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Supporting information  
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Discrimination of cellular developmental states focusing on glycan transformation and membrane dynamics by using BODIPY-tagged lactosyl ceramide

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We carried out several experiments that support our findings.

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## Preparation of a vector pCX4 bsr/hygro LifeAct-eGFP.

The plasmid expressing LifeAct-eGFP (pEGFP-N1-LifeAct) was obtained from S. Fukuhara (National Cerebral and Cardiovascular Center Research Institute, Japan). The plasmid used for preparing the retrovirus vector expressing LifeAct-eGFP (pCX4 bsr/hygro LifeAct-eGFP) was constructed by introducing the LifeAct-eGFP fragment (amplified by polymerase chain reaction from pEGFP-N1-LifeAct) into the *HpaI* site of pCX4 bsr or pCX4 hygro using the In-Fusion HD cloning kit (TaKaRa Bio, Otsu, Japan). The primers used are listed in Supplementary Table S1.

### Table S1

```
295 pEGFP pCX4 Hpa F
GCGGTAACAATTGTTTCAGATCCGCTAGCGCTACCGGTC
296 pEGFP pCX4 Hpa R
TAGCTTAAGTTAGTTGTA AACCTCTACAAATGTGGTATGG
```



Current Data Parameters  
NAME YH01036--1  
EXPNO 20  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20071113  
Time 21.02  
INSTRUM av500  
PROBHD 5 mm CPTXI 1H-  
PULPROG zg30  
TD 65536  
SOLVENT MeOD  
NS 128

DS 2  
SWH 10330.578 Hz  
FIDRES 0.157632 Hz  
AQ 3.1720407 sec  
RG 16  
DW 48.400 usec  
DE 6.00 usec  
TE 298.0 K  
D1 1.00000000 sec  
MCREST 0.00000000 sec  
MCWRK 0.01500000 sec

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P1 8.25 usec  
PL1 -5.70 dB  
SFO1 500.1330885 MHz

F2 - Processing parameters  
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SF 500.1300160 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00

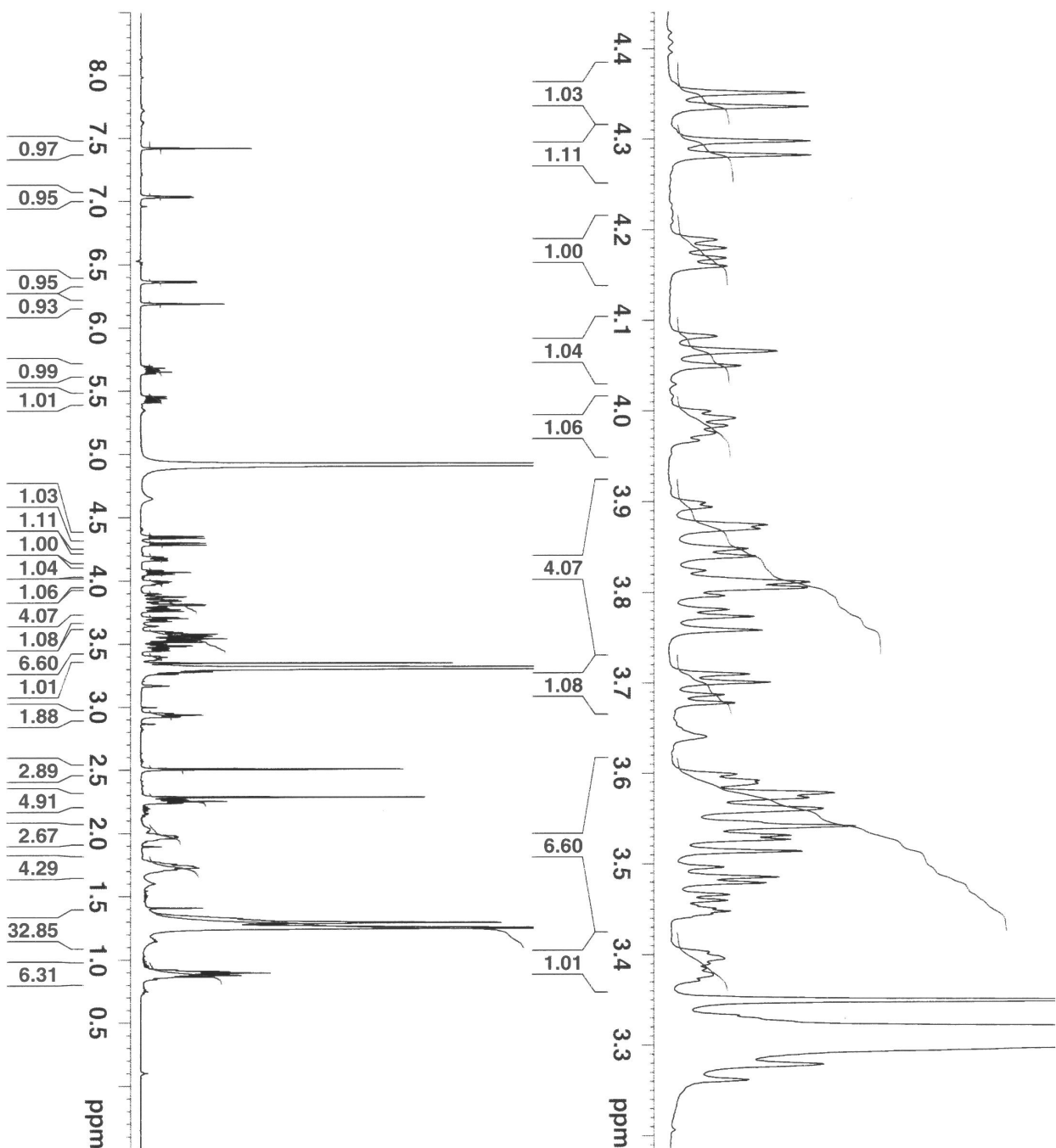
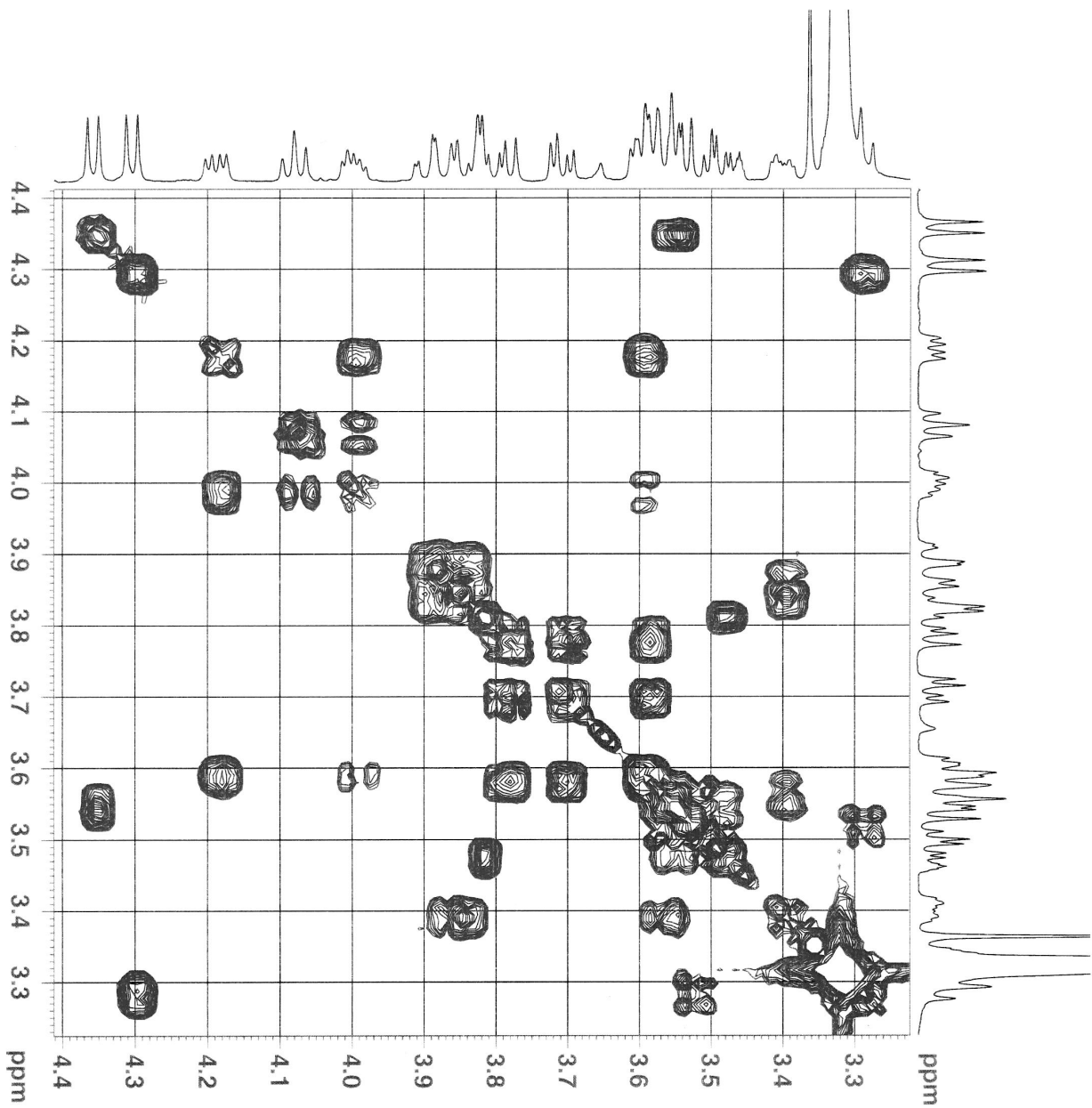


Fig. S1. <sup>1</sup>H NMR spectrum of compound 1.



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 EXPNO: 2  
 PROCNO: 1

F2 - Acquisition Parameters

Date\_ 20071113  
 Time 21.03  
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 PROBRD 5 mm CPTXI IH-  
 PULPROG zgpg30  
 TD 2048  
 SFO100 100.628150  
 SOLVENT MeD4  
 DS 16  
 SWH 5000.000 Hz  
 FIDRES 2.441406 Hz  
 AQ 0.2049500 sec  
 RG 64  
 DW 100.000 usec  
 DE 6.00 usec  
 TE 300.2 K

TR 2.02100 sec  
 DI 1.48689198 sec  
 d13 0.00000400 sec  
 d16 0.00010000 sec  
 INO 0.00019995 sec  
 MCREST 0.00000000 sec  
 MCWRR 1.48689198 sec

===== CHANNEL f1 =====

NUC1 1H  
 P1 8.25 usec  
 PL1 -5.70 dB  
 SFO1 500.1320005 MHz

===== GRADIENT CHANNEL =====

GRAMA1 SINE.100  
 GRVAM2 SINE.100  
 GRX1 0.00 %  
 GRX2 0.00 %  
 GRP1 0.00 %  
 GRP2 0.00 %  
 GRZ1 10.00 %  
 GRZ2 10.00 %  
 P16 1000.00 usec

F1 - Acquisition parameters

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 TD 512  
 SFO1 500.132 MHz  
 FIDRES 9.768067 Hz  
 SN 10.000 ppm  
 FWHM06 Gf

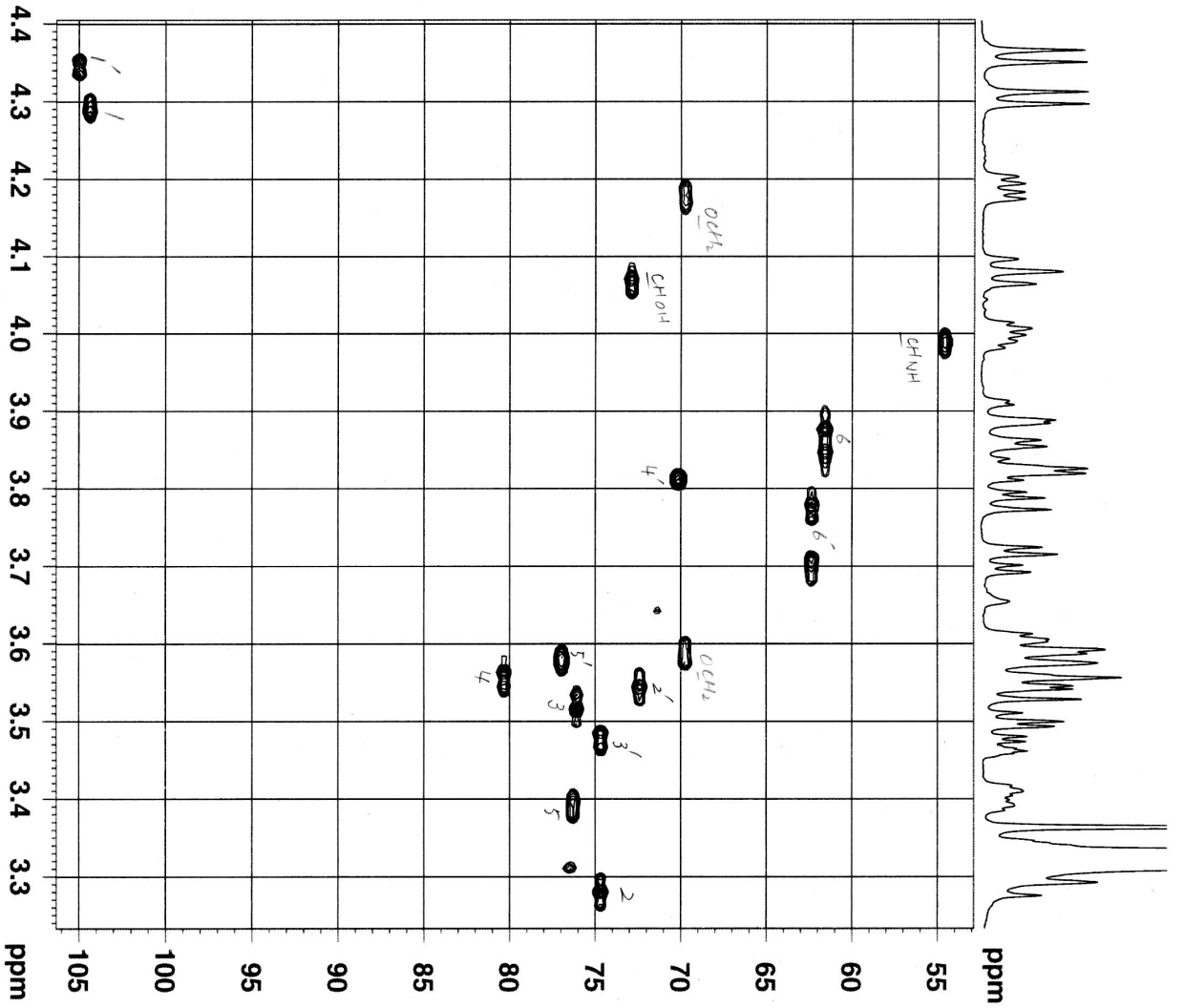
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 SF 500.1300150 MHz  
 WDM SINE  
 SSB 0  
 LB 0.00 Hz  
 GB 0  
 PC 1.40

F1 - Processing parameters

SI 1024  
 OF 500.1300126 MHz  
 SF 500.1300126 MHz  
 WDM SINE  
 SSB 0  
 LB 0.00 Hz  
 GB 0

Fig. S2.  $^1\text{H}$ - $^1\text{H}$  COSY NMR spectrum of compound 1.



Current Data Parameters  
 NAME YH0136-1  
 FREQNO 21

F2 - Acquisition Parameters  
 Date\_ Time 2011.04.22.04  
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 EUIPROC Inv1edqph  
 TD 2048  
 NS2/VRT 8692  
 DS 16  
 SFO1 500.000 MHz  
 AQ 0.2049500 sec  
 RG 29133 usec  
 DE 100.600 usec  
 TE 298.0 K

===== CHANNEL F1 =====  
 NUCL1 1H  
 P1 8.25 usec  
 PL1 -5.70 dBc  
 SFO1 500.1320005 MHz

===== CHANNEL F2 =====  
 CPDPRG2 gapp  
 NUCL2 13C  
 P2 15.00 usec  
 PL2 0.00 dBc  
 SFO2 125.7672208 MHz

===== GRADIENT CHANNEL =====  
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 GPC3 0.00 %  
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 GPC7 0.00 %  
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 GPC18 0.00 %  
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 GPC25 0.00 %  
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 GPC32 0.00 %  
 GPC33 0.00 %  
 GPC34 0.00 %  
 GPC35 0.00 %  
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 GPC37 0.00 %  
 GPC38 0.00 %  
 GPC39 0.00 %  
 GPC40 0.00 %  
 GPC41 0.00 %  
 GPC42 0.00 %  
 GPC43 0.00 %  
 GPC44 0.00 %  
 GPC45 0.00 %  
 GPC46 0.00 %  
 GPC47 0.00 %  
 GPC48 0.00 %  
 GPC49 0.00 %  
 GPC50 0.00 %  
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 GPC67 0.00 %  
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 GPC72 0.00 %  
 GPC73 0.00 %  
 GPC74 0.00 %  
 GPC75 0.00 %  
 GPC76 0.00 %  
 GPC77 0.00 %  
 GPC78 0.00 %  
 GPC79 0.00 %  
 GPC80 0.00 %  
 GPC81 0.00 %  
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 GPC84 0.00 %  
 GPC85 0.00 %  
 GPC86 0.00 %  
 GPC87 0.00 %  
 GPC88 0.00 %  
 GPC89 0.00 %  
 GPC90 0.00 %  
 GPC91 0.00 %  
 GPC92 0.00 %  
 GPC93 0.00 %  
 GPC94 0.00 %  
 GPC95 0.00 %  
 GPC96 0.00 %  
 GPC97 0.00 %  
 GPC98 0.00 %  
 GPC99 0.00 %  
 GPC100 0.00 %

F1 - Acquisition parameters  
 NS 2  
 NDS 51.2  
 SFO1 125.7672 MHz  
 FIDRES 41.785745 Hz  
 FWHM 0.20 Hz  
 EQ 0.00 Hz  
 WDM 0.00 Hz  
 WNW 0.00 Hz  
 WVB 0.00 Hz  
 GB 0.00 Hz  
 PC 1.40

F2 - Processing parameters  
 SI 32768  
 SF 125.7672 MHz  
 SFO 125.7672 MHz  
 WDM 0.00 Hz  
 WNW 0.00 Hz  
 WVB 0.00 Hz  
 GB 0.00 Hz  
 PC 1.40

Fig. S3. HSQC NMR spectrum of compound 1.



Current Data Parameters  
NAME: YH101346-1  
PROCNO: 21

F2 - Acquisition Parameters  
Time: 500.126  
Date\_Time: 3/26/08  
INSTRUM: spect  
PROBHD: 5 mm CPXI H-  
PULPROG: InvSgpphprnd4  
SOLVENT: MeOD  
NS: 32  
DS: 2  
SWH: 500.116 Hz  
FIDRES: 2.441405 Hz  
AQ: 0.2649590 sec  
RG: 32768  
DM: 100.000 usec  
TE: 298.0 K

----- CHANNEL f1 -----  
NUC1: <sup>1</sup>H  
P1: 8.25 usec  
P2: 16.50 usec  
SFO1: 500.1320005 MHz

----- CHANNEL f2 -----  
NUC2: <sup>13</sup>C  
P1: 15.00 usec  
P2: -5.90 dB  
SFO2: 125.7691072 MHz

----- CHANNEL f3 -----  
SFO3: 125.7691072 MHz

----- CHANNEL f4 -----  
SFO4: 125.7691072 MHz

----- CHANNEL f5 -----  
SFO5: 125.7691072 MHz

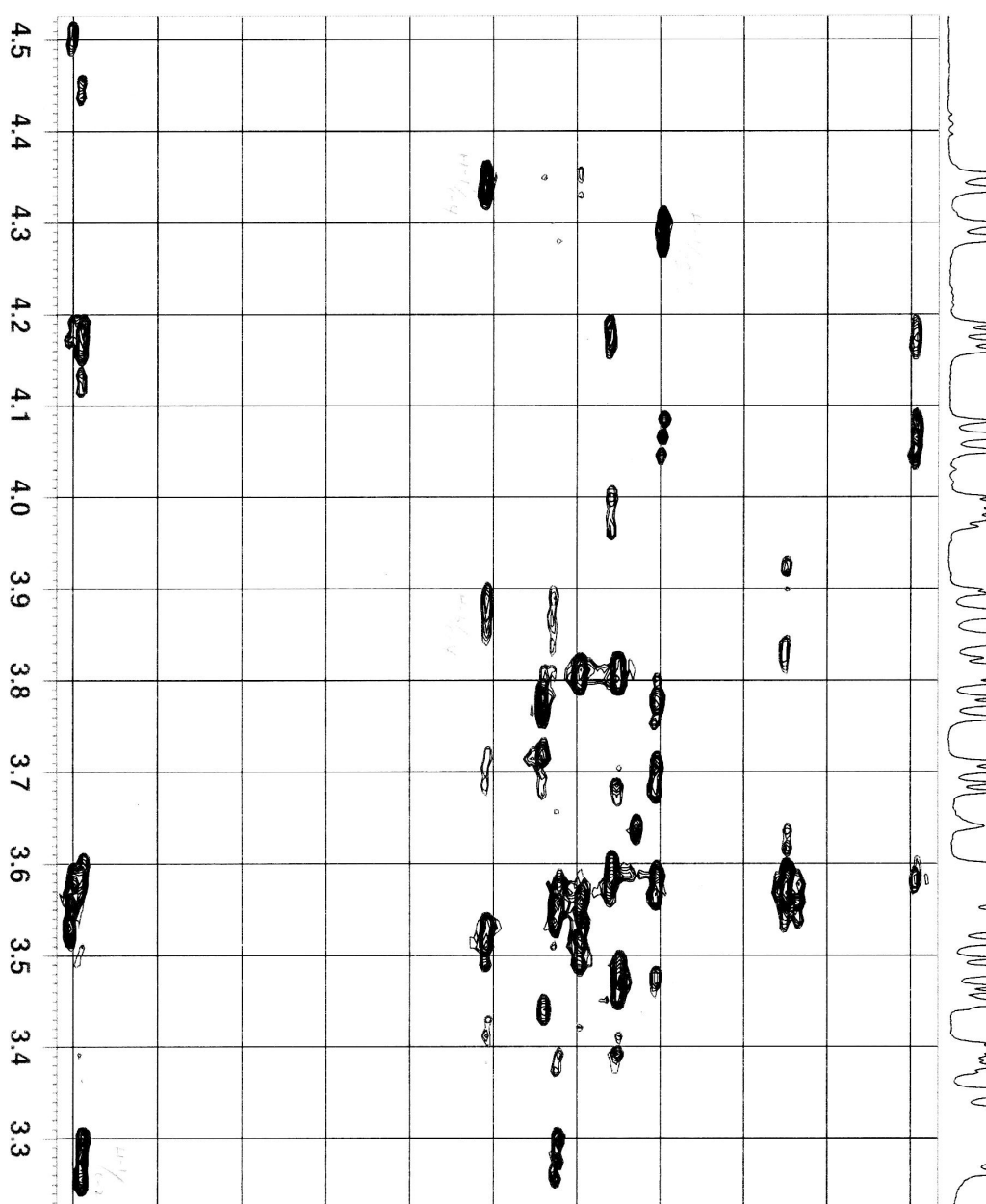
----- CHANNEL f6 -----  
SFO6: 125.7691072 MHz

----- CHANNEL f7 -----  
SFO7: 125.7691072 MHz

----- CHANNEL f8 -----  
SFO8: 125.7691072 MHz

----- CHANNEL f9 -----  
SFO9: 125.7691072 MHz

----- CHANNEL f10 -----  
SFO10: 125.7691072 MHz



F1 - Acquisition Parameters  
ND0: 2  
NUC1: <sup>1</sup>H  
SFO1: 500.1320005 MHz  
FIDRES: 4.9139220 Hz  
SW: 200.027 ppm  
FNUC1: <sup>13</sup>C

F2 - Processing Parameters  
SI: 1024  
SF: 500.1300163 MHz  
SINE: SINE  
LB: 0.00 Hz  
GB: 0  
PC: 1.40

F3 - Processing Parameters  
SI: 1024  
SF: 125.7691072 MHz  
SINE: SINE  
LB: 0.00 Hz  
GB: 0  
PC: 1.40

F4 - Processing Parameters  
SI: 1024  
SF: 125.7691072 MHz  
SINE: SINE  
LB: 0.00 Hz  
GB: 0  
PC: 1.40

F5 - Processing Parameters  
SI: 1024  
SF: 125.7691072 MHz  
SINE: SINE  
LB: 0.00 Hz  
GB: 0  
PC: 1.40

F6 - Processing Parameters  
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SF: 125.7691072 MHz  
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PC: 1.40

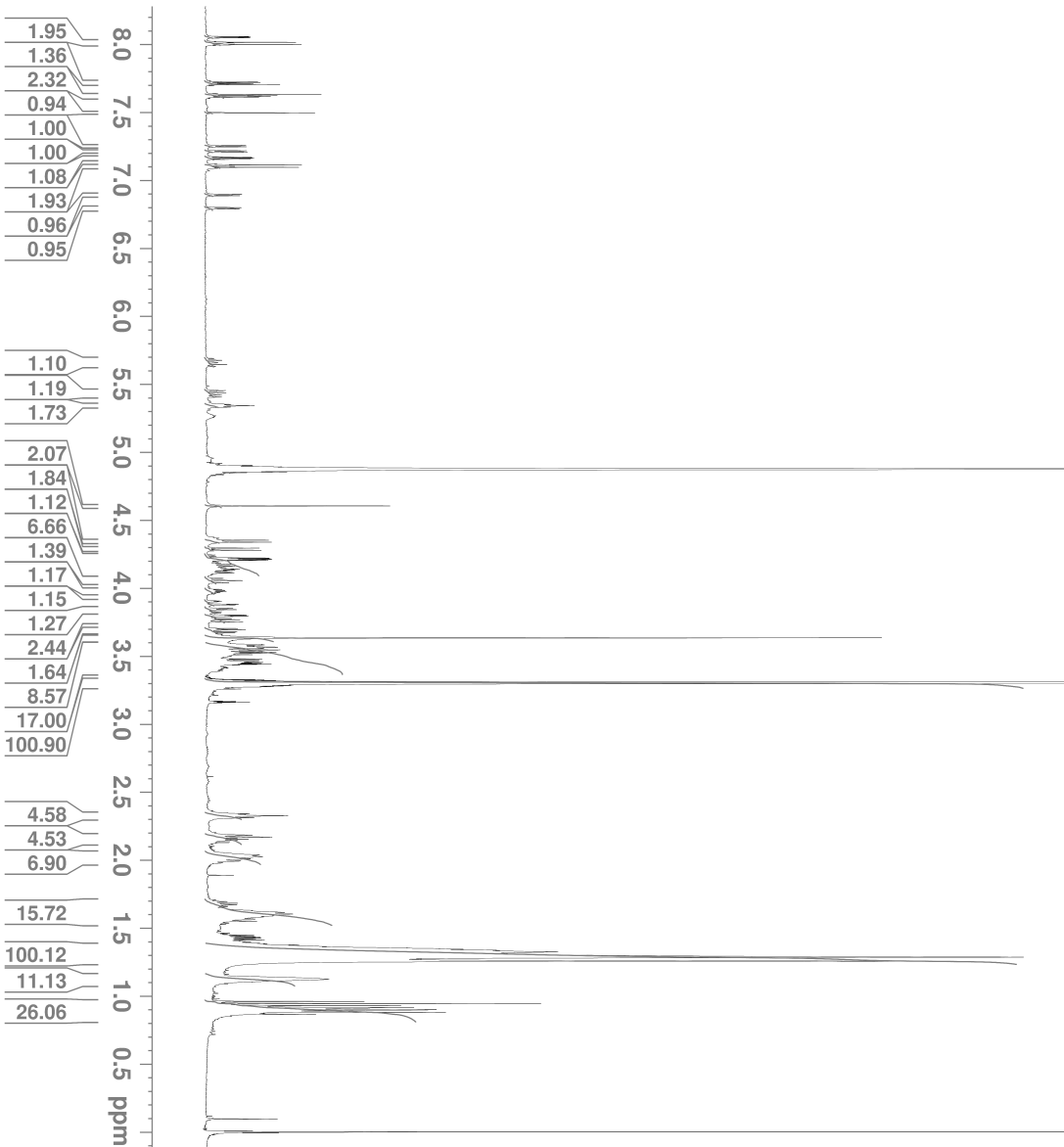
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LB: 0.00 Hz  
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PC: 1.40

F8 - Processing Parameters  
SI: 1024  
SF: 125.7691072 MHz  
SINE: SINE  
LB: 0.00 Hz  
GB: 0  
PC: 1.40

F9 - Processing Parameters  
SI: 1024  
SF: 125.7691072 MHz  
SINE: SINE  
LB: 0.00 Hz  
GB: 0  
PC: 1.40

F10 - Processing Parameters  
SI: 1024  
SF: 125.7691072 MHz  
SINE: SINE  
LB: 0.00 Hz  
GB: 0  
PC: 1.40

Fig. S4. HMBC NMR spectrum of compound 1.



Current Data Parameters  
NAME LaccerbODIPYTRX2  
EXPNO 10  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20200421  
Time 16.45  
INSTRUM spect  
PROBHD 5 mm PABBO BB/  
PULPROG zg30  
TD 65536  
SOLVENT MeOD  
NS 64  
DS 2  
SWH 10000.000 Hz  
FIDRES 0.152588 Hz  
AQ 3.2767999 sec  
RG 119.97  
DW 50.000 usec  
DE 6.50 usec  
TE 297.4 K  
D1 1.00000000 sec  
TD0 1

==== CHANNEL f1 =====  
SFO1 500.1330885 MHz  
NUC1 1H  
P1 12.00 usec  
PLW1 15.50000000 W

F2 - Processing parameters  
SI 65536  
SF 500.1300137 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00

Fig. S5. <sup>1</sup>H NMR spectrum of compound 2.

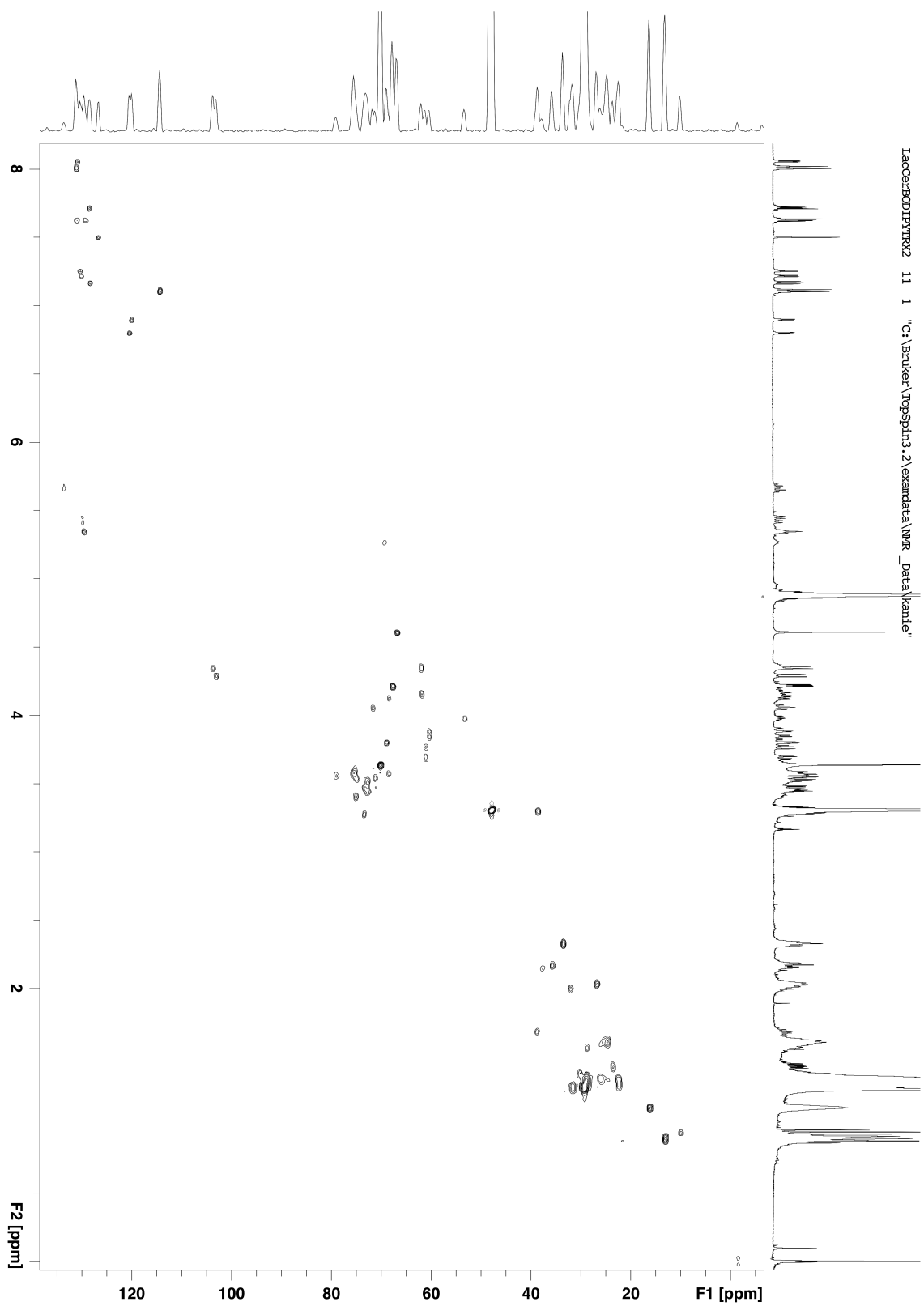


Fig. S6. HSQC NMR spectrum of compound 2.





Current Data Parameters  
NAME SphBODIPy  
EXPNO 10  
PROCNO 1

F2 - Acquisition Parameters

Date\_ 20200422

Time 17.18

INSTRUM spect

PROBHD 5 mm PABBO BB/

PULPROG zg30

TD 65536

SOLVENT CDCl3

NS 64

DS 2

SWH 10000.000 Hz

FIDRES 0.152588 Hz

AQ 3.2767999 sec

RG 185.59

DW 50.000 usec

DE 6.50 usec

TE 297.1 K

D1 1.00000000 sec

TD0 1

==== CHANNEL f1 =====

SFO1 500.1330885 MHz

NUC1 1H

P1 12.00 usec

PLW1 15.50000000 W

F2 - Processing parameters

SI 65536

SF 500.1300124 MHz

WDW EM

SSB 0

LB 0.30 Hz

GB 0

PC 1.00

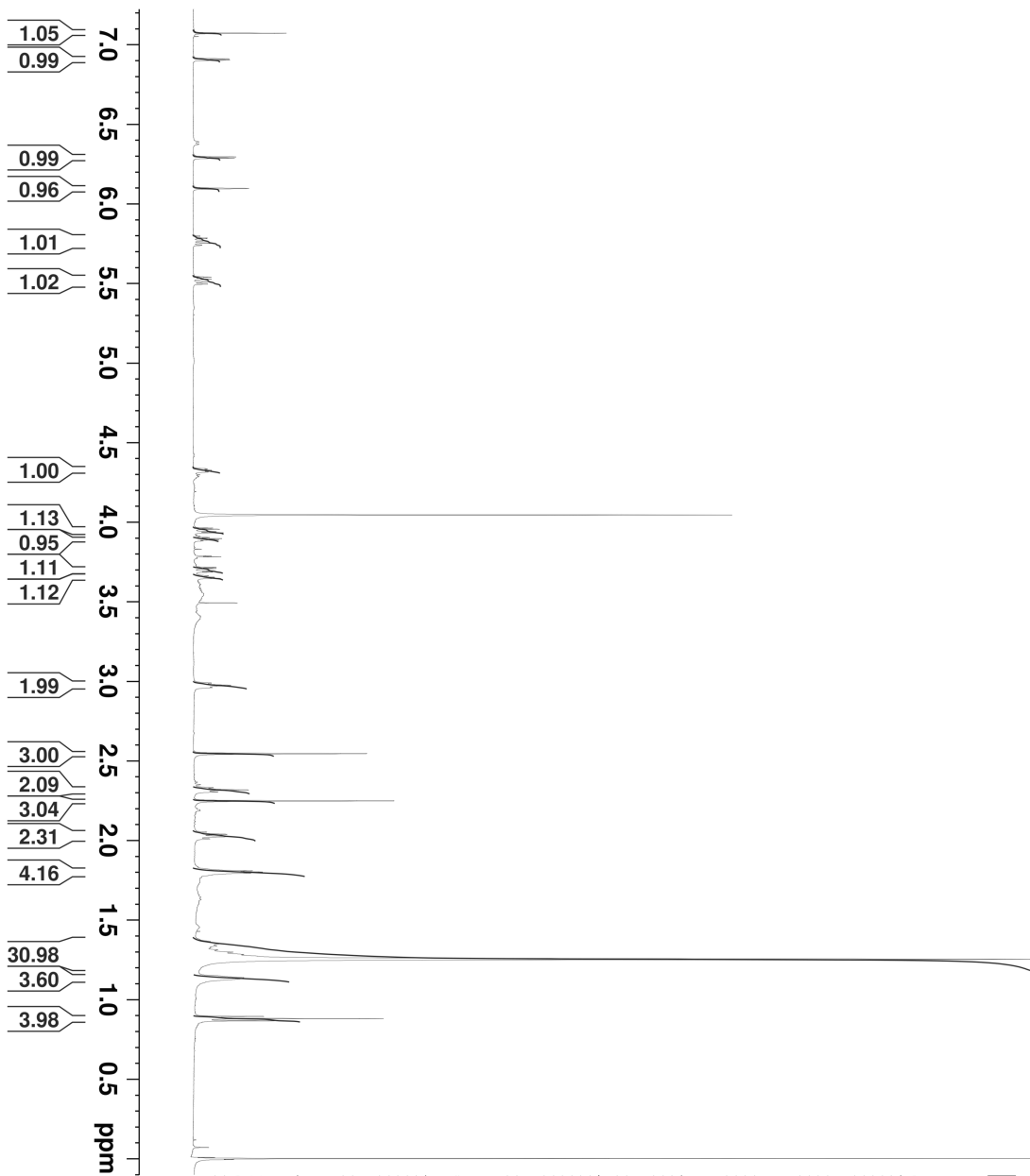
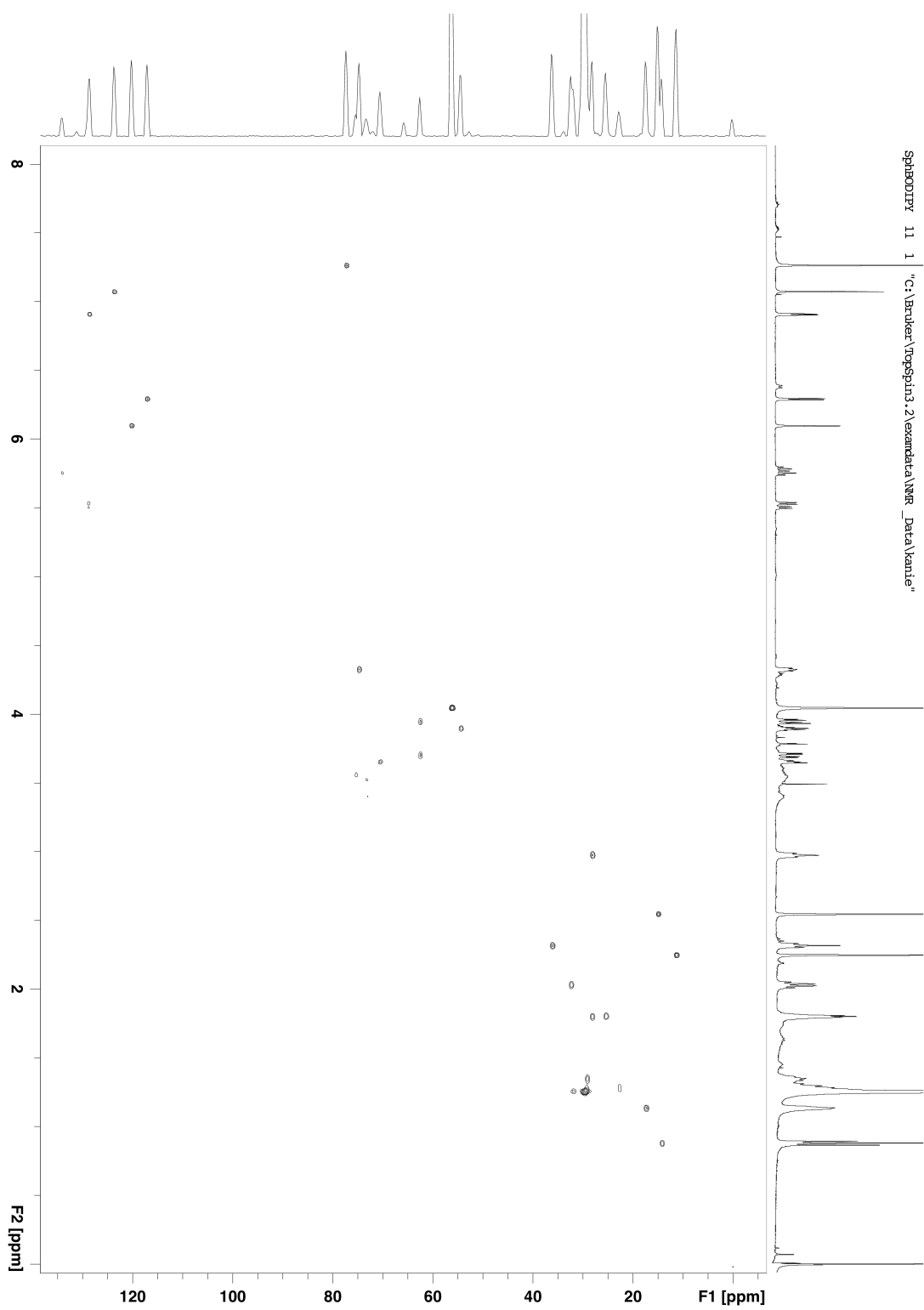


Fig. S7. <sup>1</sup>H NMR spectrum of compound 3.

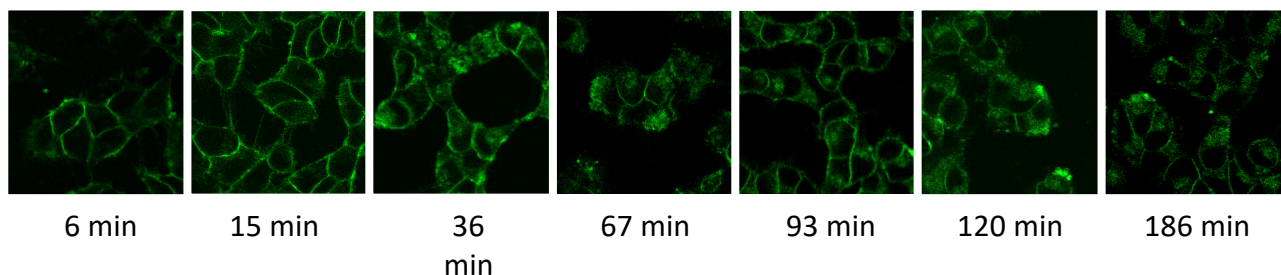


**Fig. S8. HSQC NMR spectrum of compound 3.**

## Time study showing the internalisation speed difference depending on differentiation states.

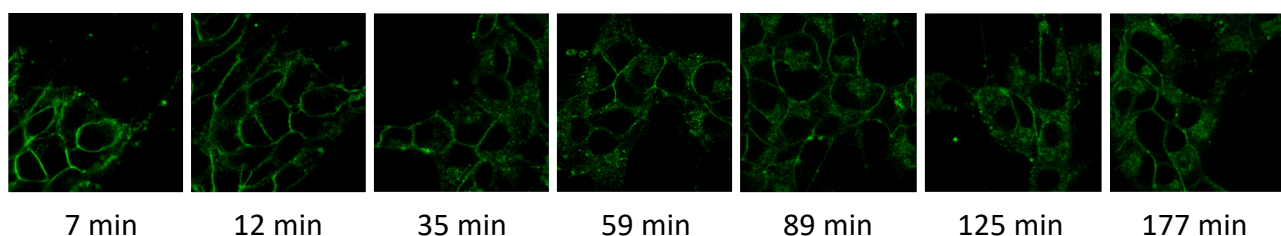
Fluorescence derived from compound **1** could be monitored over a day. Here we show the localisation changes from pulse introduction of compound **1** over approximately three hours. The fluorescence of plasma membranes in undifferentiated PC12D cells almost disappeared after 15 minutes (Fig. S1A). In differentiated cells, the fluorescence of plasma membranes disappeared after approximately 60 minutes (Fig. S1B). The difference in internalisation rates indicates that the fluorescent probe density on the plasma membrane changed during the FRAP experiment over time.

### A Undifferentiated cells; NGF(-)



### B Differentiated cells; NGF(+)

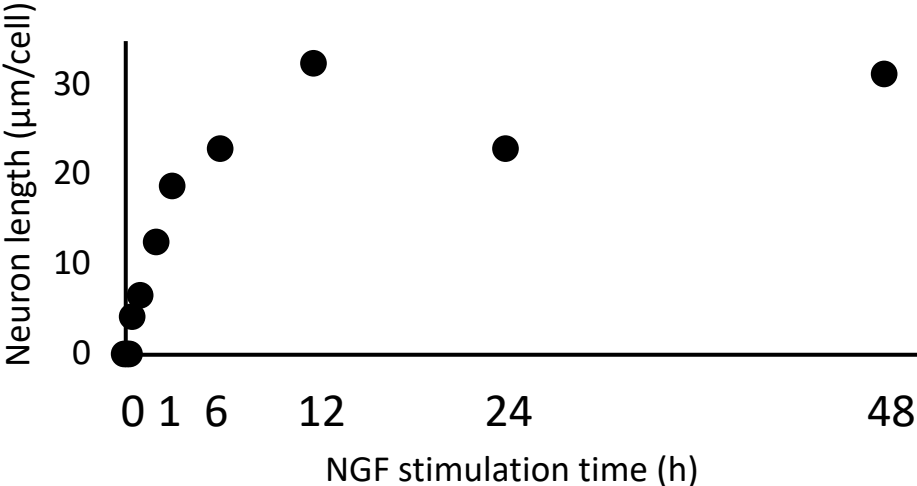
72 h incubation after NGF treatment



**Fig. S9.** Visualisation of the recycling process of the fluorescent probe.

**Time course study of nerve-like projection extension on NGF treatment**

PC12D neurite outgrowth was induced by NGF stimulation and the process was observed over 48 hours. Neurite length in differential images was analysed by using Image J with Neuron J plug-in. A remarkable neurite outgrowth was observed after NGF stimulation and the length of the neurite was increased in a time dependent manner (Fig. S1).



**Fig. S10.** The average length of neuron-like cells after NGF stimulation.