Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

Synthesis of Hydrophilic Caged DAG-lactones for Chemical Biology Applications

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I. General information

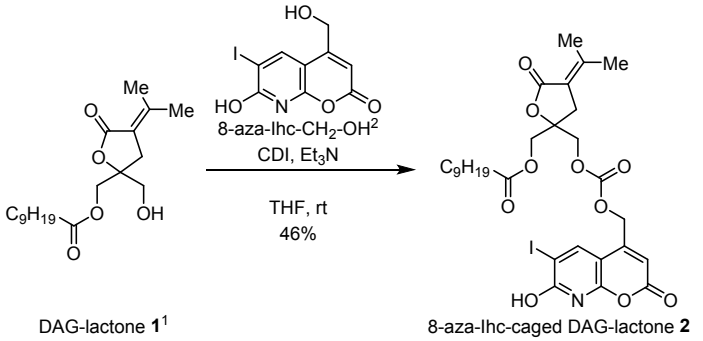
I-I. General methods

All reactions were performed using commercially supplied reagents and solvents in dried glassware under an atmosphere of nitrogen unless otherwise noted. CH_2Cl_2 was used after being distilled from CaH_2. Analytical thin-layer chromatography (TLC, Merck $60F_{254}$) was performed on precoated silica-gel plates and was visualized by fluorescence quenching under UV light and staining with phosphomolybdic acid and ninhydrin. Flash column chromatography was conducted using Silica-gel 60 N (Kanto Chemical Co.).

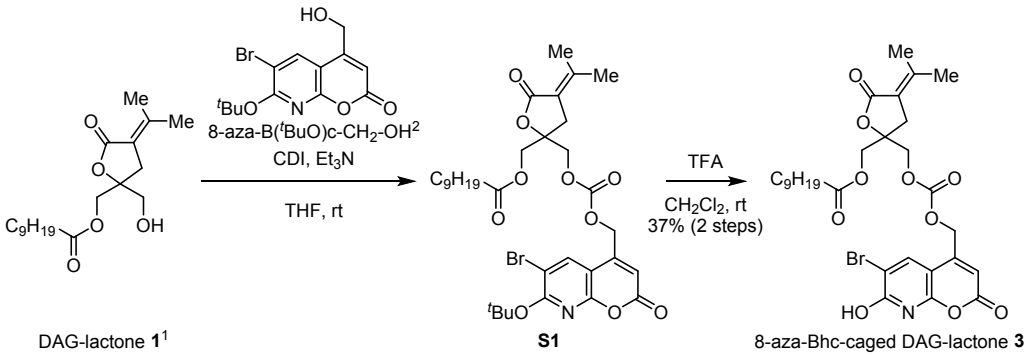
I-II. Characterization Data

^1H NMR (400 or 500 MHz) and ^{13}C NMR (126 MHz) spectra were obtained with a Bruker Avance II spectrometer with a CryoProbe. All NMR measurements were performed at 25 ºC using DMSO-d_6, CDOD, $CDCl_3$ and THF-d_8 as solvents. Chemical shifts are reported in δ (ppm) relative to Me_4Si or the solvent peak as an internal reference. Infrared (IR) spectra were measured on a JASCO FT/IR 4100, and are recorded as wavelength (cm^{-1}). The absorbance spectra (UV/Vis) and fluorescence spectra (FL) were measured with a JASCO V-650 spectrophotometer and a JASCO FP-750 spectrofluorophotometer, respectively. High-resolution mass spectra (HRMS) were recorded on a Bruker Daltonics micrOTOF (ESI-MS) spectrometer under positive (ESI^+) and negative (ESI^-) electrospray ionization.

II. Experimental procedures



(2-(((((7-Hydroxy-6-iodo-2-oxo-2H-pyrano[2,3-b]pyridin-4-yl)methoxy)carbonyl)oxy)methyl)5-oxo-4-(propn-2-ylidene)tetrahydrofuran-2-yl)methyl decanoate (**2**); DAG-lactone **1**[1] (24.4 mg, 0.0717 mmol) was added at 0 ºC to a solution (A) of CDI (11.4 mg, 0.0703 mmol) in THF (0.400 mL), and the mixture was stirred at 0 ºC for 1 h. A solution (B) of 8-aza-Ihc-CH_2OH[2] (22.5 mg, 0.0705 mmol) and Et_3N (30.0 μL, 0.215 mmol) in THF (0.6 mL) was prepared in a separate flask. After 1 h, solution A was added to solution B by syringe. The mixture was then stirred at room temperature for 9 h. The reaction mixture was concentrated with reduced pressure to give the crude compound. Purification by flash column chromatography over silica gel with $CHCl_3$-MeOH (7:1) gave the title compound (**2**) (22.2 mg, 46% yield) as a white solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.88 (m, 3H), 1.28 (m, 12H), 1.61 (m, 2H), 1.91 (s, 3H), 2.28 (s, 3H), 2,34 (m, 2H), 2.78 (m, 2H), 4.22 (m, 2H), 4.33 (s, 1H), 5.22 (s, 2H), 6.29 (s, 1H), 8.18 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 14.1, 20.1, 22.7, 24.7, 24.8, 29.1, 29.2, 29.4, 31.8, 32.5, 34.0, 64.4, 65.0, 69.2, 78.4, 80.0, 102.0, 108.8, 117.7, 144.3, 147.3, 152.9, 153.9, 155.7, 157.2, 160.7, 168.1, 173.1; HRMS (ESI), *m*/*z* calcd for $C_{29}H_{35}INO_{10}$ $[M-H]^-$ 684.1311, found 684.1309.



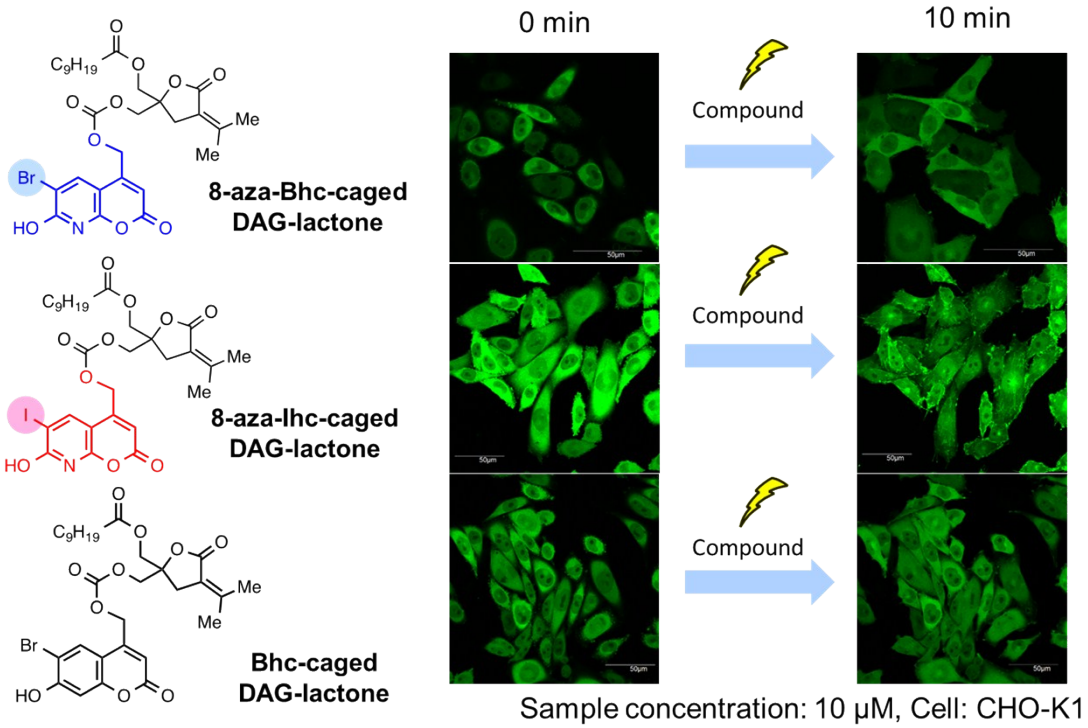
(2-(((((6-Bromo-7-hydroxy-2-oxo-2H-pyrano[2,3-b]pyridin-4-yl)methoxy)carbonyl)oxy)methyl)-5-oxo-4-(propan-2-ylidene)tetrahydrofuran-2-yl)methyl decanoate (**3**): The DAG-lactone **1**[1] (23.8 mg, 0.0700 mmol) was added to a solution of CDI (11.4 mg, 0.0705 mmol) in THF (0.400 mL), and the mixture was stirred at 0 ºC for 1 h. Subsequently, 8-aza-B(^{t}BuO)c-CH_2OH[2] (22.9 mg, 0.0700 mmol) and Et_3N (24.4 μL, 0.175 mmol) in THF (0.400 mL) were added to the above solution, and the mixture was stirred at room temperature for 15 h. The reaction mixture was concentrated under

reduced pressure, and purification by flash column chromatography over silica gel with hexane-EtOAc (2:1) gave the compound **S1** (29.4 mg, 61% yield). TFA (0.420 mL) was added to a solution of compound **S1** (29.4 mg, 0.0424 mmol) in CH_2Cl_2 (0.140 mL), and the mixture was stirred at room temperature for 1 h. Purification by flash column chromatography over silica gel with $CHCl_3$-MeOH (7:1) gave the title compound (**3**) (16.5 mg, 61% yield) as a white solid; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.88 (m, 3H), 1.27 (m, 12H), 1.60 (m, 2H), 1.90 (s, 3H), 2.28 (s, 3H), 2.32 (m, 2H), 2.78 (m, 2H), 4.19 (m, 2H), 4.33 (s, 1H), 5.21 (s, 2H), 6.31 (s, 1H), 7.98 (s, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 14.1, 20.1, 22.6, 24.7, 24.8, 29.1, 29.2, 29.4, 31.8, 32.5, 34.0, 64.4, 65.0, 69.2, 78.4, 99.5, 101.1, 109.2, 117.7, 137.5, 152.9, 153.9, 154.6, 168.0, 173.1; HRMS (ESI), *m*/*z* calcd for $C_{29}H_{35}BrNO_{10}$ $[M-H]^-$636.1450, found 636.1451.

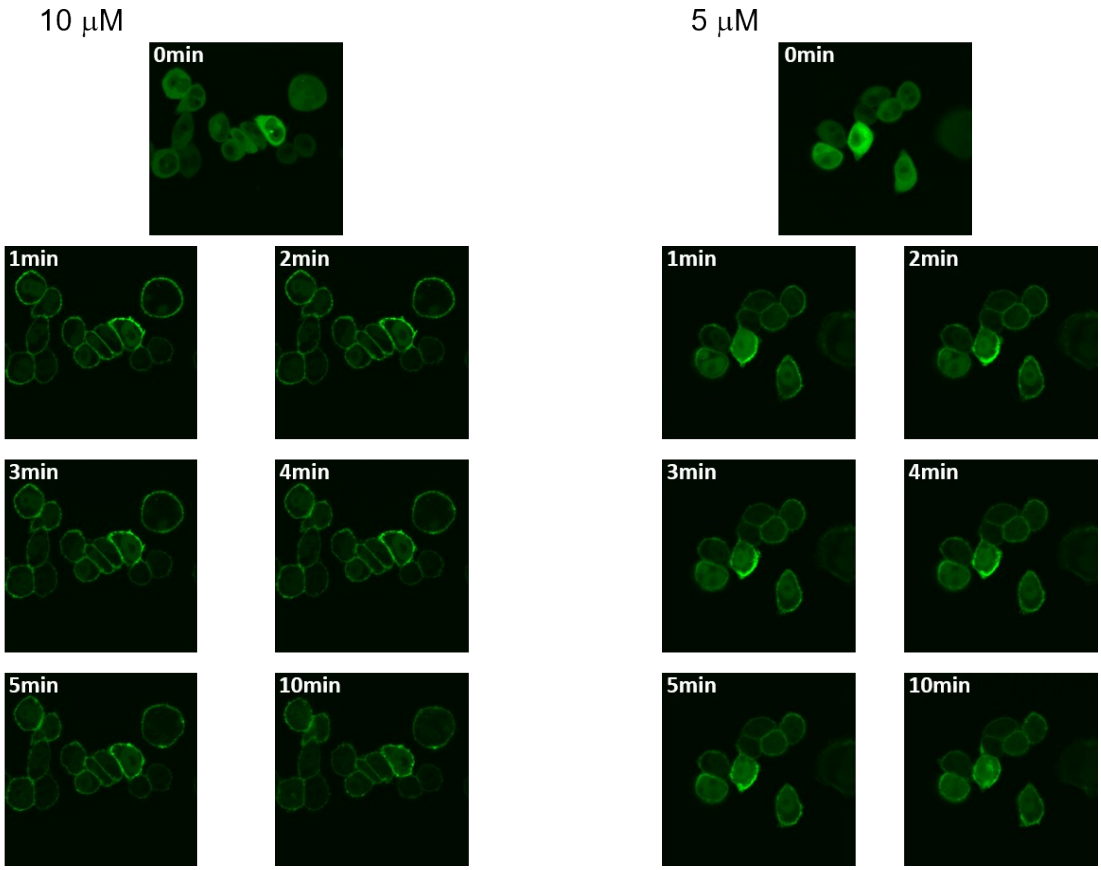
III. References

1. Malolanarasimhan, K.; Kedei, N.; Sigano, D. M.; Kelley, J. A.; Lai, C. C.; Lewin, N. E.; Surawski, R. J.; Pavlyukovets, V. A.; Garfield, S. H.; Wincovitch, S.; Blumberg, P. M.; Marquez, V. E. Conformationally Constrained Analogues of Diacylglycerol (DAG). 27. Modulation of Membrane Translocation of Protein Kinase C (PKC) Isozymes α and δ by Diacylglycerol Lactones (DAG-lactones) Containing Rigid-Rod Acyl Groups. *J. Med. Chem.* **2007**, *50*, 962−978.
2. Narumi, T.; Takano, H.; Ohashi, N.; Suzuki, A.; Furuta, T.; Tamamura, H. Isostere-Based Design of 8-Azacoumarin-Type Photolabile Protecting Groups: A Hydrophilicity-Increasing Strategy for Coumarin-4-ylmethyls. *Org. Lett.* **2014**, *16*, 1184−1187.

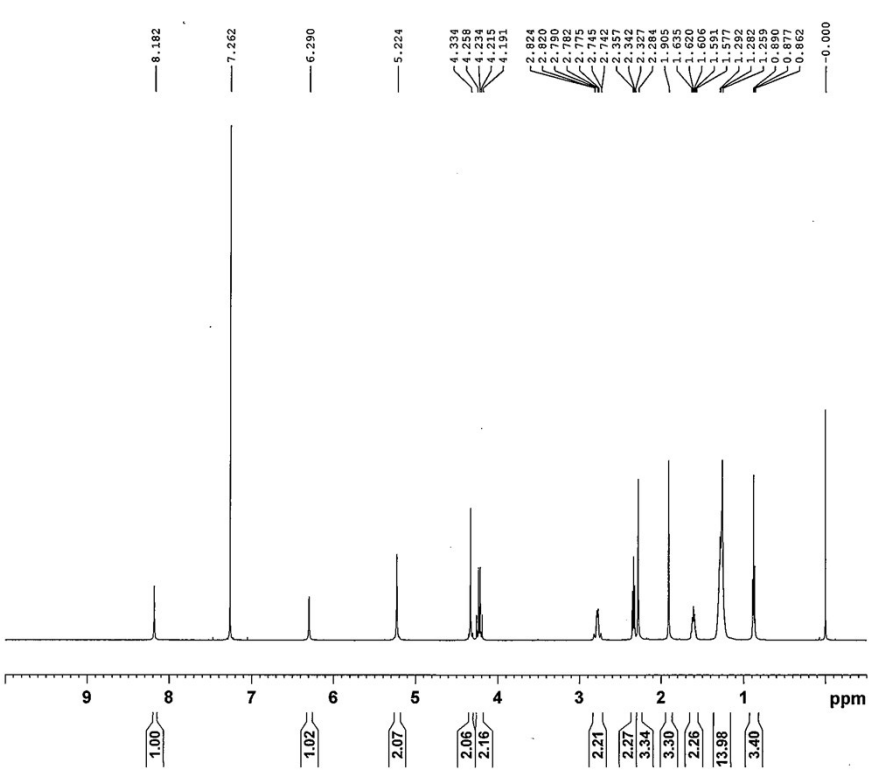
IV. Translocation observation of PKCδ with GFP (another data for the reproducibility)



V. Translocation data using DAG-lactone (1) (10 μM and 5 μM)



VI. ^{1}H NMR and ^{13}C NMR charts



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NS	16
DS	2
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FIDRES	0.157632 Hz
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RG	14.3
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1
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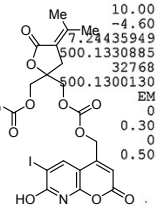
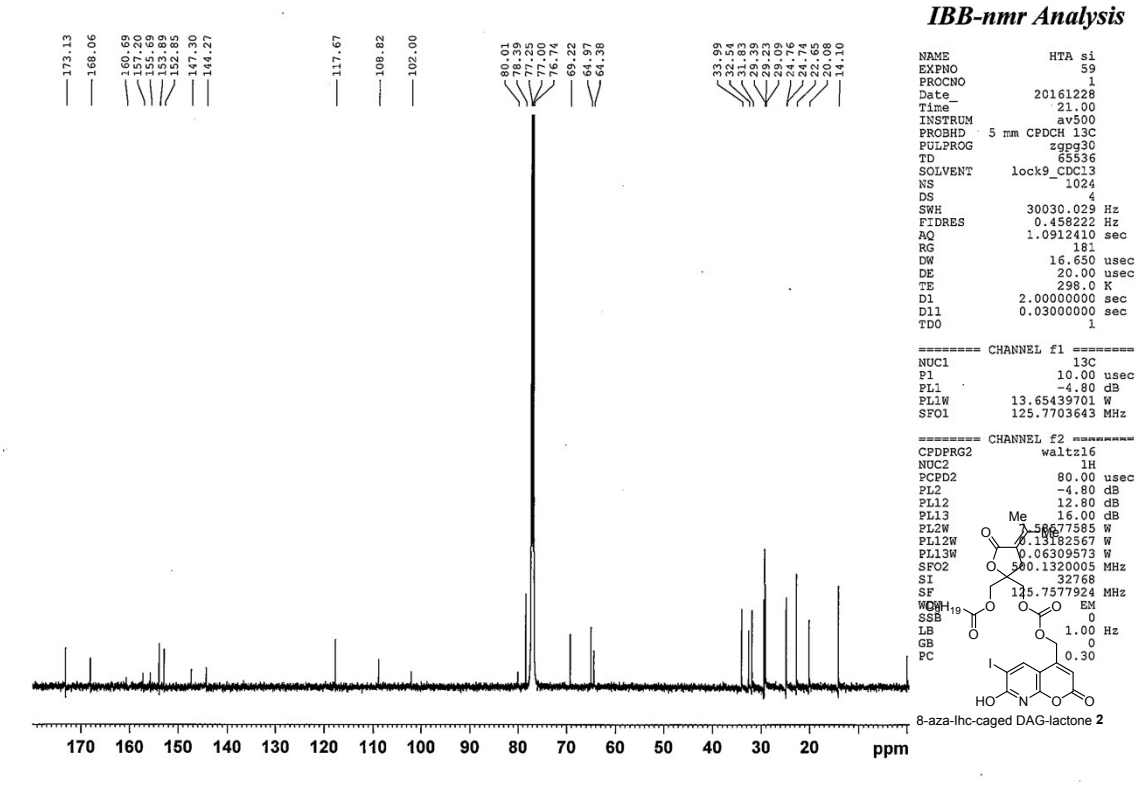


8-aza-Ihc-caged DAG-lactone **2**


IBB-nmr Analysis
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160.69
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147.30
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77.00
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33.99
32.54
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29.23
29.09
24.76
24.74
22.65
20.08
14.10
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PROCNO 1
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Time 21.00
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TD 65536
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PL12 12.80 dB
PL13 16.00 dB
PL2W 0.58677585 W
PL12W 0.13182567 W
PL13W 0.06309573 W
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SI 32768
SF 125.7577924 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 0.30
Me
Me
C15H31
I
HO
N
O
O
O
O
O
O
O
O
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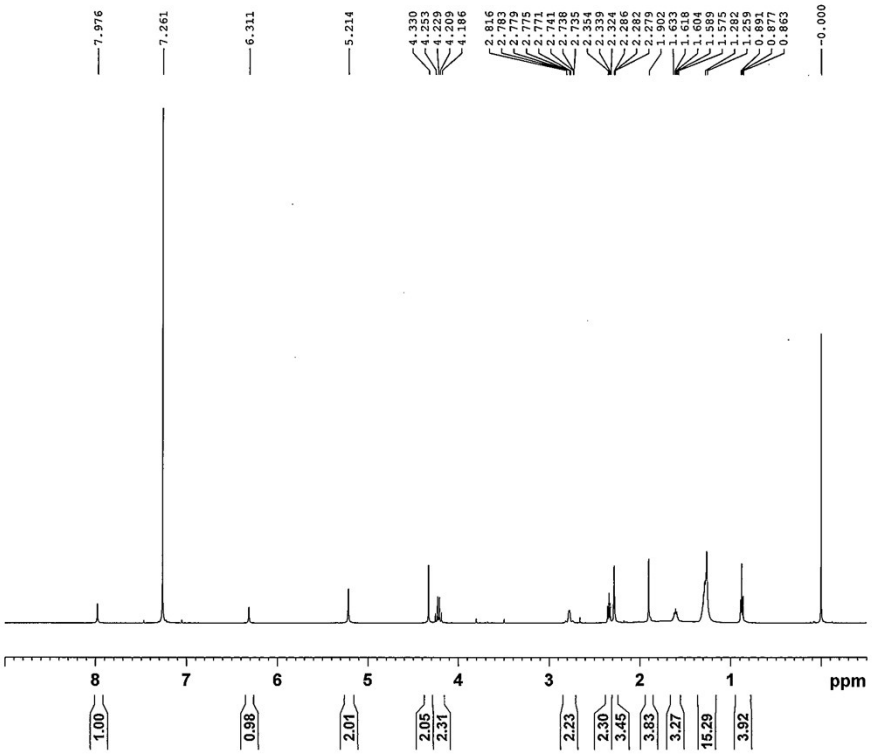

IBB-nmr Analysis

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NS	16
DS	2
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FIDRES	0.157632 Hz
AQ	3.1720407 sec
RG	28.5
DW	48.400 usec
DE	6.00 usec
TE	298.0 K
D1	1.00000000 sec
TD0	1
======== CHANNEL f1 ========	
NUC1	1H
P1	10.00 usec
PL1	-4.60 dB
PL1W	7.24435949 W
SFO1	500.1330885 MHz
SI	32768
SF	500.1300135 MHz
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SSB	0
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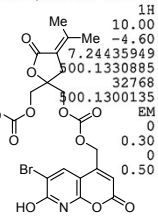
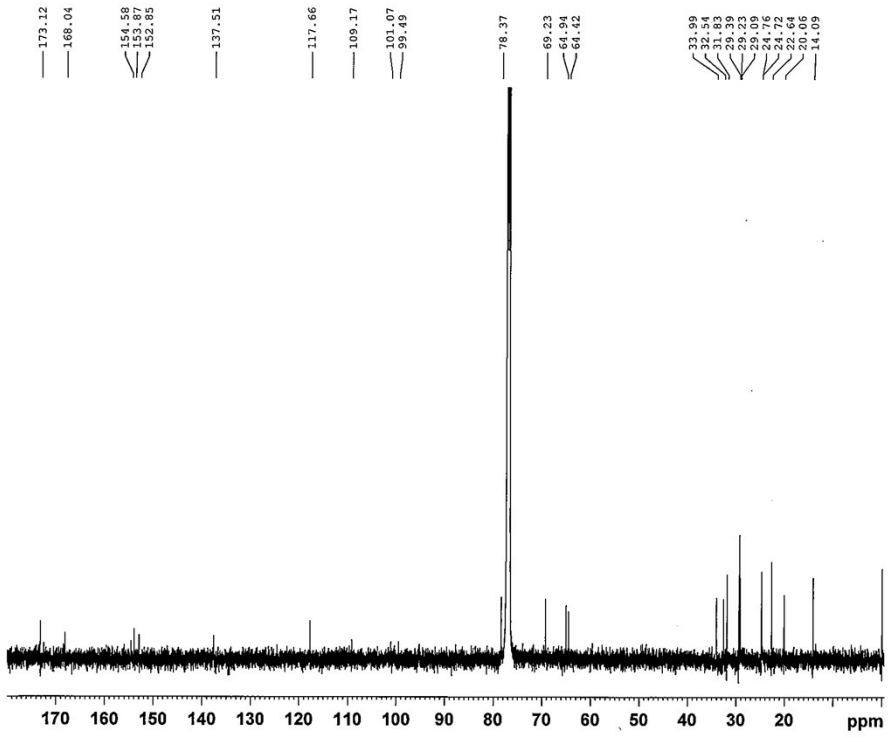


8-aza-Bhc-caged DAG-lactone **3**



IBB-nmr Analysis

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RG	228.1
DW	16.650 usec
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TE	298.0 K
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TD0	1
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SFO1	125.7703643 MHz
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NUC2	1H
PCPD2	80.00 usec
PL2	-4.80 dB
PL12	12.80 dB
PL13	16.00 dB
PL2W	17.58477585 W
PL12W	0.13182567 W
PL13W	0.06309573 W
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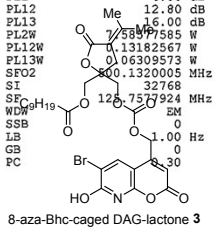

8-aza-Bhc-caged DAG-lactone **3**