

Supplementary Material

Ligand induced activation of human TRPM2 requires the terminal ribose of ADPR and involves Arg 1433 and Tyr 1349

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Supplementary Figure S1. Synthesis of methyl-ADP and ¹H-NMR data. p2

Supplementary Figure S2. Synthesis of THF-ADP and ¹H-NMR data. p3

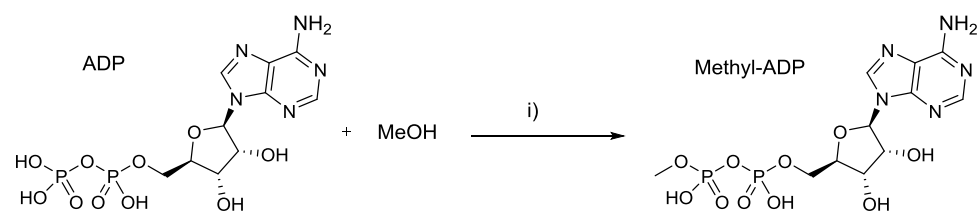
Supplementary Figure S3. Synthesis and purification of α -1''-O-methyl-ADPR and β -1''-O-methyl-ADPR and chromatographic data. p4

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Supplementary Table S1. Possible templates for modelling ADPR into the TRPM2 Nudix domain binding site. p6

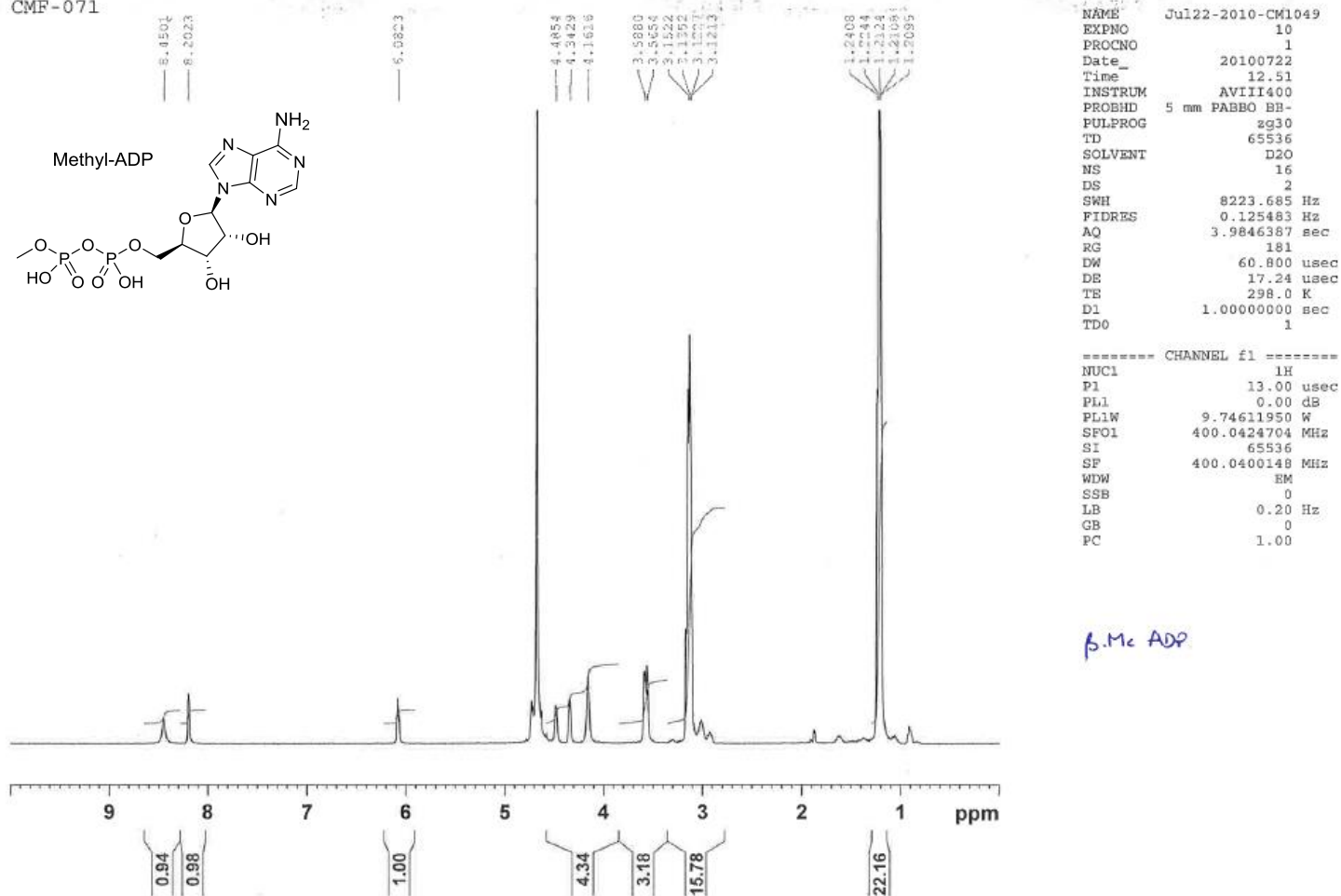
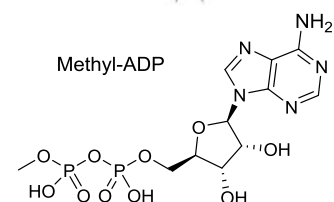
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Synthesis of methyl-ADP



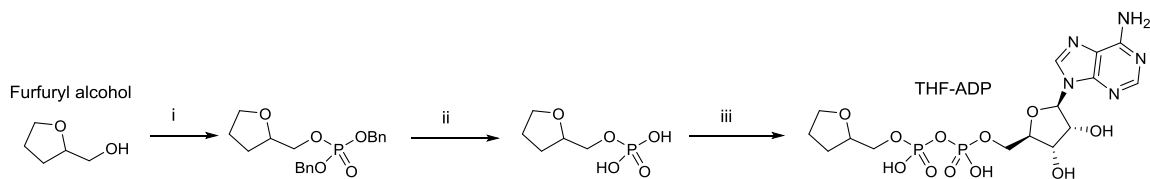
i) *N,N*-Dicyclohexylcarbodiimide (DCC),

CMF-071

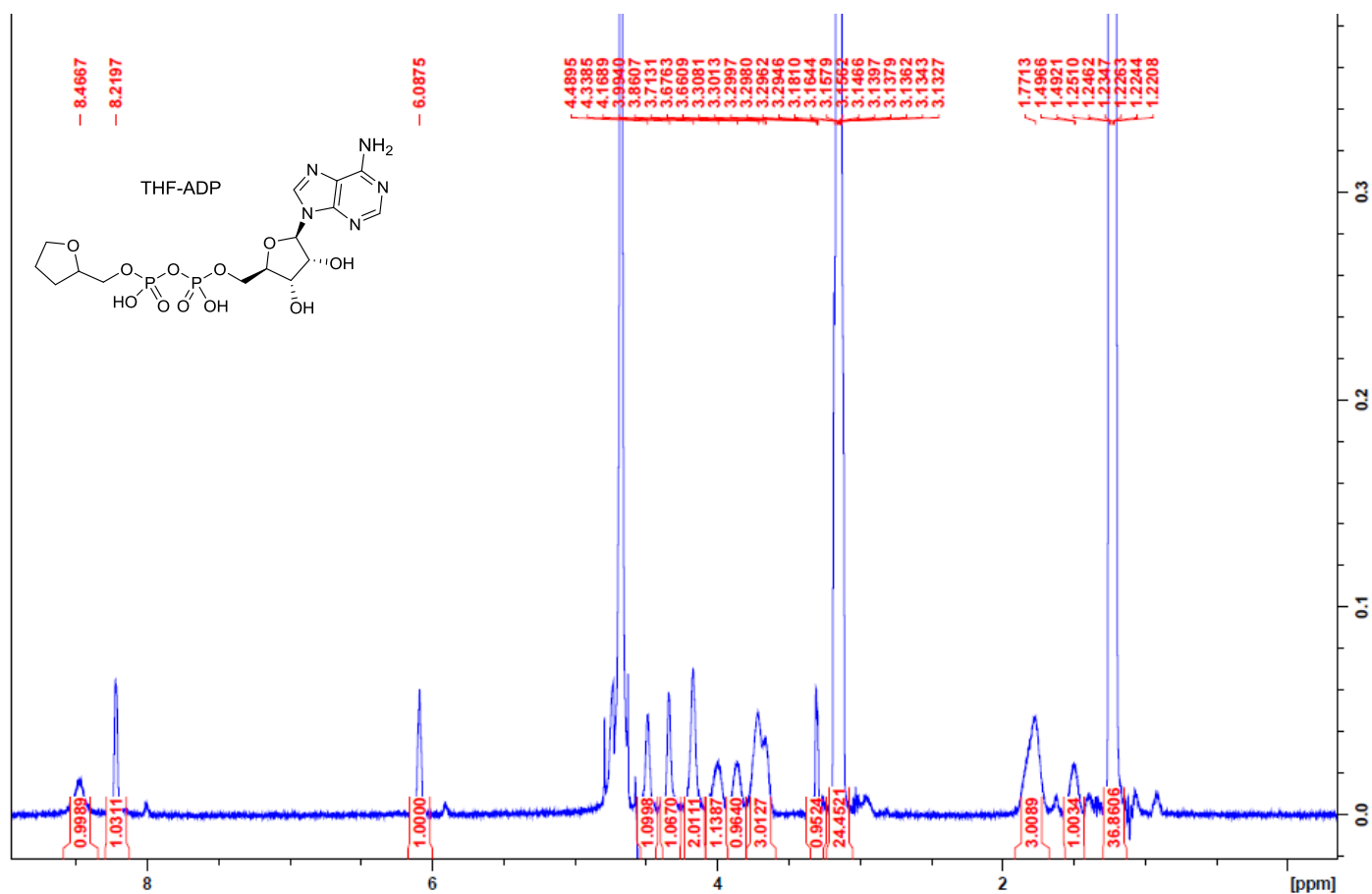


Supplementary Figure S1. Synthesis of methyl-ADP and ¹H-NMR data.

Synthesis of THF-ADP

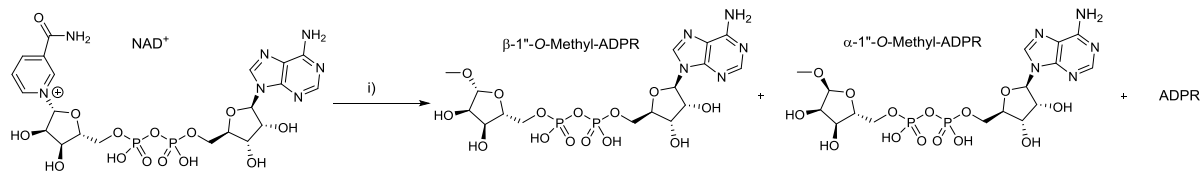


i) a) $(iPr)_2NP(OBn)_2$, 1-*H*-tetrazole, DCM b) mCPBA; ii) $Pd(OH)_2/C$, cyclohexene, MeOH-H₂O (10:1 v/v); iii) AMP-morpholidate, MgSO₄, 0.2 M MnCl₂ in formamide



Supplementary Figure S2. Synthesis of THF-ADP and ¹H-NMR data.

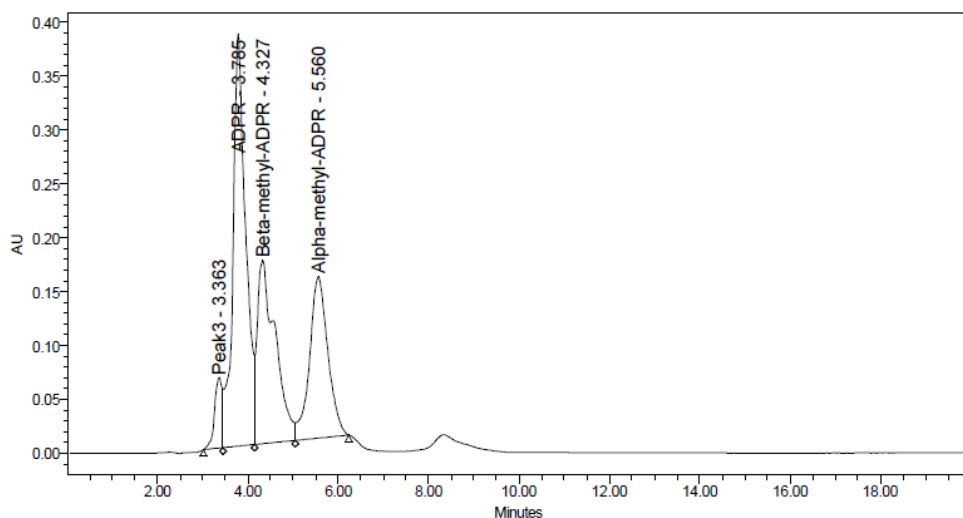
Synthesis of α - and β -1''-O-methyl-ADPR



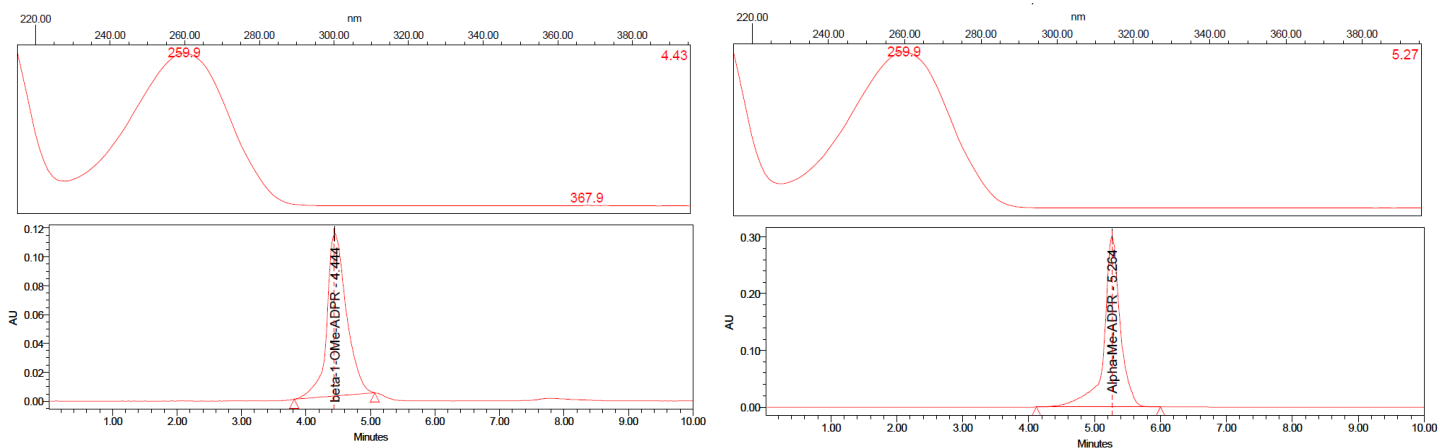
i) MeOH-Na₂HPO₄ (0.05 M, aq.) (1:2 v/v, 3mL), 60 °C

HPLC of crude mixture containing α -1''-O-methyl-ADPR, β -1''-O-methyl-ADPR and ADPR.

SAMPLE INFORMATION			
Sample Name:	JMS 1077 NMR	Acquired By:	Joanna
Sample Type:	Unknown	Date Acquired:	10/02/2015 1:25:55 PM
Vial:	10	Acq. Method Set:	RP18 LC
Injection #:	1	Date Processed:	07/09/2016 2:51:41 PM
Injection Volume:	10.00 ul	Processing Method:	Methyl Tiazo Riboside Phospat
Run Time:	20.0 Minutes	Channel Name:	WVInCh1
Sample Set Name:	JMS 1077 isocratic	Proc. Chnl. Descr.:	PDA 259.1 nm



Separation of the desired products after semi-preparative HPLC:

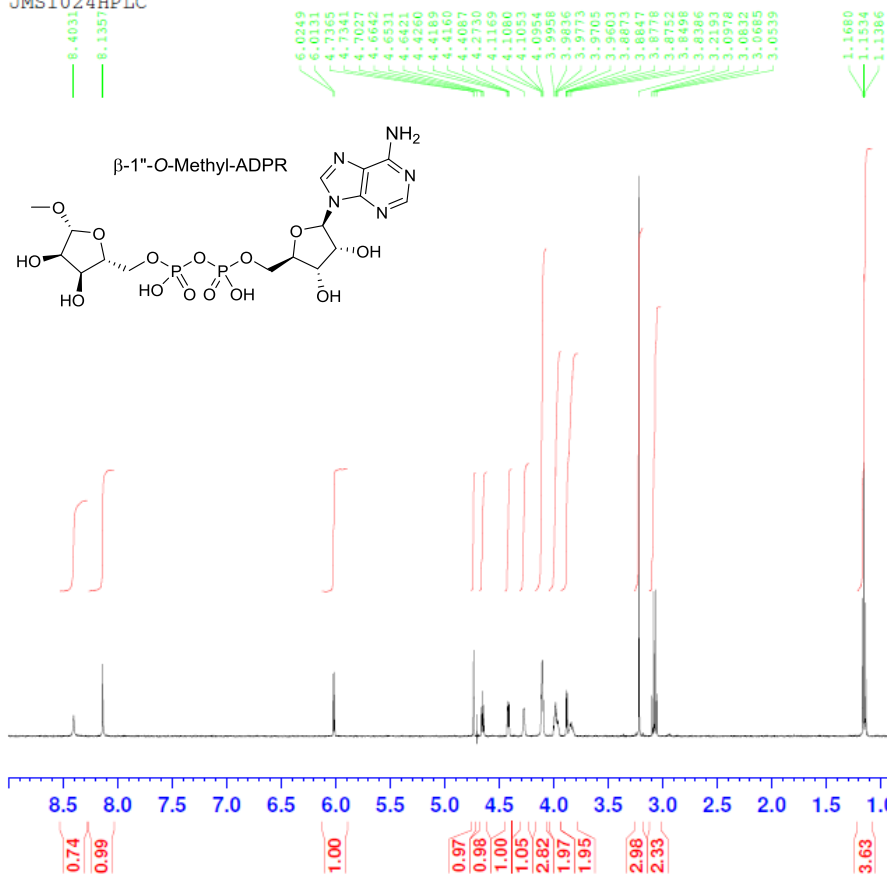


PDA Result Table									
Name	RT	Area	Purity (#1) Angle	Purity (#1) Threshold	Purity Flag	Match (#1) Spect. Name	Match (#1) Angle	Match (#1) Threshold	PDA Match Flag
1	beta-1-OMe-ADPR	4.444	2381448		No				No
2	Alpha-Me-ADPR	5.264			No				No

PDA Result Table									
Name	RT	Area	Purity (#1) Angle	Purity (#1) Threshold	Purity Flag	Match (#1) Spect. Name	Match (#1) Angle	Match (#1) Threshold	PDA Match Flag
1	Alpha-Me-ADPR	5.264	5188645		No				No

Supplementary Figure S3. Synthesis and separation of α -1''-O-methyl-ADPR and β -1''-O-methyl-ADPR and chromatographic data.

JMS1024HPLC



```

NAME      JMS1024HPLC
EXPNO     26
PROCNO    1
Date_     20140905
Time      9.05
INSTRUM   spect
PROBHD    5 mm PABBO BB-
PULPROG   ledpppg2s1d
TD        65536
SOLVENT   D2O
NS         16
DS         2
SWH        10330.578 Hz
FIDRES     0.157632 Hz
AQ         3.1719923 sec
RG         161
DW         48.400 usec
DE         9.73 usec
TE         295.2 K
D1         4.00000000 sec
D16        0.00020000 sec
D20        0.05000000 sec
D21        0.00500000 sec
TD0        1
    
```

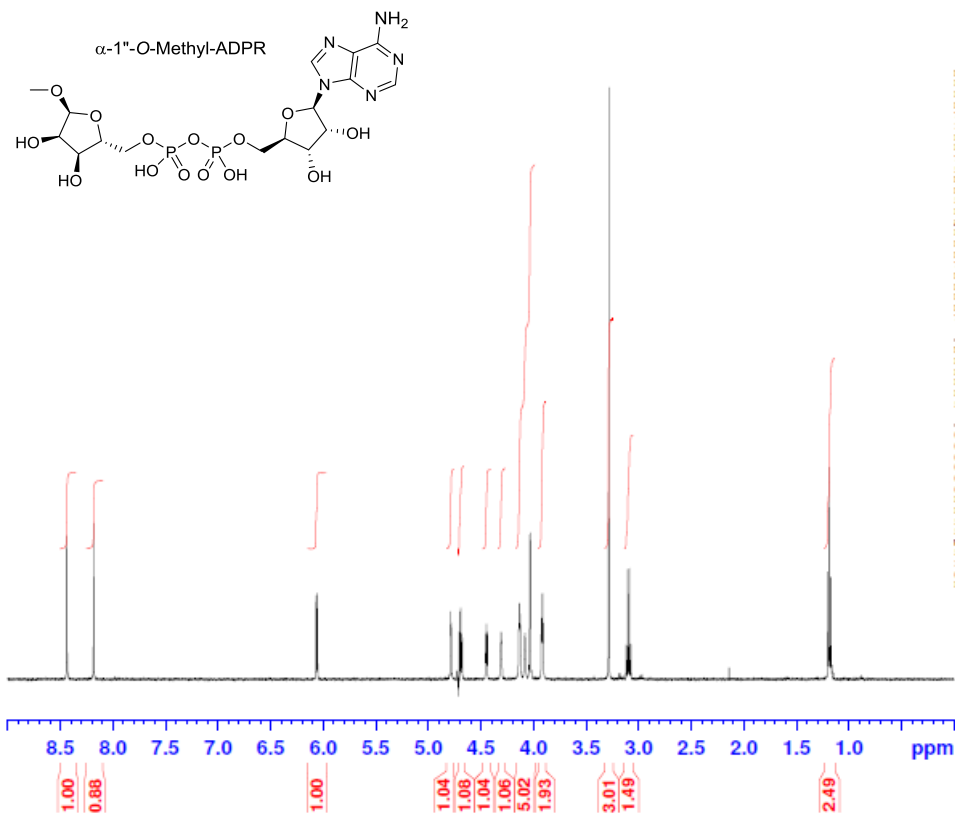
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----- CHANNEL f1 -----
NUC1      1H
P1        10.00 usec
P2        20.00 usec
PL1       -0.12 dB
PL1W      19.35150909 W
SFO1      500.1330885 MHz
    
```

```

----- GRADIENT CHANNEL -----
GPNAM6    sine.100
GPNAM7    sine.100
GPNAM8    sine.100
GPZ6      95.00 %
GPZ7      -17.13 %
GPZ8      -13.17 %
P19       600.00 usec
P30       2000.00 usec
SI        32768
SF        500.1300000 MHz
WDW       EM
SSB       0
LB        0.30 Hz
GB        0
PC        1.00
    
```

JMS1077 Methyl 1 alpha GF HPLC



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NAME      JMS1077 Methyl 1 alpha GF HPLC
EXPNO     10
PROCNO    1
Date_     20150216
Time      15.28
INSTRUM   spect
PROBHD    5 mm PABBO BB-
PULPROG   ledpppg2s1d
TD        65536
SOLVENT   D2O
NS         112
DS         2
SWH        10330.578 Hz
FIDRES     0.157632 Hz
AQ         3.1719923 sec
RG         362
DW         48.400 usec
DE         9.73 usec
TE         295.2 K
D1         4.00000000 sec
D16        0.00020000 sec
D20        0.05000000 sec
D21        0.00500000 sec
TD0        1
    
```

```

----- CHANNEL f1 -----
NUC1      1H
P1        10.00 usec
P2        20.00 usec
PL1       -0.12 dB
PL1W      19.35150909 W
SFO1      500.1330885 MHz
    
```

```

----- GRADIENT CHANNEL -----
GPNAM6    sine.100
GPNAM7    sine.100
GPNAM8    sine.100
GPZ6      95.00 %
GPZ7      -17.13 %
GPZ8      -13.17 %
P19       600.00 usec
P30       2000.00 usec
SI        32768
SF        500.1300000 MHz
WDW       EM
SSB       0
LB        0.30 Hz
GB        0
PC        1.00
    
```

Supplementary Figure S4. ¹H-NMR data for α-1''-O-methyl-ADPR and β-1''-O-methyl-ADPR.

PDB Structure	Species	Protein	Reference
1G9Q	<i>Escherichia coli</i>	ADPRase	[18]
1MK1	<i>Mycobacterium tuberculosis</i>	ADPRase	[19]
1V8L	<i>Thermus thermophilus</i>	ADPRase	[20]
2QJO	<i>Synechocystis</i> sp. PCC6803	NMN adenyltransferase/ADPRase	[25]
3GZ8	<i>Shewanella oneidensis</i>	Transcriptional Regulator	[28]

Supplementary Table S1. Possible templates for modelling ADPR into the TRPM2 Nudix domain binding site.

Mutagenesis primers

T1347V for	TTCGGACCCAACCACGTGCTGTACCCCATGGTC
T1347V rev	GACCATGGGGTACAGCACGTGGTTGGGTCCGAA
Y1349F for	CCCAACCACACGCTGTTCCCATGGTCACGCGG
Y1349F rev	CCGCGTGACCATGGGGAACAGCGTGTGGTTGGG
L1381I for	GTGGTGAAGCTCCCTATCTCCGAGCACTGGGCC
L1381I rev	GGCCCAGTGCTCGGAGATAGGGAGCTTCACCAC
R1433M for	TACATGGATGACCCGATGAACACGGACAATGCC
R1433M rev	GGCATTGTCCGTGTTTCATCGGGTCATCCATGTA
Y1485F for	CGCATCCCCTCTTCGCGAACCACAAGACC
Y1485F rev	GGTCTTGTGGTTCGCGAAGAGTGGGATGCG

Sequencing primers

CMVfor	CGCAAATGGGCGGTAGGCGTG
TRPM2.5	GTCATCACCATCGGAGTCGC
TRPM2.7	CACCTTGCTCTACCTGTACGA
TRPM2.9	CTCTGCCTGTTTCGCCTACGTG
TRPM2.11	CTGCAGCTCTTCATCAAGAGG
TRPM2.13	CAGGCCGGGTTGCCCTGAAC
TRPM2.15	GGGAGACACCCTGGAGCCAC

Supplementary Table S2. Primers used for QuikChange mutagenesis and sequencing of the coding sequence for TRPM2