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Synthesis, structure and cytotoxicity of novel tetrazolo[1′,5′-*c*]-fused 3-aza-A-homosteroids

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Contents

General Experimental S1
NMR spectral data of compounds **2a,b** and **3c** S4
1D NMR spectra S4
2D NMR spectra S10
Mass-spectra S15
IR data S18
X-ray crystal data S20
Calculations S36
Biological *in vitro* study S37
References S39

General Experimental

General

All reagents and solvents were purchased from commercial sources and used without further purification. Specific rotations were measured in $CHCl_3$ (**2a, 2b**) and DMF (**3c**) (*c* 0.1) at 20 °C with an AA-65 (Optical Activity) polarimeter. NMR spectra were recorded on a Bruker Avance III-400 spectrometer at 400.13 (^{1}H NMR) and 100.61 MHz (^{13}C NMR). Chemical shifts in ^{1}H and ^{13}C{^{1}H} NMR spectra were measured relative to the residual proton and carbon signals of the deuterated solvent ($CDCl_3$, or DMSO-d_6) and were referenced to that of $SiMe_4$ ($\delta = 0$). The mass spectra were obtained on a Bruker MaXis instrument. IR spectra were recorded on a Perkin Elmer Spectrum BX spectrometer in disk KBr. Melting points (m.p.) were determined on a Wägetechnik Rapido PHMK micro hot stage. The reactions were monitored by TCL on Kieselgel-G (Merck Si 254F) layers (0.25 mm thick) with solvent system: chloroform/methanol (10:1, v/v). Spots were visualized by spraying with a 5% solution of 4-methoxybenzaldehyde in ethanol acidified with sulfuric acid, followed by heating at 100–120°C for 2–3 min. Flash column chromatography was performed on silica gel 5-25μm using hexane/ethyl acetate and chloroform solvent systems as an eluent.

*Synthesis of tetrazoles (**2a-c**, **3a-c**)*

Steroids (**1a-c**, 10.4 mmol) were dissolved in dioxane (60 mL) and NaN_3 (57.3 mmol) and silicon tetrachloride (20.8 mmol) were added. The reaction mixture was stirred at room temperature for 10 days. Then it was poured into a mixture of ice and water (250 mL) and neutralized by adding of sodium carbonate to pH = 6-8. The crystalline precipitate was filtered off, washed thoroughly with water and dried. Pure product was obtained by flash column chromatography on silica gel using hexane/ethyl acetate in different concentrations and $CHCl_3$ as an eluent.

*17β-Hydroxy-3-aza-A-homo-androst-5-eno[3,4-d]tetrazole **2a***

Yield 1.6 g, 47%. M.p. 273-275°C. $[\alpha]_D^{20}$ = +93 (c 0.1, in $CHCl_3$). IR spectrum, ν, cm^{-1}: 3418 (C^{17}-OH). Mass spectrum, m/z (ESI): 351.2166 $[M + Na]^+$. $C_{19}H_{28}N_4O$. Calculated M: 328.2363. ^1H NMR (δ, ppm, $CDCl_3$) 5.71 (dd, *J* = 5.4, 2.2 Hz, C^6H), 4.59 – 4.51 (m, C^2H), 4.12 (dd, *J* = 14.3, 11.6 Hz, C^2H), 3.81 (d, *J* = 16.4 Hz, C^4H), 3.70 (t, *J* = 8.5 Hz, $C^{17}H$), 3.58 (d, *J* = 16.4 Hz, C^4H), 2.28 (dd, *J* = 15.9, 6.9 Hz, C^1H), 2.17 – 2.05 (m, $C^{16}H$, C^7H), 2.00 – 1.89 (m, C^1H, $C^{11}H$), 1.72 – 1.63 (m, $C^{12}H$, C^8H, $C^{15}H$, C^7H), 1.62 – 1.54 (m, $C^{12}H$), 1.54 – 1.46 (m, $C^{16}H$), 1.39 – 1.25 (m, C^9H, $C^{15}H$), 1.16 (s, $C^{11}H$, $C^{19}H_3$), 1.10 – 1.00 (m, $C^{14}H$), 0.82 (s, $C^{18}H_3$). ^{13}C NMR (δ, ppm, $CDCl_3$) δ 154.1 ($C^{tetr.}$), 136.0 (C^5), 129.2 (C^6), 81.6 (C^{17}), 51.4 (C^{14}), 43.8 (C^2), 43.7 (C^9), 42.8 (C^{13}), 40.1 (C^{10}), 36.5 (C^{11}), 34.0 (C^1), 31.7 (C^8), 30.8 (C^7), 30.5 (C^{16}), 28.3 (C^4), 23.3 (C^{15}), 21.9 (C^{19}), 21.1 (C^{12}), 11.1 (C^{18}).

*20-Oxo-3-aza-A-homo-pregn-5-eno[3,4-d]tetrazole **2b***

Yield 1.0 g, 32%. M.p. 197-200°C. $[\alpha]_D^{20}$ = +34 (c 0.1, in $CHCl_3$). IR spectrum, ν, cm^{-1}: 1703 (C^{20}=O). Mass spectrum, m/z (ESI): 355.2514 $[M+H]^+$. $C_{21}H_{30}N_4O$. Calculated M: 354.2612. ^1H NMR (δ, ppm, $CDCl_3$) 5.76 – 5.59 (m, C^6H), 4.59 – 4.47 (m, C^2H), 4.11 (dd, *J* = 14.3, 11.5 Hz, C^2H), 3.77 (d, *J* = 16.4 Hz, C^4H), 3.56 (d, *J* = 16.4 Hz, C^4H), 2.56 – 2.50 (m, $C^{17}H$), 2.29 – 2.22 (m, C^1H), 2.22 – 2.14 (m, $C^{16}H$), 2.12 (s, $C^{21}H_3$), 2.11 – 2.08 (m, C^7H, $C^{12}H$), 1.96 – 1.90 (m, C^1H), 1.71 – 1.67 (m, $C^{15}H$, $C^{16}H$), 1.67 – 1.64 (m, $C^{11}H$), 1.63 – 1.60 (m, C^7H), 1.60 – 1.58 (m, C^8H), 1.57 – 1.51 (m, $C^{11}H$), 1.50 – 1.43 (m, $C^{12}H$), 1.34 (td, *J* = 11.0, 4.2 Hz, C^9H), 1.30 – 1.24 (m, $C^{14}H$), 1.24 – 1.20 (m, $C^{15}H$), 1.13 (s, $C^{19}H_3$), 0.66 (s, $C^{18}H_3$). ^{13}C NMR (δ, ppm, $CDCl_3$) 209.2 (C^{20}), 154.1 ($C^{tetr.}$), 135.9 (C^5), 129.2 (C^6), 63.4 (C^{17}), 56.8 (C^{14}), 43.8 (C^{13}), 43.7 (C^2), 43.5 (C^9), 40.0 (C^{10}), 38.6 (C^{12}), 34.0 (C^1), 31.6 (C^8), 31.5 (C^{21}), 31.1 (C^7), 28.3 (C^4), 24.3 (C^{15}), 22.9 (C^{16}), 21.8 (C^{19}), 21.4 (C^{11}), 13.3 (C^{18}).

21-Acetoxy-11β,17α-dihydroxy-20-oxo-3-aza-A-homo-pregn-4-eno[3,4-d]tetrazole ***3c***

Yield 1.3 g, 39%. M.p. 268-270°C. $[\alpha]_D^{20}$ = +71 (c 0.1, in DMF). IR spectrum, ν, cm^{-1}: 3444, 3307 (OH), 1747 (C=O) 1725 (C=O), 1654 (C^4=C^5). Mass spectrum, m/z (ESI): 467,2270 $[M+Na]^+$. $C_{23}H_{32}N_4O_5$. Calculated M: 444,2372. ^{1}H NMR (δ, ppm, DMSO-d_6) 6.35 (s, C^4H), 5.09 (d, *J* = 17.5 Hz, C^{21}H), 4.73 (d, *J* = 17.5 Hz, C^{21}H), 4.66 – 4.55 (m, C^2H), 4.54 – 4.44 (m, C^2H), 4.29 – 4.20 (m, C^{11}H), 2.69 – 2.57 (m, C^6H), 2.56 – 2.45 (m, C^{16}H), 2.40 – 2.28 (m, C^1H, C^6H), 2.13 – 2.07 (m, C^1H, $C^{23}H_3$), 2.06 – 1.95 (m, C^7H, C^8H), 1.95 – 1.89 (m, C^{12}H), 1.73 – 1.61 (m, C^{12}H, C^{14}H, C^{15}H), 1.51 – 1.41 (m, C^9H, C^{16}H), 1.39 (s, C^{15}H, $C^{19}H_3$), 1.08 – 1.00 (m, C^7H), 0.78 (s, $C^{18}H_3$). ^{13}C NMR (δ, ppm, DMSO) 205.7 (C^{20}), 170.20 (C^{22}), 162.9 (C^5), 151.7 ($C^{tetr.}$), 103.1 (C^4), 89.0 (C^{17}), 67.9 (C^{21}), 67.0 (C^{11}), 52.0 (C^{14}), 47.3 (C^{13}), 45.4 (C^9), 44.6 (C^{10}), 43.20 (C^2), 39.2 (C^{12}), 35.5 (C^6), 34.3 (C^7), 33.5 (C^{16}), 32.0 (C^1), 31.4 (C^8), 24.5 (C^{23}), 23.8 (C^{15}), 20.9 (C^{19}), 17.0 (C^{18}).

NMR spectral data of compounds **2a,b** and **3c**

1D NMR spectra

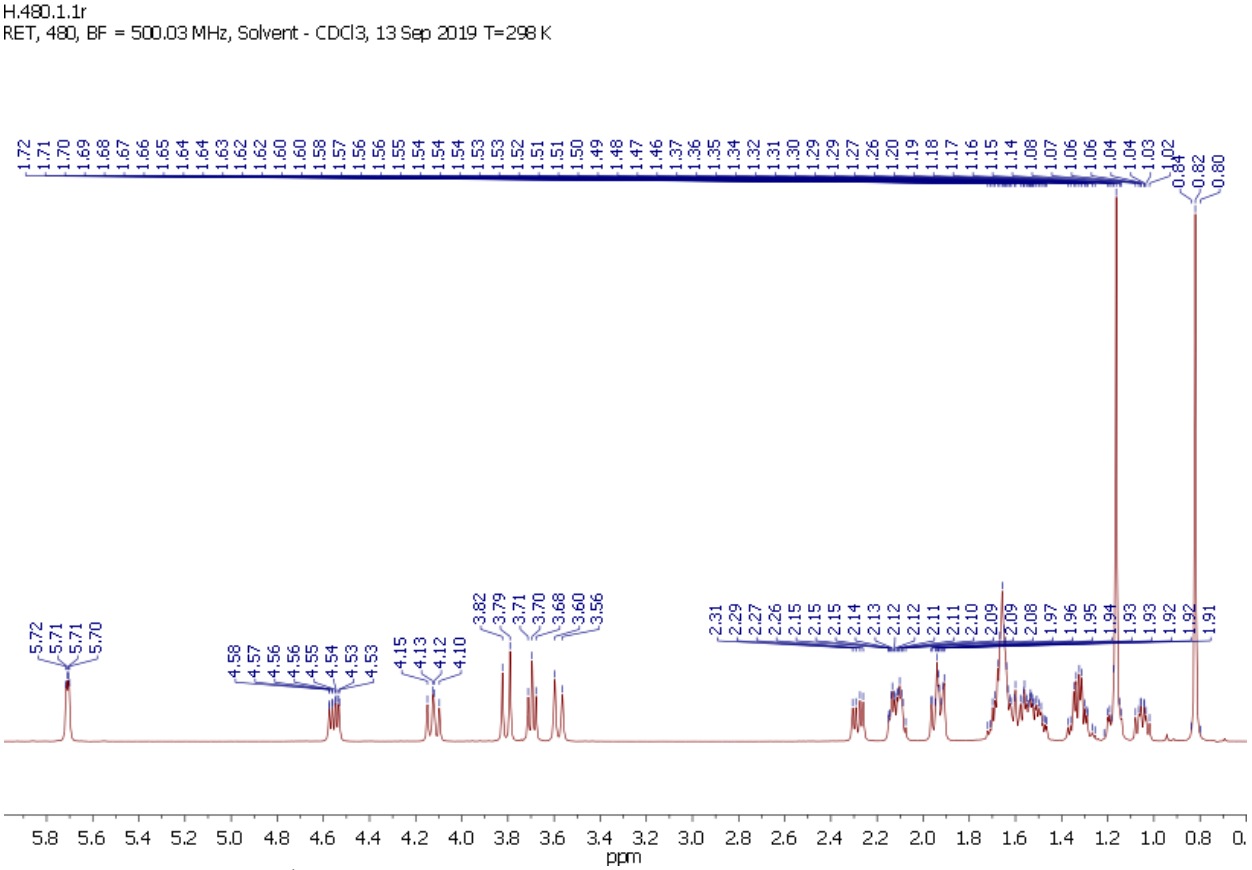

Figure S1 Spectrum ^{1}H NMR of Compound 2a.

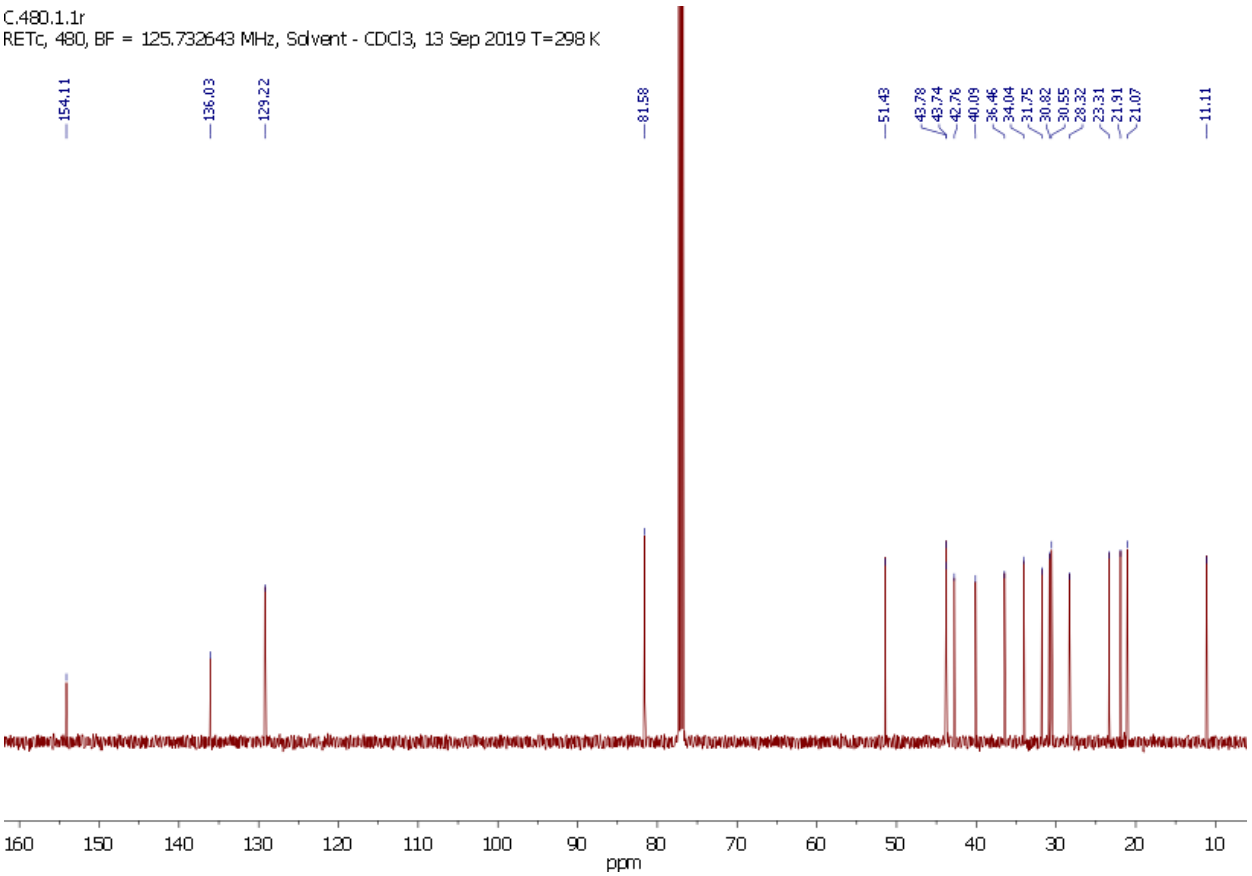

Figure S2 Spectrum ^{13}C NMR of Compound 2a.

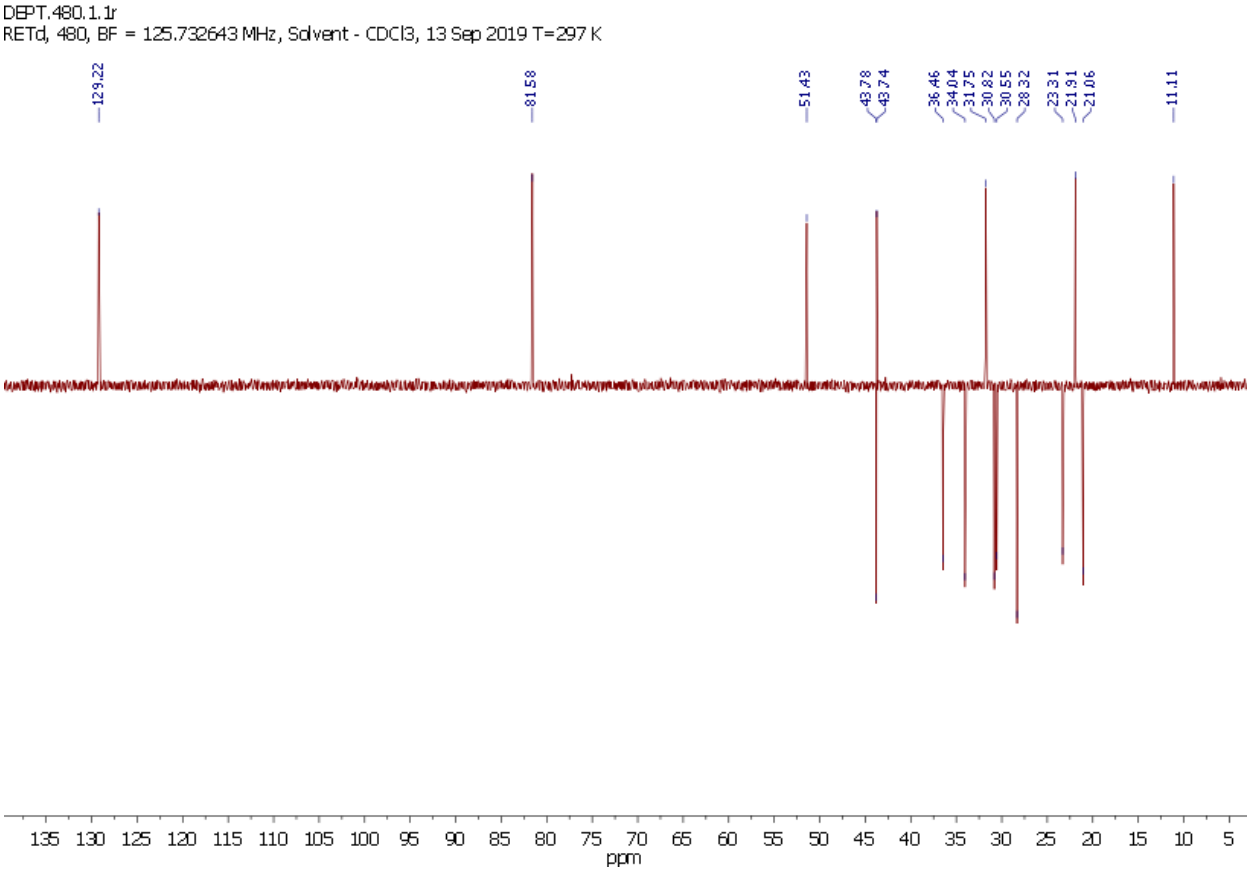

Figure S3 DEPT of Compound 2a.

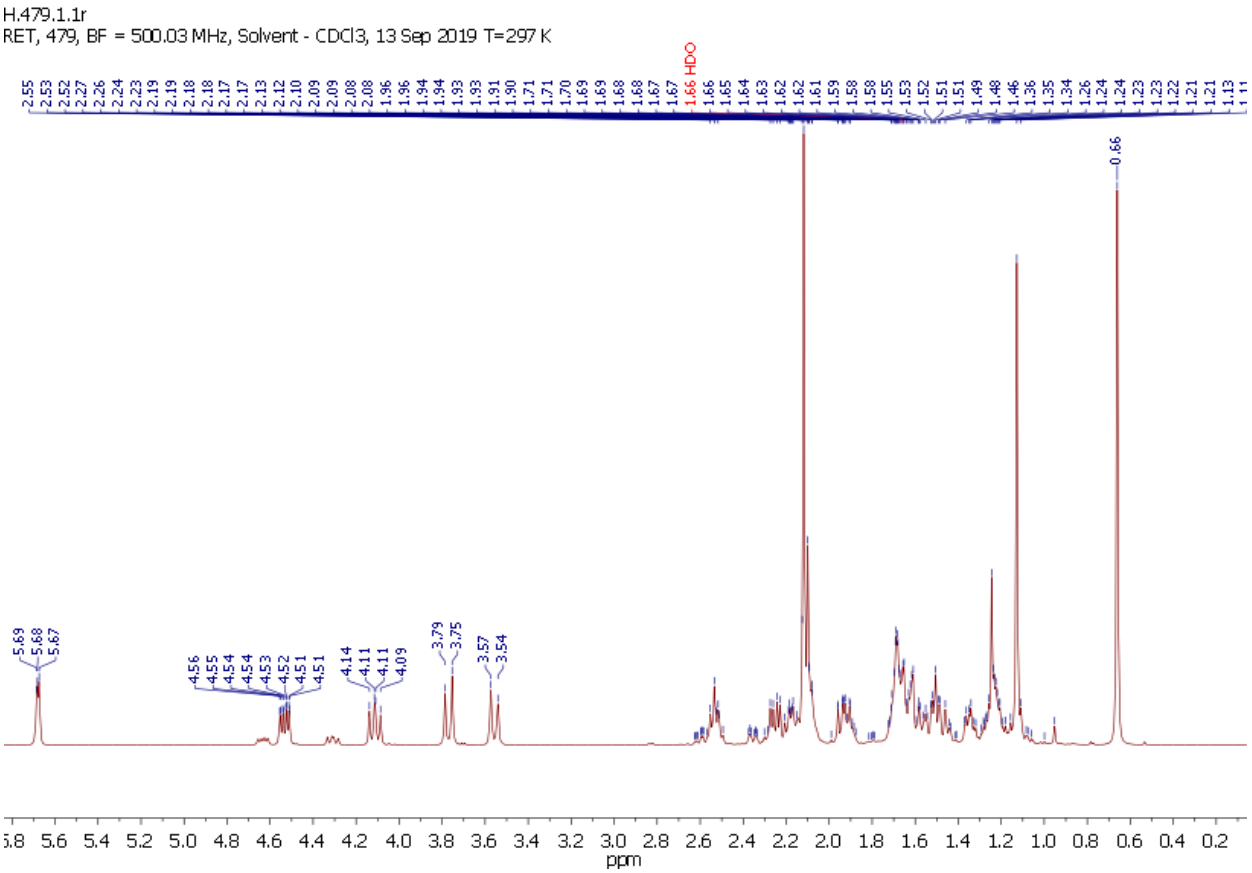

Figure S4 Spectrum ^{1}H NMR of Compound 2b.

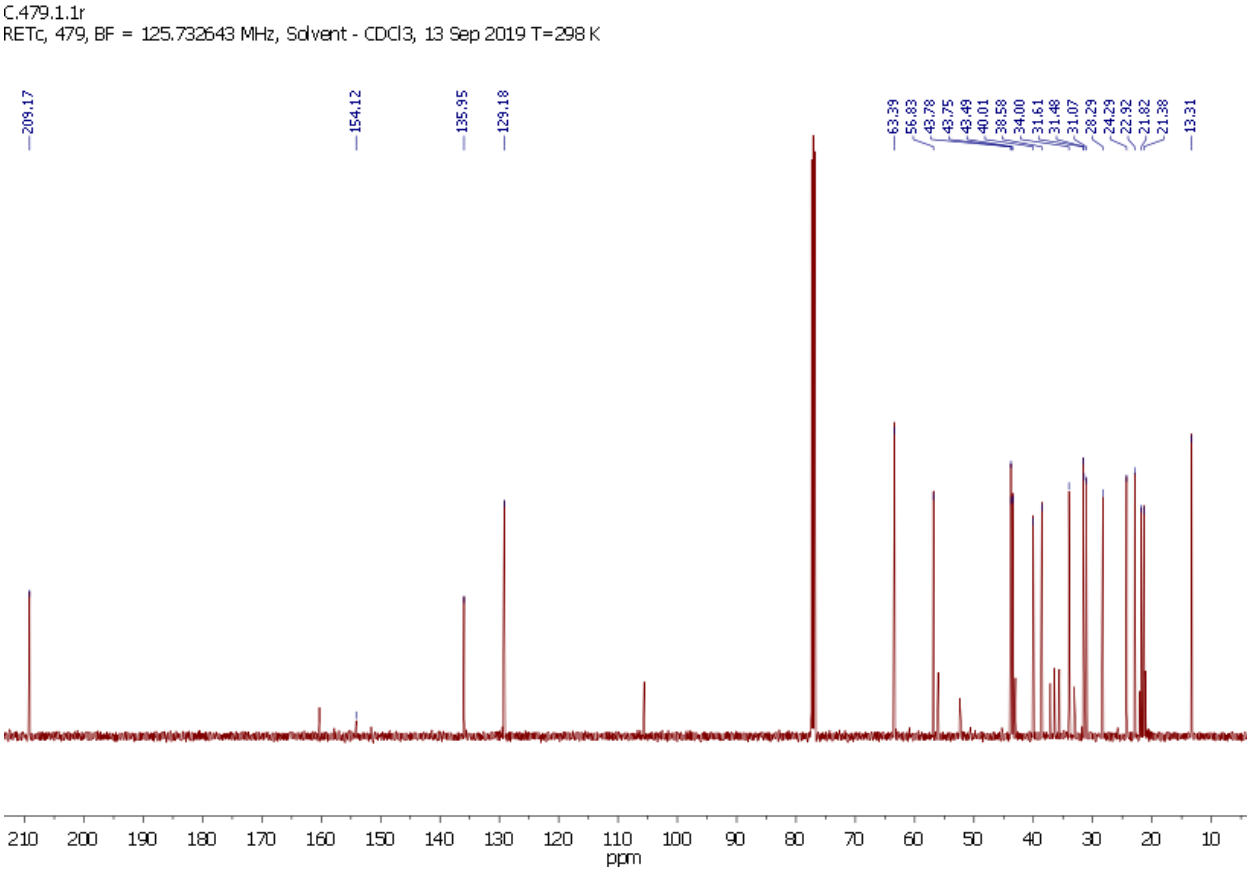

Figure S5 Spectrum ^{13}C NMR of Compound 2b.

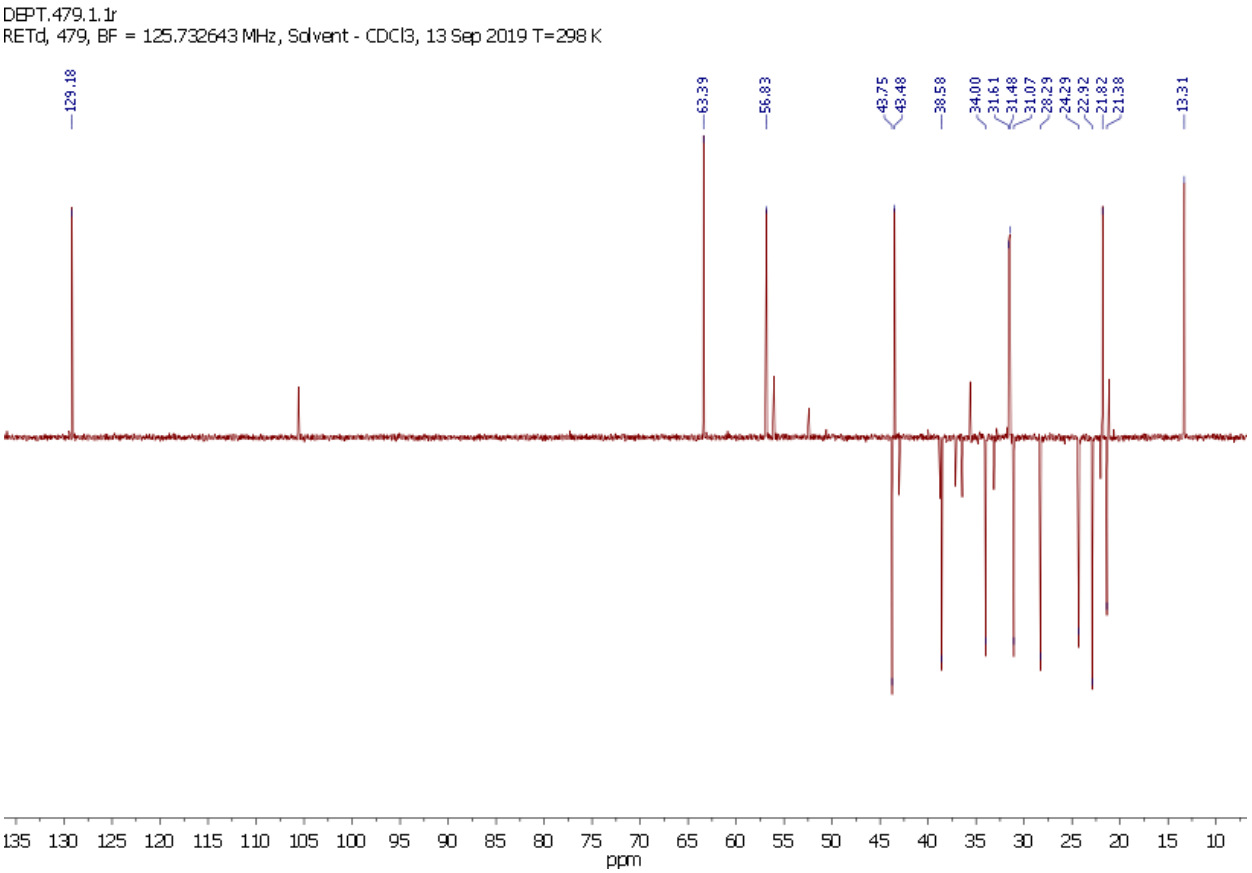

Figure S6 DEPT of Compound 2b.

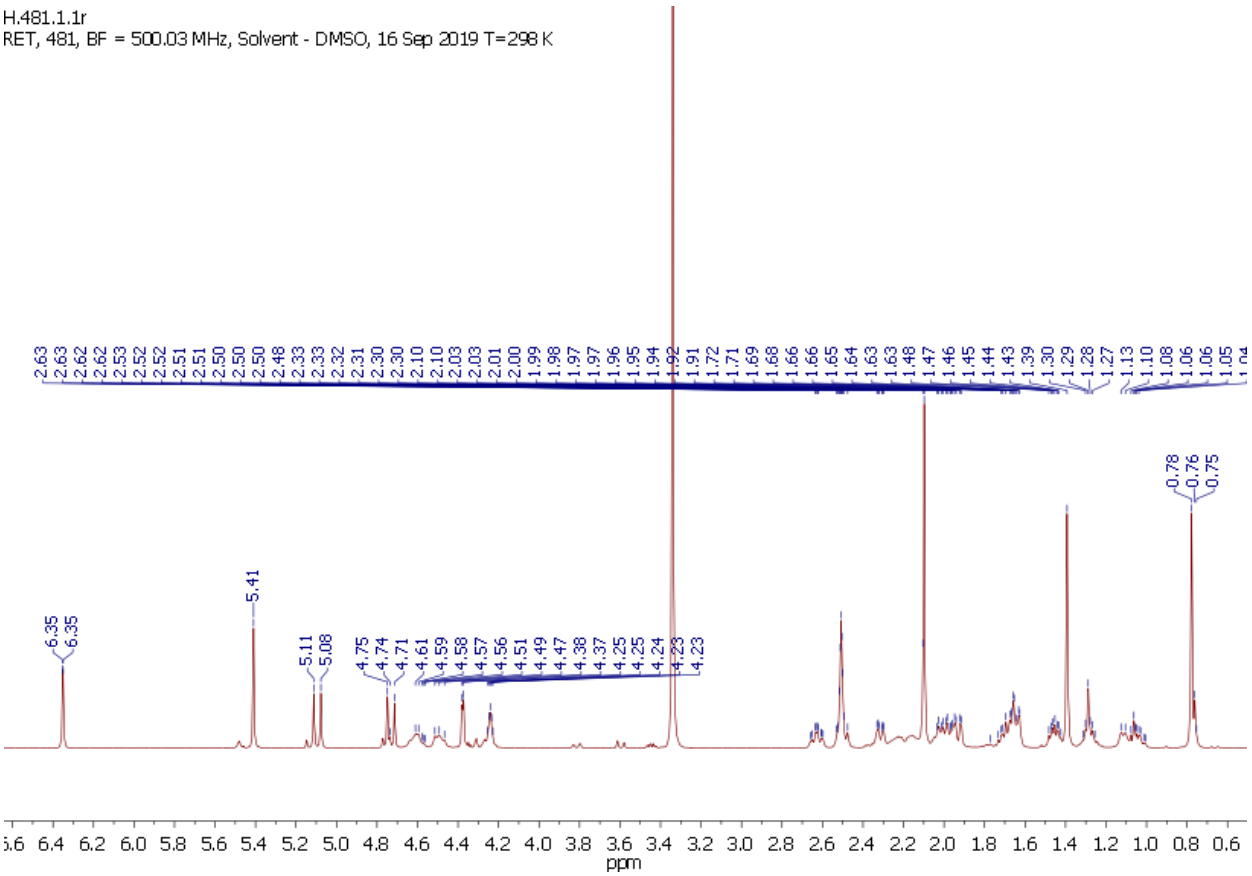

Figure S7 Spectrum ^{1}H NMR of Compound 3c.

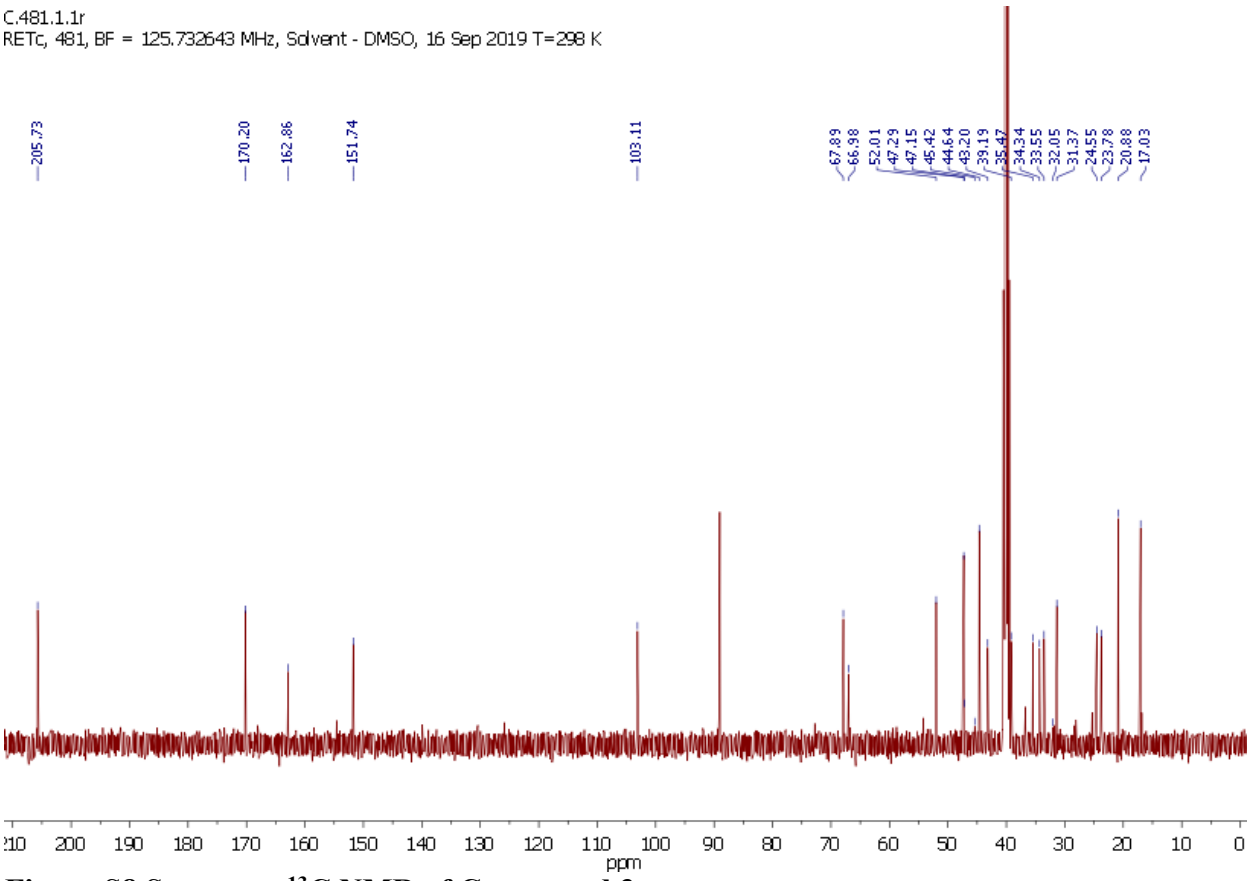

Figure S8 Spectrum ^{13}C NMR of Compound 3c.

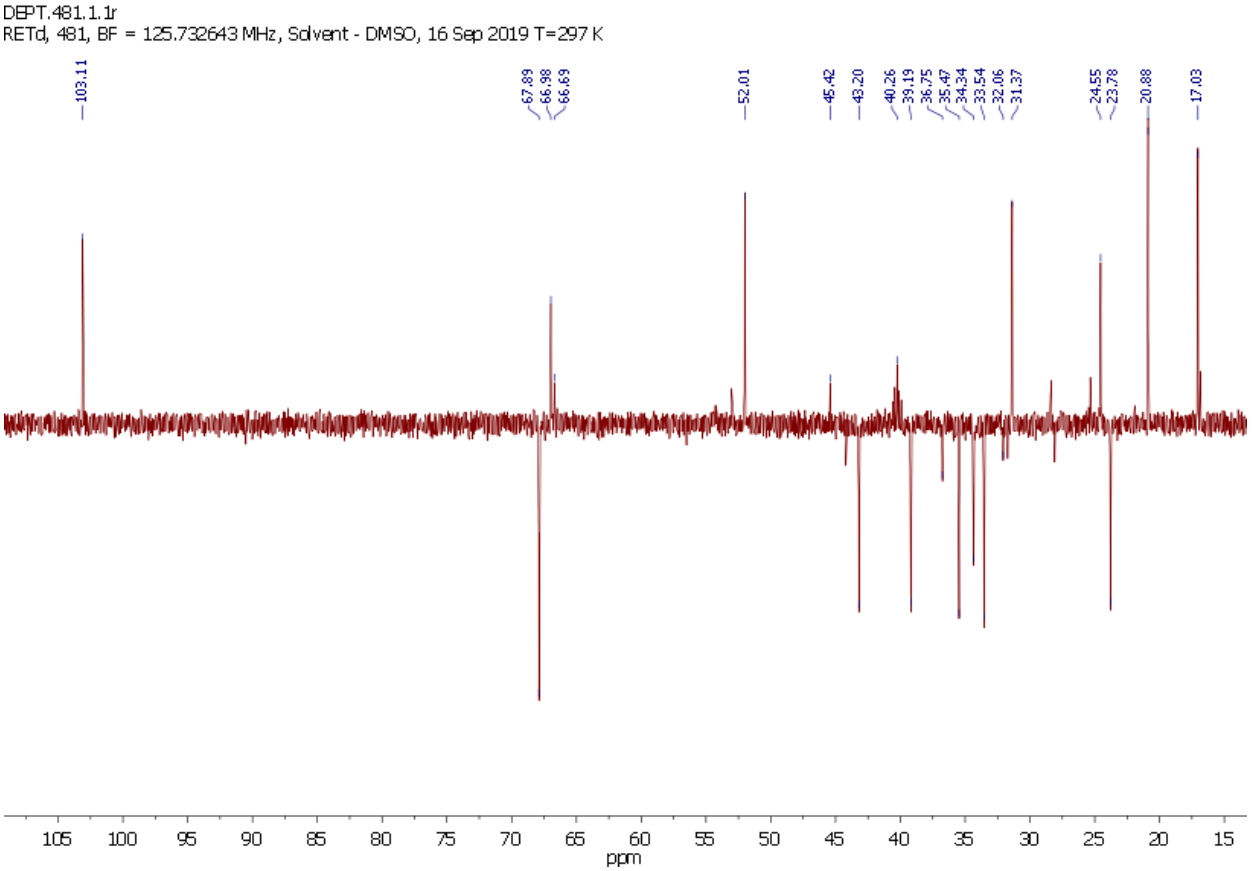

Figure S9 DEPT of Compound 3c.

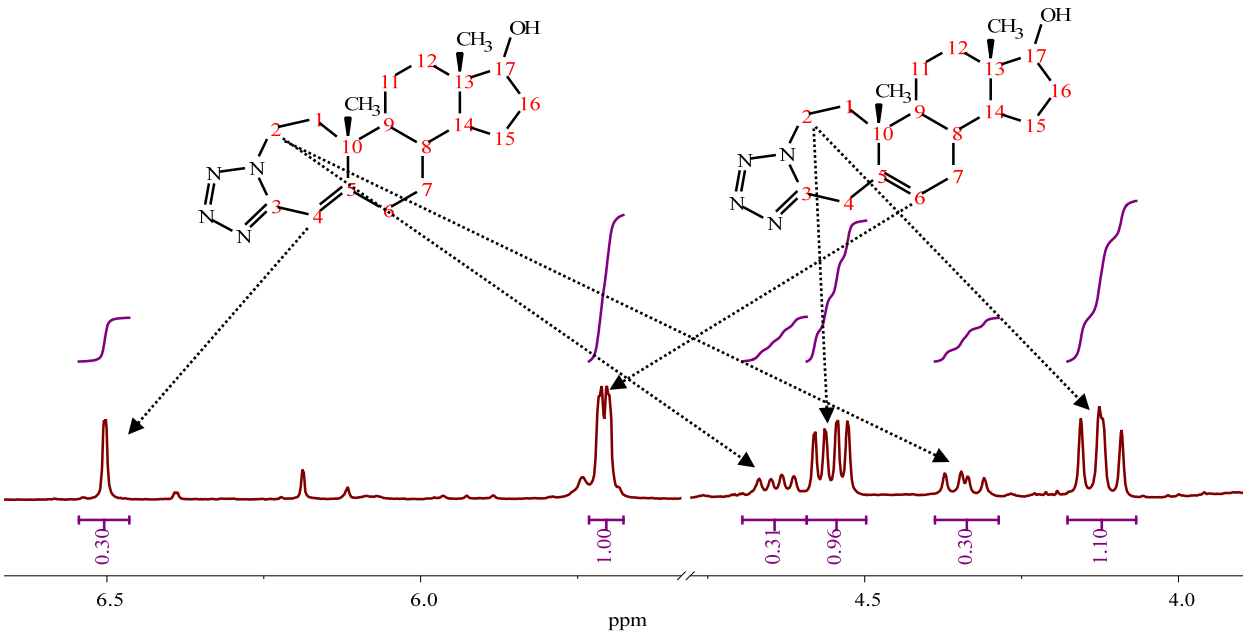

Figure S10 Fragment of ^{1}H NMR spectrum of reaction mass contained isomeric products 2a, 3a.

As can be seen here, on the example of testosterone conversion products, characteristic signals of the C$^{2}H_{2}$ and C*H*=C protons of isomers **2a**, **3a** are present in the ^{1}H NMR spectrum of the reaction mixture.

2D NMR spectra

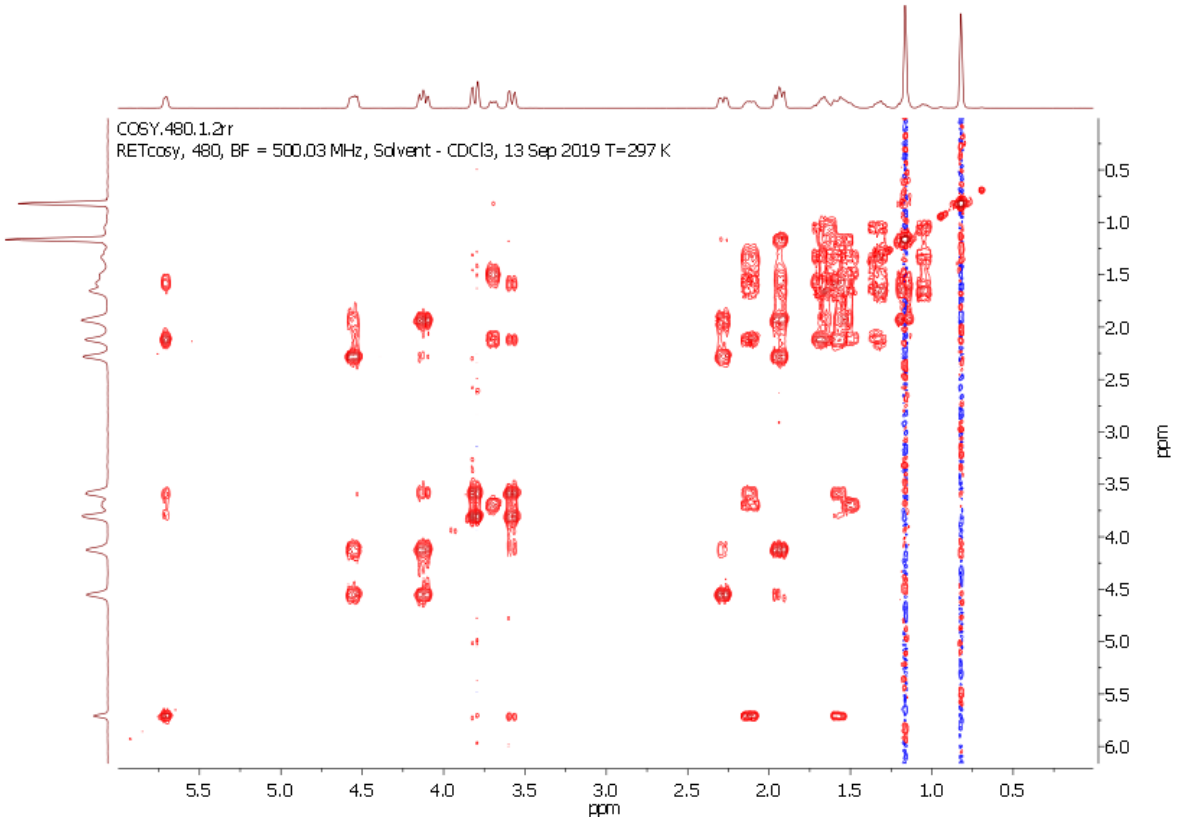

Figure S11 COSY of Compound 2a.

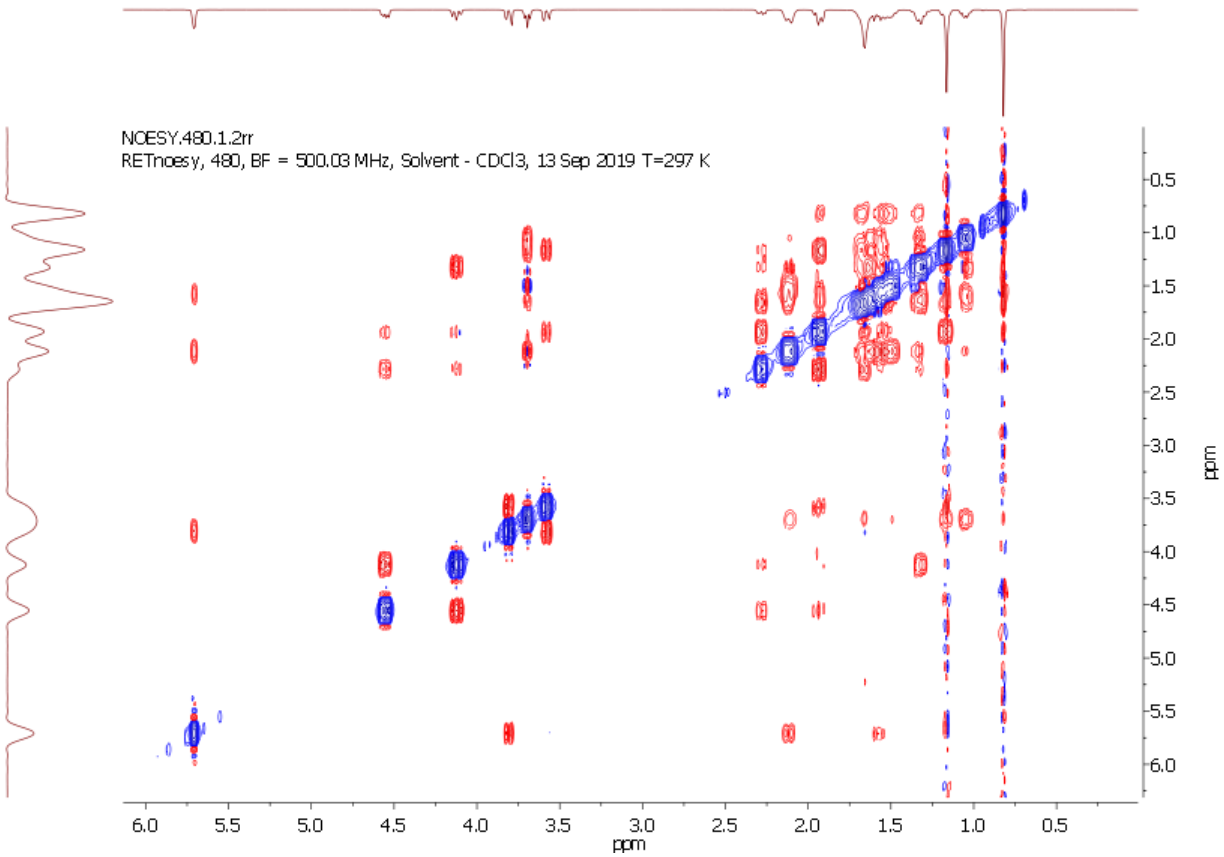

Figure S12 NOESY of Compound 2a.

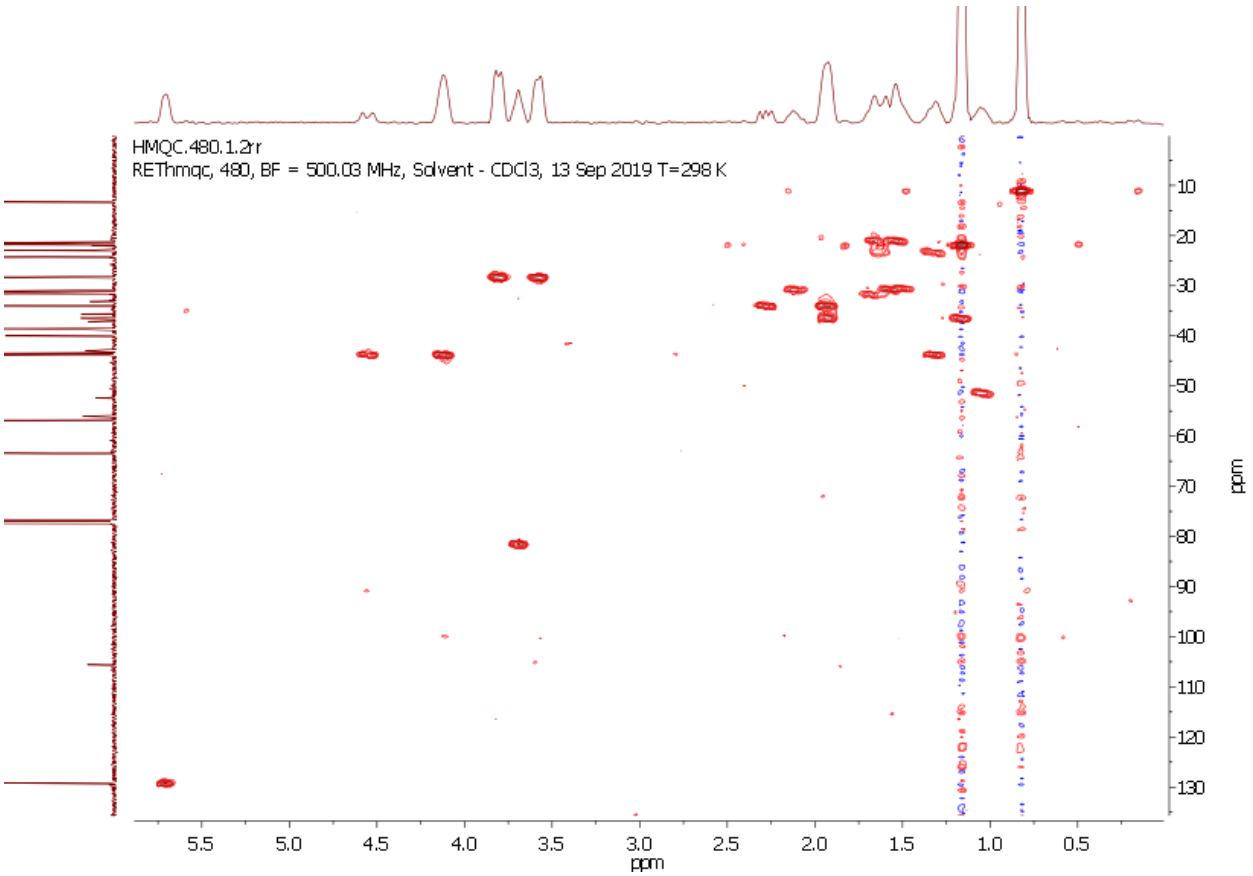

Figure S13 Spectrum HMQC of Compound 2a.

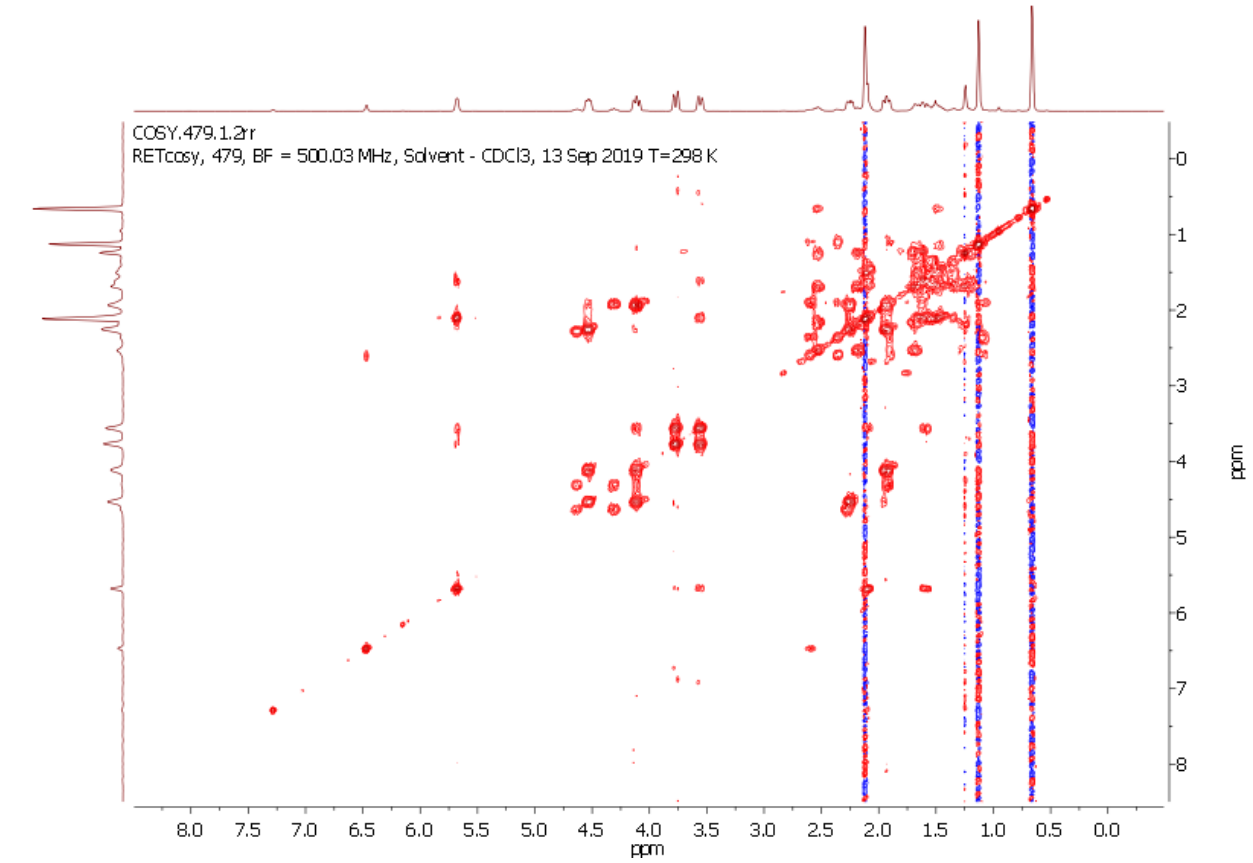

Figure S14 COSY of Compound 2b.

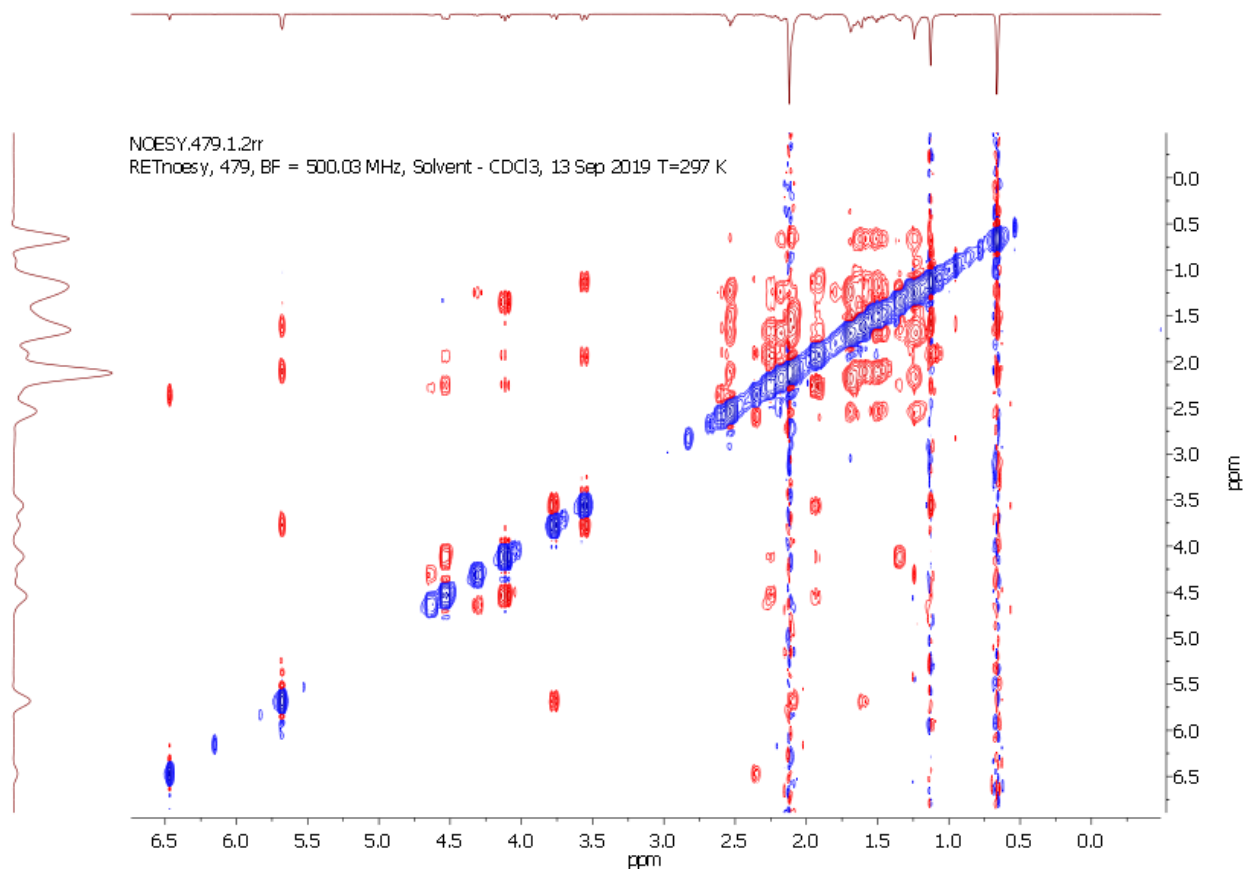

Figure S15 NOESY of Compound 2b.

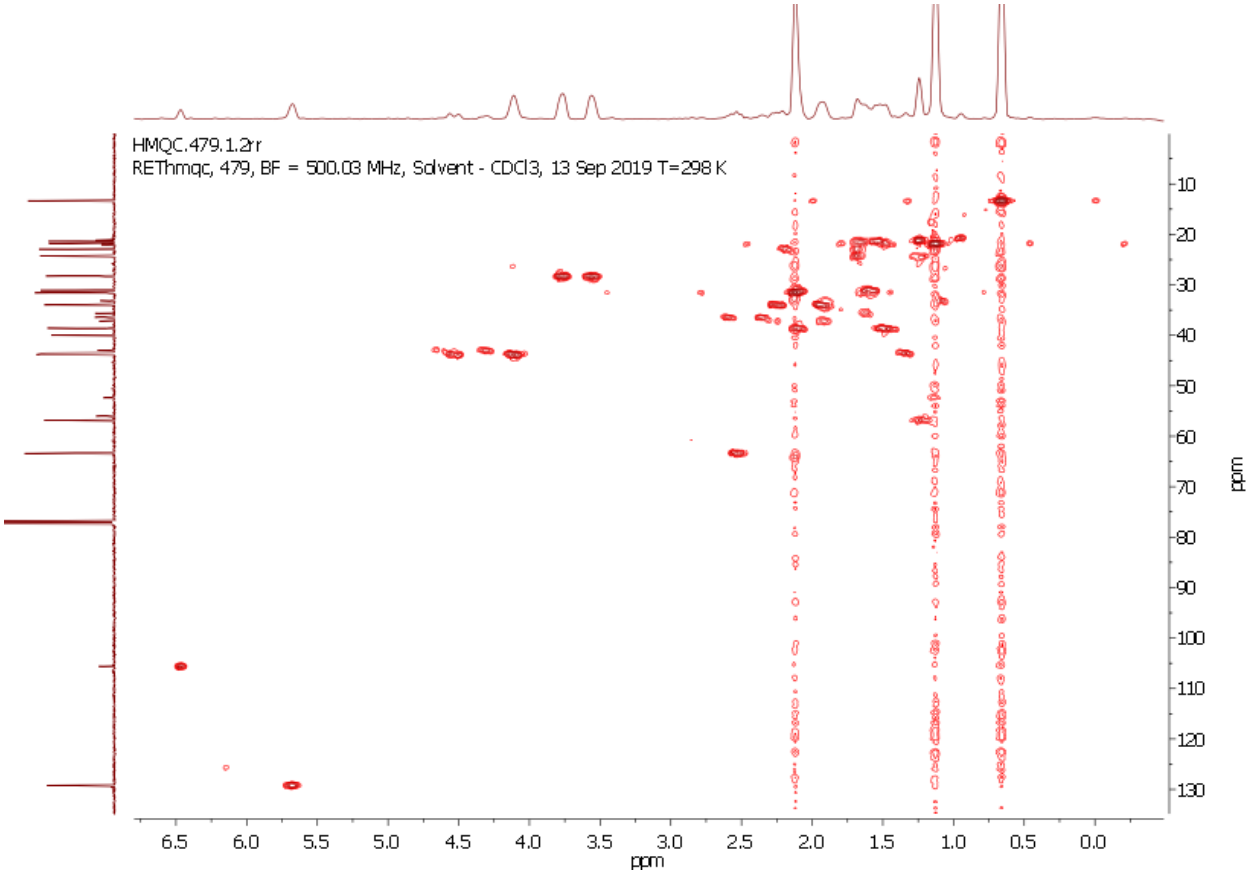

Figure S16 Spectrum HMQC of Compound 2b.

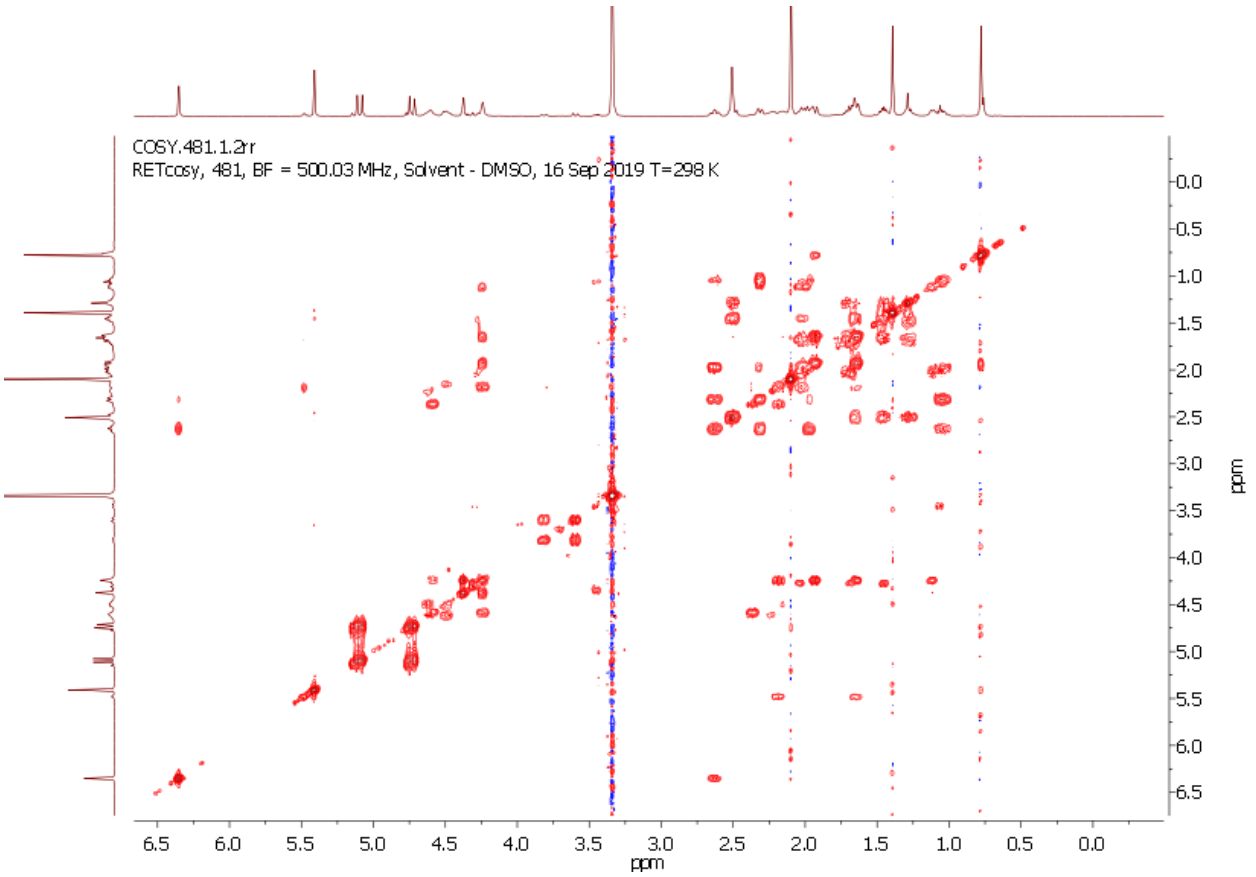

Figure S17 COSY of Compound 3c.

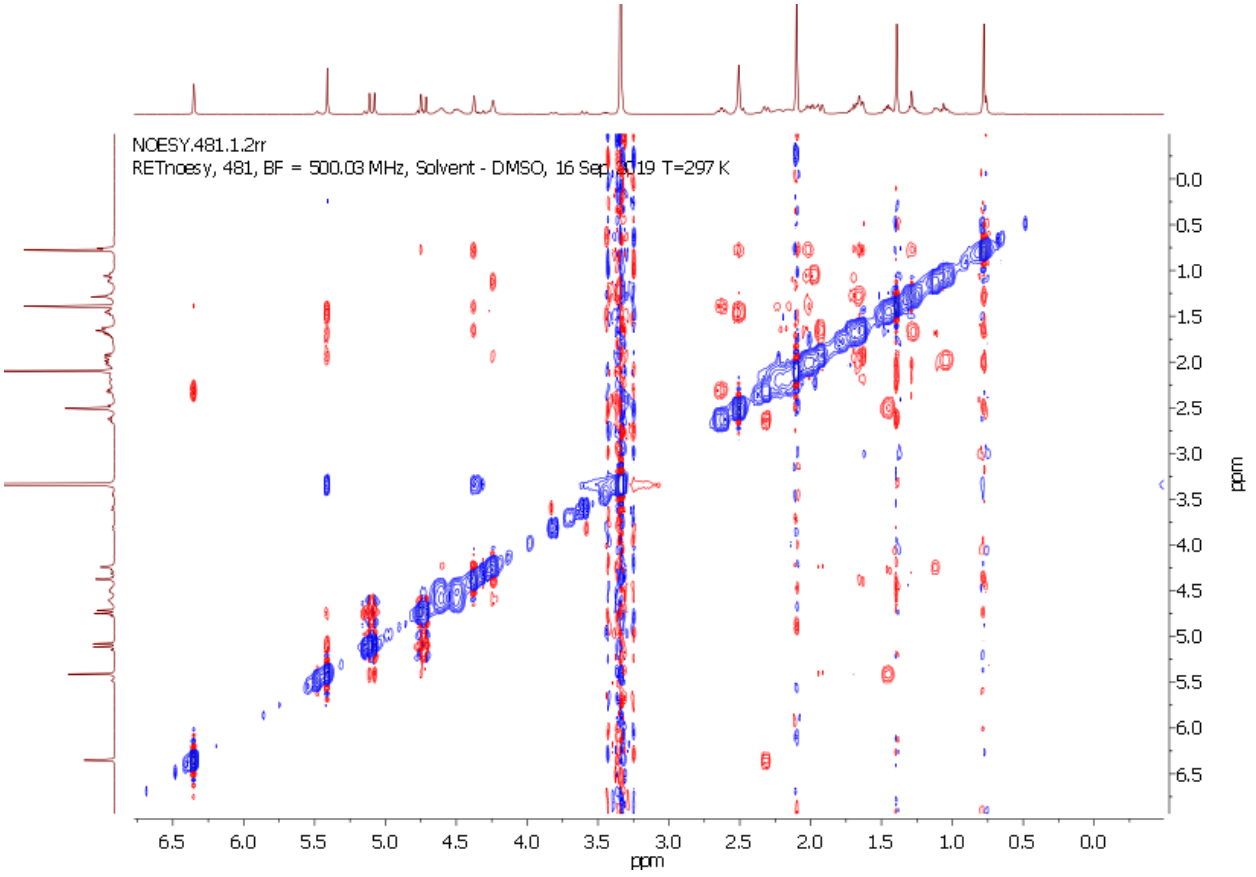

Figure S18 NOESY of Compound 3c.

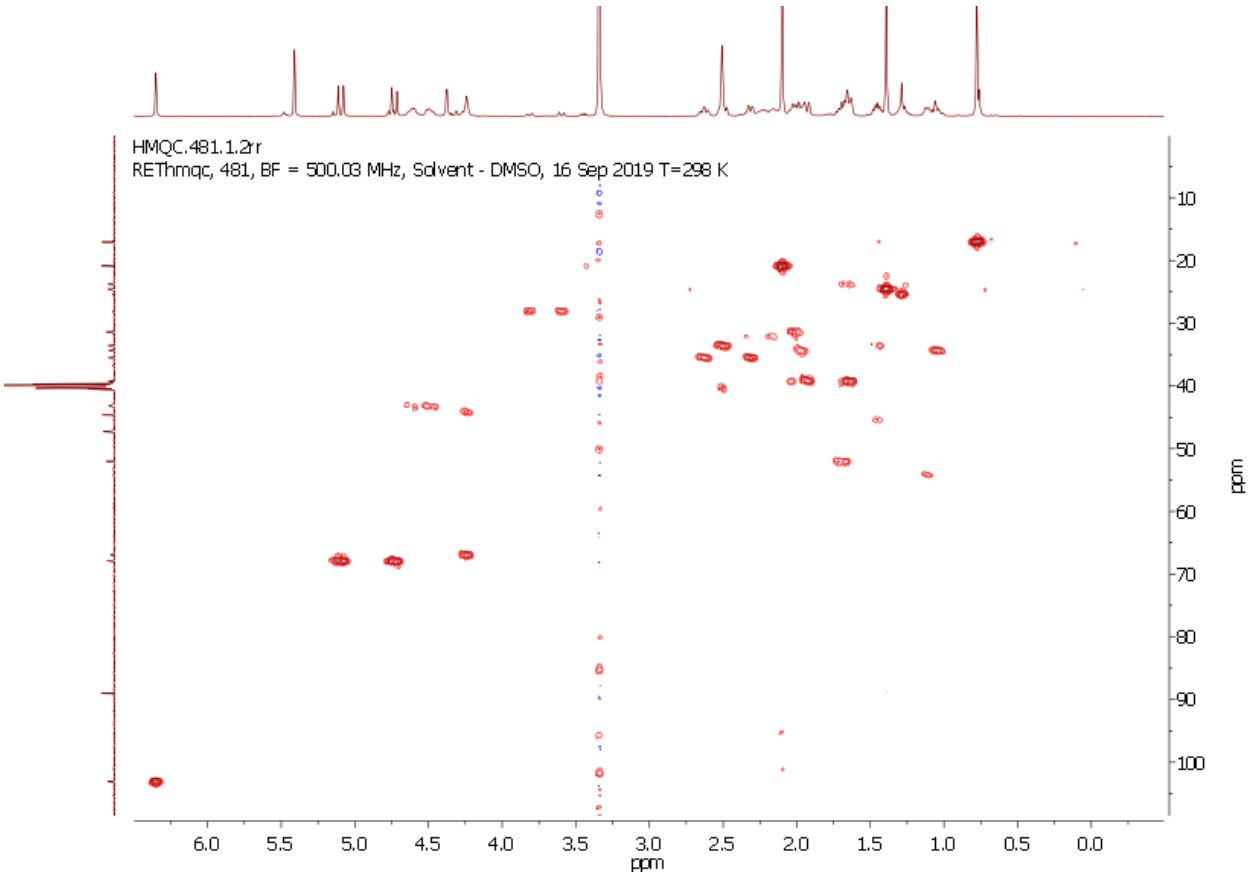

Figure S19 Spectrum HMQC of Compound 3c.

Mass-spectra

The HR-ESI mass-spectra were obtained on a BRUKER maxis spectrometer equipped with an electrospray ionization (ESI) source; methanol was used as the solvent. The instrument was operated in positive mode using an m/z range of 50–1200. The capillary voltage of the ion source was set at 4000 V The nebulizer gas pressure was 1.0 bar, and the drying gas flow was 4.0 dm^3 min^{-1}.

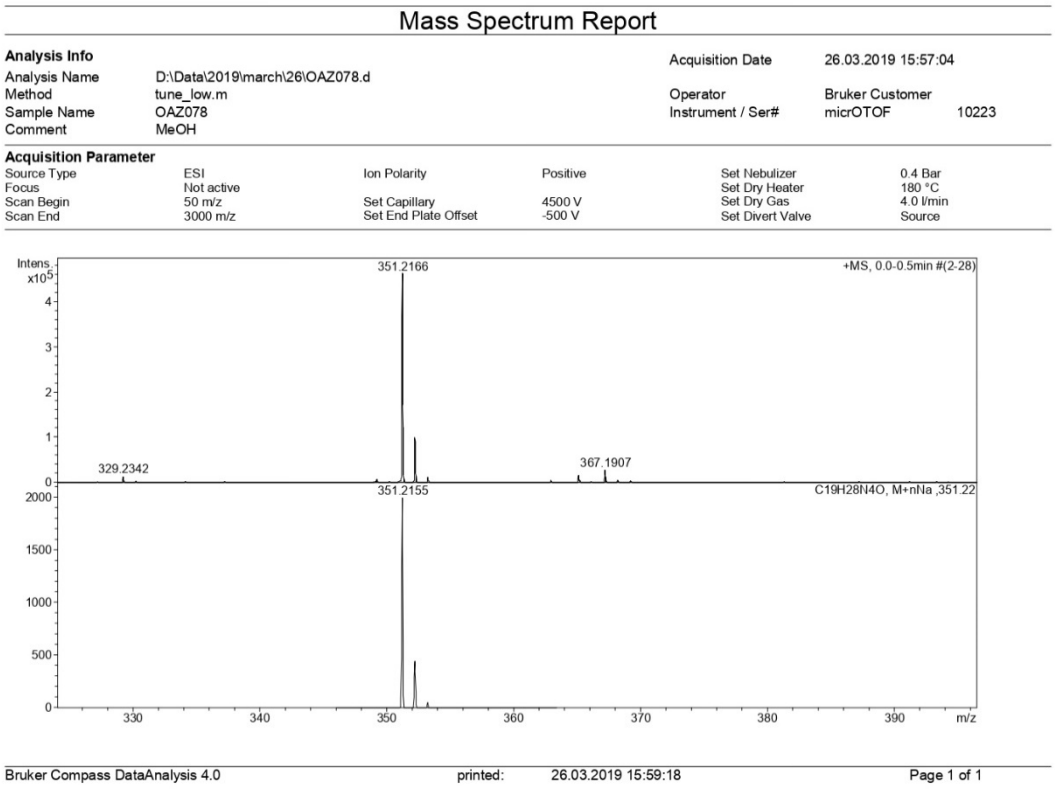

Figure S20 The HR-ESI mass-spectrum of Compound 2a.

Display Report

Analysis Info

Analysis Name	D:\Data\Work\2019\February\07\2_000001.d	Acquisition Date	07-Feb-19 11:51:04
Method	tune_low_pos.m	Operator	BDAL@DE
Sample Name	2_	Instrument / Ser#	maXis 62
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	1.0 Bar
Focus	Active			Set Dry Heater	180 °C
Scan Begin	110 m/z	Set Capillary	4500 V	Set Dry Gas	4.0 l/min
Scan End	1500 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source

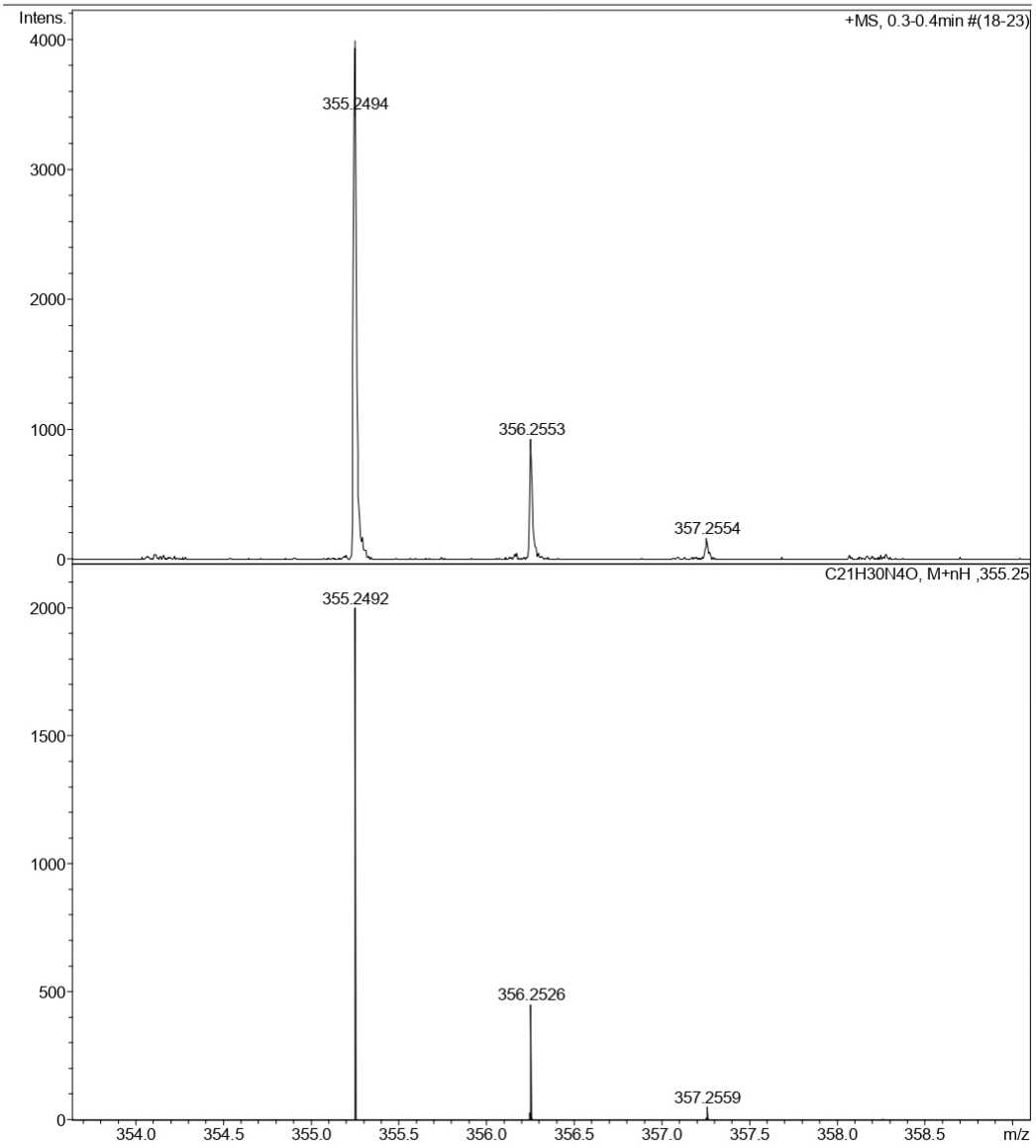

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Figure S21 The HR-ESI mass-spectrum of Compound 2b.

Mass Spectrum Report

Analysis Info

Analysis Name	D:\Data\Data\2019\april\29\2\2_000001.d	Acquisition Date	30.04.2019 10:54:02
Method	tune_low.m	Operator	Bruker Customer
Sample Name		Instrument / Ser#	micrOTOF 10223
Comment			

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
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Scan End	3000 m/z	Set End Plate Offset	-500 V	Set Divert Valve	Source

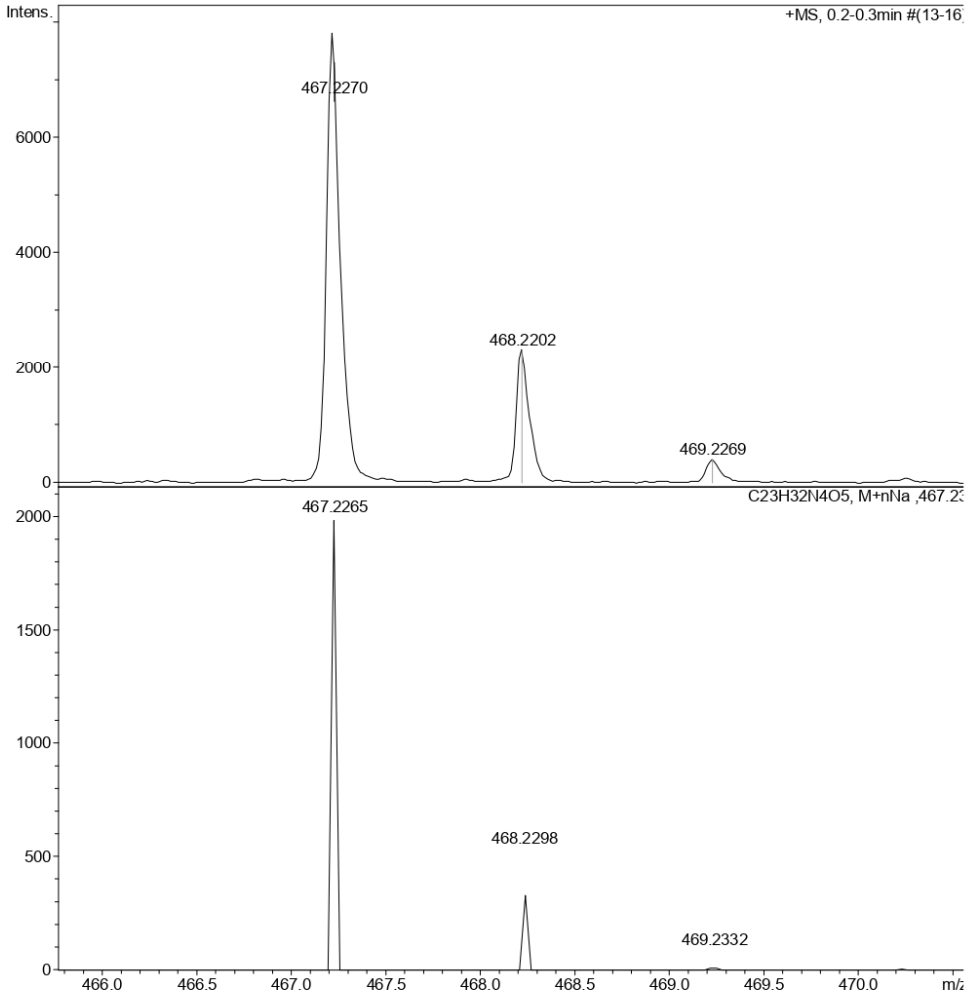

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Figure S22 The HR-ESI mass-spectrum of Compound 3c.

IR data

IR spectra were recorded on a Perkin Elmer Spectrum BX spectrometer in disk KBr.

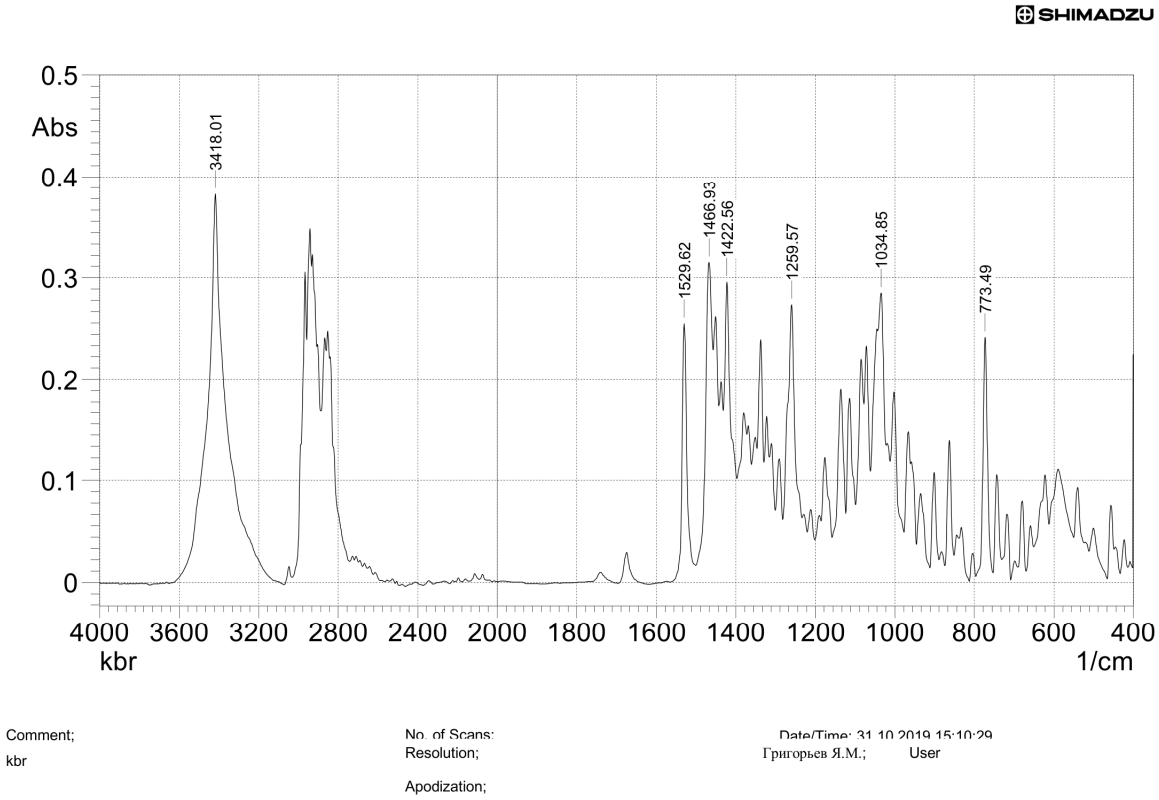


Figure S23 IR spectrum of Compound 2a.

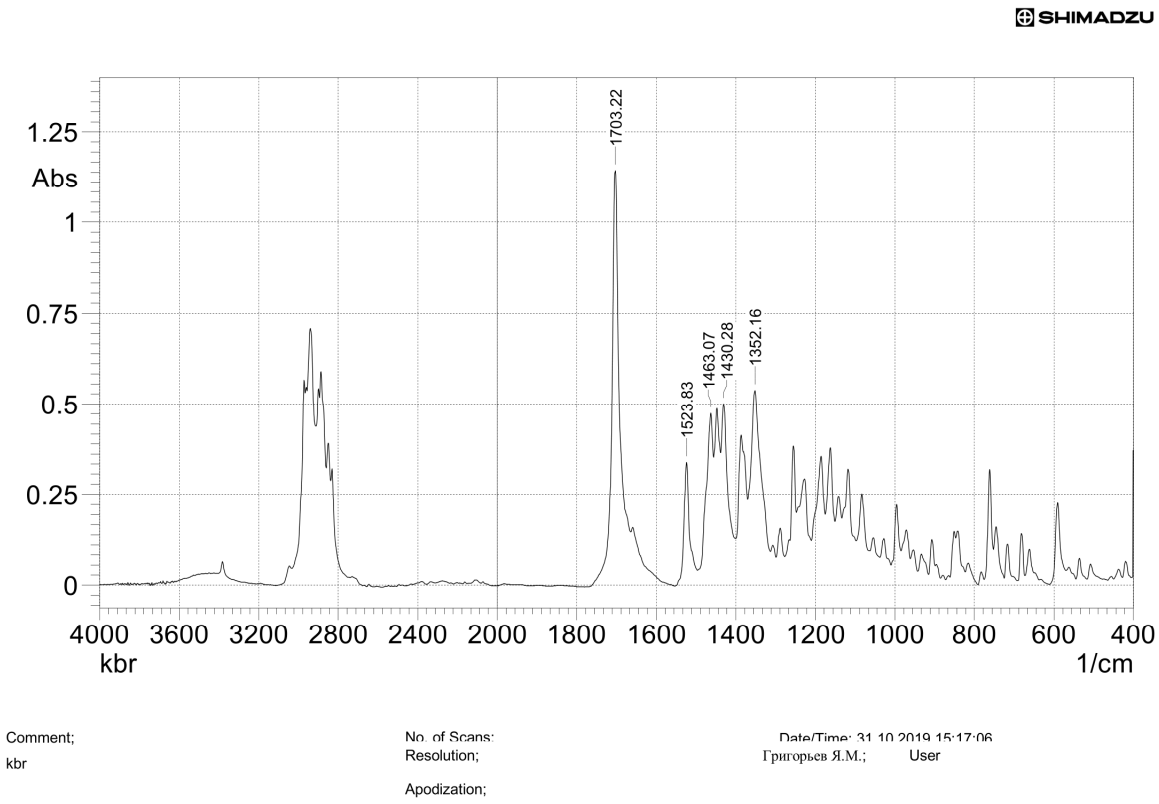


Figure S24 IR spectrum of Compound 2b.

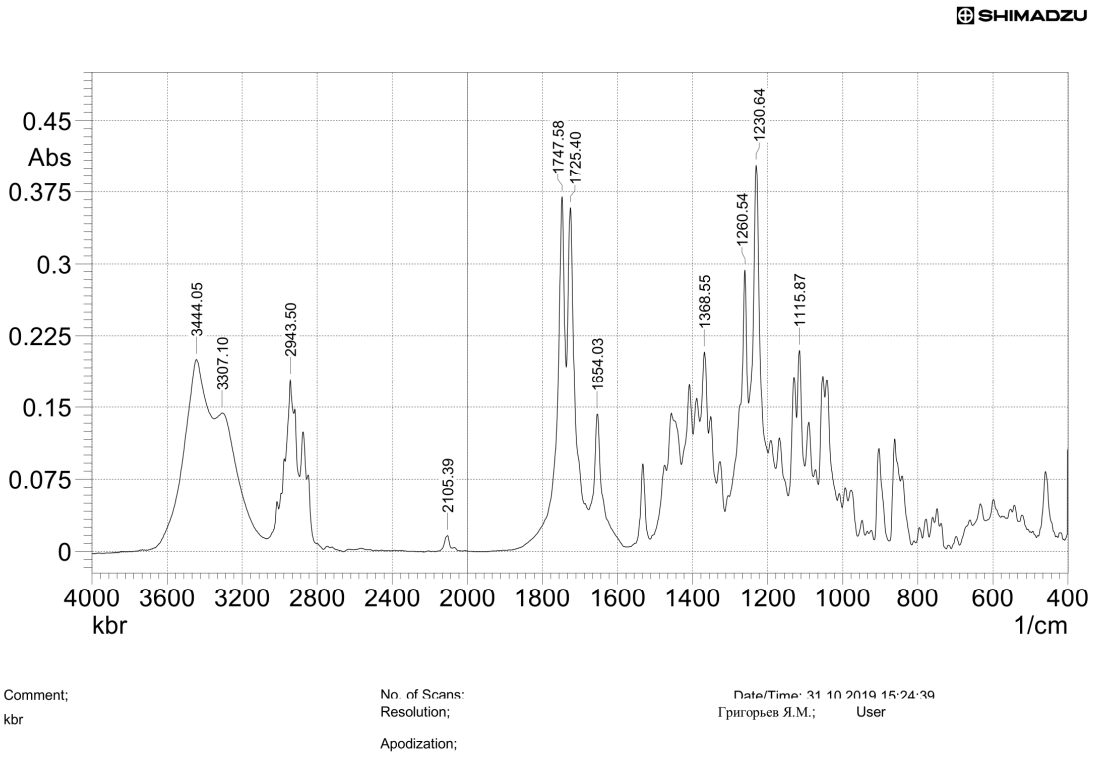


Figure S25 IR spectrum of Compound 3c.

X-ray crystal data

The unit cell parameters for compounds obtained are given in Table S3. A SC-XRD experiments were carried out using Agilent Technologies «Xcalibur» and Rigaku Oxford Diffraction «XtaLAB Supernova» diffractometers. Diffraction experiments were collected at the temperature of 100K using monochromated Mo*Kα* and Cu*Kα* radiation. The unit cell parameters (Table 1) were refined by least square techniques in the 2θ range of 5–55 degrees for Mo*Kα* and 8–148 for Cu*Kα.* Structures were solved by the Charge Flipping and Intrinsic Phasing methods using Superflip[S1-S3] and SHELXT,[S4] respectively, and refined by means of the SHELXL[S5] program incorporated in the OLEX2[S6] program package. Empirical absorption correction was applied in CrysAlisPro (Agilent Technologies, 2018) program complex using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm. Supplementary crystallographic data for this paper have been deposited at Cambridge Crystallographic Data Centre (CCDC 1936462, 1938453, 1938454) and can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif.

Table S1. The unit cell parameters for tetrazolo-steroids **2a, 2b, 3c.**

Identification code	compound **2a**	compound **2b**	compound **3c**
Empirical formula	$C_{19}H_{28}N_4O$	$C_{21}H_{30}N_4O$	$C_{23}H_{32}N_4O_5$
Formula weight	328.45	354.49	444.52
Temperature/K	100(2)	109(2)	100(2)
Crystal system	monoclinic	orthorhombic	orthorhombic
Space group	$P2_1$	$P2_12_12_1$	$P2_12_12_1$
a/Å	6.19730(10)	7.63990(10)	7.72085(11)
b/Å	12.7562(3)	11.4831(2)	14.1374(2)
c/Å	10.9121(3)	21.3820(3)	20.5233(2)
α/°	90	90	90
β/°	98.905(2)	90	90
γ/°	90	90	90
Volume/Å^3	852.25(3)	1875.84(5)	2240.18(5)
Z	2	4	4
ρ_{calc}g/cm^3	1.280	1.255	1.318
μ/mm^1	0.639	0.619	0.094
F(000)	356.0	768.0	952.0
Crystal size/mm^3	0.29 × 0.26 × 0.11	0.33 × 0.24 × 0.21	0.5 × 0.3 × 0.25
Radiation	CuKα (λ = 1.54184)	CuKα (λ = 1.54184)	MoKα (λ = 0.71073)
2Θ range for data collection/°	8.202 to 148.458	8.27 to 147.914	5.638 to 54.998
Index ranges	-6 ≤ h ≤ 7, -15 ≤ k ≤ 15, -13 ≤ l ≤ 13	-5 ≤ h ≤ 9, -14 ≤ k ≤ 14, -25 ≤ l ≤ 26	-10 ≤ h ≤ 10, -17 ≤ k ≤ 18, -26 ≤ l ≤ 26
Reflections collected	7687	12453	26864
Independent reflections	3427 [R_{int} = 0.0373, R_{sigma} = 0.0453]	3772 [R_{int} = 0.0349, R_{sigma} = 0.0303]	5136 [R_{int} = 0.0288, R_{sigma} = 0.0214]
Data/restraints/parameters	3427/1/221	3772/0/239	5136/0/294
Goodness-of-fit on F^2	1.060	1.052	1.042
Final R indexes [I>=2σ (I)]	R_1 = 0.0396, wR_2 = 0.1038	R_1 = 0.0448, wR_2 = 0.1200	R_1 = 0.0377, wR_2 = 0.0952
Final R indexes [all data]	R_1 = 0.0413, wR_2 = 0.1056	R_1 = 0.0471, wR_2 = 0.1227	R_1 = 0.0407, wR_2 = 0.0970
Largest diff. peak/hole / e Å^{-3}	0.16/-0.23	0.38/-0.20	0.56/-0.22
Flack parameter	0.3(4)	0.0(5)	-0.6(3)
CCDC number	1938454	1938453	1936462

X-ray crystal data of compound 2a (8930-12762-ret)

(17β-hydroxy-3-aza-A-homo-androst-5-eno[3,4-*d*]tetrazole)

8930-12762-ret CCDC 1938453

Summary of Data CCDC 193845

Compound Name: 8930-12762-ret

Formula: C19 H28 N4 O

Unit Cell Parameters: a 6.1973(1) b 12.7562(2) c 10.9121(3) P21

Table S2 Compound 2a (8930-12762-ret).

CheckCIF/PLATON report

Structure factors have been supplied for datablock(s) 8930-12762-ret

THIS REPORT IS FOR GUIDANCE ONLY. IF USED AS PART OF A REVIEW PROCEDURE FOR PUBLICATION, IT SHOULD NOT REPLACE THE EXPERTISE OF AN EXPERIENCED CRYSTALLOGRAPHIC REFEREE.

No syntax errors found. CIF dictionary Interpreting this report

Datablock: 8930-12762_plate

Bond precision:	C-C = 0.0033 A	Wavelength=1.54184	
Cell:	a=6.1973(1)	b=12.7562(3)	c=10.9121(3)
	alpha=90	beta=98.905(2)	gamma=90

Temperature: 100 K

	Calculated	Reported
Volume	852.25(3)	852.25(3)
Space group	P 21	P 1 21 1
Hall group	P 2yb	P 2yb
Moiety formula	C19 H28 N4 O	C19 H28 N4 O
Sum formula	C19 H28 N4 O	C19 H28 N4 O
Mr	328.45	328.45
Dx,g cm-3	1.280	1.280
Z	2	2

Mu (mm-1)	0.639	0.639
F000	356.0	356.0
F000’	356.96	
h,k,lmax	7,15,13	7,15,13
Nref	3468[1815]	3427
Tmin,Tmax	0.831,0.932	0.624, 1.000
Tmin’	0.831	

Correction method= # Reported T Limits: Tmin=0.624 Tmax=1.000

AbsCorr = MULTI-SCAN

Data completeness = 1.89/0.99 Theta(max) = 74.229

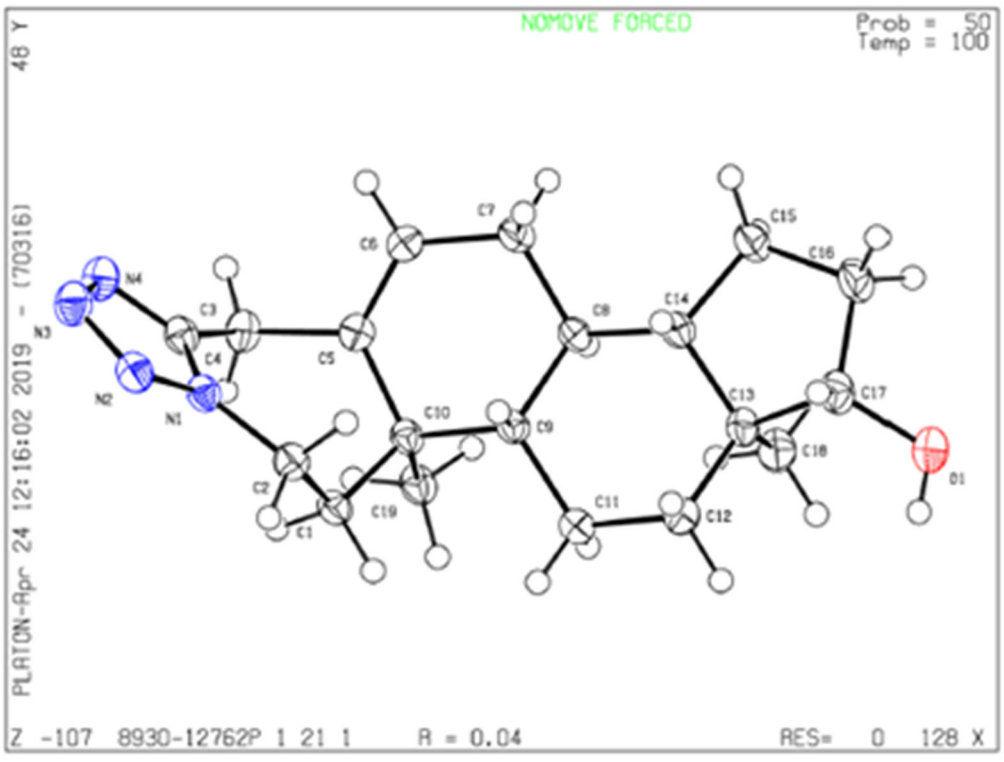
R(reflections)= 0.0396(3270) wR2(reflections)= 0.1056(3427)

S = 1.060 Npar= 221

The following ALERTS were generated. Each ALERT has the format

test-name_AL ERT_alert-type_alert-level.

Click on the hyperlinks for more details of the test.



X-ray crystal data of Compound 2b (8930-11438-ret)

(*20-oxo-3-aza-A-homo-pregn-5-eno[3,4-d]tetrazole*)

8930-11438-ret CCDC 1938453

Summary of Data CCDC 1938453

Compound Name: 8930-11438-ret
Formula: C21 H30 N4 O
Unit Cell Parameters: a 7.6399(1) b 11.4831(2) c 21.3820(3) P 21 21 21

Table S3 Compound 2b (8930-11438-ret).

CheckCIF/PLATON report

Structure factors have been supplied for datablock(s) 8930-11438-ret

THIS REPORT IS FOR GUIDANCE ONLY. IF USED AS PART OF A REVIEW PROCEDURE FOR PUBLICATION, IT SHOULD NOT REPLACE THE EXPERTISE OF AN EXPERIENCED CRYSTALLOGRAPHIC REFEREE.

No syntax errors found. CIF dictionary Interpreting this report

Datablock: 8930-11438-ret

Bond precision: C-C = 0.0035 A Wavelength=1.54184

Cell: a=7.6399(1) b=11.4831(2) c=21.3820(3)

alpha=90 beta=90 gamma=90 Temperature: 109 K

	Calculated	Reported
Volume	1875.84(5)	1875.84(5)
Space group	P 21 21 21	P 21 21 21
Hall group	P 2ac 2ab	P 2ac 2ab
Moiety formula	C21 H30 N4 O	C21 H30 N4 O
Sum formula	C21 H30 N4 O	C21 H30 N4 O
Mr	354.49	354.49
Dx,g cm-3	1.255	1.255
Z	4	4
Mu (mm-1)	0.619	0.619

F000	768.0	768.0
F000’	770.05	
h,k,lmax	9,14,26	9,14,26
Nref	3808[2196]	3772
Tmin,Tmax	0.837,0.878	0.882,1.000
Tmin’	0.815	

Correction method= # Reported T Limits: Tmin=0.882 Tmax=1.000

AbsCorr = MULTI-SCAN

Data completeness= 1.72/0.99 Theta(max)= 73.957

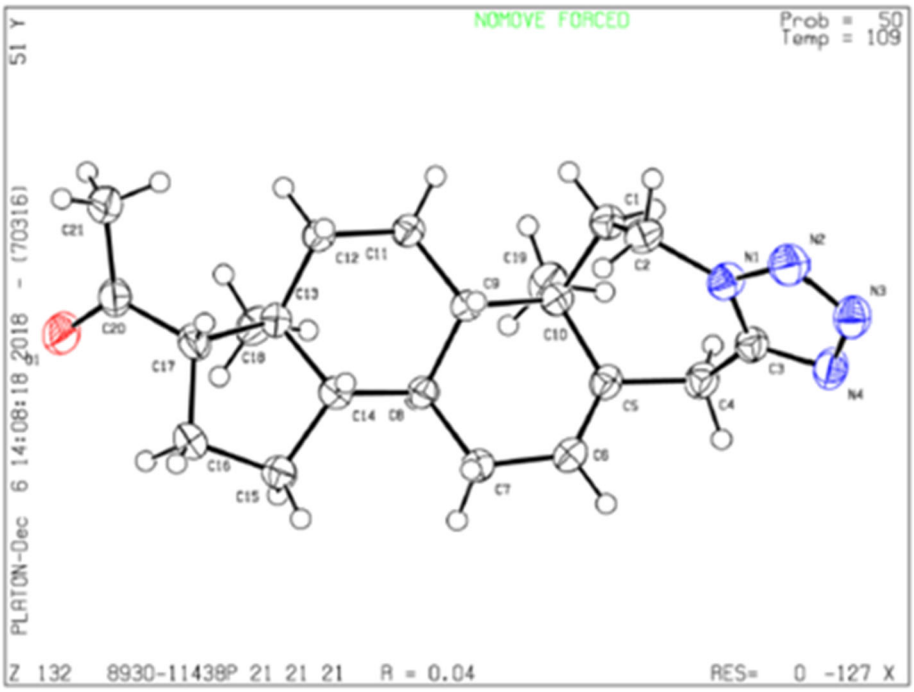
R(reflections)= 0.0448(3561) wR2(reflections)= 0.1227(3772)

S = 1.052 Npar= 239

The following ALERTS were generated. Each ALERT has the format

test-name_ALERT_alert-type_alert-level.

Click on the hyperlinks for more details of the test.



X-ray crystal data of Compound 3c (8930-12821-ret)

21-acetoxy-11β,17α-dihydroxy-20oxo-3-aza-A-homo-pregn-4-eno[3,4-d]tetrazole

8930-12821-ret CCDC 1936462

Summary of Data CCDC 1936462

Compound Name:
Formula: C23 H32 N4 O5
Unit Cell Parameters: a 7.172085(1) b 13.74(2) c 20.5233(3) P 21 21 21

Table S4 Compound 3c (8930-12821-ret).

checkCIF/PLATON report

Structure factors have been supplied for datablock(s) 8930-12821- ret

THIS REPORT IS FOR GUIDANCE ONLY. IF USED AS PART OF A REVIEW PROCEDURE FOR PUBLICATION, IT SHOULD NOT REPLACE THE EXPERTISE OF AN EXPERIENCED CRYSTALLOGRAPHIC REFEREE.

No syntax errors found. CIF dictionary Interpreting this report

Datablock: 8930-12821 ret

Bond precision:	C-C = 0.0030 A	Wavelength=0.71073	
Cell:	a=7.72085(11)	b=14.1374(2)	c=20.5233(2)
	alpha=90	beta=90	gamma=90
Temperature:	100 K		
	Calculated	Reported	
Volume	2240.17(5)	2240.18(5)	
Space group	P 21 21 21	P 21 21 21	
Hall group	P 2ac 2ab	P 2ac 2ab	
Moiety formula	C23 H32 N4 O5	C23 H32 N4 O5	
Sum formula	C23 H32 N4 O5	C23 H32 N4 O5	
Mr	444.53	444.52	
Dx,g cm-3	1.318	1.318	
Z	4	4	

Mu (mm-1)	0.094	0.094
F000	952.0	952.0
F000’	952.43	
h,k,lmax	10,18,26	10,18,26
Nref	5139[2928]	5136
Tmin,Tmax	0.967,0.977	0.989, 1.000
Tmin’	0.954	

Correction method = # Reported T Limits: Tmin=0.989 Tmax=1. 000

AbsCorr = MULTI-SCAN

Data completeness= 1.75/1.00 Theta(max)= 27.499

R(reflections)= 0.0377(4832) wR2(reflections)= 0.0970(5136)

S = 1.042 Npar= 294

The following ALERTS were generated. Each ALERT has the format

test-name_ALERT_alert-type_alert-level.

Click onthe hyperlinks for more details of the test.

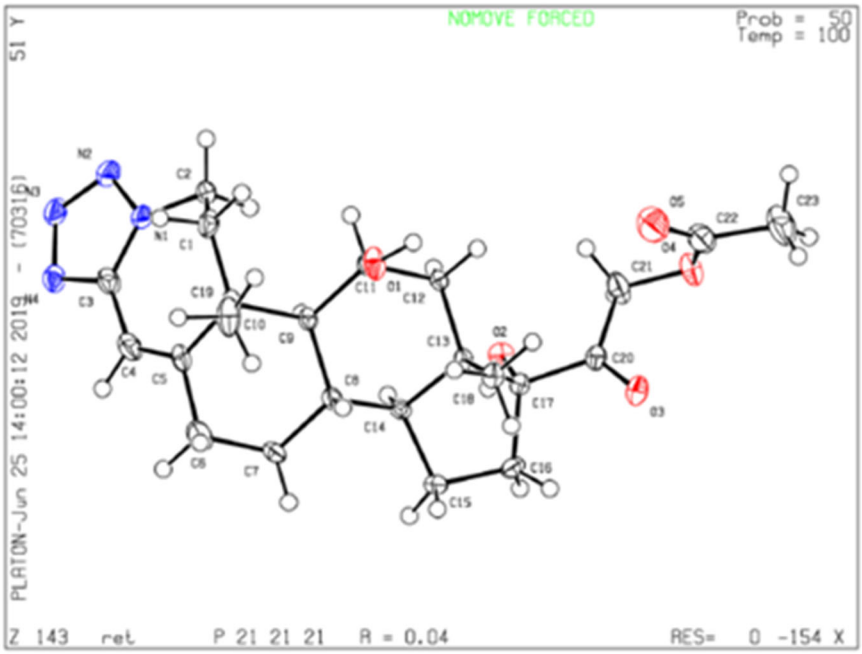


Table S5 LAST.CIF of Compound 2a

17β-hydroxy-3-aza-A-homo-androst-5-eno[3,4-*d*]tetrazole.

Data_ 8930-12762-ret CCDC 1938453

Audit creation-data 24-04-2019

List of Runs (angles in degrees, time in seconds):

#	Type	Start	End	Width	t~exp~	\w	\q	\k	\f	Frames
1	\w	-38.00	9.00	0.50	0.20	--	-38.82	110.00	-95.00	94
2	\w	-73.00	-47.00	0.50	0.20	--	-38.82	110.00	-95.00	52
3	\w	110.00	136.00	0.50	0.25	--	107.00	110.00	-95.00	52
4	\w	69.00	111.00	0.50	0.25	--	107.00	-110.00	169.00	84
5	\w	-85.00	-53.00	0.50	0.20	--	-38.82	-54.00	150.00	64
6	\w	-25.00	1.00	0.50	0.20	--	-38.82	32.00	-90.00	52
7	\w	-63.00	-27.00	0.50	0.20	--	-38.82	-54.00	-120.00	72
8	\w	3.00	28.00	0.50	0.20	--	-38.82	32.00	-90.00	50
9	\w	-55.00	-7.00	0.50	0.20	--	-38.82	54.00	-90.00	96
10	\w	-99.00	-74.00	0.50	0.20	--	-38.82	-54.00	-120.00	50
11	\w	31.00	105.00	0.50	0.20	--	38.82	43.00	90.00	148
12	\w	12.00	66.00	0.50	0.20	--	38.82	77.00	-180.00	108
13	\w	70.00	101.00	0.50	0.20	--	38.82	77.00	-180.00	62
14	\w	49.00	88.00	0.50	0.20	--	38.82	21.00	-30.00	78
15	\w	-7.00	29.00	0.50	0.20	--	38.82	-110.00	169.00	72
16	\w	56.00	81.00	0.50	0.20	--	38.82	-138.00	-90.00	50
17	\w	121.00	157.00	0.50	0.25	--	107.00	133.00	150.00	72
18	\w	85.00	116.00	0.50	0.25	--	107.00	61.00	-120.00	62
19	\w	70.00	100.00	0.50	0.25	--	107.00	133.00	150.00	60

20 \w 113.00 157.00 0.50 0.25 -- 107.00 53.00 90.00 88

21 \w 98.00 152.00 0.50 0.25 -- 107.00 37.00 60.00 108

22 \w 43.00 71.00 0.50 0.25 -- 107.00 -61.00 150.00 56

23 \w 122.00 157.00 0.50 0.25 -- 107.00 69.00 -90.00 70

24 \w 88.00 114.00 0.50 0.25 -- 107.00 69.00 -90.00 52

25 \w 63.00 89.00 0.50 0.25 -- 107.00 -93.00-150.00 52

26 \w 82.00 157.00 0.50 0.25 -- 107.00 93.00 -60.00 150

27 \w 35.00 61.00 0.50 0.25 -- 107.00 -93.00-150.00 52

28 \w 100.00 157.00 0.50 0.25 -- 107.00 45.00-180.00 114

29 \w 113.00 154.00 0.50 0.25 -- 107.00 93.00 -30.00 82

30 \w 53.00 112.00 0.50 0.25 -- 107.00-133.00-180.00 118

31 \w 47.00 113.00 0.50 0.25 -- 107.00-133.00 0.00 132

32 \w 123.00 149.00 0.50 0.25 -- 107.00 61.00-120.00 52

33 \w 79.00 124.00 0.50 0.25 -- 107.00 -61.00 150.00 90

34 \w 49.00 124.00 0.50 0.25 -- 107.00 -53.00 -60.00 150

35 \w 88.00 138.00 0.50 0.25 -- 107.00 110.00 -95.00 100

36 \w -49.00 -23.00 0.50 0.20 -- -38.82 -21.00 30.00 52

37 \w -29.00 13.00 0.50 0.20 -- -38.82 32.00 -30.00 84

38 \w -91.00 -65.00 0.50 0.20 -- -38.82 -21.00 30.00 52

39 \w -40.00 2.00 0.50 0.20 -- -38.82 54.00-150.00 84

40 \w 80.00 106.00 0.50 0.20 -- 38.82 32.00-180.00 52

41 \w 63.00 100.00 0.50 0.20 -- 38.82 77.00-120.00 74

42 \w 18.00 44.00 0.50 0.20 -- 38.82 -32.00 -30.00 52

43 \w 36.00 87.00 0.50 0.20 -- 38.82-138.00 90.00 102

44 \w 9.00 37.00 0.50 0.20 -- 38.82-104.00 -60.00 56

45 \w 12.00 38.00 0.50 0.20 -- 38.82 77.00-120.00 52

46 \w 71.00 125.00 0.50 0.25 -- 107.00 133.00 -60.00 108

47 \w 96.00 123.00 0.50 0.25 -- 107.00-133.00 30.00 54

48 \w 38.00 93.00 0.50 0.25 -- 107.00-111.00-180.00 110

49 \w 56.00 99.00 0.50 0.25 -- 107.00-133.00 150.00 86

50 \w 44.00 69.00 0.50 0.25 -- 107.00-133.00 30.00 50

51 \w 81.00 106.00 0.50 0.25 -- 107.00-133.00 -30.00 50

52 \w 52.00 137.00 0.50 0.25 -- 107.00 -86.00 -30.00 170

53 \w 127.00 153.00 0.50 0.25 -- 107.00 37.00 120.00 52

54 \w 132.00 157.00 0.50 0.25 -- 107.00 45.00 0.00 50

55 \w 98.00 149.00 0.50 0.25 -- 107.00 45.00 150.00 102

56 \w 98.00 124.00 0.50 0.25 -- 107.00 45.00 0.00 52

57 \w 89.00 115.00 0.50 0.25 -- 107.00 -45.00-180.00 52

58 \w 114.00 140.00 0.50 0.25 -- 107.00 37.00-150.00 52

59 \w 59.00 85.00 0.50 0.25 -- 107.00 -69.00 150.00 52

60 \w 89.00 115.00 0.50 0.25 -- 107.00 -69.00 150.00 52

61 \w 35.00 119.00 0.50 0.25 -- 107.00 -22.00 -90.00 168

62 \w 51.00 117.00 0.50 0.25 -- 107.00 -30.00 150.00 132

63 \w 40.00 65.00 0.50 0.25 -- 107.00 -93.00 30.00 50

64 \w 78.00 103.00 0.50 0.25 -- 107.00 -93.00 30.00 50

65 \w 43.00 69.00 0.50 0.25 -- 107.00 -45.00-180.00 52

66 \w 122.00 148.00 0.50 0.25 -- 107.00 93.00 0.00 52

67 \w 132.00 157.00 0.50 0.25 -- 107.00 30.00 60.00 50

68 \w 81.00 106.00 0.50 0.25 -- 107.00 93.00 0.00 50

Table S6 LAST.CIF of Compound 2b

20-oxo-3-aza-A-homo-pregn-5-eno[3,4-*d*]tetrazole.

Data _8930-11438-ret

Audit creation-data 06-12-2018

List of Runs (angles in degrees, time in seconds):

#	Type	Start	End	Width	t~exp~	\w	\q	\k	\f	Frames
1	\w	6.00	40.00	0.50	0.20	--	-38.82	6.00	-96.00	68
2	\w	-66.00	-21.00	0.50	0.20	--	-38.82	6.00	-96.00	90
3	\w	-91.00	-48.00	0.50	0.20	--	-38.82	141.00	-103.00	86
4	\w	26.00	61.00	0.50	0.20	--	38.82	-144.00	110.00	70
5	\w	70.00	95.00	0.50	0.20	--	38.82	-144.00	110.00	50
6	\w	72.00	113.00	0.50	0.27	--	107.00	-6.00	86.00	82
7	\w	73.00	107.00	0.50	0.27	--	107.00	128.00	84.00	68
8	\w	42.00	68.00	0.50	0.27	--	107.00	-6.00	86.00	52
9	\w	86.00	116.00	0.50	0.27	--	107.00	-128.00	10.00	60
10	\w	112.00	153.00	0.50	0.27	--	107.00	128.00	84.00	82
11	\w	64.00	104.00	0.50	0.27	--	107.00	-144.00	110.00	80
12	\w	63.00	100.00	0.50	0.27	--	107.00	144.00	164.00	74
13	\w	-55.00	-7.00	0.50	0.20	--	-38.82	43.00	-30.00	96
14	\w	-64.00	-39.00	0.50	0.20	--	-38.82	126.00	-4.00	50
15	\w	-38.00	-12.00	0.50	0.20	--	-38.82	32.00	90.00	52
16	\w	-1.00	26.00	0.50	0.20	--	-38.82	43.00	-30.00	54
17	\w	-30.00	51.00	0.50	0.20	--	38.82	-32.00	-120.00	162
18	\w	62.00	88.00	0.50	0.20	--	38.82	65.00	-150.00	52
19	\w	-29.00	-3.00	0.50	0.20	--	38.82	-6.00	86.00	52

20 \w 31.00 56.00 0.50 0.20 -- 38.82-141.00-161.00 50

21 \w 44.00 69.00 0.50 0.20 -- 38.82-128.00 10.00 50

22 \w 28.00 53.00 0.50 0.20 -- 38.82 65.00-150.00 50

23 \w 89.00 153.00 0.50 0.27 -- 107.00 45.00 -90.00 128

24 \w 45.00 91.00 0.50 0.27 -- 107.00-133.00-150.00 92

25 \w 97.00 122.00 0.50 0.27 -- 107.00-133.00-150.00 50

26 \w 38.00 112.00 0.50 0.27 -- 107.00-111.00-150.00 148

27 \w 70.00 120.00 0.50 0.27 -- 107.00 -53.00 -60.00 100

28 \w 40.00 122.00 0.50 0.27 -- 107.00 -37.00-180.00 164

29 \w 69.00 95.00 0.50 0.27 -- 107.00 141.00-103.00 52

30 \w -72.00 -28.00 0.50 0.20 -- -38.82 -54.00-120.00 88

31 \w 15.00 41.00 0.50 0.20 -- 38.82 -43.00 -60.00 52

32 \w 30.00 56.00 0.50 0.20 -- 38.82 32.00-180.00 52

33 \w 89.00 115.00 0.50 0.27 -- 107.00 111.00 -30.00 52

34 \w 113.00 139.00 0.50 0.27 -- 107.00 178.00 30.00 52

35 \w 41.00 122.00 0.50 0.27 -- 107.00 -37.00 -90.00 162

36 \w 36.00 95.00 0.50 0.27 -- 107.00 178.00 30.00 118

37 \w 94.00 145.00 0.50 0.27 -- 107.00 30.00 30.00 102

38 \w 43.00 69.00 0.50 0.27 -- 107.00-111.00 -90.00 52

39 \w 87.00 113.00 0.50 0.27 -- 107.00-111.00 -90.00 52

40 \w 90.00 127.00 0.50 0.27 -- 107.00 -61.00 30.00 74

41 \w 48.00 87.00 0.50 0.27 -- 107.00 -61.00 30.00 78

42 \w 24.00 49.00 0.50 0.20 -- 38.82-128.00 10.00 50

43 \w -53.00 -27.00 0.50 0.20 -- -38.82 43.00 90.00 52

44 \w -55.00 -28.00 0.50 0.20 -- -38.82 -32.00 90.00 54

45 \w -17.00 25.00 0.50 0.20 -- -38.82 43.00 90.00 84

46 \w -102.00 -72.00 0.50 0.20 -- -38.82 -77.00-120.00 60

47 \w -95.00 -69.00 0.50 0.20 -- -38.82 -32.00 90.00 52

48 \w -97.00 -13.00 0.50 0.20 -- -38.82 -77.00-150.00 168

49 \w -55.00 -18.00 0.50 0.20 -- -38.82 -77.00-120.00 74

50 \w -31.00 24.00 0.50 0.20 -- -38.82 43.00 150.00 110

51 \w -15.00 10.00 0.50 0.20 -- 38.82-104.00 120.00 50

52 \w 27.00 53.00 0.50 0.20 -- 38.82 -43.00 -30.00 52

53 \w 39.00 71.00 0.50 0.20 -- 38.82-138.00-150.00 64

54 \w -23.00 12.00 0.50 0.20 -- 38.82 -43.00 -30.00 70

Table S7 LAST.CIF of Compound 3c

21-acetoxy-11β,17α-dihydroxy-20-oxo-3-aza-A-homo-pregn-4-eno[3,4-*d*]tetrazole.

data_ **8930-12821** -ret CCDC **1936462**

audit_creation_date 25-06-2019

List of Runs (angles in degrees, time in second:)

The symmetry employed for this shelxl refinement is uniquely defined by the following loop, which should always be used as a source of symmetry information in preference to the above space-group names. They are only intended as comments.

#__ type_ start__ end____ width___ exp.time_

1 omega -23.00 19.00 1.0000 90.0000

omega____ theta____ kappa____ phi_____ frames

- -25.2748 -19.0000 90.0000 42

#__ type_ start__ end____ width___ exp.time_

2 omega 0.00 98.00 1.0000 90.0000

omega____ theta____ kappa____ phi_____ frames

- 26.3686 19.0000 90.0000 98

#__ type_ start__ end____ width___ exp.time_

3 omega 36.00 101.00 1.0000 90.0000

omega____ theta____ kappa____ phi_____ frames

- 26.3686 178.0000 120.0000 65

#__ type_ start__ end____ width___ exp.time_

4 omega -73.00 5.00 1.0000 90.0000

omega____ theta____ kappa____ phi_____ frames

- -25.2748 -77.0000 30.0000 78

#__ type_ start__ end____ width___ exp.time_

5 omega -94.00 5.00 1.0000 90.0000

omega____ theta____ kappa____ phi_____ frames

- -25.2748 -77.0000 150.0000 99

#__ type_ start__ end____ width___ exp.time_

6 omega -91.00 -10.00 1.0000 90.0000

omega____ theta____ kappa____ phi_____ frames

- -25.2748 -57.0000 -150.0000 81

#__ type_ start__ end____ width___ exp.time_

7 omega -59.00 -4.00 1.0000 90.0000

omega____ theta____ kappa____ phi_____ frames

- -25.2748 -57.0000 -90.0000 55

#__ type_ start__ end____ width___ exp.time_

8 omega 19.00 67.00 1.0000 90.0000

omega____ theta____ kappa____ phi_____ frames

- 26.3686 -99.0000 150.0000 48

#__ type_ start__ end____ width___ exp.time_

9 omega -19.00 13.00 1.0000 90.0000

omega____ theta____ kappa____ phi_____ frames

- 26.3686 -99.0000 0.0000 32

Calculations

Quantum-chemical calculations were performed using Gaussian 16 software.[S7] Firstly, geometry of isomers **2** and **3** in gas phase was optimized with M06-2X meta-hybrid density functional[S8] and 6-31+G(d,p) basis set followed by frequency calculation in order to make sure the necessary stationary points had been found and to obtain zero-point energy (ZPE) as well. ZPE scaling factor for M06-2X/6-31+G(d,p) method was taken from.[S9] Next, single-point calculation for the optimized geometries employing Moeller-Plesset 2nd order perturbation theory (MP2)[S10] with full electronic correlation and extended 6-311++G(d,p) basis-set was also performed. The final energy E_{corr} was obtained from equation:

$$E_{corr}=E_{el}+a*ZPE(calc),$$

where E_{el} is electronic energy, ZPE(calc) is the calculated zero-point energy and a=0.967 is a scaling factor.

Table S8. The results of DFT and MP2 calculations of energies of compounds **2a-c** and **3a-c**.

Isomer	M06-2X/6-31+G(d,p)		MP2/6-311++G(d,p)	
	$E_{corr.}$, a.u.	$E_{rel.}$, kcal	Ecorr., a.u.	$E_{rel.}$, kcal
2a	-1034.249012	0	-1032.354810	0
3a	-1034.248491	0.33	-1032.355046	-0.15
2b	-1111.608835	0	-1109.566253	0
3b	-1111.608209	0.39	-1109.565863	0.24
2c	-1489.753714	0	-1487.161781	0
3c	-1489.756155	-1.53	-1487.165064	-2.06

Biological *in vitro* study

Cell viability was evaluated by Hoechst/PI staining by standard method as previously described.[S11] Human larynx carcinoma (Hep2), human breast adenocarcinoma (MCF-7), human hepatocellular carcinoma (HepG2) and human embryonic kidney (Hek293) cell lines were seeded on 96-well plates at 6 10^3 cells per well and cultured in Iscove's Modified Dulbecco's Medium (IMDM, pH = 7.4) supplemented with a 10% fetal bovine serum under a humidified atmosphere (5% CO_2 and 95% air) at 37 °C. After 24 hours cells were treated with compounds **2a, 2b, 3c** dissolved in DMSO. Serial dilutions were prepared in IMDM medium in the concentration range of 1-100 μM. For identification of live, apoptotic and dead cells, treated cells and control cells were stained after 48 hours with a mixture of fluorescent dyes Hoechst 33342 (Sigma-Aldrich, USA) and propidium iodide (Invitrogen, USA) for 30 min at 37 °C. IN Cell Analyzer 2200 (GE Healthcare, UK) was used to perform automatic imaging of four fields per well under 200× magnification, in bright-field and fluorescence channels. IN Cell Investigator image analysis software (GE Healthcare, UK) was used to determine live, apoptotic and dead cells among the whole population.

Table S9 Cytotoxic activity (expressed by IC_{50}) of the compounds **2a,b** and **3c** against Hep2, MCF-7, HepG2 and Hek293 cell lines.

Compound	IC_{50} (μM)			
	Hep2	MCF-7	HepG2	Hek293
2a	42.9 ± 2.7	>100	>50	>50
2b	>100	>100	>50	>50
3c	>100	>100	>50	>50
Carboplatin	16.8 ± 0.2	38.9 ± 2.0	–	–

All the data shown are mean of three wells. The quantitative data were expressed as the mean ± standard deviation (SD). The half maximal inhibitory concentration (IC_{50}) was defined as drug concentration that reduces the number of living cells by 50% and calculated from curves constructed by plotting cell survival (%) versus drug concentration (μM).

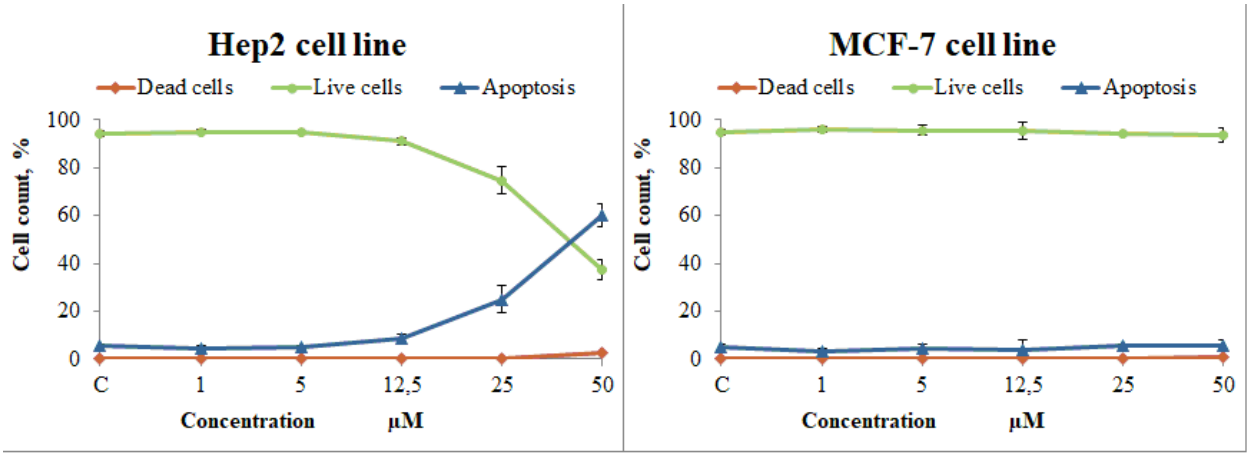


Figure S26. Effect of the compound **2a** on the viability of Hep2 cells determined after 48 h by dual staining with Hoechst 33342/propidium iodide.

References

S1. L. Palatinus and G. Chapuis, *J. Appl. Crystallogr*., 2007, **40**, 786.

S2. L. Palatinus, A. van der Lee, *J. Appl. Crystallogr.,* 2008, **41**, 975.

S3. L. Palatinus, S. J. Prathapa, S. van Smaalen, *J. Appl. Crystallogr.,* 2012, **45**, 575.

S4. G.M. Sheldrick, *Acta Crystallogr.*, 2015, **A71**, 3.

S5. G.M. Sheldrick, *Acta Crystallogr.*, 2015, **C71**, 3.

S6. O.V. Dolomanov, L. J. Bourhis, R. J. Gildea, J. A.K. Howard and H. Puschmann, *J. Appl. Crystallogr.,* 2009, **42**, 339.

S7. M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. V. Marenich, J. Bloino, B. G. Janesko, R. Gomperts, B. Mennucci, H. P. Hratchian, J. V. Ortiz, A. F. Izmaylov, J. L. Sonnenberg, D. Williams-Young, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. J. Bearpark, J. J. Heyd, E. N. Brothers, K. N. Kudin, V. N. Staroverov, T. A. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. P. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C. Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman, and D. J. Fox, *Gaussian 16, Revision C.01*, Gaussian, Wallingford, CT, 2016.

S8. Y. Zhao and D. G. Truhlar, *Theor. Chem. Acc.,* 2008, **120**, 215.

S9. I. M. Alecu, J. Zheng, Y. Zhao and D. G. Truhlar, *J. Chem. Theory Comput.,* 2010, **6**, 2872.

S10. M. J. Frisch, M. Head-Gordon and J. A. Pople, *Chem. Phys. Lett.,* 1990, **166**, 275.

S11. A. V. Artem'ev, J. A. Eremina, E. V. Lider, O. V. Antonova, E. V. Vorontsova and I. Yu. Bagryanskaya, *Polyhedron,* 2017, **138**, 218.