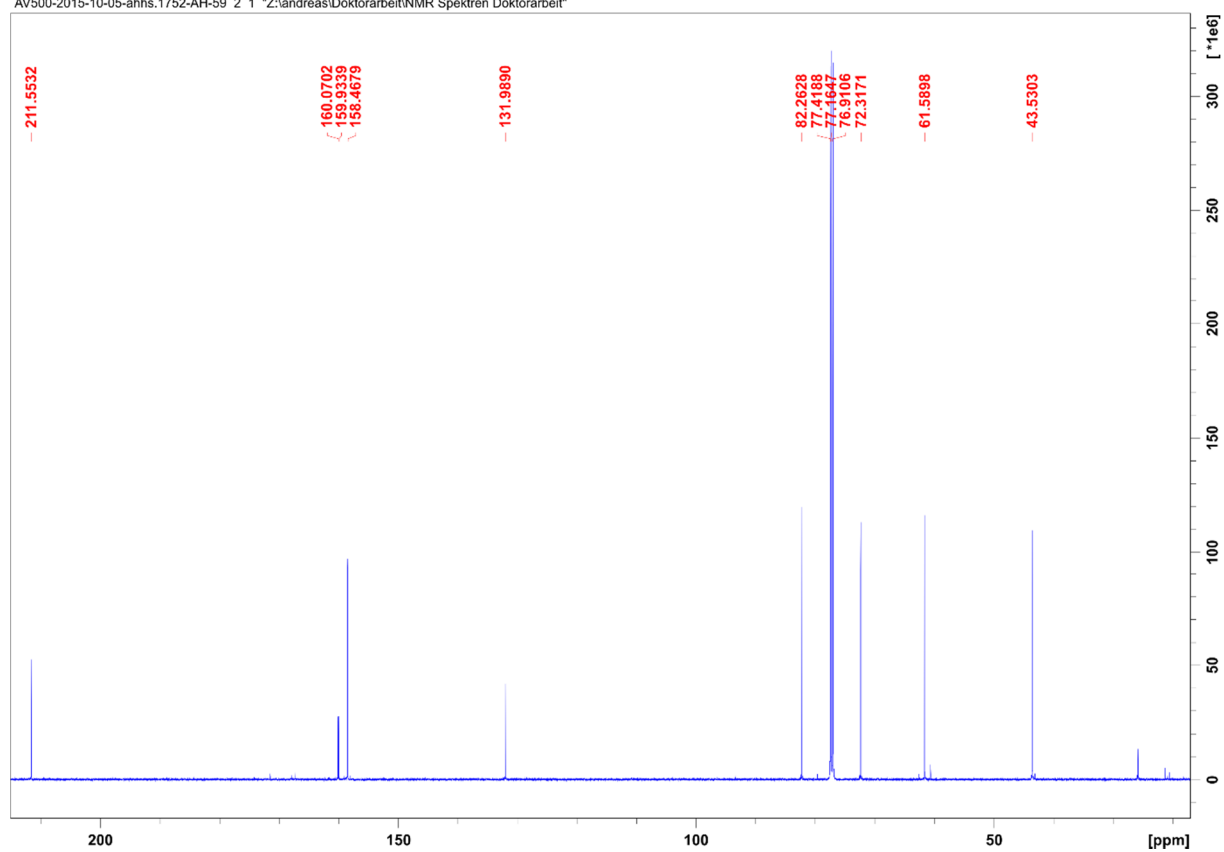
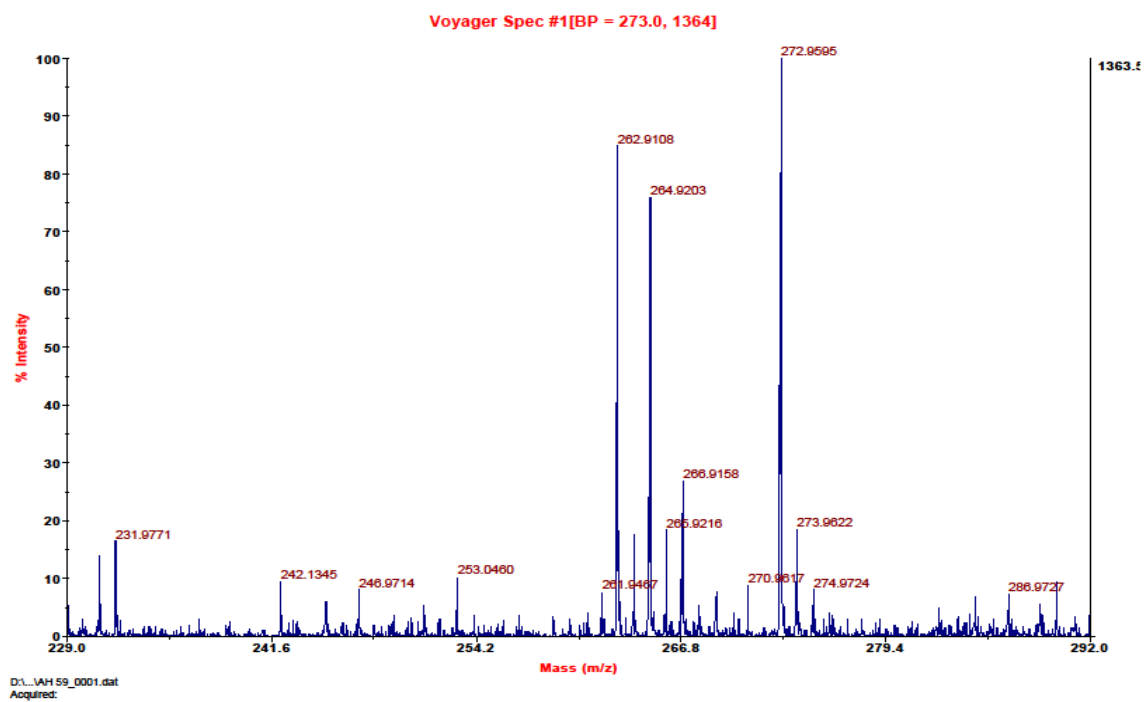


Figure S16: MALDI-spectrum



Analytics of 1β-(2,4-dichloropyrimidin-5-yl)-1,2-dideoxy-D-ribofuranose (7)

Figure S17: ¹H-spectrum

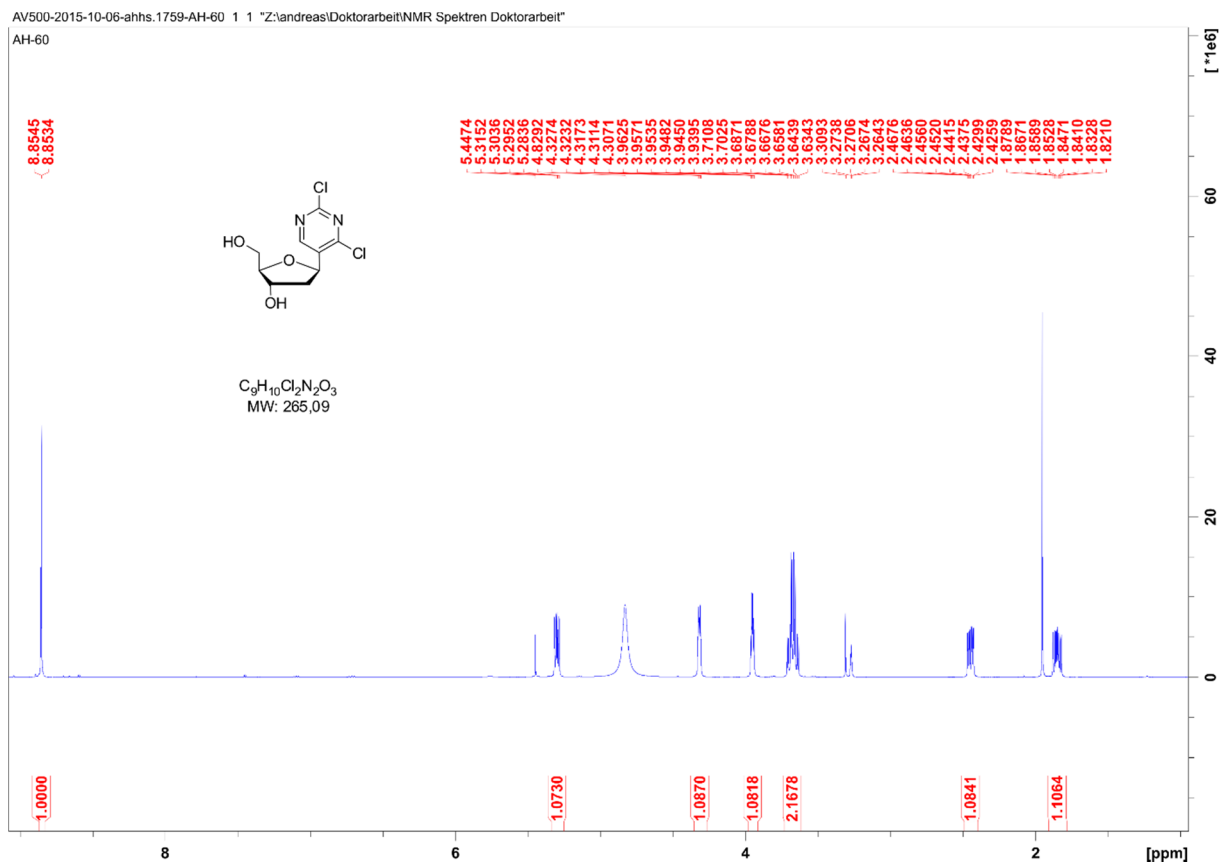


Figure S18: ^{13}C -spectrum

"AV500-2015-01-26-ahhs.8090-AH-28 Reduktion recy" 2 1 "Z:\andreas\Doktorarbeit\NMR Spektren Doktorarbeit"

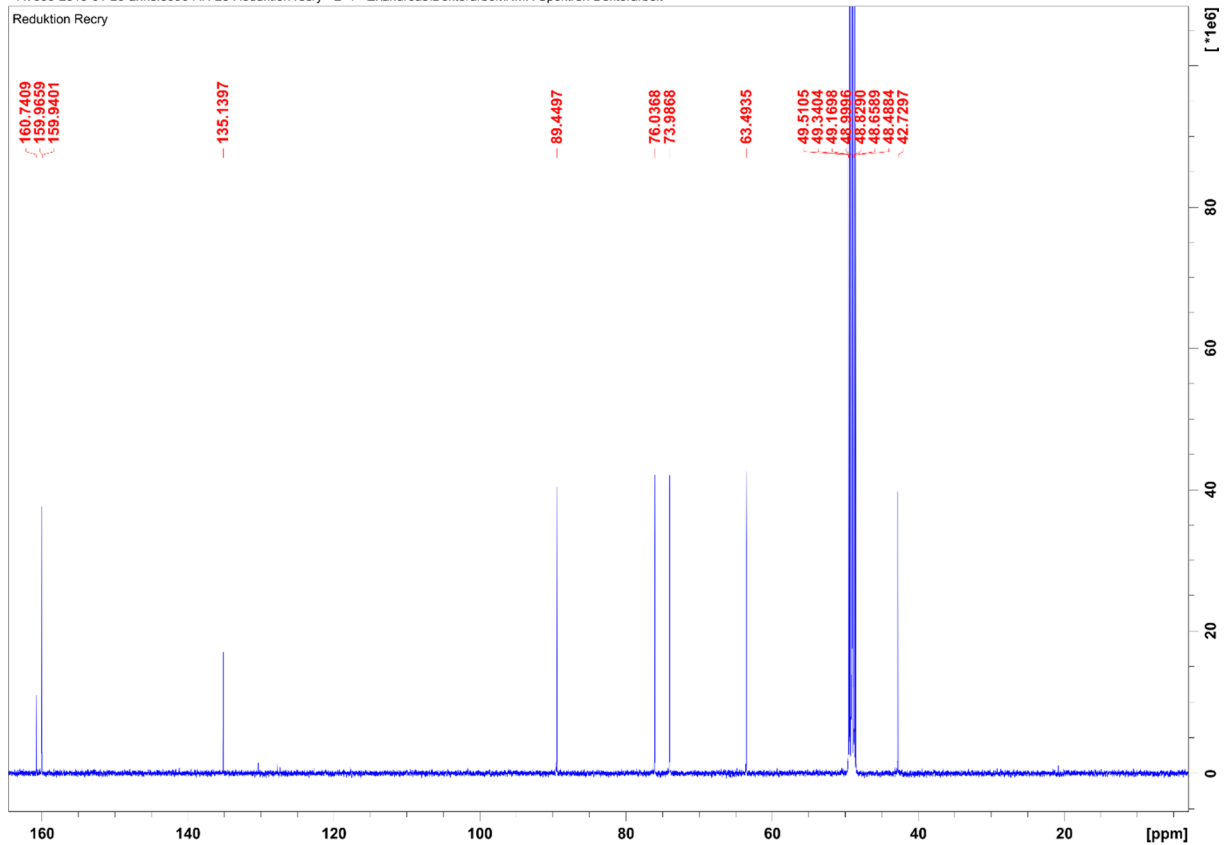
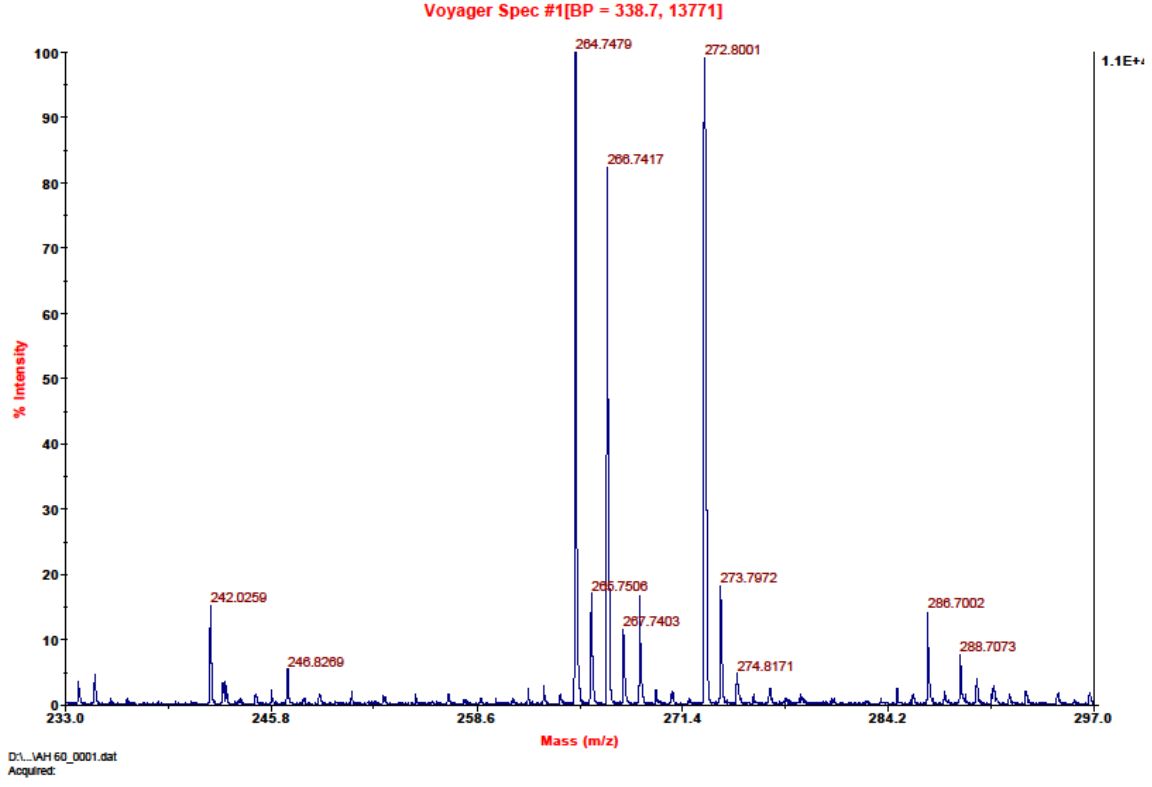


Figure S19: MALDI-spectrum



Analytics of 1β-(2,4-dichloropyrimidin-5-yl)-1,2-dideoxy-5-O-(tert-butyl-dimethylsilyl)-D-ribofuranose (8)

Figure S20: ¹H-spectrum

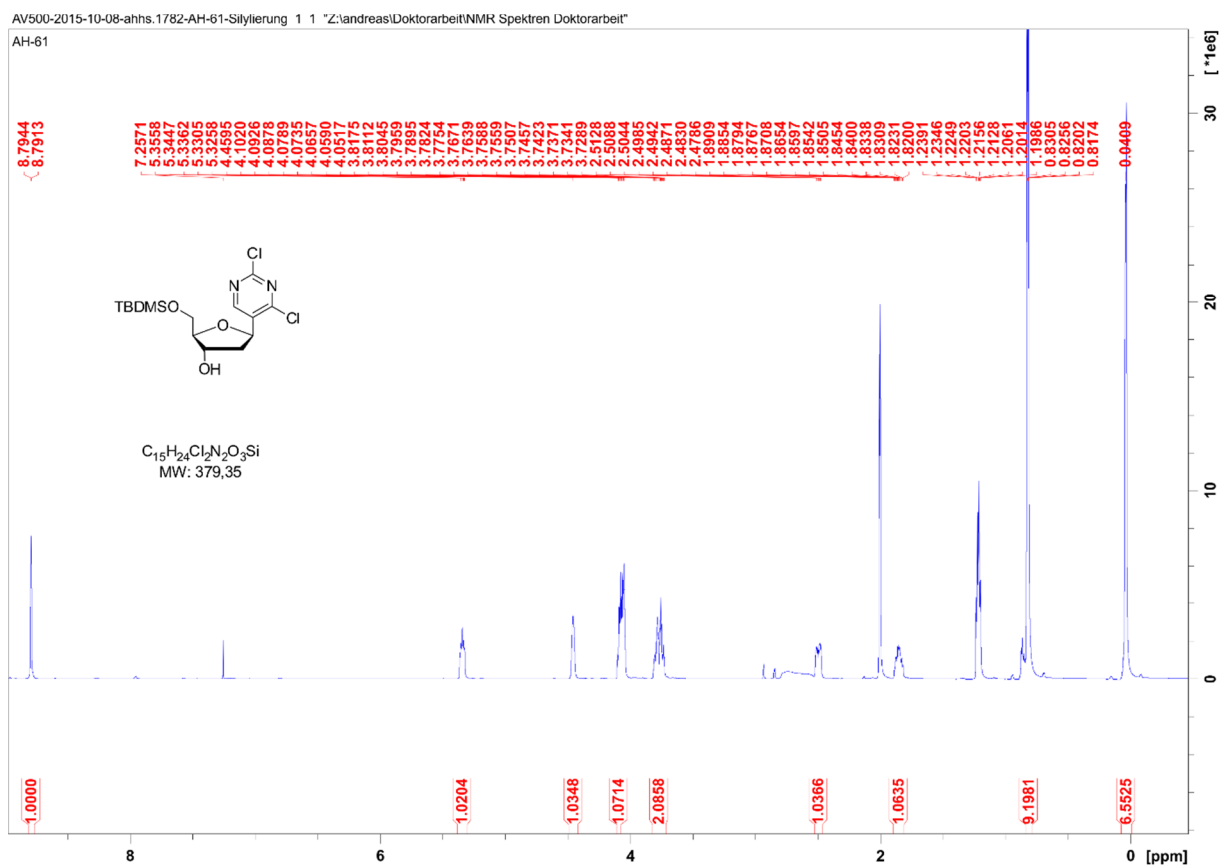


Figure S21: ¹³C-spectrum

AV500-2015-10-16-ahhs.11871-AH-61-Silylierung 2 1 "Z:\andreas\Doktorarbeit\NMR Spektren Doktorarbeit"

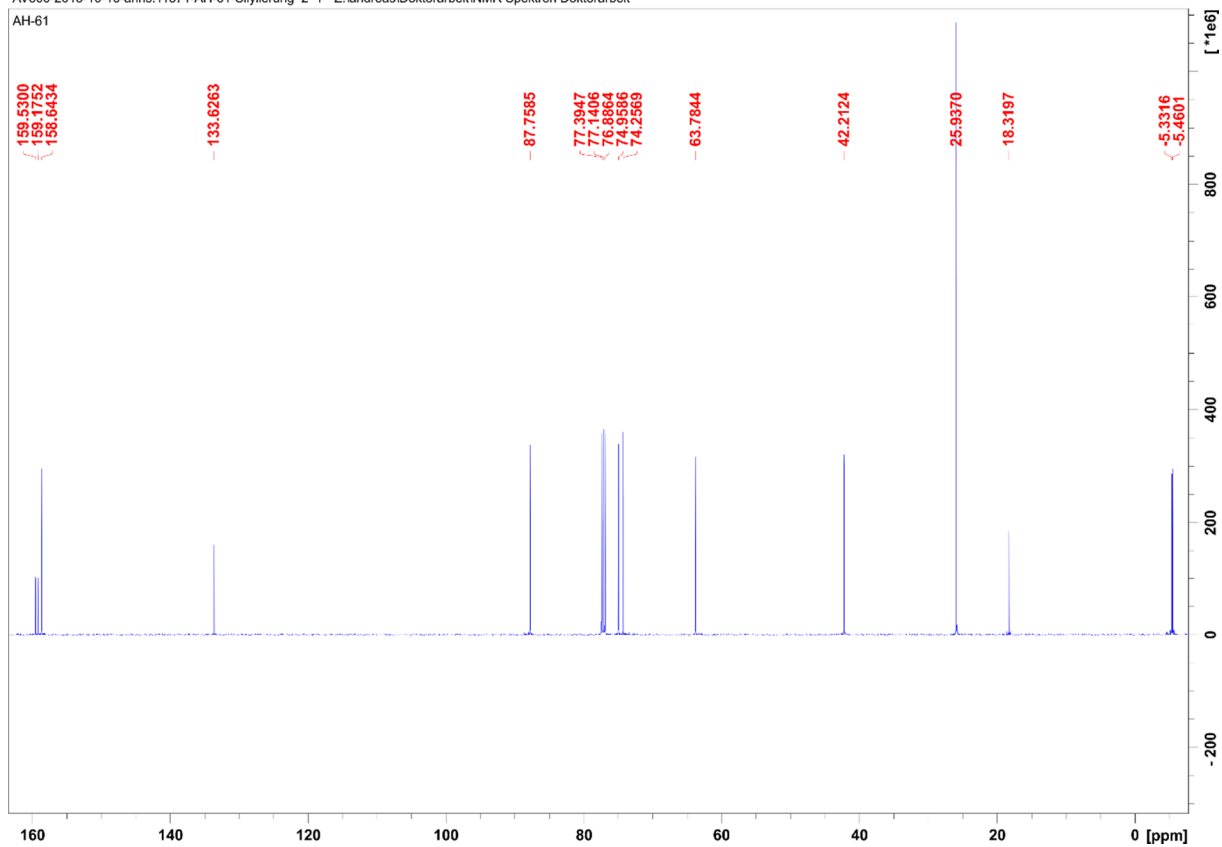
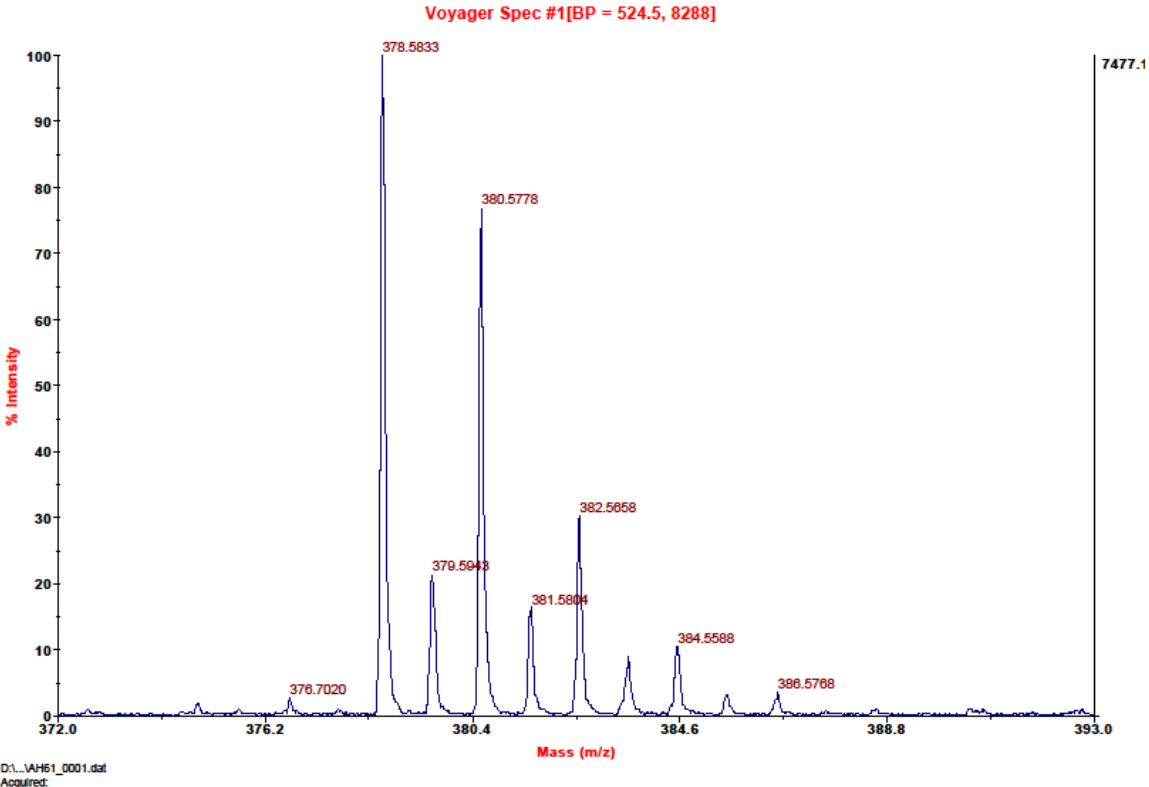


Figure S22: MALDI-spectrum



Analytics of 1β-[2,4-bis(benzoylamino)pyrimidin-5-yl]-1,2-dideoxy-5-O-(tert-butyl-dimethylsilyl)-D-ribofuranose (9)

Figure S23: ¹H-spectrum

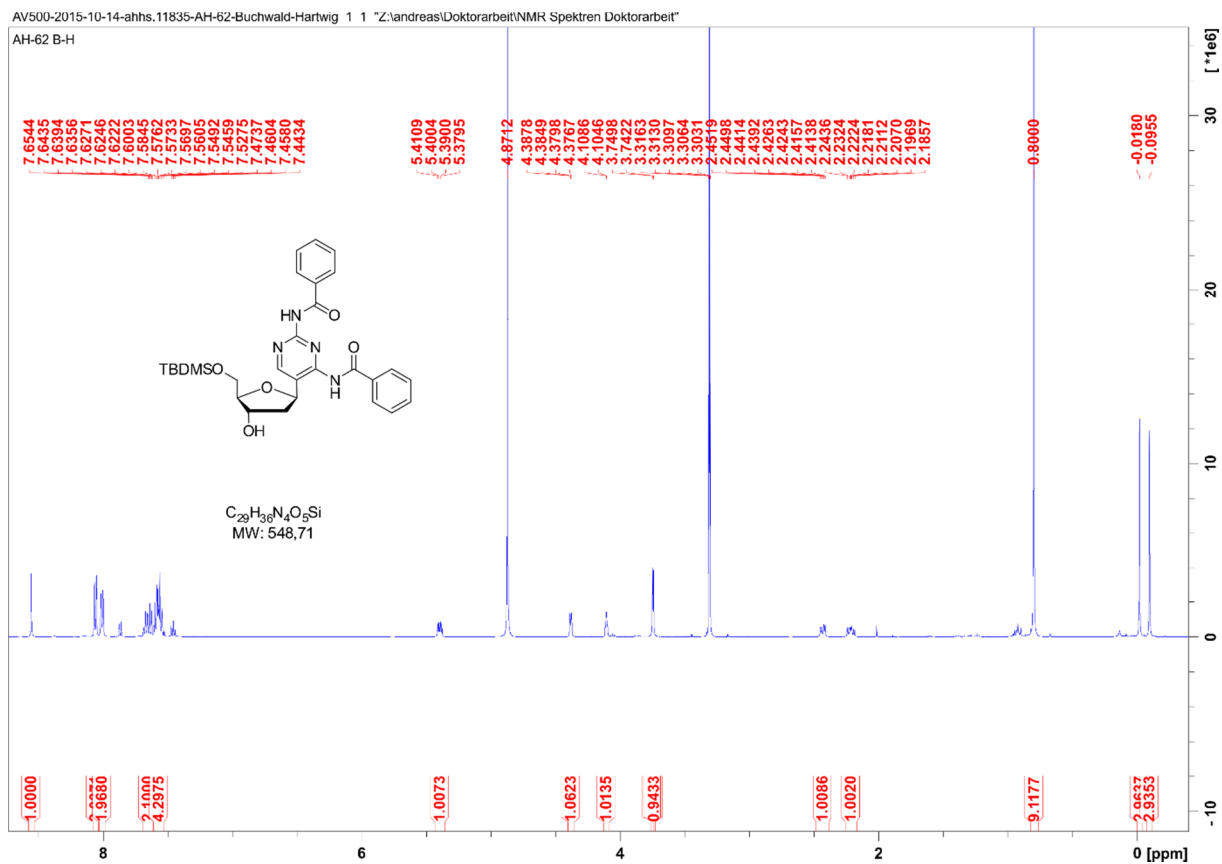


Figure S24: ¹³C-spectrum

AV500-2015-10-14-ahhs.11835-AH-62-Buchwald-Hartwig 2 1 "Z:\andreas\Doktorarbeit\NMR Spektren Doktorarbeit"

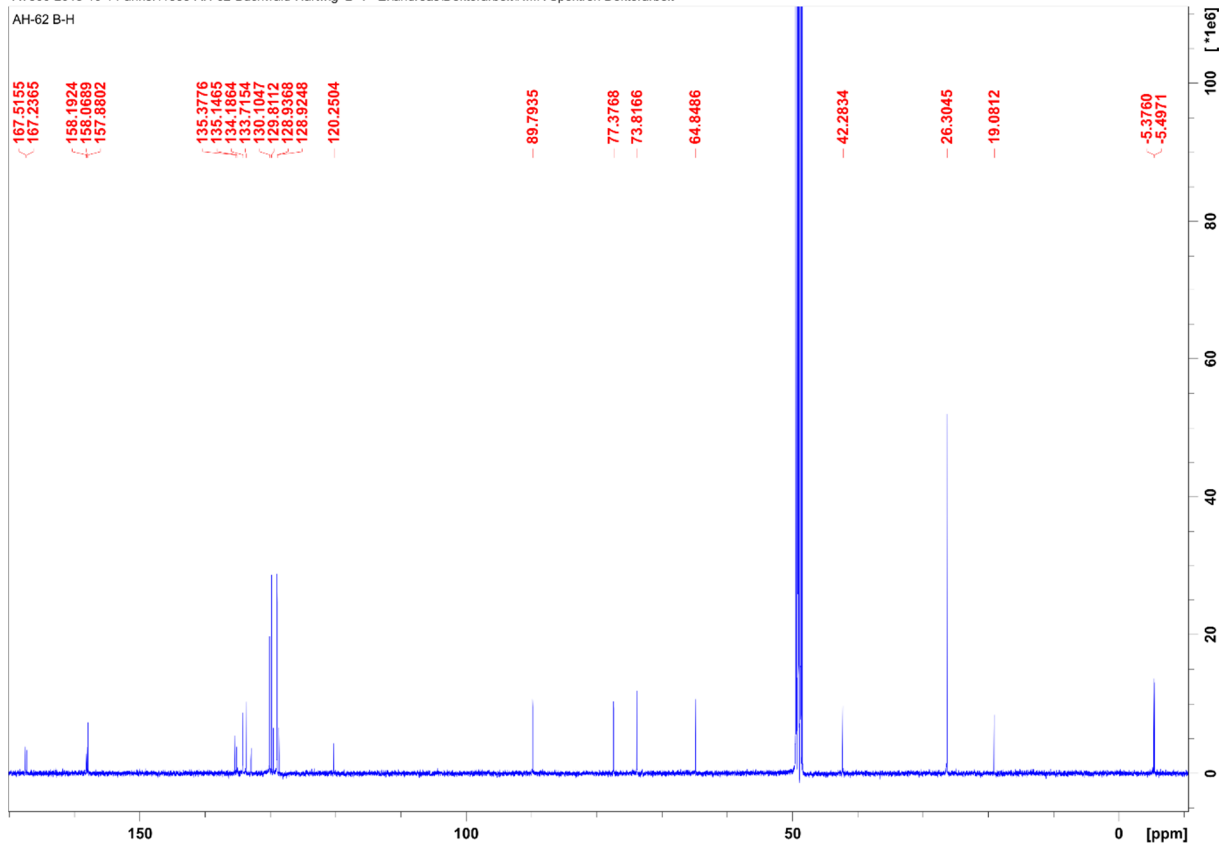
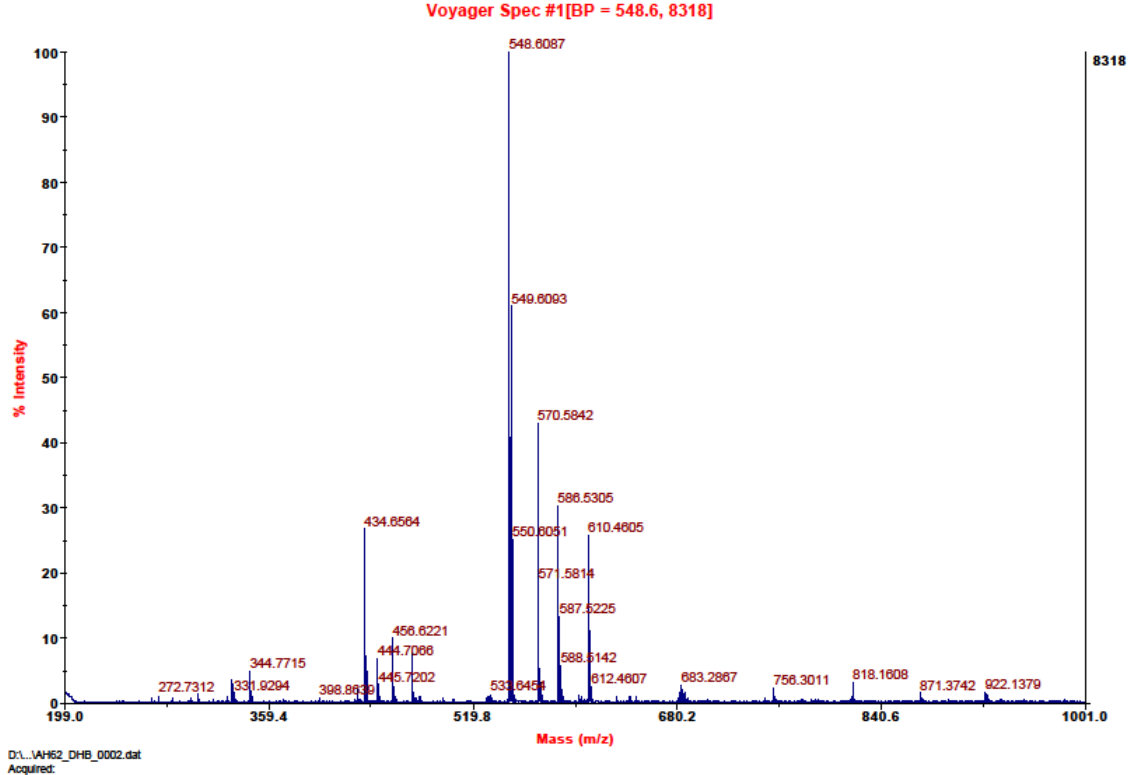


Figure S25: MALDI-spectrum



Analytics of 1 β -[2,4-bis(benzoylamino)pyrimidin-5-yl]-1,2-dideoxy-D-ribofuranose (10)

Figure S26: ^1H -spectrum

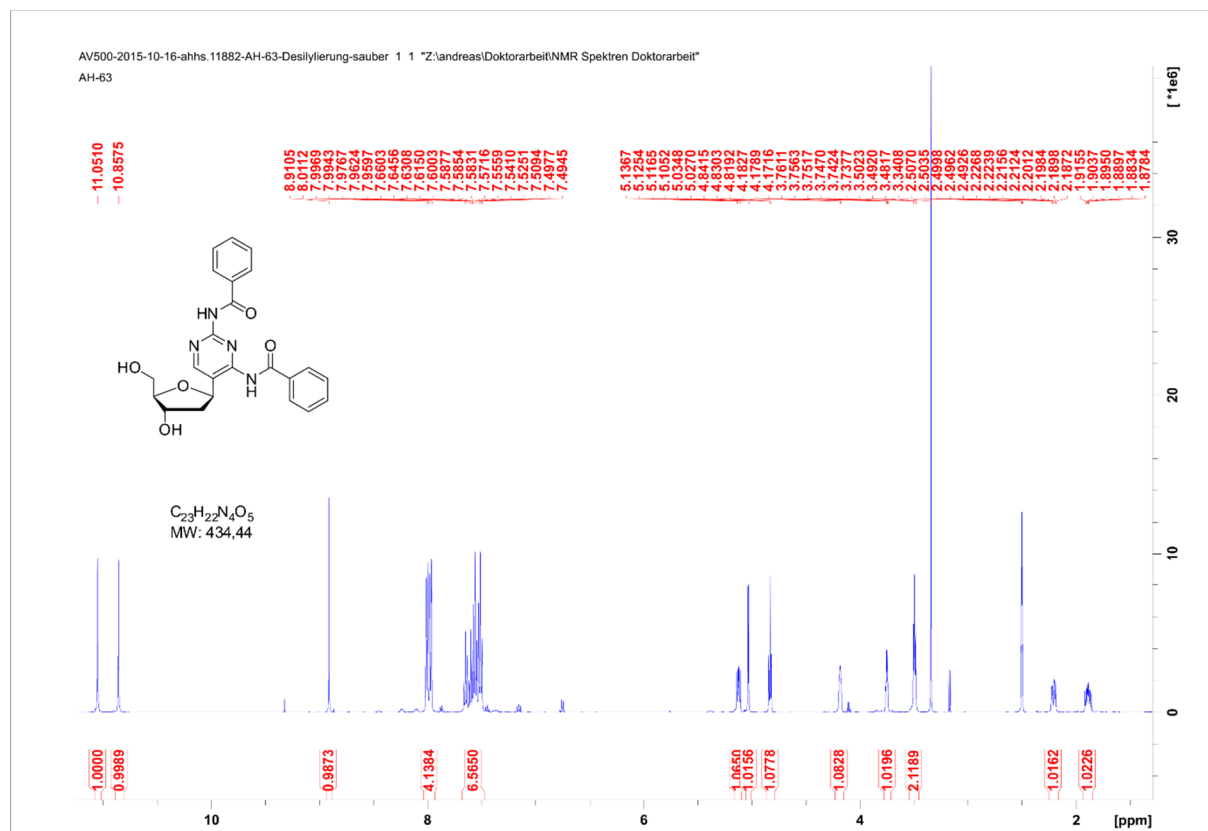


Figure S27: ^{13}C -spectrum

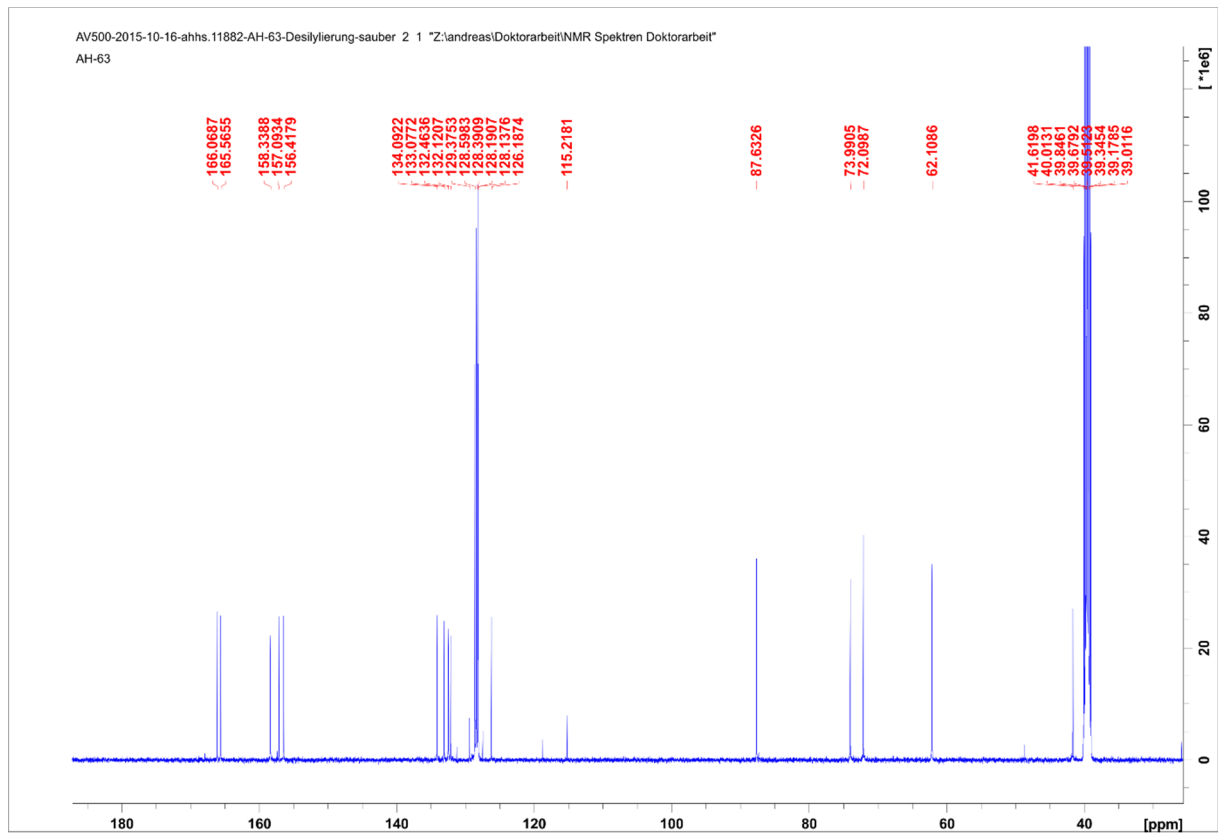
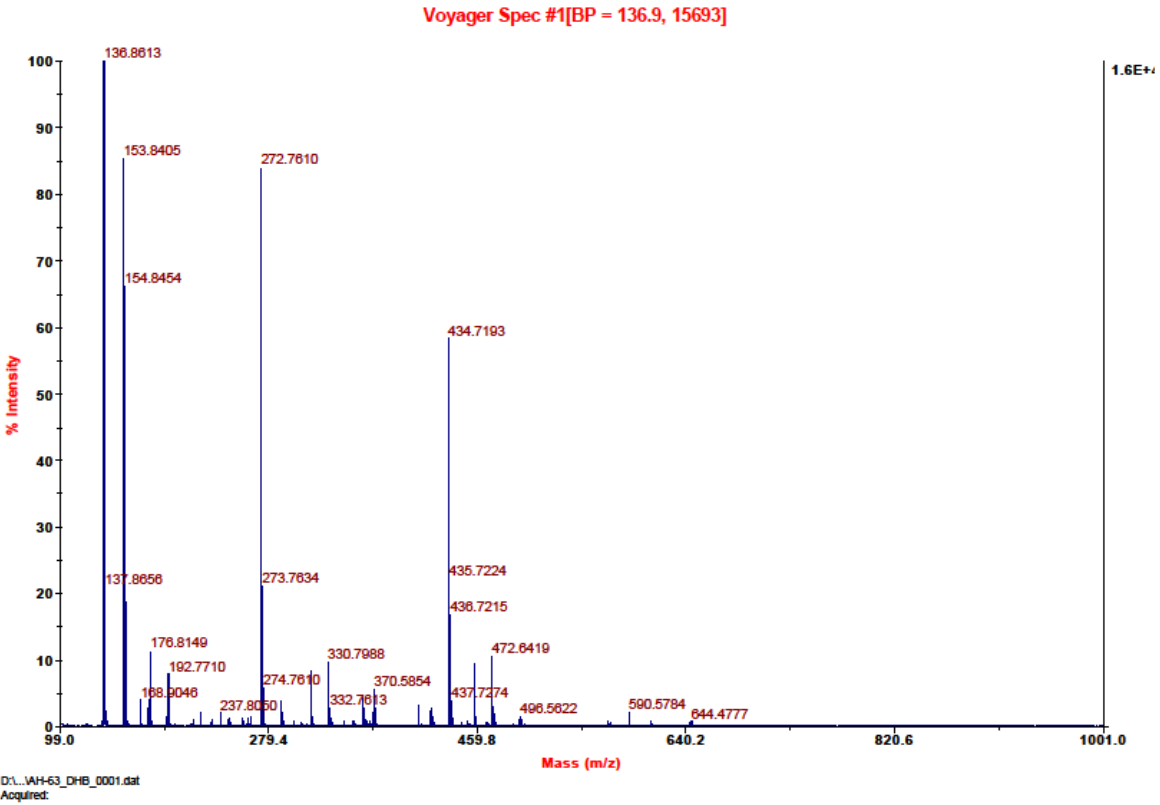


Figure S28: MALDI-spectrum



Analytics of 1β-[2,4-bis(benzoylamino)pyrimidin-5-yl]-1,2-dideoxy-5-O-(4,4'-dimethoxytriphenylmethyl)-D-ribofuranose (11)

Figure S29: ¹H-spectrum

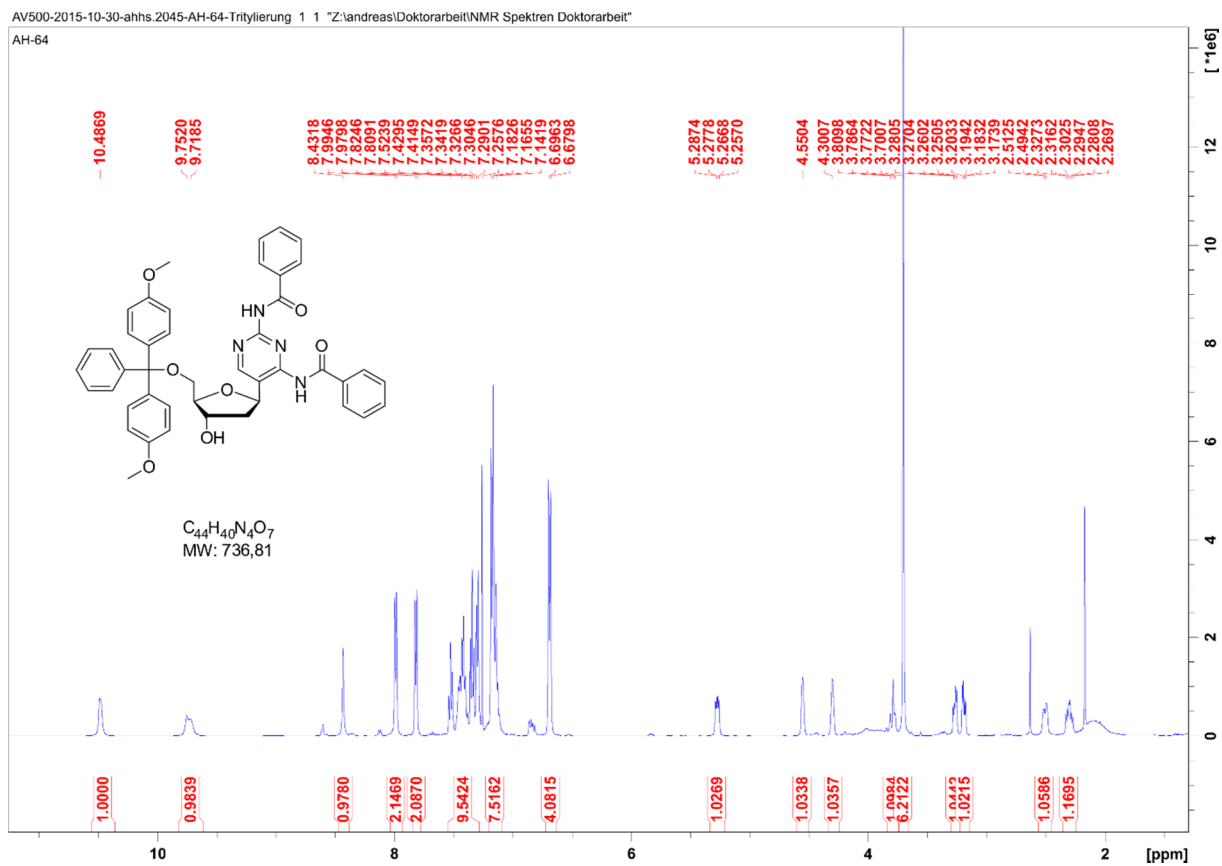


Figure S30: ¹³C-spectrum

AV500-2015-10-30-ahhs.2045-AH-64-Tritylierung 2 1 "Z:\andreas\Doktorarbeit\NMR Spektren Doktorarbeit"

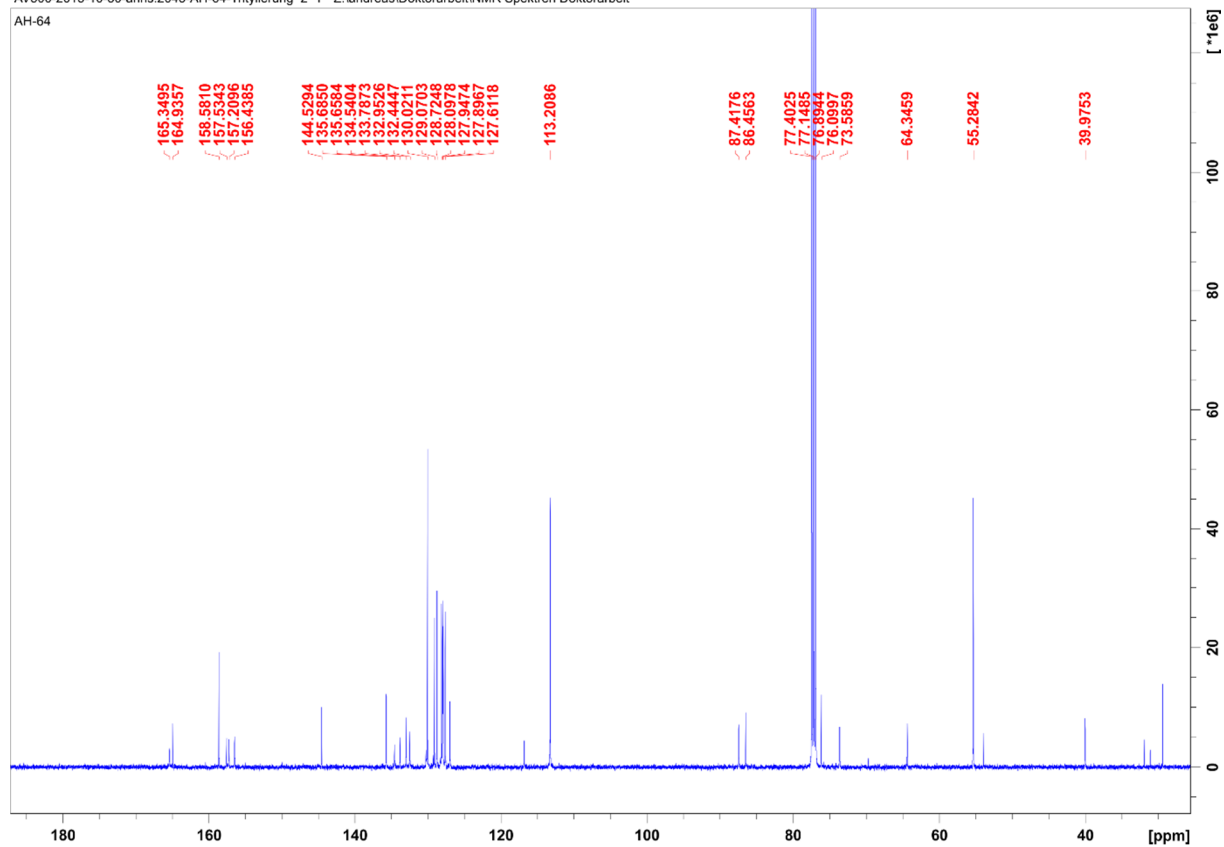
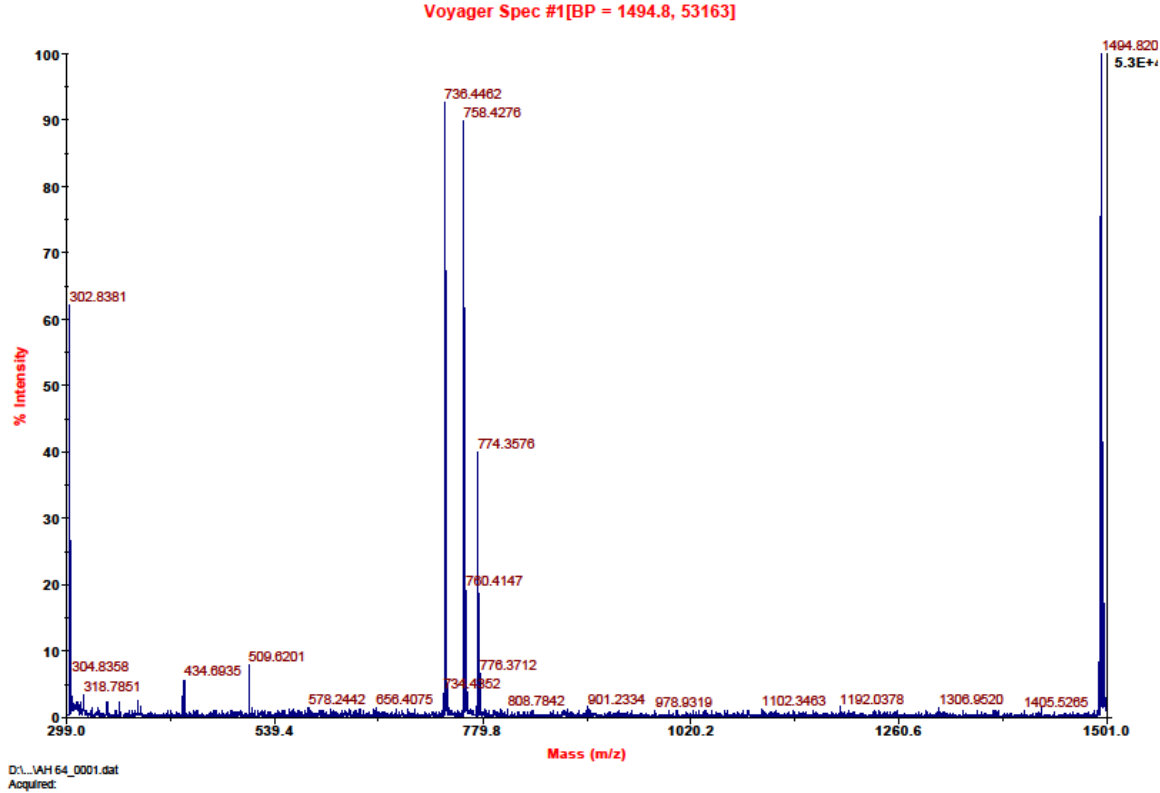


Figure S31: MALDI-spectrum



Analytics of 1β-[2,4-bis(benzoylamino)pyrimidin-5-yl]-1,2-dideoxy-5-O-(4,4'-dimethoxytriphenylmethyl)-D-ribofuranose-3-[(2-cyanoethyl)(N,N-diisopropyl)]phosphoramidite (12)

Figure S32: ¹H-spectrum

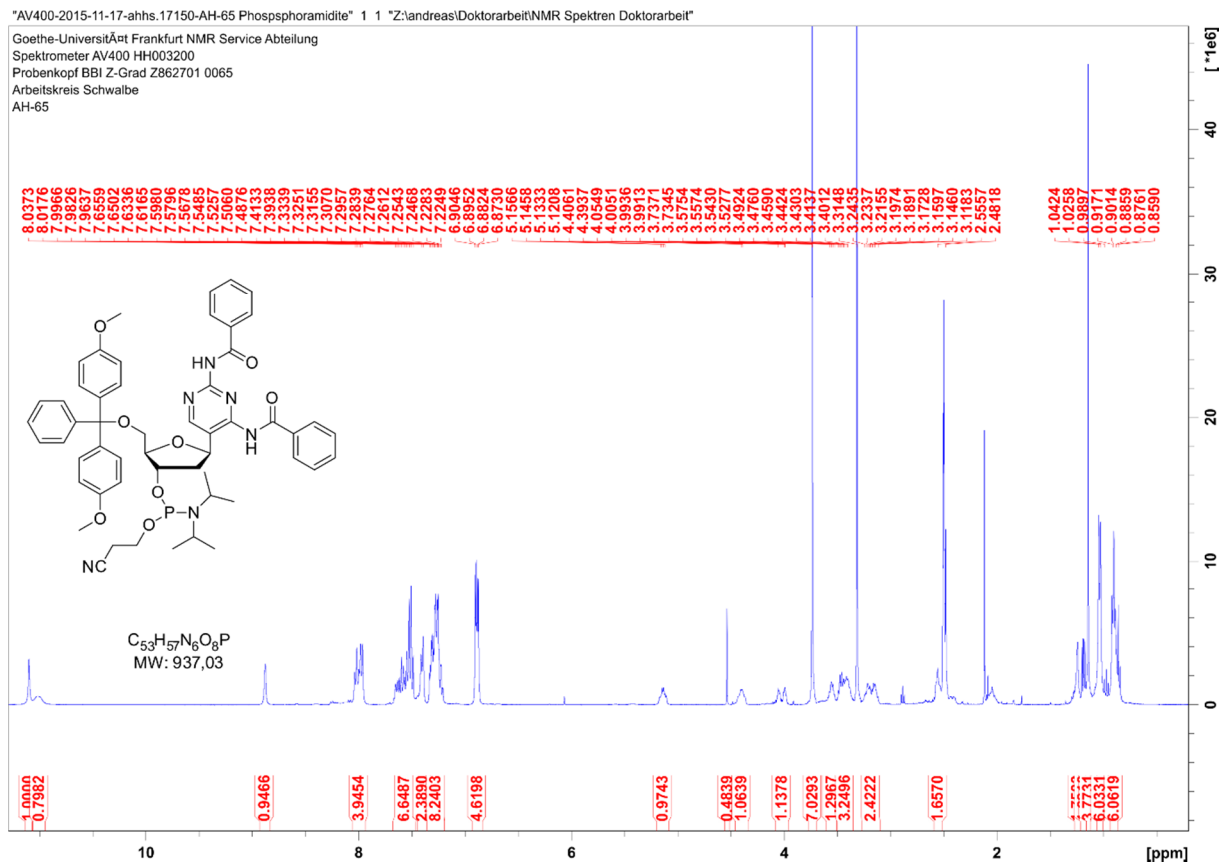


Figure S33: ^{31}P -spectrum

"AV500-2015-11-17-ahhs.12305-AH-65-Phosphoramidite 2" 2 1 "Z:\landreas\Doktorarbeit\NMR Spektren Doktorarbeit"

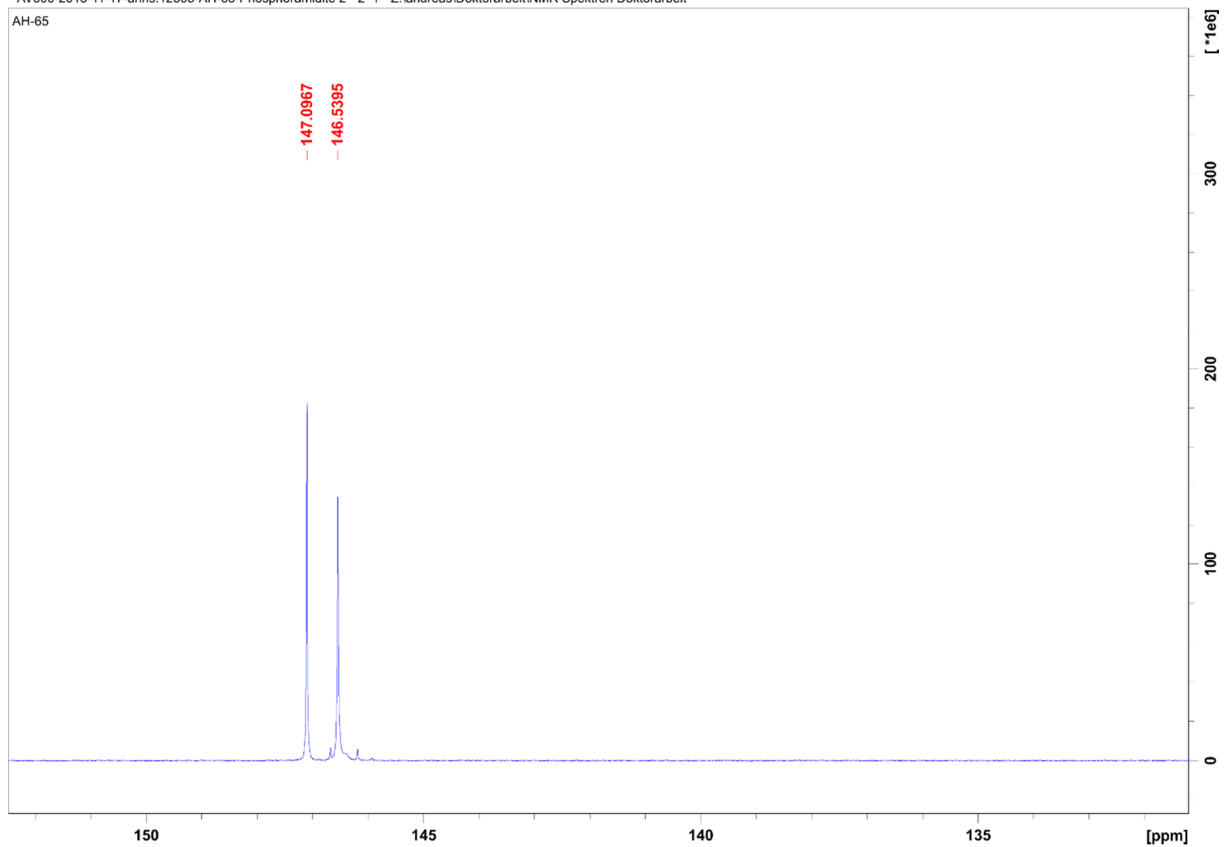
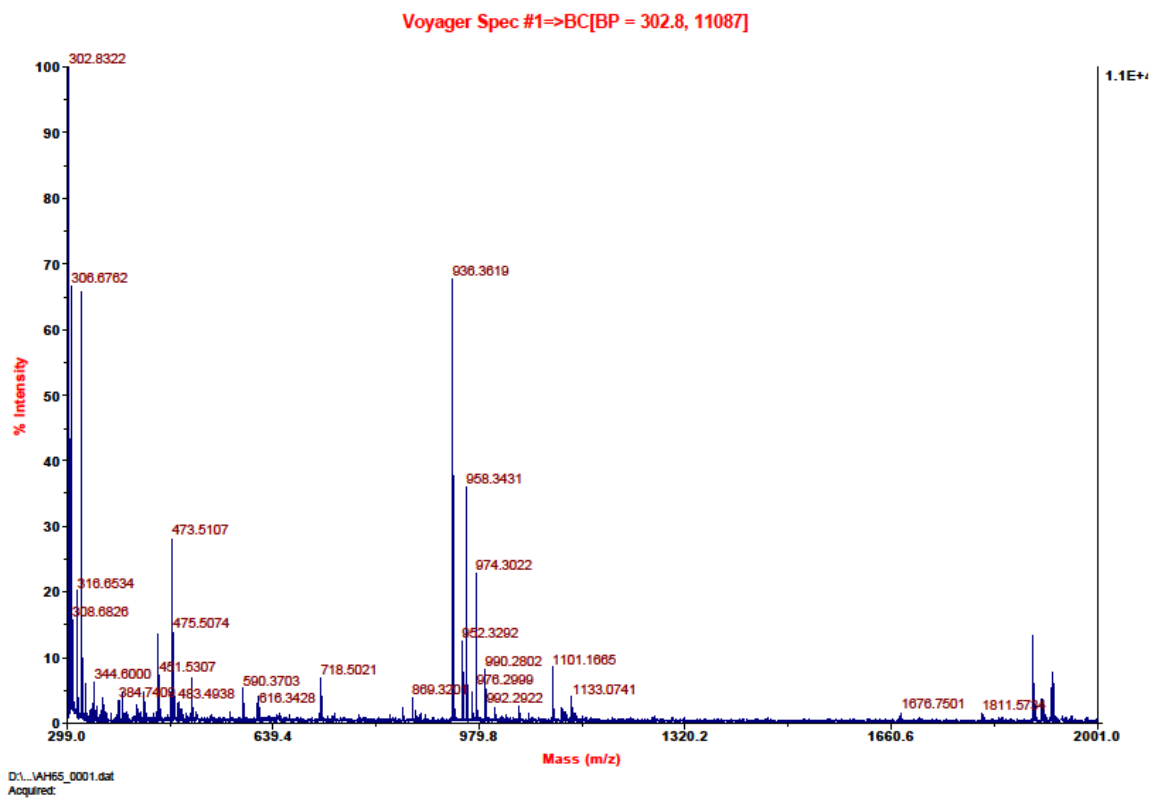


Figure S34: MALDI-spectrum



Analytics of *N*-(4-chloro-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)-2,2-dimethylpropionamide (14)

Figure S35: ¹H-spectrum

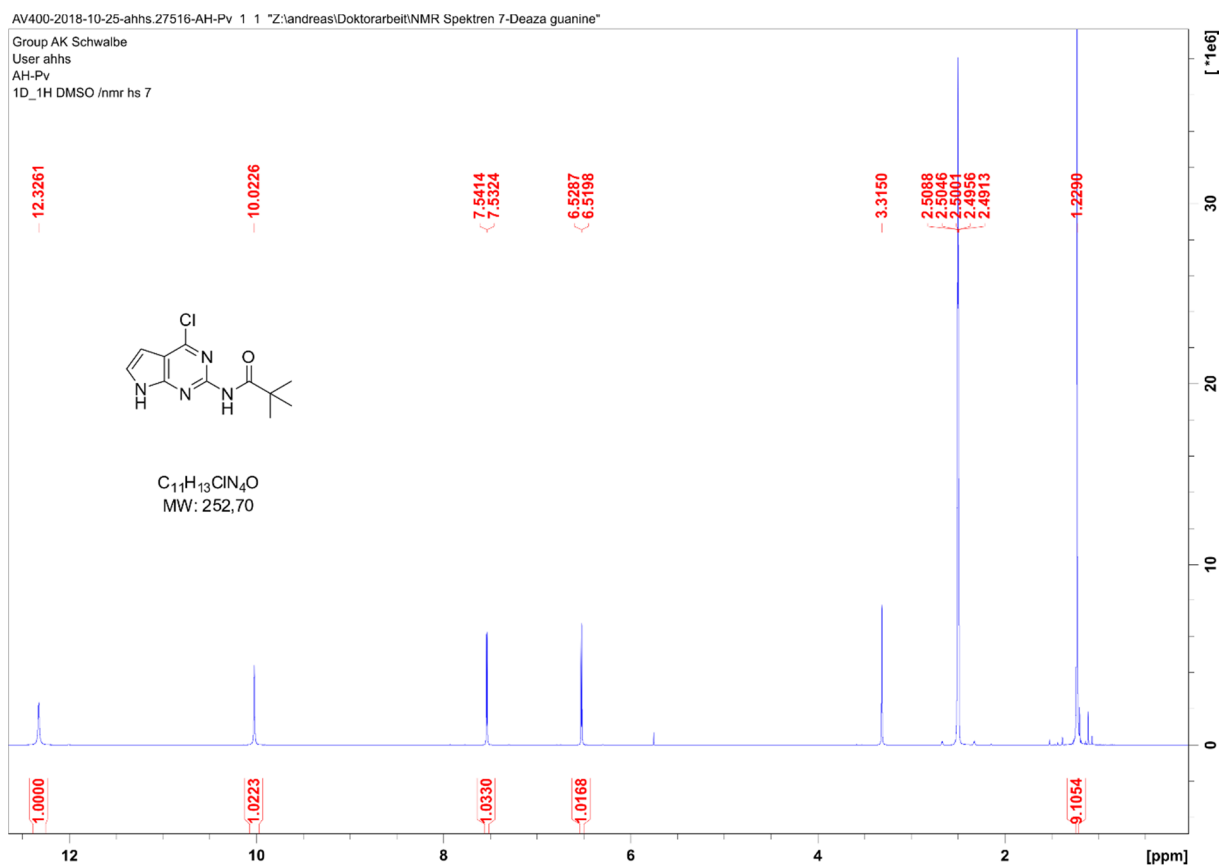


Figure S36: ¹³C-spectrum

AV500-2018-10-17-ahhs.26475-AH-Pv 2 1 "Z:\andreas\Doktorarbeit\NMR Spektren 7-Deaza guanine"

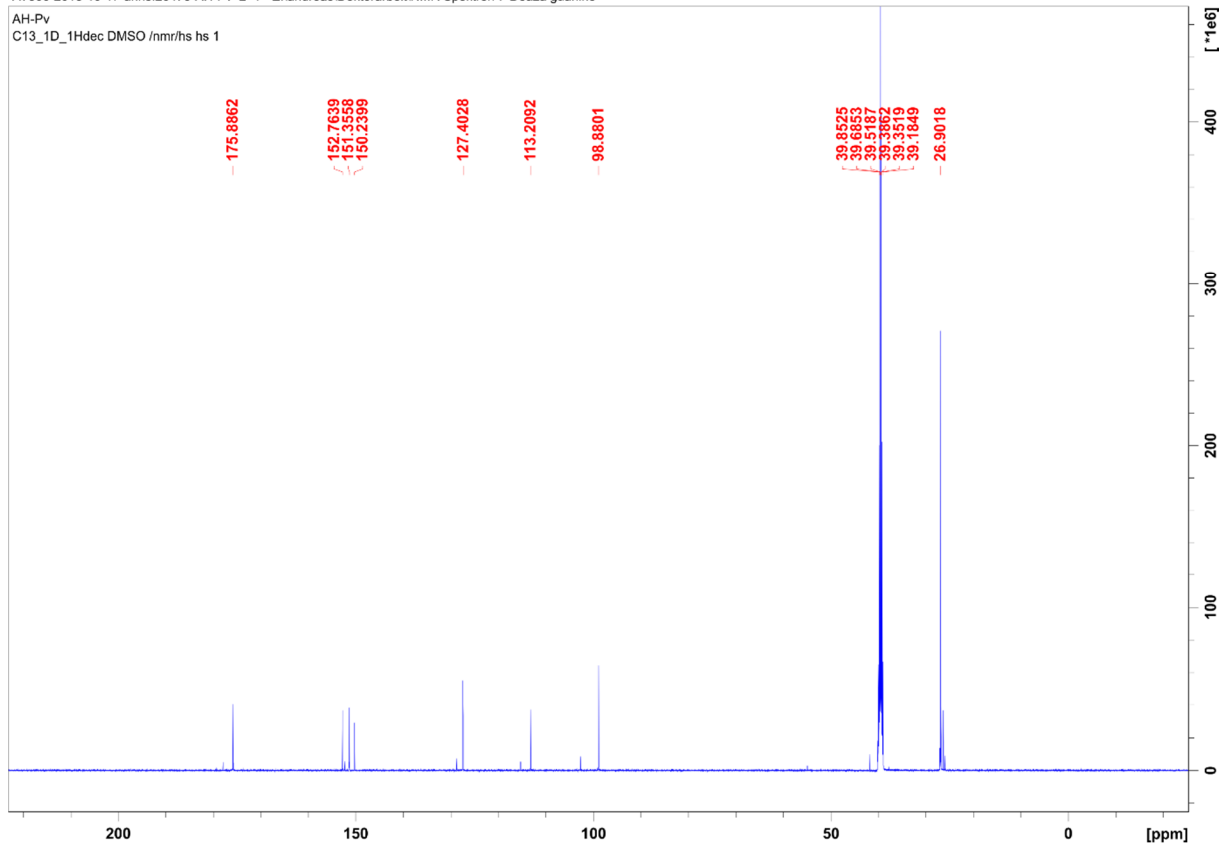
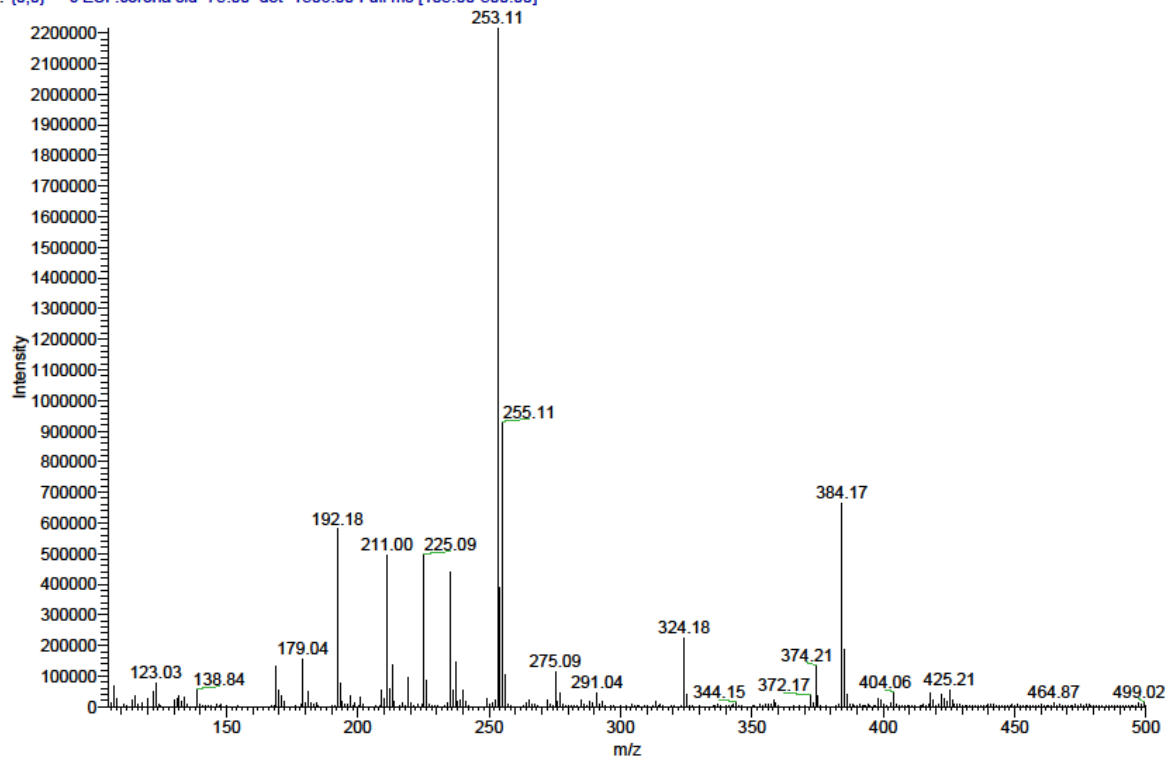


Figure S37: ESI-spectrum

C:\Xcalibur\data\AH-152

11/9/2016 2:12:54 PM

AH-152 #39-43 RT: 0.65-0.72 AV: 5 SB: 9 0.33-0.46 NL: 2.22E6
T: {0,0} + c ESI Icorona sid=75.00 det=1506.00 Full ms [105.00-500.00]



Analytics of *N*-(4-chloro-5-iodo-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl)-2,2-dimethylpropionamide (15)

Figure S38: ¹H-spectrum

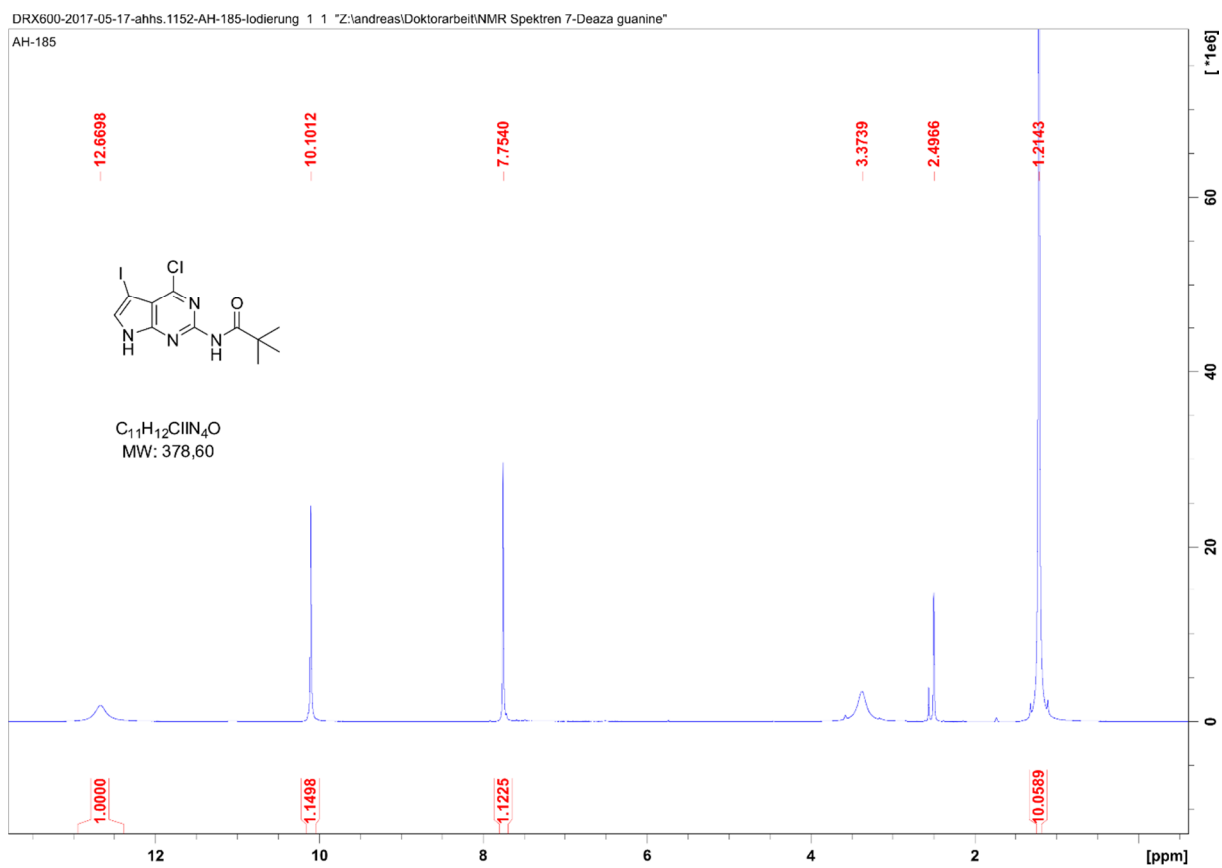


Figure S39: ¹³C-spectrum

DRX600-2017-05-17-ahhs.1152-AH-185-Iodierung 2 1 "Z:\andreas\Doktorarbeit\NMR Spektren 7-Deaza guanine"

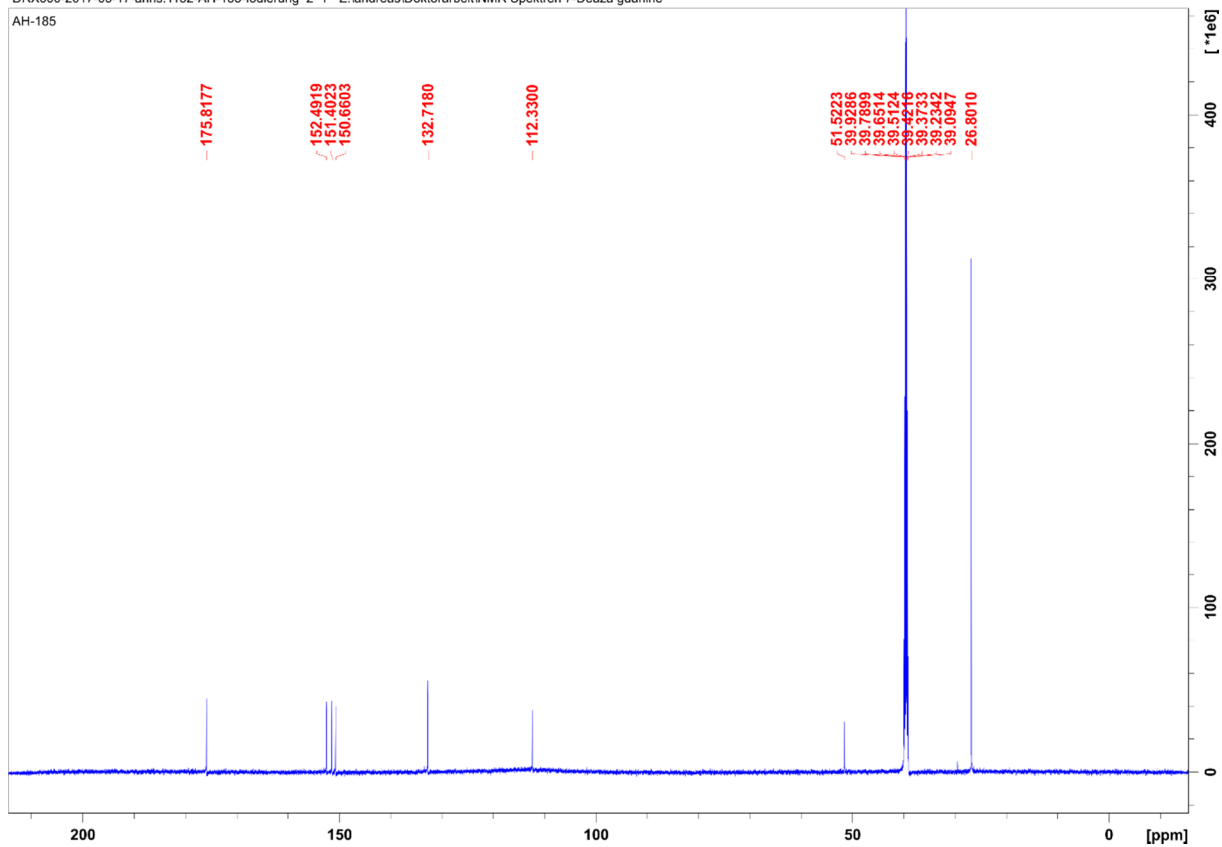
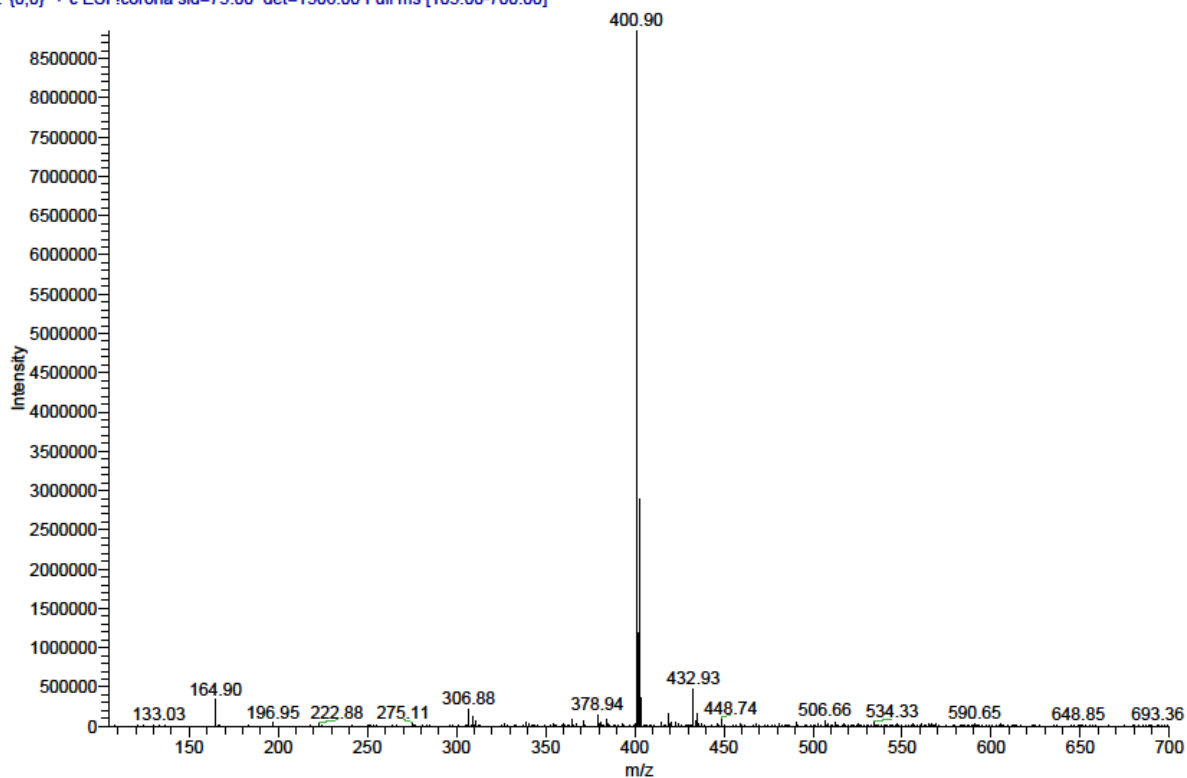


Figure S40: ESI-spectrum

C:\Xcalibur\data\AH-153

11/10/2016 3:01:20 PM

AH-153 #41-44 RT: 0.69-0.74 AV: 4 SB: 8 0.07-0.19 NL: 8.85E6
T: {0,0} + c ESI Icorona sid=75.00 det=1506.00 Full ms [105.00-700.00]



Analytics of 4-chloro-5-iodo-2-pivaloylamino-7-[(2,3,5-tri-*O*-benzoyl)- β -D-ribofuranosyl]-7*H*-pyrrolo[2,3-*d*]pyrimidine (16)

Figure S41: ^1H -spectrum

DRX600-2018-09-19-ahhs.3439-AH-Zucker 1 1 "Z:\andreas\ Doktorarbeit\ NMR Spektren 7-Deaza guanine"

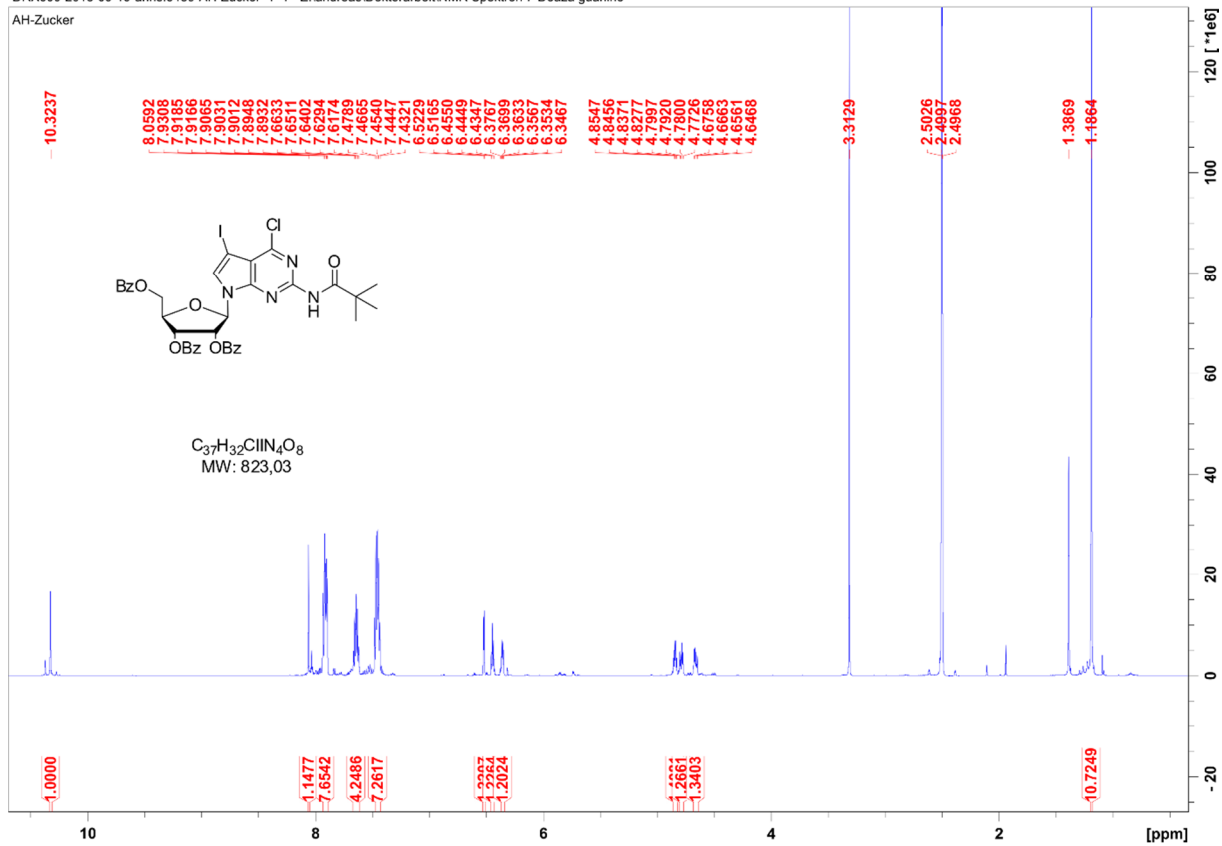


Figure S42: ¹³C-spectrum

DRX600-2018-09-19-ahhs.3439-AH-Zucker 2 1 "Z:\andreas\Doktorarbeit\NMR Spektren 7-Deaza guanine"

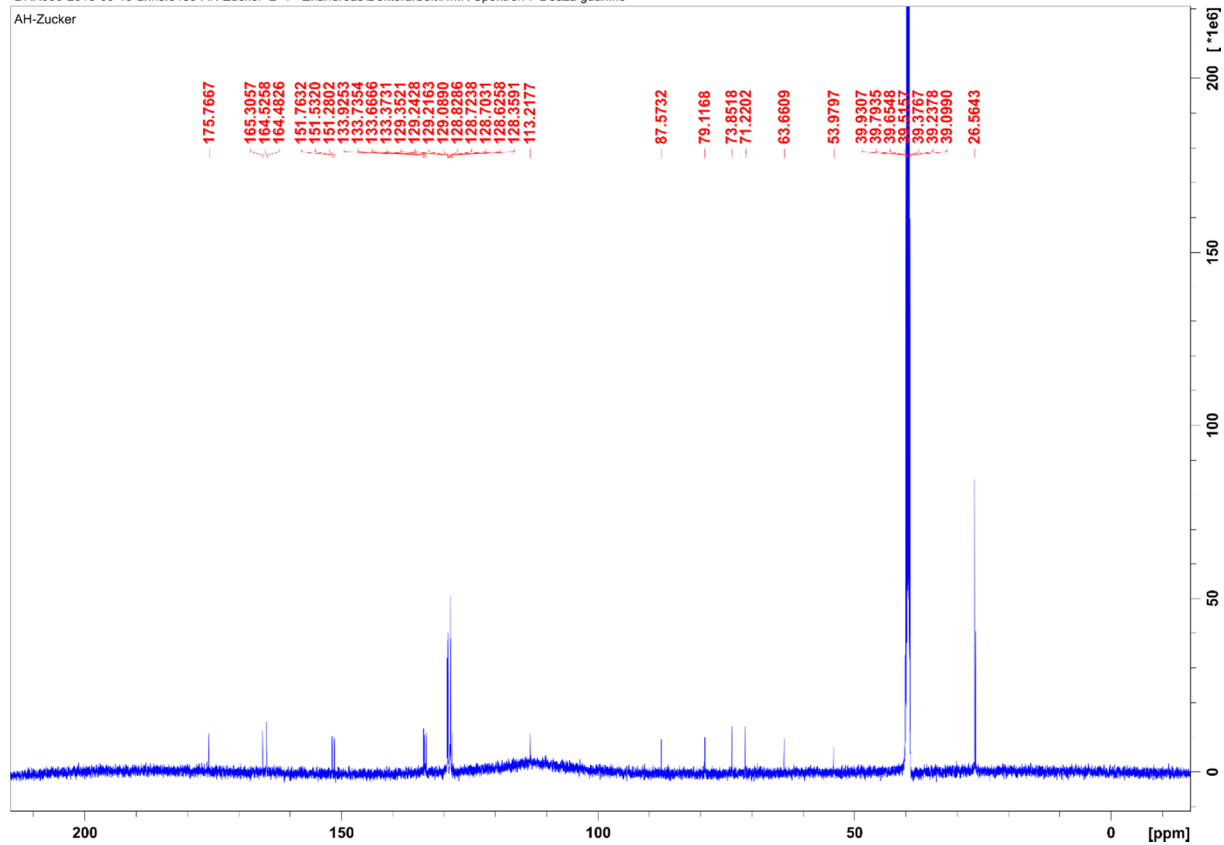
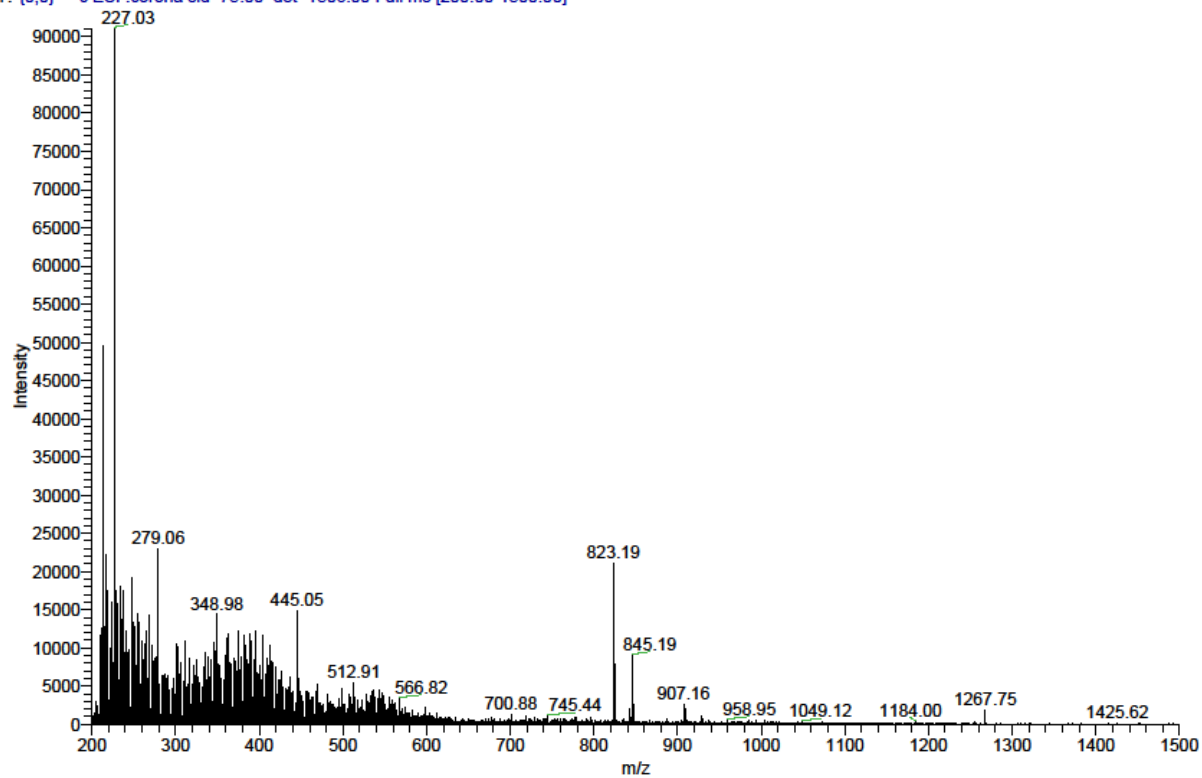


Figure S43: ESI-spectrum

C:\Xcalibur\data\AH-161-1

12/15/2016 11:11:50 AM

AH-161-1 #33-43 RT: 0.56-0.73 AV: 11 NL: 9.11E4
T: {0,0} + c ESI !corona sid=75.00 det=1506.00 Full ms [200.00-1500.00]



Analytics of 2-amino-5-iodo-3,7-dihydro-7-(β -D-ribofuranosyl)-4H-pyrrolo-[2,3-d]pyrimidin-4-one (17)

Figure S44: ^1H -spectrum

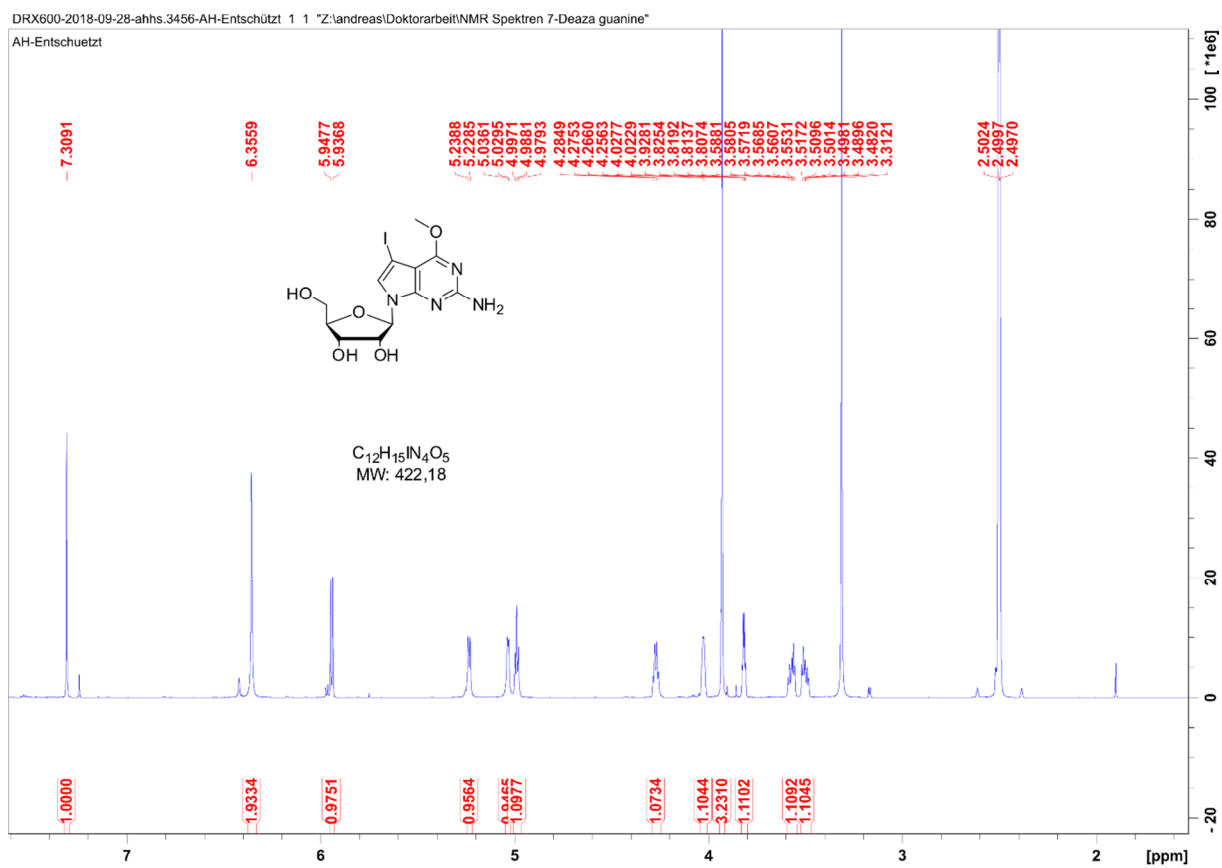


Figure S45: ¹³C-spectrum

DRX600-2018-09-28-ahhs.3456-AH-Entschützt 2 1 "Z:\andreas\Doktorarbeit\NMR Spektren 7-Deaza guanine"

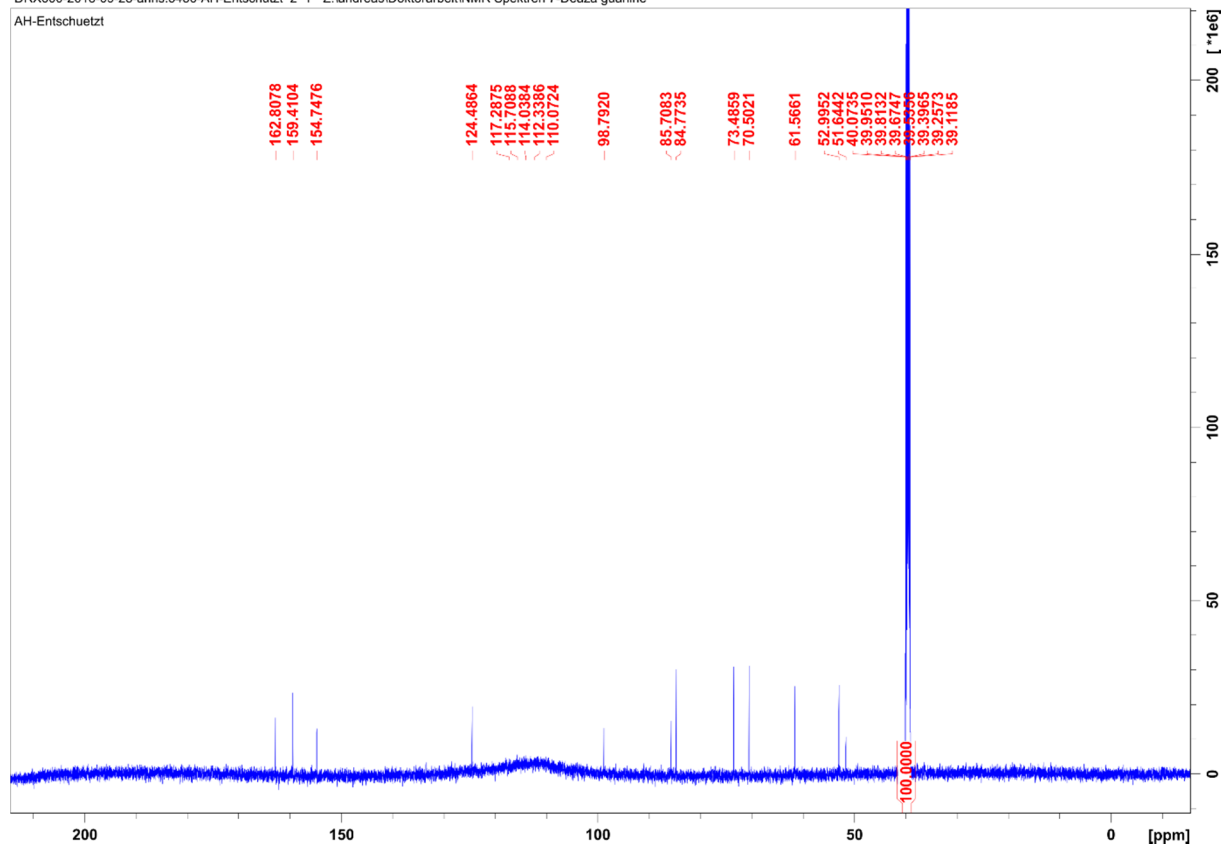
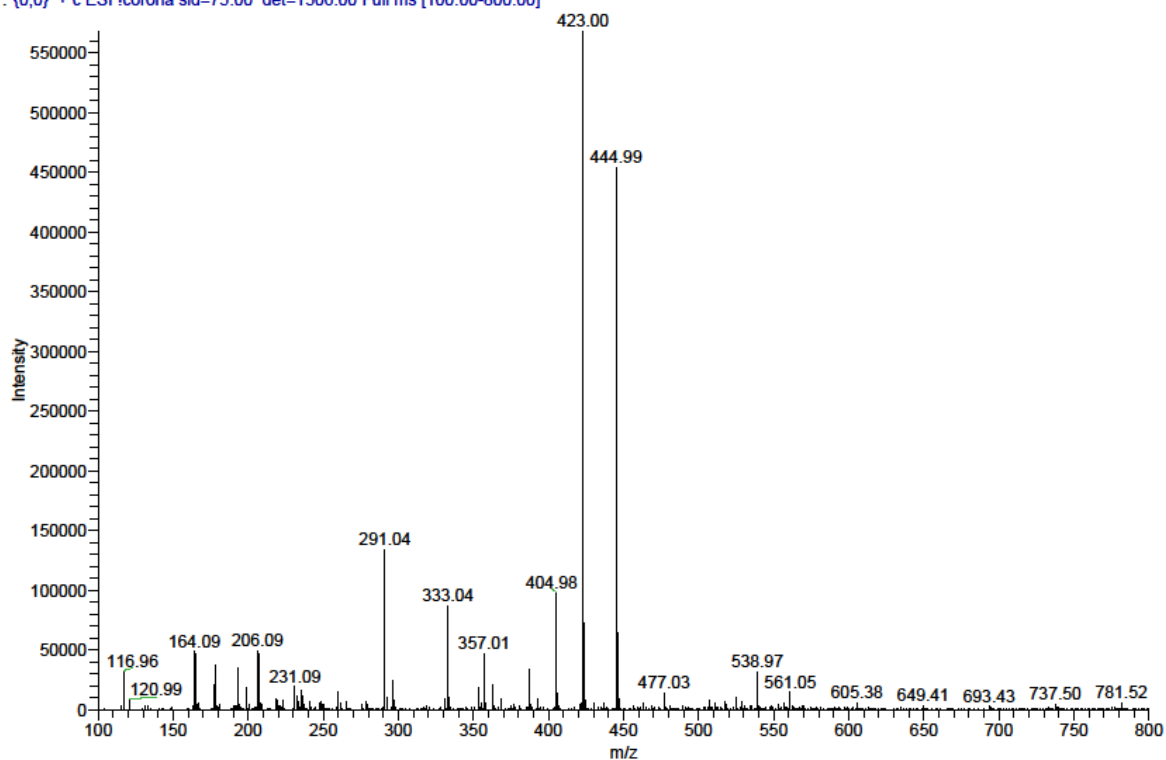


Figure S46: ESI-spectrum

C:\Xcalibur\data\AH-164-1

1/16/2017 2:18:03 PM

AH-164-1 #34-43 RT: 0.57-0.72 AV: 10 SB: 12 0.09-0.28 NL: 5.68E5
T: {0,0} + c ESI Icorona sid=75.00 det=1506.00 Full ms [100.00-800.00]



Analytics of 1,7-dihydro-5-iodo-4-methoxy-7-(β-D-ribofuranosyl)-2H-pyrrolo[2,3-d]pyrimidin-2-amine (18)

Figure S47: ¹H-spectrum

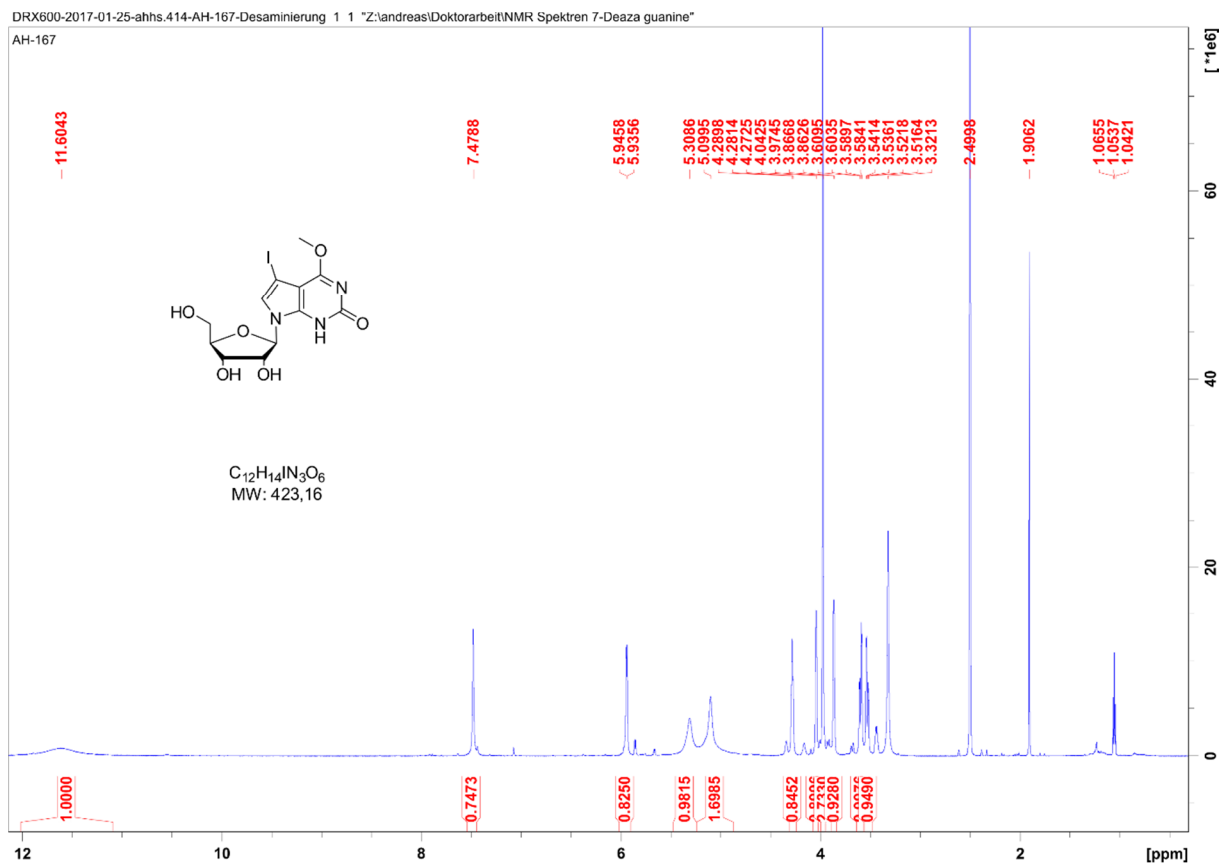


Figure S48: ¹³C-spectrum

"AV500-2017-04-18-ahhs.19558-AH-178 Desaminierung" 2 1 "Z:\andreas\Doktorarbeit\NMR Spektren 7-Deaza guanine"

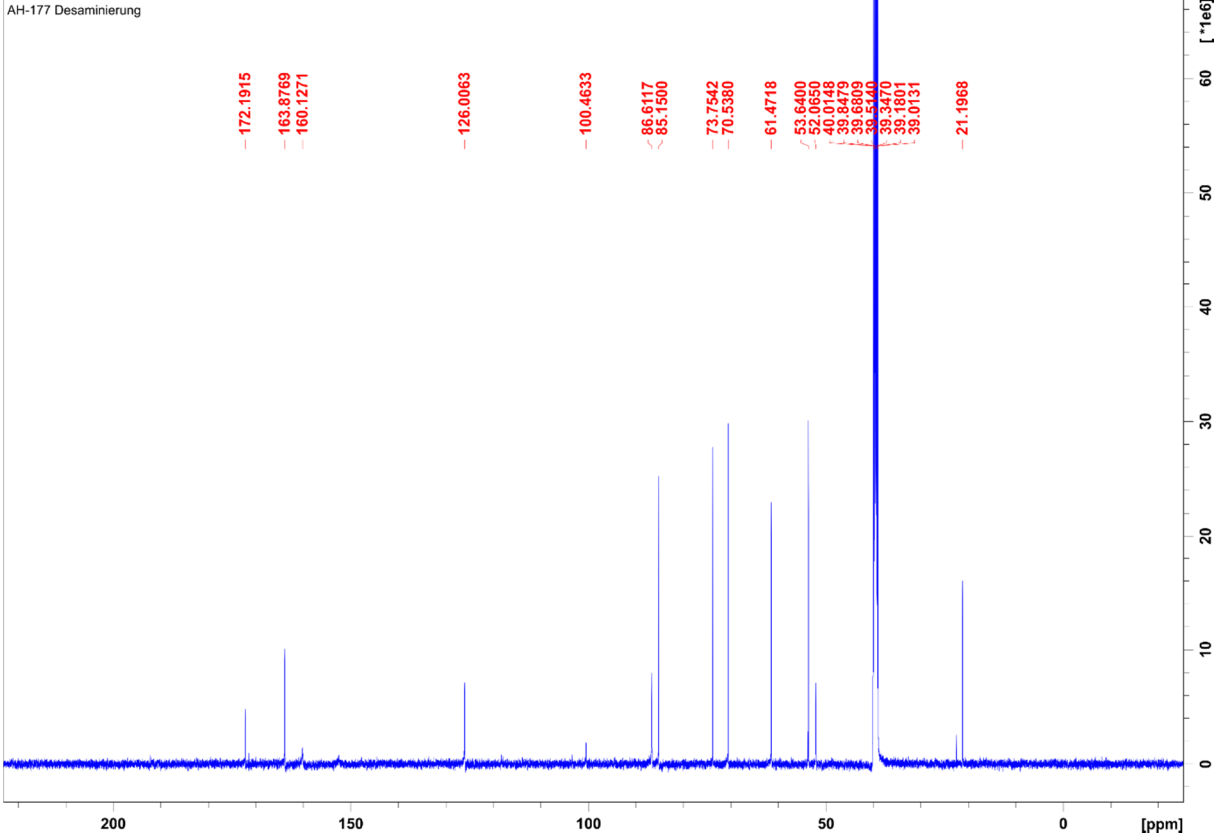
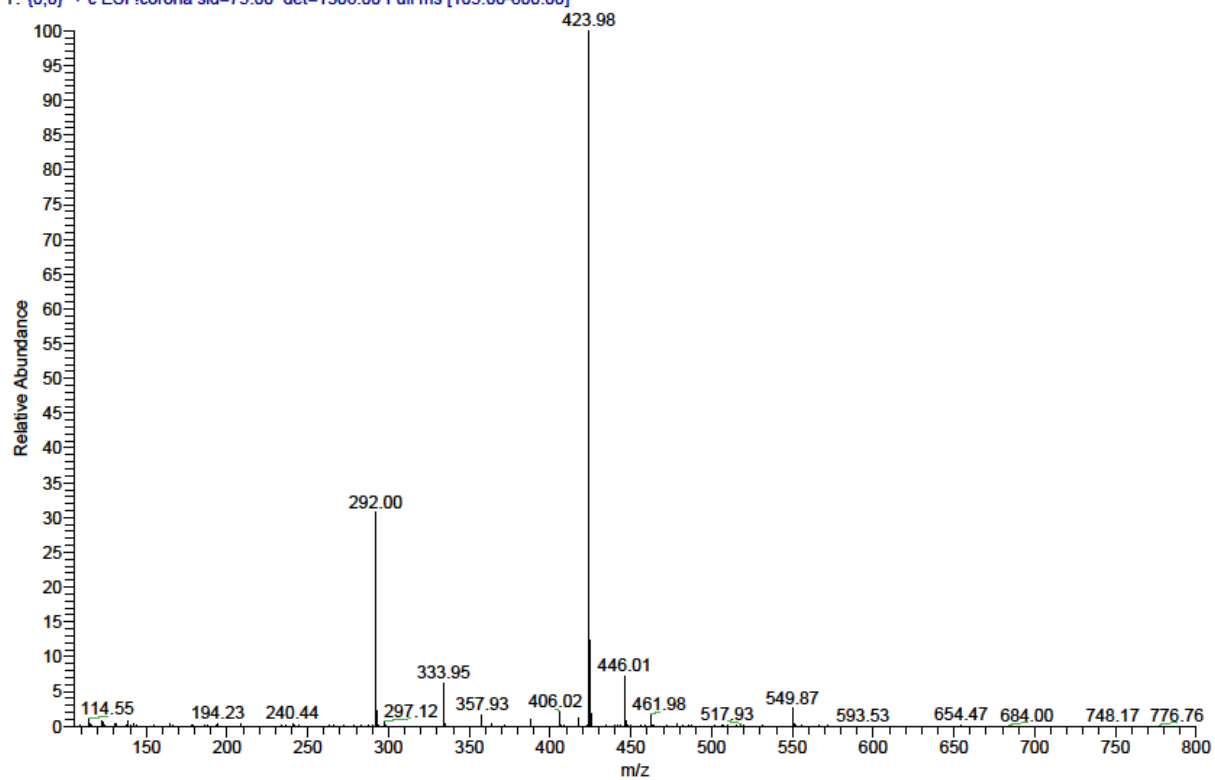


Figure S49: ESI-spectrum

C:\Xcalibur\data\AH262

2/26/2018 2:52:47 PM

AH262 #37-42 RT: 0.63-0.72 AV: 6 SB: 17 0.14-0.42 NL: 3.21E6
T: {0,0} + c ESI Icorona sid=75.00 det=1306.00 Full ms [105.00-800.00]



Analytics of 1,7-dihydro-5-(octa-1,7-diynyl)-4-methoxy-7-(β-D-ribofuranosyl)-2H-pyrrolo[2,3-d]pyrimidin-2-amine (19)

Figure S50: ¹H-spectrum

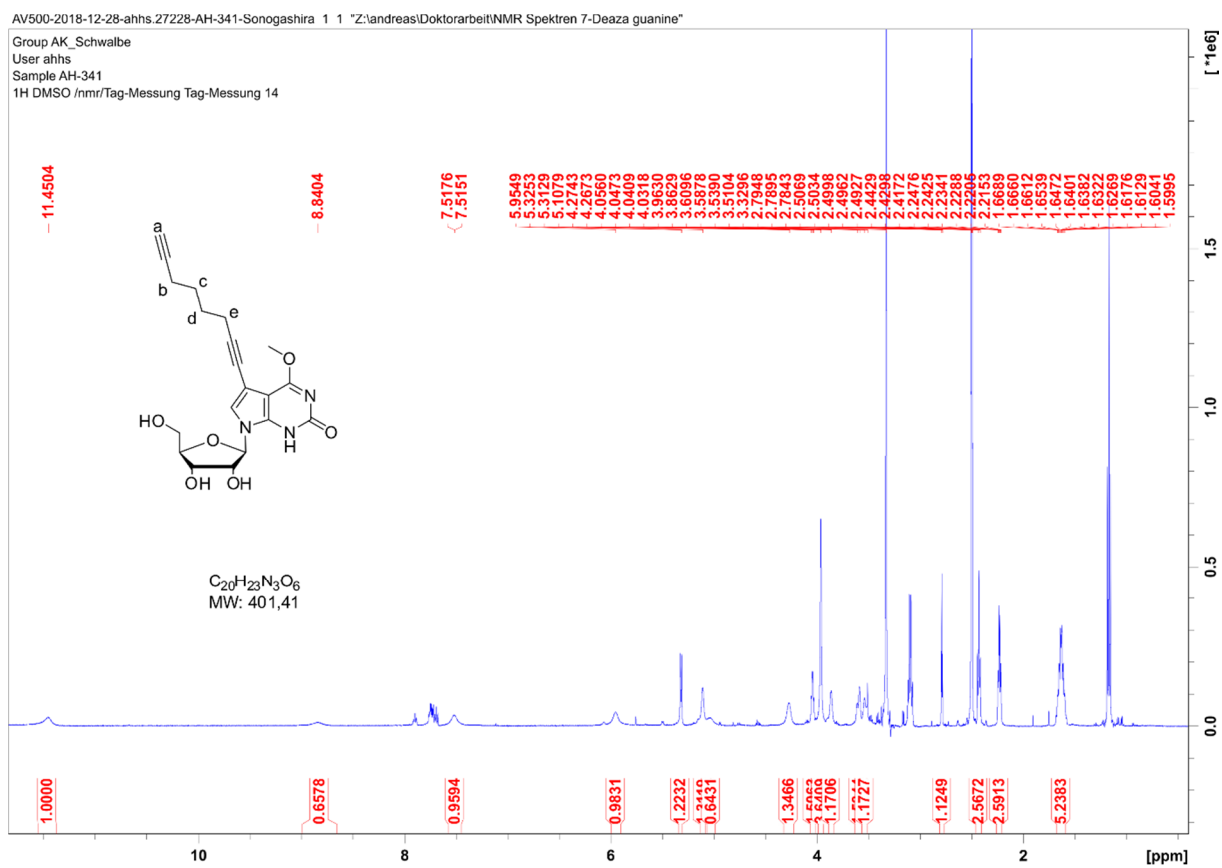


Figure S51: 13C-spectrum

AV500-2018-12-28-ahhs.27229-AH-341-Sonogashira 2 1 "Z:\andreas\Doktorarbeit\NMR Spektren 7-Deaza guanine"

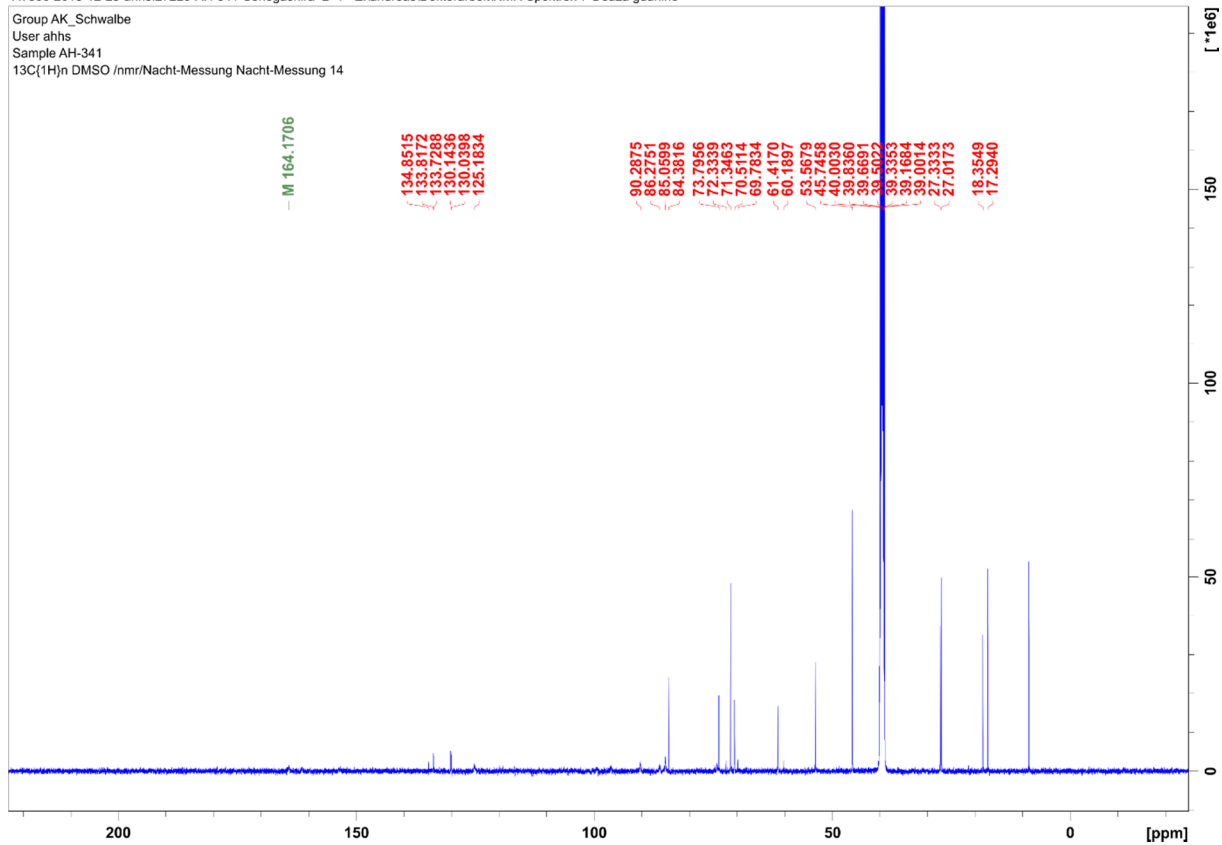
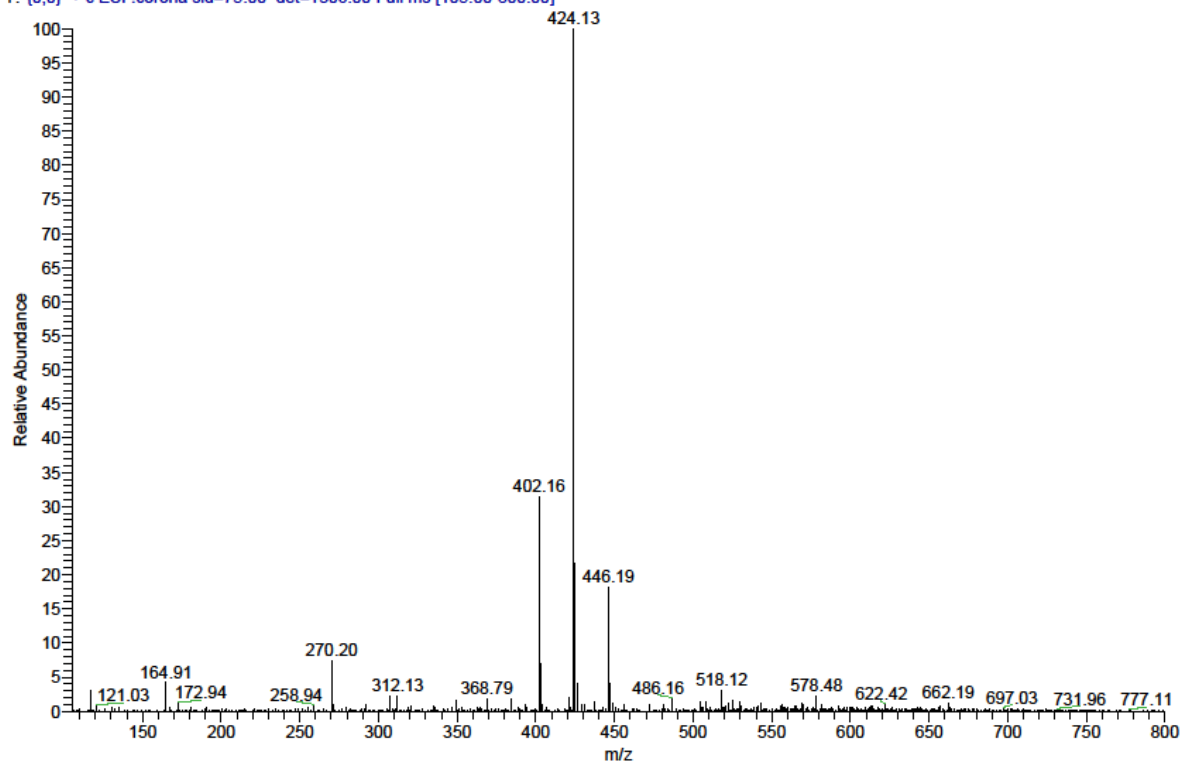


Figure S52: ESI-spectrum

C:\Xcalibur\data\AH-340-3

12/19/2018 10:31:00 AM

AH-340-3 #28-42 RT: 0.47-0.72 AV: 15 SB: 12 0.05-0.25 NL: 3.96E5
T: {0,0} + c ESI Icorona sid=75.00 det=1306.00 Full ms [105.00-800.00]



Analytics of 5-(octa-1,7-diynyl)-7-(β-D-ribofuranosyl)-1,3,7-trihydro-2H,4H-pyrrolo-[2,3-d]pyrimidin-2,4-dione (20)

Figure S53: ¹H-spectrum

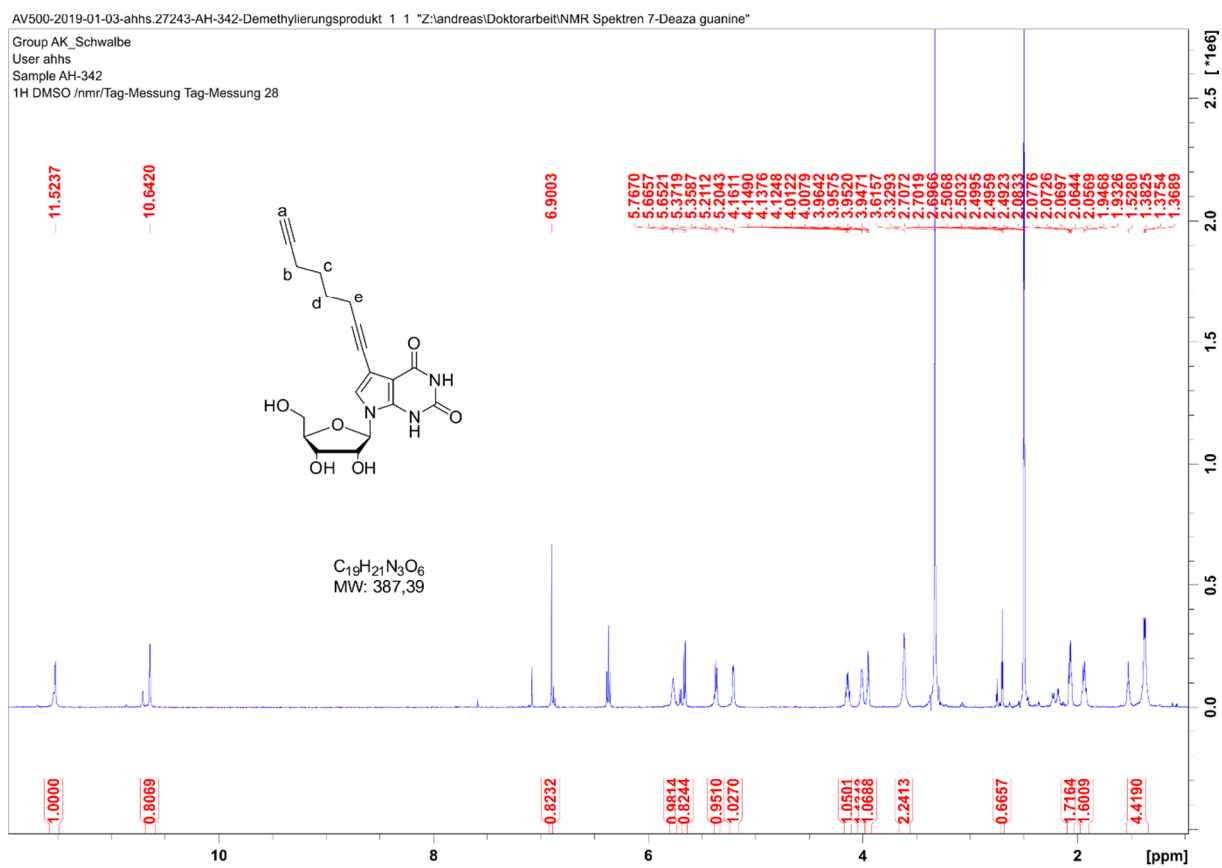


Figure S54: ¹³C-spectrum

AV500-2019-01-03-ahhs.27243-AH-342-Demethylierungsprodukt 2 1 "Z:\landreas\Doktorarbeit\NMR Spektren 7-Deaza guanine"

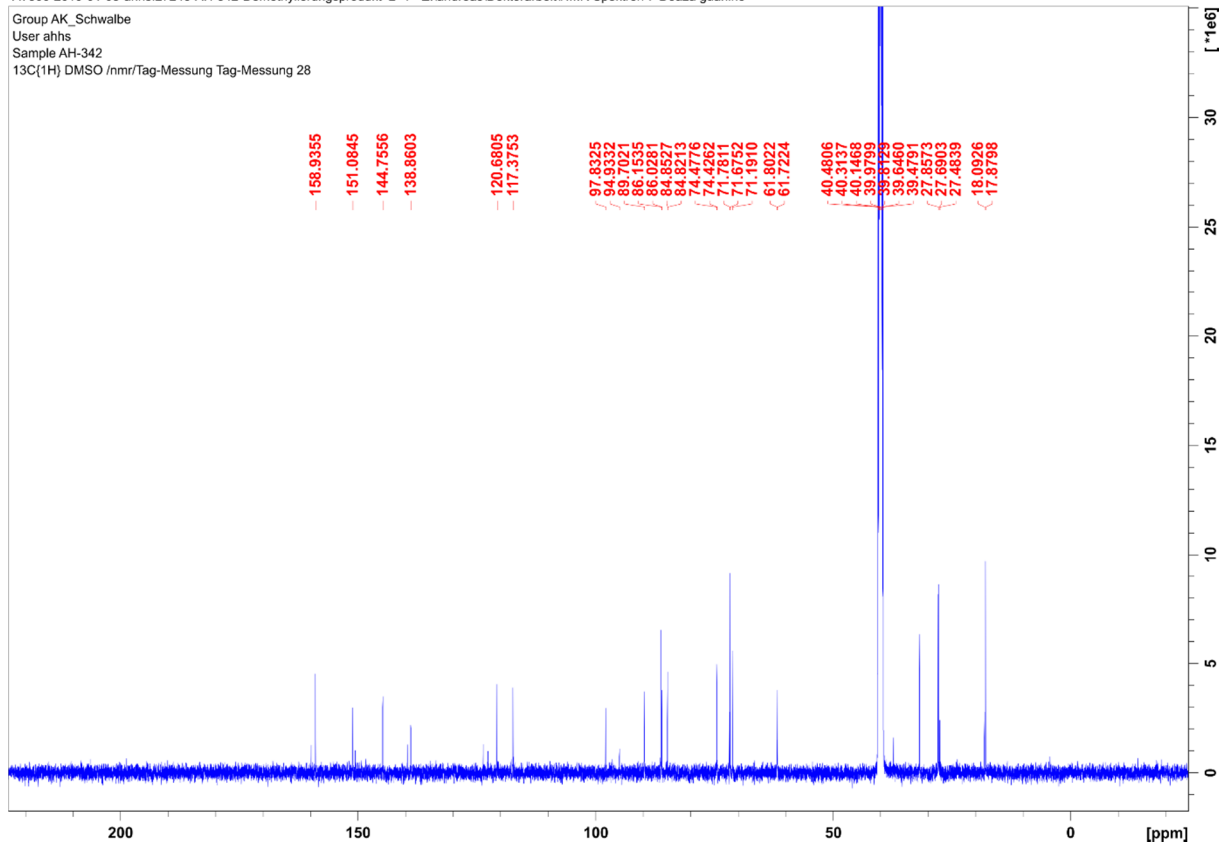


Figure S55: ESI-spectrum

C:\Xcalibur\data\AH-342

2/15/2019 8:25:49 AM

AH-342 #45-49 RT: 0.76-0.83 AV: 5 SB: 6 1.02-1.10 NL: 4.77E5
T: {0,1} - c ESI !corona sid=75.00 det=1506.00 Full ms [105.00-800.00]

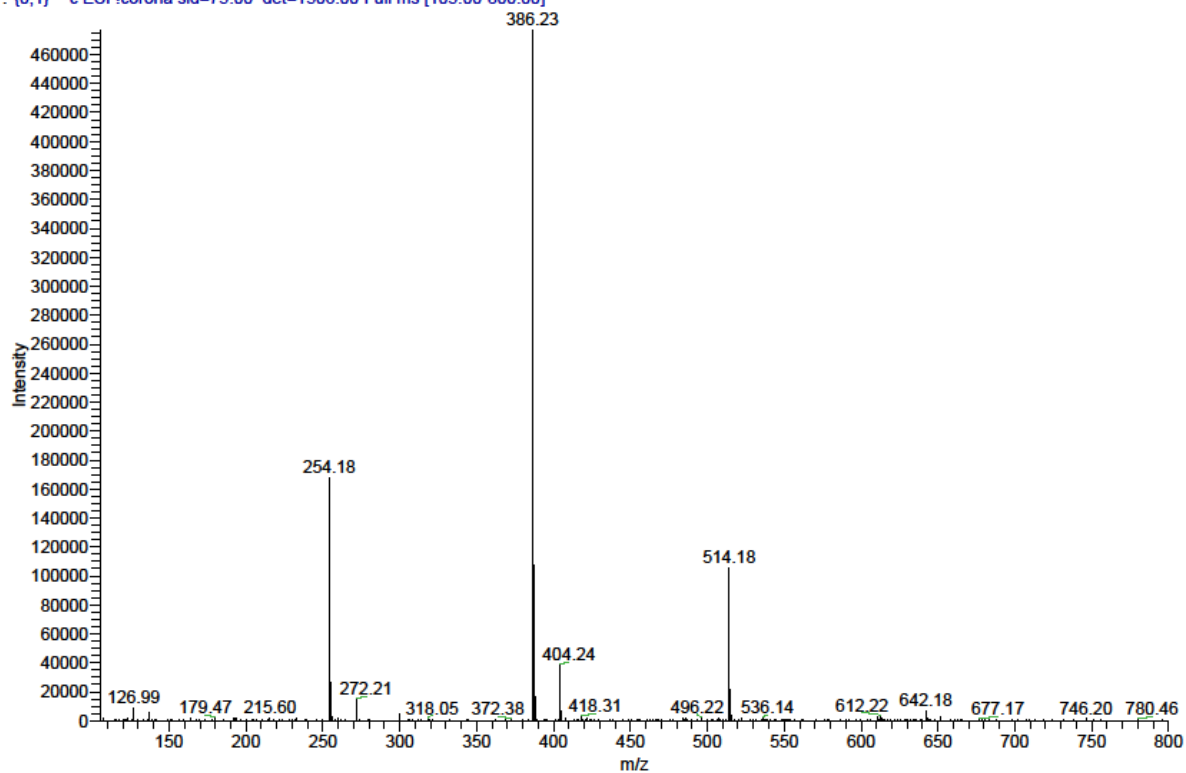


Figure S57: ³¹P-spectrum

AV400-2019-02-12-ahhs.29058-AH-343a 22.2 min TP 1 1 *Z:\andreas\Doktorarbeit\NMR Spektren 7-Deaza guanine"

Group AK_Schwalbe
User ahhs
Sample AH-343a 22.2
31P(1H) D2O /nmr Tag-Messung 10

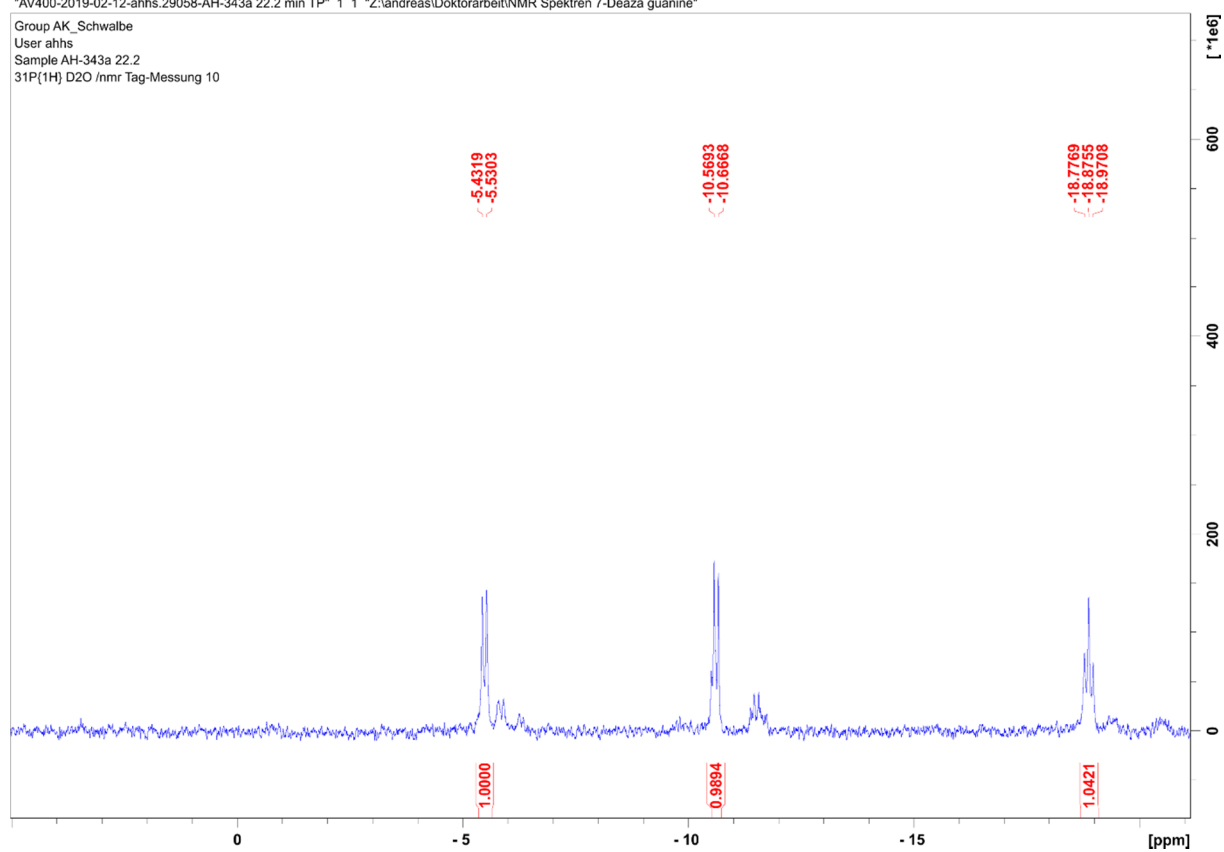


Figure S58: ^{31}P -HMBC-spectrum

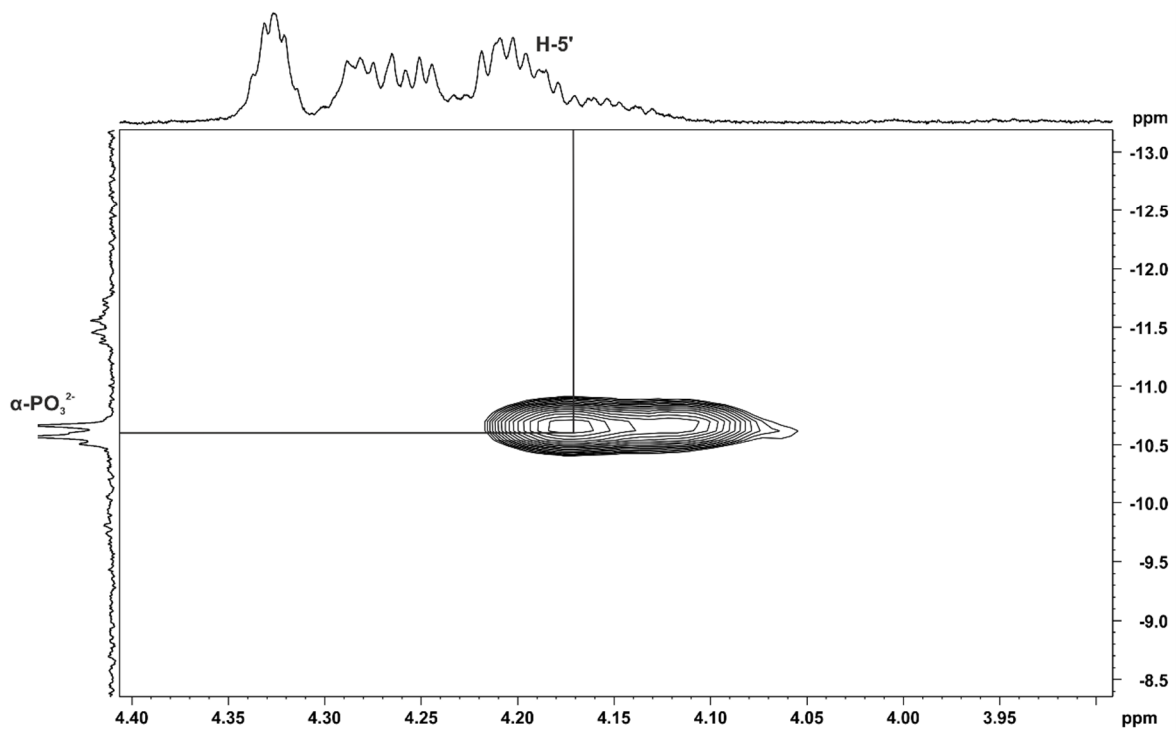


Figure S59: MALDI-spectrum

C:\User\...\2019\190111\AH-TP-6_D6

1/11/2019 12:26:41 PM

AH-TP-6.2 mit HCCA gemessen.

AH-TP-6_D6 #1-20 RT: 0.00-1.31 AV: 20 NL: 7.70E5
T: FTMS + p MALDI Full ms [600.00-800.00]

