Supporting Information

Structure-Guided Design of *N*-Phenyl Tertiary Amines as Transrepression-Selective Liver X Receptor Modulators with Anti-inflammatory Activity

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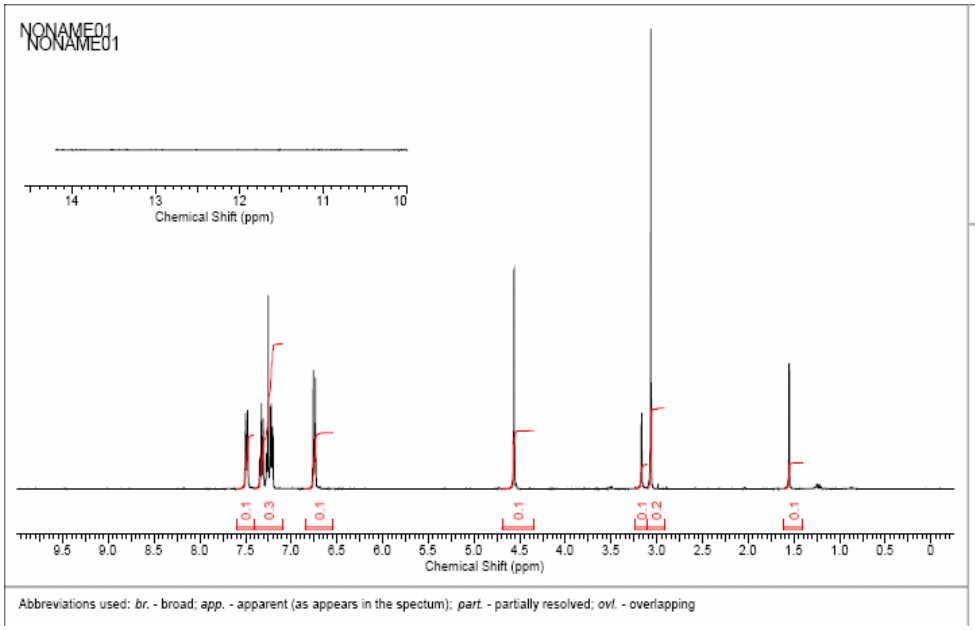
Contents:

1) NMR Spectra

Compound 4:



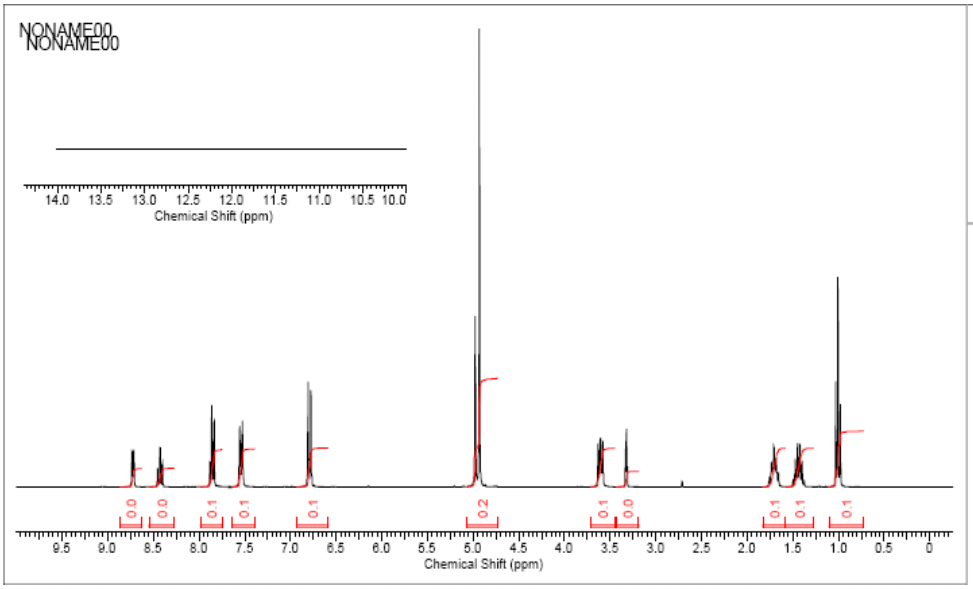
Nucleus	1H
Frequency (MHz)	399.77
Solvent	CHLOROFORM-d
Sweep Width (Hz)	6000.60
Number of Transients	16
Receiver Gain	50.00
Pulse Sequence	s2pul
Temperature (degree C)	29.000
Date	Mar 13 2001

Table of Peaks

No.	(ppm)	(Hz)
1	1.55	621.1
2	3.07	1226.0
3	3.17	1266.5
4	4.56	1823.9
5	6.73	2692.3
6	6.76	2701.5
7	7.22	2886.3
8	7.25	2898.9
9	7.31	2921.1
10	7.33	2928.6
11	7.48	2988.6
12	7.50	2997.4

Abbreviations used: *br.* - broad; *app.* - apparent (as appears in the spectum); *part.* - partially resolved; *ovl.* - overlapping

Compound 8:

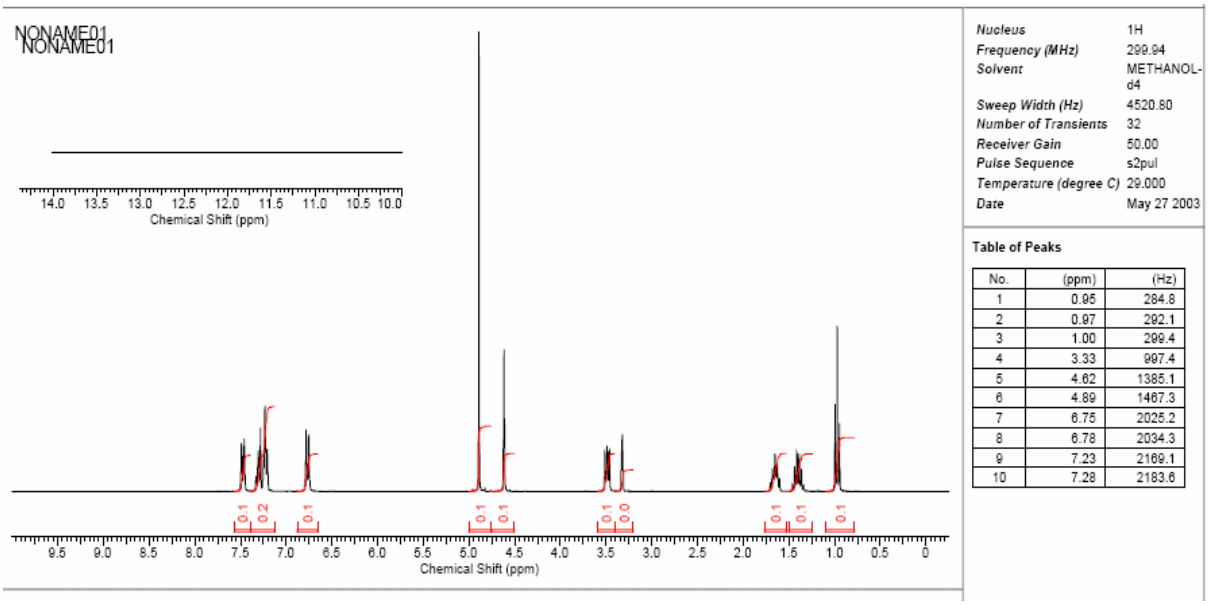


Nucleus	1H
Frequency (MHz)	299.94
Solvent	METHANOL-d4
Sweep Width (Hz)	4520.80
Number of Transients	32
Receiver Gain	46.00
Pulse Sequence	s2pul
Temperature (degree C)	29.000
Date	Mar 25 2003

Table of Peaks

No.	(ppm)	(Hz)
1	0.98	295.0
2	1.01	302.3
3	1.03	309.6
4	3.32	997.2
5	4.93	1479.4
6	4.98	1493.2
7	6.78	2032.4
8	6.81	2041.5
9	7.53	2257.2
10	7.55	2266.0
11	7.83	2349.9
12	7.86	2357.7

Compound 5:

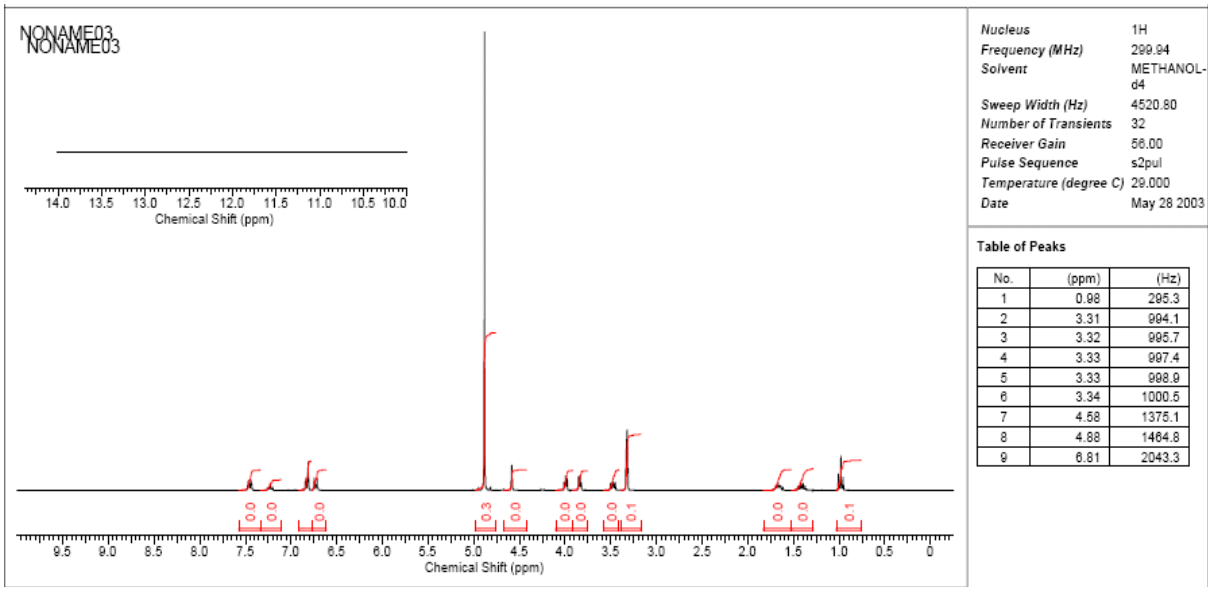


Nucleus	1H
Frequency (MHz)	299.94
Solvent	METHANOL-d4
Sweep Width (Hz)	4520.80
Number of Transients	32
Receiver Gain	50.00
Pulse Sequence	s2pul
Temperature (degree C)	29.000
Date	May 27 2003

Table of Peaks

No.	(ppm)	(Hz)
1	0.95	284.8
2	0.97	292.1
3	1.00	299.4
4	3.33	997.4
5	4.62	1385.1
6	4.89	1467.3
7	6.75	2025.2
8	6.78	2034.3
9	7.23	2169.1
10	7.28	2183.6

Compound 7:

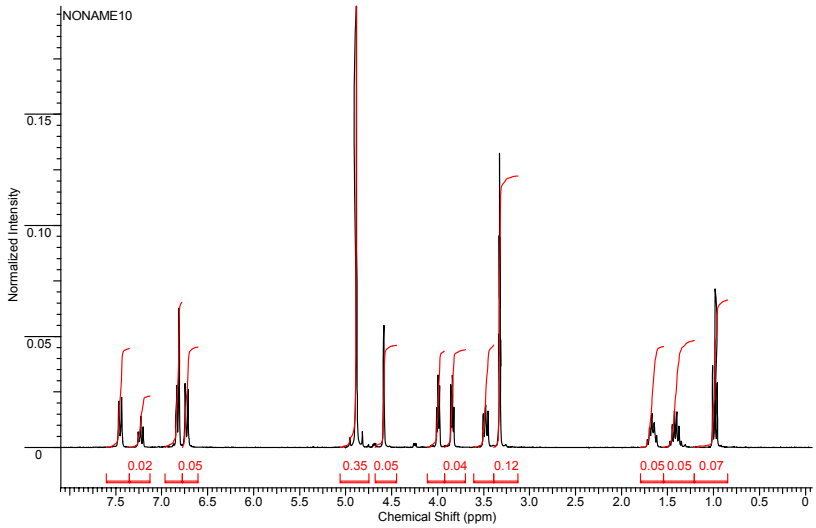


Nucleus	1H
Frequency (MHz)	299.94
Solvent	METHANOL-d4
Sweep Width (Hz)	4520.80
Number of Transients	32
Receiver Gain	56.00
Pulse Sequence	s2pul
Temperature (degree C)	29.000
Date	May 28 2003

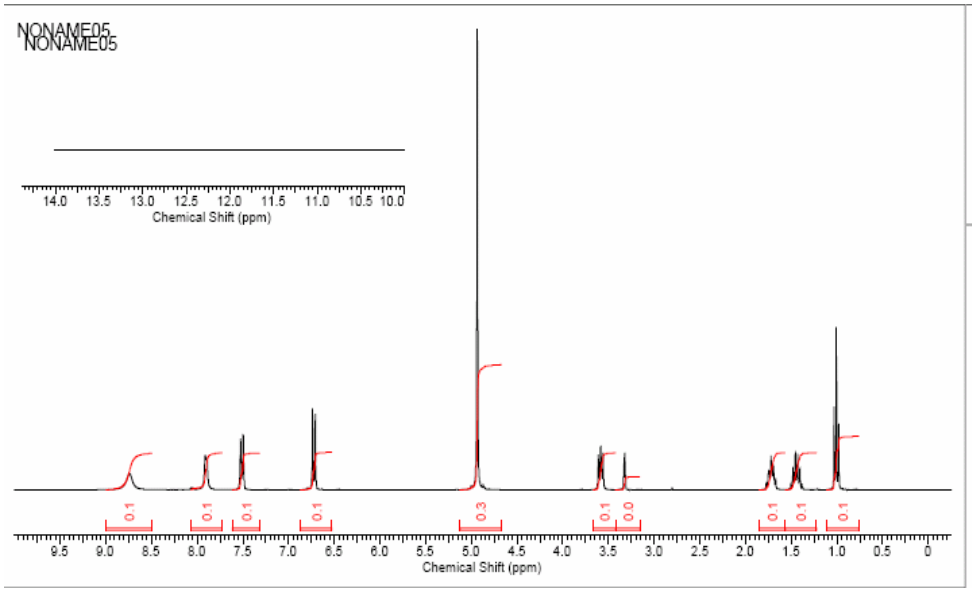
Table of Peaks

No.	(ppm)	(Hz)
1	0.98	295.3
2	3.31	994.1
3	3.32	995.7
4	3.33	997.4
5	3.33	998.9
6	3.34	1000.5
7	4.58	1375.1
8	4.88	1464.8
9	6.81	2043.3

Expanded vertical axis:



Compound 9:

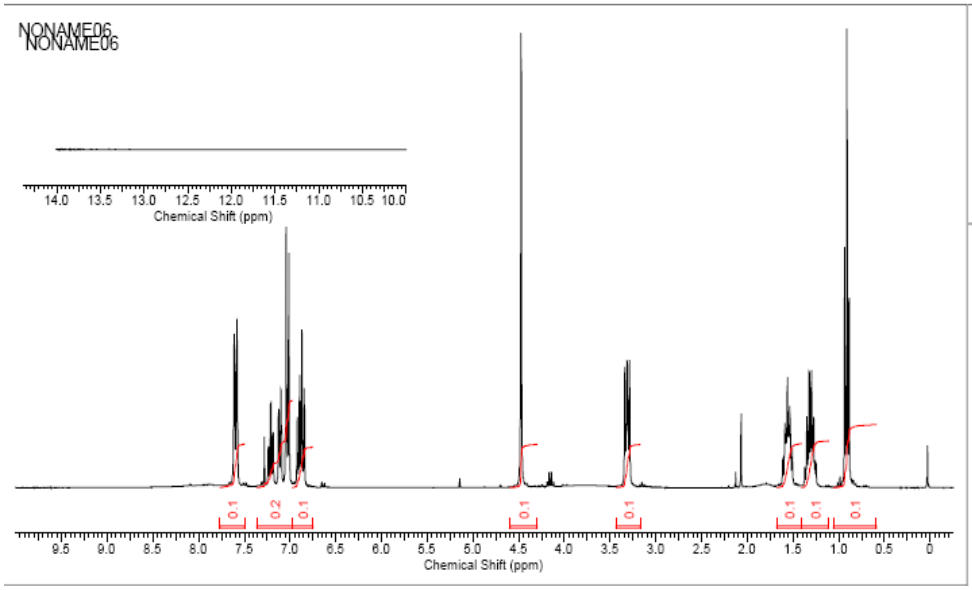


Nucleus	1H
Frequency (MHz)	299.94
Solvent	METHANOL-d4
Sweep Width (Hz)	4520.80
Number of Transients	32
Receiver Gain	46.00
Pulse Sequence	s2pul
Temperature (degree C)	29.000
Date	May 8 2003

Table of Peaks

No.	(ppm)	(Hz)
1	0.98	295.1
2	1.01	302.6
3	1.03	309.9
4	4.94	1480.7
5	6.71	2011.7
6	6.74	2021.0
7	7.49	2247.9

Compound 10:

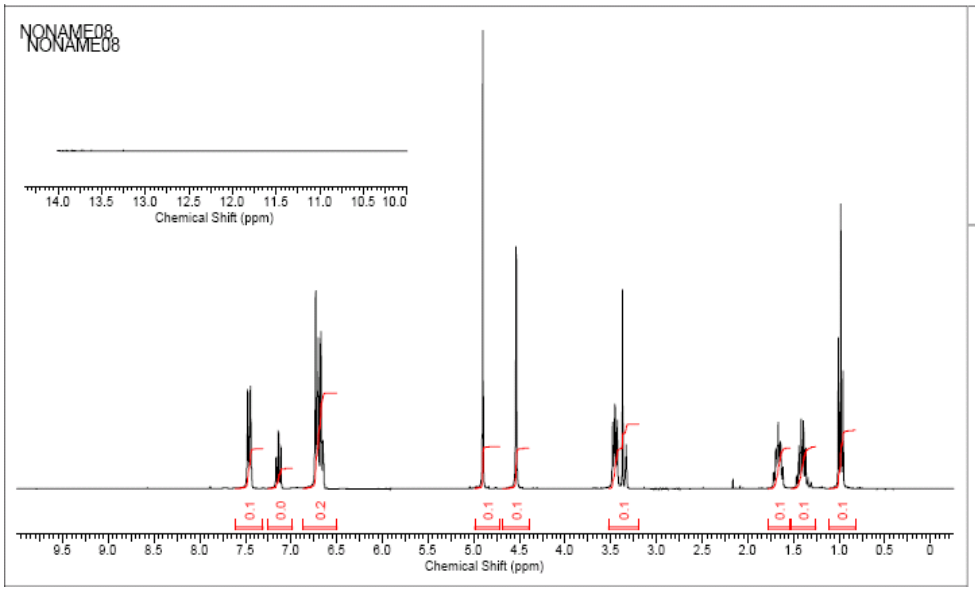


Nucleus	1H
Frequency (MHz)	299.93
Solvent	CHLOROFORM-d
Sweep Width (Hz)	4520.80
Number of Transients	32
Receiver Gain	44.00
Pulse Sequence	s2pul
Temperature (degree C)	29.000
Date	Mar 26 2003

Table of Peaks

No.	(ppm)	(Hz)
1	0.89	266.8
2	0.91	274.1
3	0.94	281.4
4	1.30	389.8
5	1.33	397.4
6	3.29	986.5
7	3.32	994.4
8	3.34	1002.1
9	4.48	1342.2
10	6.87	2061.0
11	7.01	2103.4
12	7.04	2112.4
13	7.58	2273.8
14	7.61	2282.3

Compound 11:

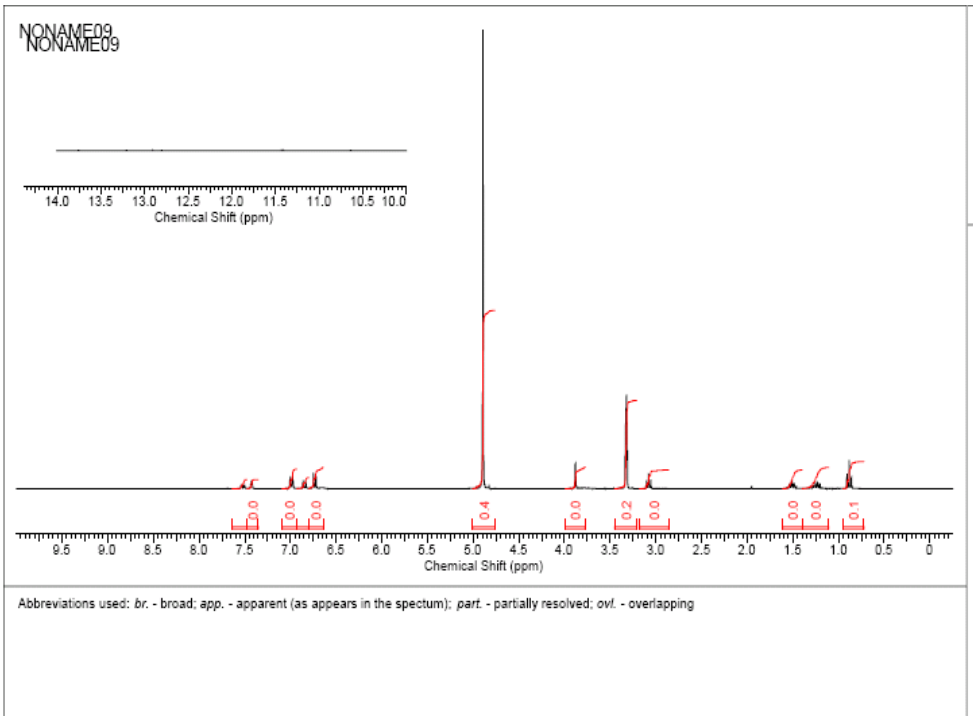


Nucleus	1H
Frequency (MHz)	299.94
Solvent	METHANOL-d4
Sweep Width (Hz)	4520.80
Number of Transients	32
Receiver Gain	44.00
Pulse Sequence	s2pul
Temperature (degree C)	29.000
Date	Apr 15 2003

Table of Peaks

No.	(ppm)	(Hz)
1	0.96	288.2
2	0.99	295.5
3	1.01	302.8
4	3.37	1011.9
5	3.45	1036.3
6	4.54	1360.5
7	4.90	1470.2
8	6.67	2001.5
9	6.70	2009.6
10	6.73	2018.7
11	7.44	2232.5
12	7.47	2241.3

Compound 12:



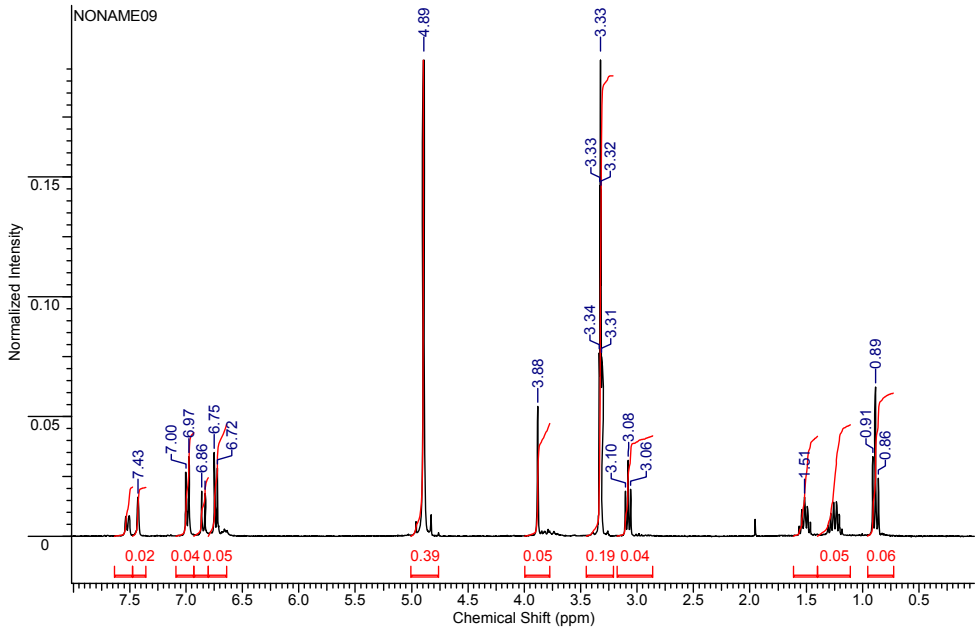
Nucleus	1H
Frequency (MHz)	299.94
Solvent	METHANOL-d4
Sweep Width (Hz)	4520.80
Number of Transients	32
Receiver Gain	56.00
Pulse Sequence	s2pul
Temperature (degree C)	29.000
Date	Jun 19 2003

Table of Peaks

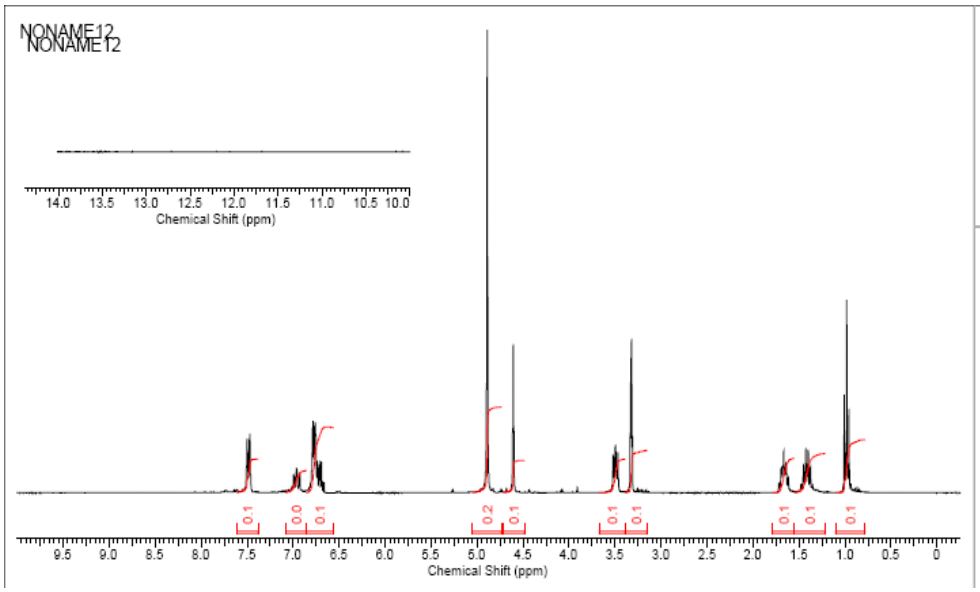
No.	(ppm)	(Hz)
1	0.86	258.3
2	0.89	265.6
3	0.91	272.9
4	1.51	454.1
5	3.06	916.9
6	3.08	924.0
7	3.10	931.1
8	3.31	994.1
9	3.32	995.7
10	3.33	997.4
11	3.33	998.9
12	3.34	1000.5
13	3.88	1164.3
14	4.89	1467.7
15	6.72	2015.7
16	6.75	2024.1
17	6.83	2049.7
18	6.86	2058.2
19	6.97	2091.6
20	7.00	2100.0
21	7.43	2227.6

Abbreviations used: *br.* - broad; *app.* - apparent (as appears in the spectum); *part.* - partially resolved; *ovl.* - overlapping

Expanded vertical axis:



Compound 13:

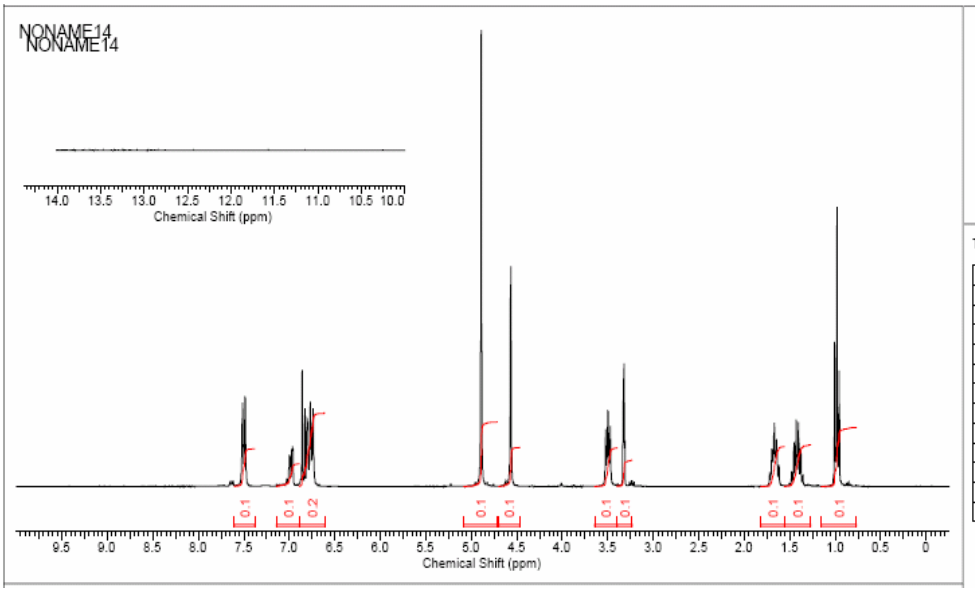


Nucleus	1H
Frequency (MHz)	299.94
Solvent	METHANOL d4
Sweep Width (Hz)	4520.80
Number of Transients	32
Receiver Gain	56.00
Pulse Sequence	s2pul
Temperature (degree C)	29.000
Date	May 14 200

Table of Peaks

No.	(ppm)	(Hz)
1	0.96	288.2
2	0.99	295.5
3	1.01	302.8
4	3.32	995.7
5	3.33	997.4
6	3.33	998.9
7	4.61	1381.9
8	4.89	1466.7

Compound 14:

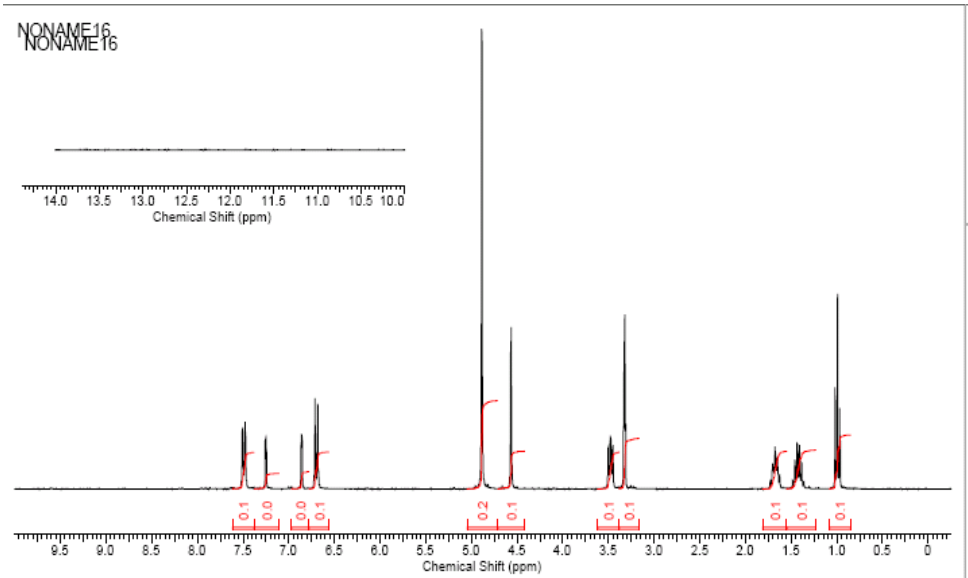


Nucleus	1H
Frequency (MHz)	299.94
Solvent	METHANOL-d4
Sweep Width (Hz)	4520.80
Number of Transients	32
Receiver Gain	52.00
Pulse Sequence	s2pul
Temperature (degree C)	29.000
Date	Aug 14 2003

Table of Peaks

No.	(ppm)	(Hz)
1	0.96	287.7
2	0.98	295.0
3	1.01	302.3
4	3.32	995.6
5	3.32	997.2
6	3.33	998.7
7	4.57	1369.7
8	4.89	1467.2
9	6.77	2030.1
10	6.86	2057.2
11	7.48	2244.9
12	7.51	2253.6

Compound 15:

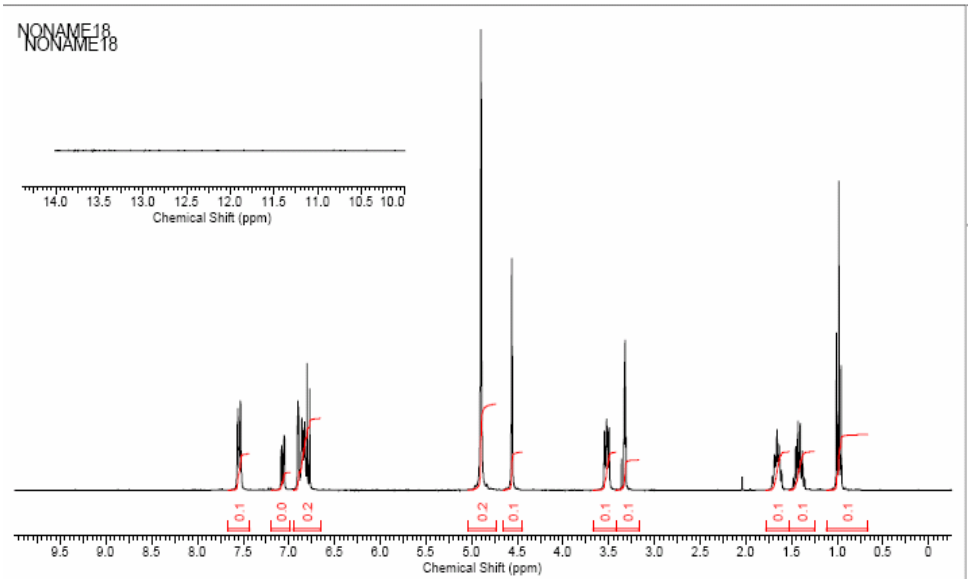


Nucleus	1H
Frequency (MHz)	299.94
Solvent	METHANOL-d4
Sweep Width (Hz)	4520.80
Number of Transients	32
Receiver Gain	56.00
Pulse Sequence	s2pul
Temperature (degree C)	29.000
Date	May 14 2003

Table of Peaks

No.	(ppm)	(Hz)
1	0.97	291.1
2	0.99	298.4
3	1.02	305.7
4	3.32	995.7
5	3.33	997.4
6	3.33	999.0
7	4.57	1369.7
8	4.89	1465.8
9	6.68	2004.0
10	6.71	2013.1

Compound 16:

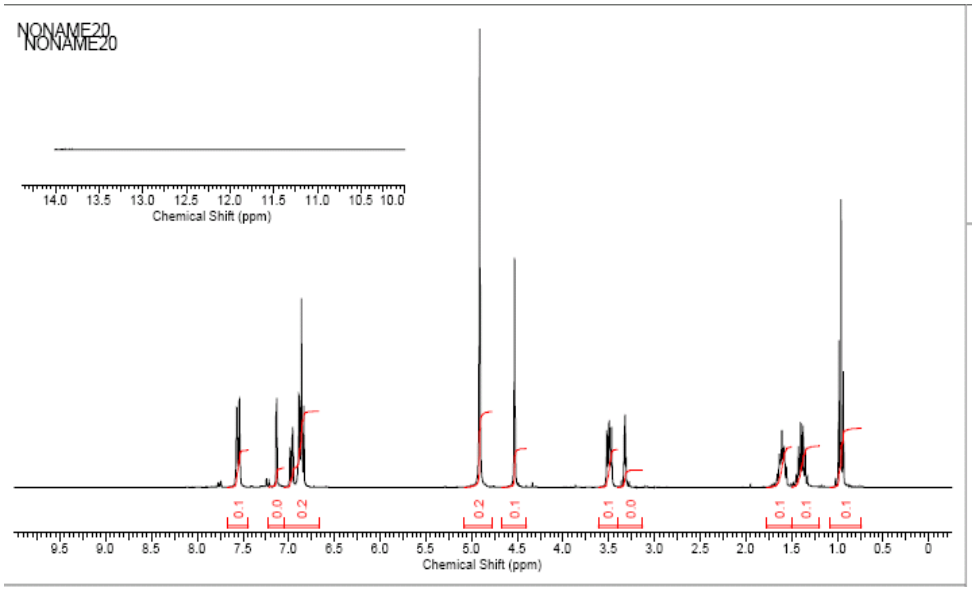


Nucleus	1H
Frequency (MHz)	299.94
Solvent	METHANOL-d4
Sweep Width (Hz)	4520.80
Number of Transients	32
Receiver Gain	52.00
Pulse Sequence	s2pul
Temperature (degree C)	29.000
Date	Aug 14 2003

Table of Peaks

No.	(ppm)	(Hz)
1	0.96	288.4
2	0.99	295.7
3	1.01	303.0
4	3.32	995.7
5	3.33	997.4
6	3.33	998.9
7	4.56	1368.1
8	4.90	1469.5
9	6.80	2039.7

Compound 17:

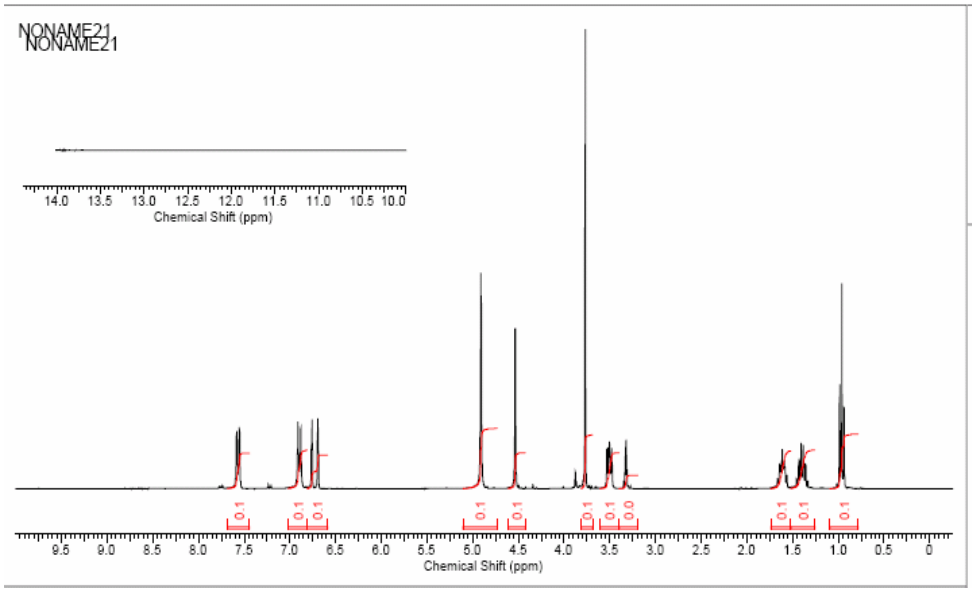


Nucleus	1H
Frequency (MHz)	299.94
Solvent	METHANOL-d4
Sweep Width (Hz)	4520.80
Number of Transients	32
Receiver Gain	50.00
Pulse Sequence	s2pul
Temperature (degree C)	29.000
Date	Aug 7 2003

Table of Peaks

No.	(ppm)	(Hz)
1	0.94	281.3
2	0.96	288.6
3	0.99	295.9
4	4.53	1358.7
5	4.91	1473.5
6	6.86	2057.8

Compound 18:

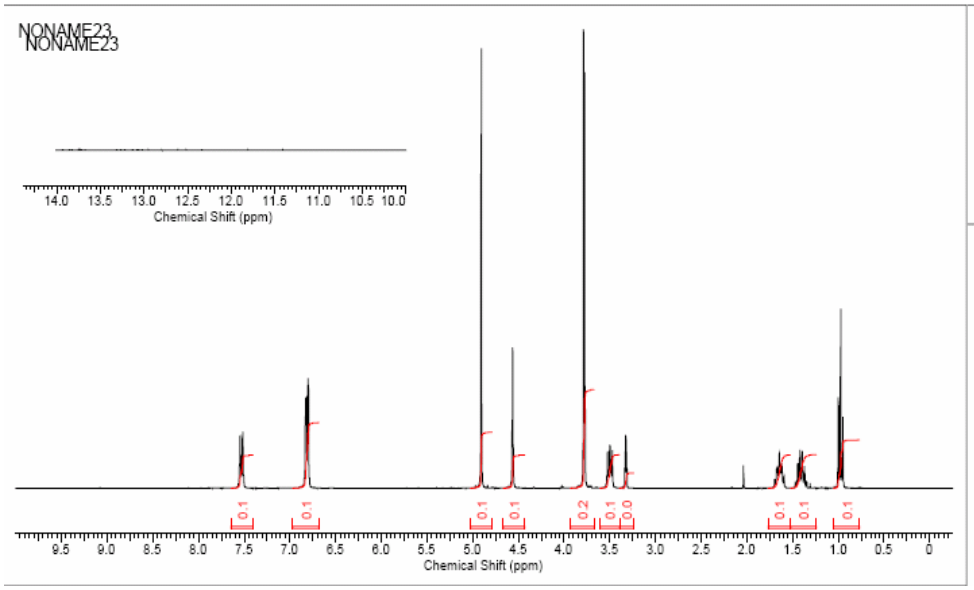


Nucleus	1H
Frequency (MHz)	299.94
Solvent	METHANOL-d4
Sweep Width (Hz)	4520.80
Number of Transients	32
Receiver Gain	46.00
Pulse Sequence	s2pul
Temperature (degree C)	29.000
Date	Aug 8 2003

Table of Peaks

No.	(ppm)	(Hz)
1	0.96	289.3
2	0.99	296.6
3	3.77	1130.8
4	4.54	1360.2
5	4.91	1472.8

Compound 19:

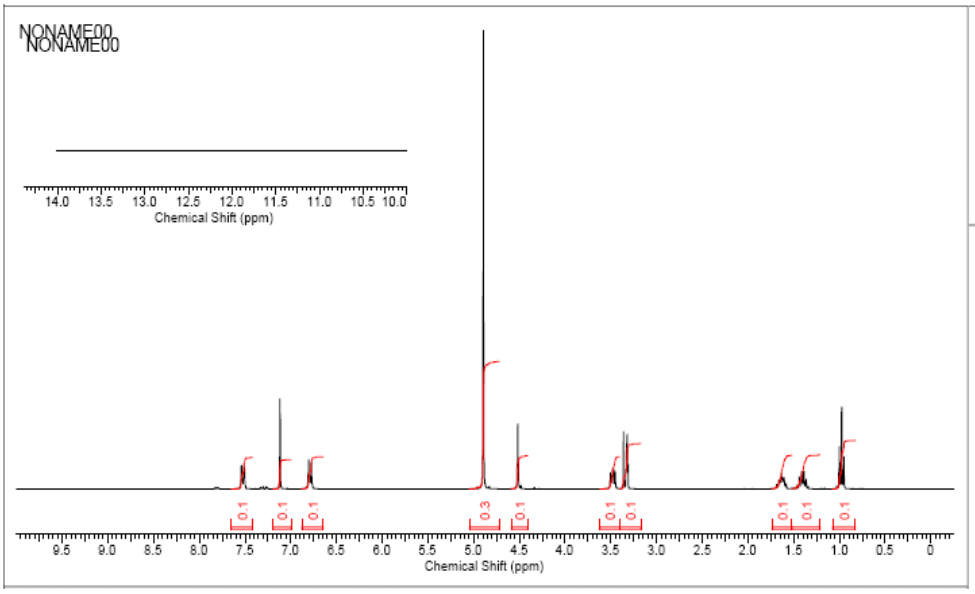


Nucleus	1H
Frequency (MHz)	299.94
Solvent	METHANOL-d4
Sweep Width (Hz)	4520.80
Number of Transients	32
Receiver Gain	56.00
Pulse Sequence	s2pul
Temperature (degree C)	29.000
Date	Oct 7 2003

Table of Peaks

No.	(ppm)	(Hz)
1	0.98	293.2
2	3.78	1132.4
3	3.79	1136.3
4	4.56	1368.8
5	4.91	1471.8
6	6.80	2039.3
7	6.80	2040.8

Compound 20:

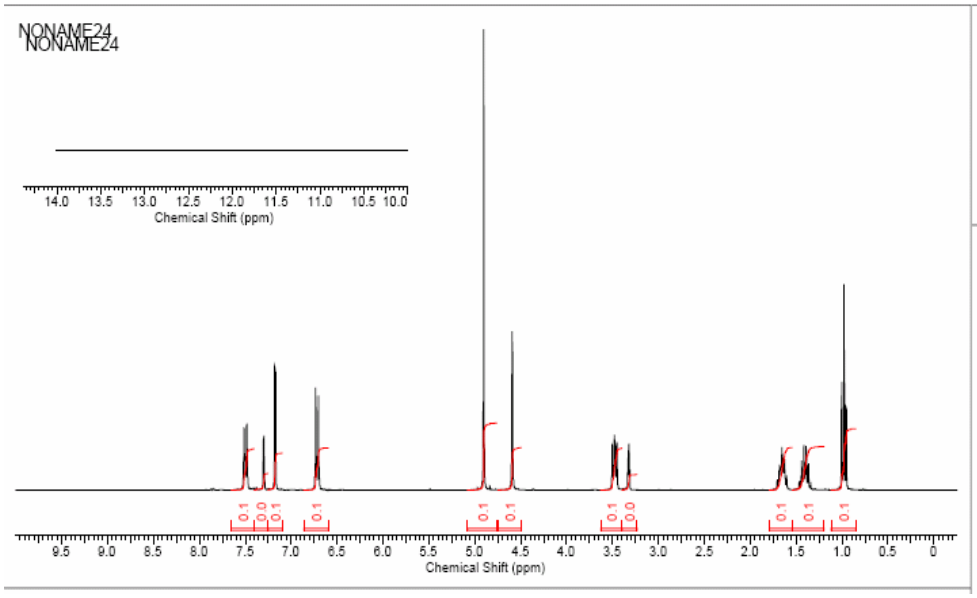


Nucleus	1H
Frequency (MHz)	299.94
Solvent	METHANOL-d4
Sweep Width (Hz)	4520.80
Number of Transients	32
Receiver Gain	56.00
Pulse Sequence	s2pul
Temperature (degree C)	29.000
Date	Sep 11 2003

Table of Peaks

No.	(ppm)	(Hz)
1	0.98	293.6
2	1.00	300.9
3	3.32	995.7
4	3.32	997.2
5	3.33	998.9
6	3.36	1009.0
7	4.52	1355.0
8	4.89	1468.0
9	7.12	2134.8

Compound 21:

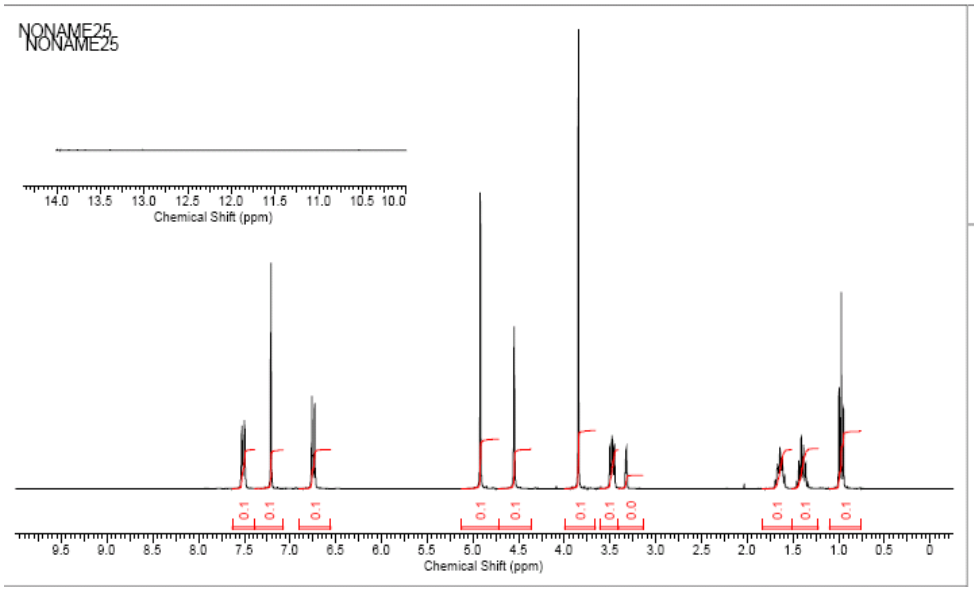


Nucleus	1H
Frequency (MHz)	299.94
Solvent	METHANOL-d4
Sweep Width (Hz)	4520.80
Number of Transients	32
Receiver Gain	46.00
Pulse Sequence	s2pul
Temperature (degree C)	29.000
Date	May 27 2003

Table of Peaks

No.	(ppm)	(Hz)
1	0.98	294.3
2	1.01	301.6
3	4.59	1377.6
4	4.90	1470.7
5	6.70	2010.1
6	6.73	2019.3
7	7.17	2150.9
8	7.18	2152.7

Compound 22:



Nucleus	1H
Frequency (MHz)	299.94
Solvent	METHANOL-d4
Sweep Width (Hz)	4520.80
Number of Transients	32
Receiver Gain	56.00
Pulse Sequence	s2pul
Temperature (degree C)	29.000
Date	Oct 7 2003

Table of Peaks

No.	(ppm)	(Hz)
1	0.98	292.6
2	1.00	299.9
3	3.85	1154.1
4	4.55	1364.6
5	4.92	1475.7
6	6.73	2018.7
7	6.76	2027.9
8	7.21	2161.8

2) Co-crystallization of GSK2186 (19) with murine LXRα and human RXRα: The mLXRα(200-445)/hRXRα(225-462) co-expression strain was made by transforming two plasmid vectors into the E. coli K12 strain HMS174(DE3) (Novagene, Madison, WI). Plasmid pLXRα(200-445) was made by inserting a PCR fragment containing the human LXRαLBD(aa200-445) ORF into the Nhe/BamHI sites of pRSETa vector (Invitrogen, Calsbad, CA). The resulting construct expresses LXRαLBD as a H6-thrombin-LXRαLBD fusion protein. Plasmid pRXRa(225-462) was constructed previously (Gampe et al., 2000, Mol. Cell 5:545-555) and is described below. The ORF containing the human RXRαLBD(225-462) was inserted into the NdeI/HindII sites of pET24a (Novagen) and from which the T7-lacO-RXRaLBD expression cassette was further subcloned between the BamHI/HindIII sites of pACYC184 (New England Biolabs). The resulting plasmid expresses untagged RXRαLBD.

To prepare N-terminal His tagged mLXRα(200-445)/hRXRα(225-462), E.Coli HMS174 cells were grown overnight at 30^{o}C in 1L of Luria-Bertani Broth(Tryptone, 10g/L; Yeast Extract, 5g/L; NaCl, 5g/L) plus 1% Glucose. As selection agents, 3.4ml of Chloramphenicol(10mg/ml) and 10ml of Carbenicillin(10mg/ml) were added. The 1L overnight was then used to inoculate a reactor for an 8L production. The reactor equipment used was a 10L Biostat B. (8L working volume(B. Braun Biotech) glass vessel with water-jacketed temperature control. Dissolved Oxygen (DO) was monitored at a setpoint of 40%. pH was controlled at 6.5 using a dropwise addition of a 30% solution of Ammonium Hydroxide. The media contained: $(NH_4)_2SO_4$, 5g/L; K_2HPO_4, 6g/L; KH_2PO_4, 3g/L; Tryptone, 10g/L; Yeast Extract, 5g/L; NaCl, 5g/L.

Components added post sterilization are 200ml of 60% Glucose, 1M $MgSO_4$.7H20, 5ml/L; 1000X Trace metals ($FeCl_2$.6H_2O, 37.8g/L; Na_2MoO_4.H_2O, 0.7g/L; $ZnSO_4$.7H_2O, 0.4g/L; $MnCl_2$.4H_2O, 0.5g/L; $CuSO_4$.5H_2O, 0.8g/L; $CoCl_2$.6H2O, 0.8g/L; H_3BO_3, 0.2g/L,) 1ml/L. The cells grew at 37^{o}C to an optical density of about 5, the temperature was then dropped to 25^{o}C and induced with 0.1mM IPTG. Induction occurred overnight for about 19hrs at which time the cell paste was recovered through centrifugation using a CARR Powerfuge.

All the purification steps were performed at 4°C. Ten grams of bacterial cells were lysed by two passages at 12,000 psi through a high pressure homogenizer (Avestin, Ottawa, Canada) in 100 mL of buffer A (50 mM Tris-HCl, pH 8.0, 200 mM NaCl, and 5% ethylene glycol) plus 1 mM DTT and 25 mM imidazole. Cell debris was removed by centrifugation at 34,000 x *g* for 1 h. The supernatant was loaded onto a Ni-NTA Agarose (Qiagen) column (bed volume 25 mL). The column was washed with buffer A plus 1 mM DTT, buffer A Plus 1 mM DTT and 50 mM imidazole, and eluted with 250 mM imidazole in buffer B (50 mM Tris-HCl, pH 8.0, and 5% ethylene glycol) plus 1 mM DTT. The Ni-NTA pool was diluted 8-fold with buffer B plus 1 mM DTT and then treated with thrombin (E/S=1/50) overnight at 4° C. The digestion mixture was loaded onto a Mono Q HR 10/10 column (Amersham Biosciences) equilibrated with buffer buffer B plus 5 mM DTT. Bound proteins were eluted with a linear gradient from 0 to 500 mM NaCl over 10 CV in buffer B plus 5 mM DTT. The Mono Q pool was applied onto a HiLoad 26/60 Superdex 200 column (Amersham Biosciences) equilibrated with buffer A plus 5 mM DTT. The column was eluted with the equilibration buffer to yield 20 mg of > 95% pure heterodimer.

The crystals were grown by vapor diffusion in sitting drops with 3 ul 6.6mg/ml protein and 3 ul well solution containing from 21%-23% PEG 3,550, 200 mM Ammonium Acetate, 100 mM Bis Tris pH 6.5 and 10 mM DTT. All operations were carried out at 4^{o}C. The crystals were small, 0.1 mm long rods, and took 3 weeks to appear. Data were collected at beam line 32 ID on a MAR CCD detector with support from the Industrial Macromolecular Crystallographic Association Collaborative Access Team (IMCA CAT). The data were processed with HKL2000 (Otwinowski,Z. and Minor, W.(1997) *Methods Enzymol*. **276**, 307-326). The Rmerge and completeness of the data were 6.4 % and 97 %, respectively. Crystals belonged to the space group C2 with cell dimensions a = 123.21, b = 90.00, c = 101.31, α = 90.00, β = 111.88, γ = 90.00 and 2 heterodimers per asymmetric unit. The structure was solved by molecular replacement using Amore (Navaza, J. (1994) *Acta Crystallogr*. **A 50**,157-163) and using starting coordinates generated from a homology model created from LXRβ (PDB id = 1P8D) and RXRα (PDB id = 1FM9). The structure was rebuilt with multiple rounds of refinement using O (Jones, T. A., Zou, J.-Y., Cowan, S. W., and Kjeldgaard, M. (1991) *Acta Crystallogr.A* **47,** 110-119**)** and CNX **(**Brunger, A. T., Adams, P. D., Clore, G. M., Delano, W. L., Gros, P., Grossekunstleve, R. W., Jiang, J. S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T., and Warren, G. L. (1998) *Acta Crystallogr.D* **54,** 905-921)**.** The model was refined to an R factor of 24 % and an R-free of 29 %. Subsequent refinement with Phenix.refine (Afonine, P.V., Grosse-Kunstleve, R.W. & Adams, P.D. (2005). CCP4 Newsl. 42, contribution 8) and model rebuilding with Coot (Emsley, P. & Cowtan, K. (2004) *Acta Crystallogr*. D. **60**, 2126- 2132) improved the R factor and R-free to 22 % and 28 %, respectively.