Supplementary Files

Supplementary Material and Methods

Synthesis of 5’-(4-fluorosulfonylbenzoyl)guanosine (FSBG)

Guanosine monohydrate (875 mg, 2.90 mmol) was co-evaporated twice with anhydrous DMF and subsequently dissolved in DMPU with gentle warming. The clear solution was cooled in an ice bath, and 4-(fluorosulfonyl)benzoyl chloride (812 mg, 3.65 mmol) was added. After 15 minutes the mixture was warmed to room temperature and stirred for another 4 hours. Petroleum ether 40/60 (50 mL) was added and a white precipitate formed. The organic layer was decanted and the residue triturated twice with a 1/1 mixture of ethyl acetate/diethyl ether (2 x 50 mL). The residue was re-crystallized from MeOH/water and further purified by C18-RP-HPLC (Phenomenex Gemini C18, pore size 110Å, particle size 5 µm, 150 x 21.2 mm, gradient 20 – 50% Acetonitrile in 0.1 % aqueous TFA, 20 mL/min) to yield the title compound as a white solid (232 mg, yield 17%) (Supplementary Figure 5).

Supplementary tables

Table S1: Virus genome used for the bioinformatics analyses.

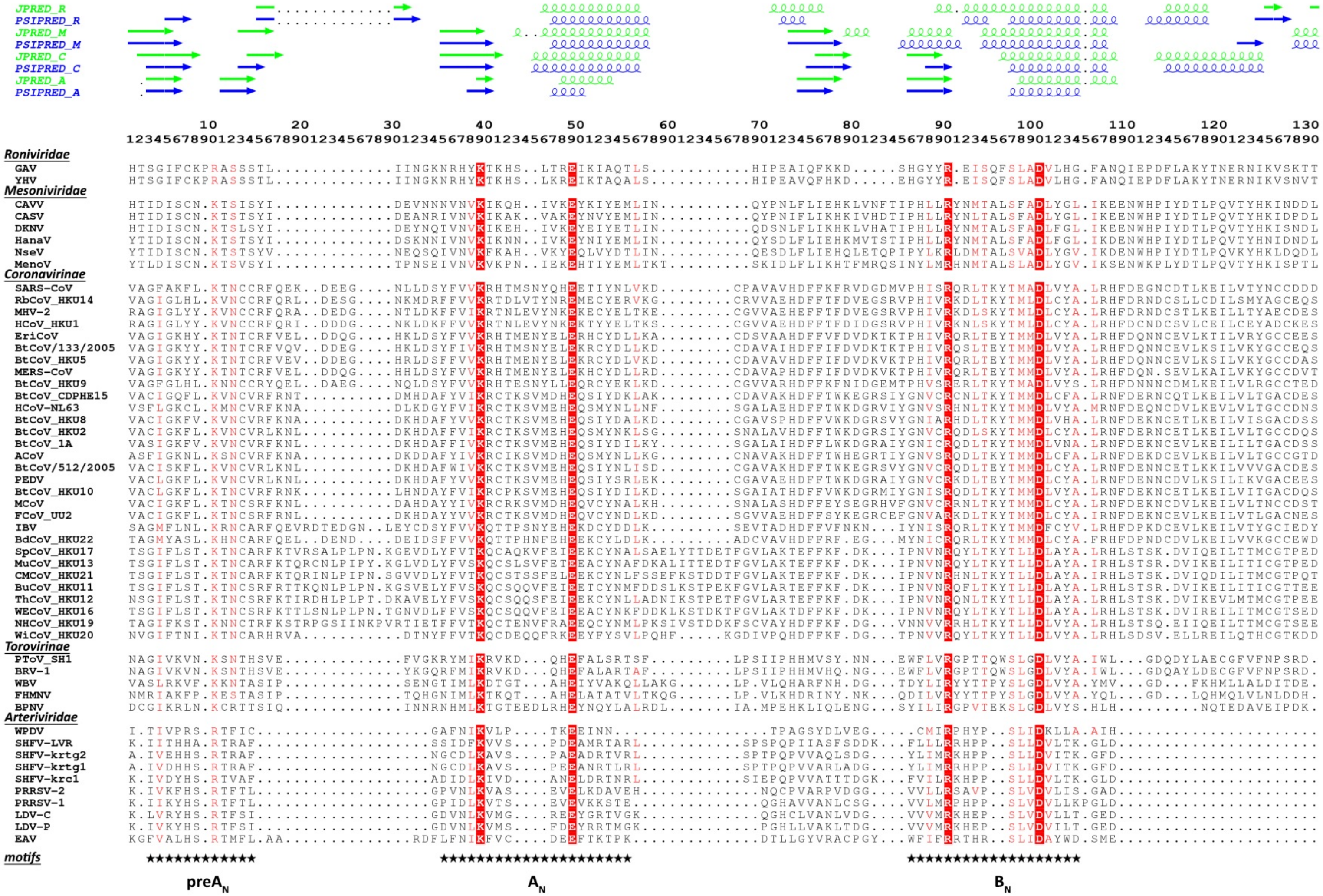
Virus name	Species	(Sub)family	Acronym	Accession number
Gill-associated virus	*Gill-associated virus*	*Roniviridae*	GAV	AF227196
Yellow head virus	to be established	*Roniviridae*	YHV	EU487200
Cavally virus	*Alphamesonivirus 1*	*Mesoniviridae*	CAVV	HM746600
Casuarina virus	to be established	*Mesoniviridae*	CASV	NC_023986
Dak Nong virus	to be established	*Mesoniviridae*	DKNV	AB753015.2
Hana virus	to be established	*Mesoniviridae*	HanaV	JQ957872
Nse virus	to be established	*Mesoniviridae*	NseV	JQ957874
Meno virus	to be established	*Mesoniviridae*	MenoV	JQ957873
SARS coronavirus Frankfurt 1	*Severe acute respiratory syndrome-related coronavirus*	*Coronavirinae*	SARS-CoV	AY291315
Rabbit coronavirus HKU14	*Betacoronavirus 1*	*Coronavirinae*	RbCoV_HKU14	JN874560
Murine hepatitis virus strain 2	*Murine coronavirus*	*Coronavirinae*	MHV-2	AF201929
Human coronavirus HKU1	*Human coronavirus HKU1*	*Coronavirinae*	HCoV_HKU1	AY884001
Betacoronavirus Erinaceus/VMC/DEU/2012	to be established	*Coronavirinae*	EriCoV	KC545383

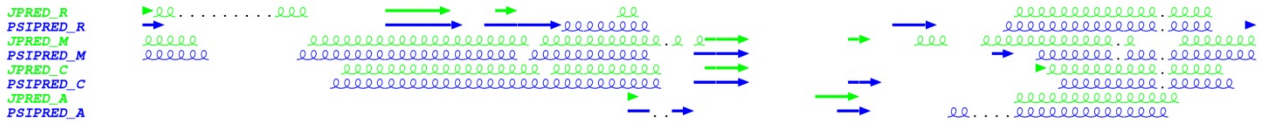
Virus name	Species	(Sub)family	Acronym	Accession number
Bat coronavirus (BtCoV/133/2005)	*Tylonycteris bat coronavirus HKU4*	*Coronavirinae*	BtCoV/133/2005	DQ648794
Bat coronavirus HKU5-1	*Pipistrellus bat coronavirus HKU5*	*Coronavirinae*	BtCoV_HKU5	EF065509
MERS coronavirus EMC/2012	to be established	*Coronavirinae*	MERS-CoV	JX869059.2
Bat coronavirus HKU9-10-2	*Rousettus bat coronavirus HKU9*	*Coronavirinae*	BtCoV_HKU9	HM211101
Bat coronavirus CDPHE15/USA/2006	to be established	*Coronavirinae*	BtCoV_CDPHE15	KF430219
Human coronavirus NL63	*Human coronavirus NL63*	*Coronavirinae*	HCoV-NL63	AY567487
Miniopterus bat coronavirus HKU8	*Miniopterus bat coronavirus HKU8*	*Coronavirinae*	BtCoV_HKU8	EU420139
Rhinolophus bat coronavirus HKU2	*Rhinolophus bat coronavirus HKU2*	*Coronavirinae*	BtCoV_HKU2	EF203064
Bat coronavirus 1A	*Miniopterus bat coronavirus 1*	*Coronavirinae*	BtCoV_1A	EU420138
Alpaca respiratory coronavirus	*Human coronavirus 229E*	*Coronavirinae*	ACoV	JQ410000
Bat coronavirus (BtCoV/512/2005)	*Scotophilus bat coronavirus 512*	*Coronavirinae*	BtCoV/512/2005	DQ648858
Porcine epidemic diarrhea virus	*Porcine epidemic diarrhea virus*	*Coronavirinae*	PEDV	KC140102
Rousettus bat coronavirus HKU10	to be established	*Coronavirinae*	BtCoV_HKU10	JQ989271
Mink coronavirus strain WD1127	to be established	*Coronavirinae*	MCoV	HM245925
Feline coronavirus UU2	*Alphacoronavirus 1*	*Coronavirinae*	FCoV_UU2	FJ938060
Infectious bronchitis virus	*Avian coronavirus*	*Coronavirinae*	IBV	KC008600
Bottlenose dolphin coronavirus HKU22	*Beluga whale coronavirus SW1*	*Coronavirinae*	BdCoV_HKU22	KF793824
Sparrow coronavirus HKU17	to be established	*Coronavirinae*	SpCoV_HKU17	JQ065045
Munia coronavirus HKU13-3514	*Munia coronavirus HKU13*	*Coronavirinae*	MuCoV_HKU13	FJ376622
Common-moorhen coronavirus HKU21	to be established	*Coronavirinae*	CMCoV_HKU21	JQ065049
Bulbul coronavirus HKU11-934	*Bulbul coronavirus HKU11*	*Coronavirinae*	BuCoV_HKU11	FJ376619.2

Virus name	Species	(Sub)family	Acronym	Accession number
Thrush coronavirus HKU12-600	*Thrush coronavirus HKU12*	*Coronavirinae*	ThCoV_HKU12	FJ376621
White-eye coronavirus HKU16	to be established	*Coronavirinae*	WECoV_HKU16	JQ065044
Night-heron coronavirus HKU19	to be established	*Coronavirinae*	NHCoV_HKU19	JQ065047
Wigeon coronavirus HKU20	to be established	*Coronavirinae*	WiCoV_HKU20	JQ065048
Porcine torovirus	*Porcine torovirus*	*Torovirinae*	PToV_SH1	NC_022787
Breda virus	*Bovine torovirus*	*Torovirinae*	BRV-1	AY427798
White bream virus	*White bream virus*	*Torovirinae*	WBV	DQ898157
Fathead minnow nidovirus	to be established	*Torovirinae*	FHMNV	GU002364.2
Ball python nidovirus	to be established	*Torovirinae*	BPNV	NC_024709
Possum nidovirus	to be established	*Arteriviridae*	WPDV	JN116253
Simian hemorrhagic fever virus	*Simian hemorrhagic fever virus*	*Arteriviridae*	SHFV-LVR	AF180391
Simian hemorrhagic fever virus	to be established	*Arteriviridae*	SHFV-krtg2	JX473847
Simian hemorrhagic fever virus	to be established	*Arteriviridae*	SHFV-krtg1	JX473848
Simian hemorrhagic fever virus	to be established	*Arteriviridae*	SHFV-krc1	HQ845737
Porcine reproductive and respiratory syndrome virus	*Porcine reproductive and respiratory syndrome virus*	*Arteriviridae*	PRRSV-2	JX138233
Porcine reproductive and respiratory syndrome virus	*Porcine reproductive and respiratory syndrome virus*	*Arteriviridae*	PRRSV-1	GU737264.2
Lactate dehydrogenase-elevating virus	*Lactate dehydrogenase-elevating virus*	*Arteriviridae*	LDV-C	L13298
Lactate dehydrogenase-elevating virus	*Lactate dehydrogenase-elevating virus*	*Arteriviridae*	LDV-P	U15146
Equine arteritis virus	*Equine arteritis virus*	*Arteriviridae*	EAV	DQ846750

Table S2. Multiple sequence alignment of NiRAN of nidoviruses (see a separate file in fasta format).

Supplementary figures


JPRED_R
PSIPRED_R
JPRED_M
PSIPRED_M
JPRED_C
PSIPRED_C
JPRED_A
PSIPRED_A
10
20
30
40
50
60
70
80
90
100
110
120
130
Roniviridae
GAV
YHV
Mesoniviridae
CAVV
CASV
DKNV
HanaV
NseV
MenoV
Coronavirinae
SARS-CoV
RbCoV_HKU14
MHV-2
HCoV_HKU1
EriCoV
BtCoV/133/2005
BtCoV_HKU5
MERS-CoV
BtCoV_HKU9
BtCoV_CDPHE15
HCoV-NL63
BtCoV_HKU8
BtCoV_HKU2
BtCoV_1A
ACoV
BtCoV/512/2005
PEDV
BtCoV_HKU10
MCoV
FCoV_UU2
IBV
BdCoV_HKU22
SpCoV_HKU17
MuCoV_HKU13
CMCoV_HKU21
BuCoV_HKU11
ThCoV_HKU12
WECoV_HKU16
NHCoV_HKU19
WiCoV_HKU20
Torovirinae
PToV_SH1
BRV-1
WBV
FHMNV
BPNV
Arteriviridae
WPDV
SHFV-LVR
SHFV-krtg2
SHFV-krtg1
SHFV-krc1
PRRSV-2
PRRSV-1
LDV-C
LDV-P
EAV
motifs
preA_N
A_N
B_N



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                        140       150       160       170       180       190       200       210       220       230
                 12345678901234567890123456789012345678901234567890123456789012345678901234567890123456789012345678901   genome coordinates (nt)
Roniviridae
GAV              WLC.........KNICNHKSCNILKMDIFDYTYTCYTKAHSFALQACSTYNFDITPDNICPEG.VYDFETFRIGT.RDPIKALNAVFYCIER.HWFFSGLS   12389-12946
YHV              WLC.........KNICNHKSCNILKMDIFDYTYTCYTKAQSFALQACTTYNFDITPDNICPEG.VYDFETYRPGN.CDPIKALNAVTYCIER.HWFSAGLS   12428-12985
Mesoniviridae
CAVV             LLKIKS.HTPSPQHTCCMLCRRFLVEFGLLLHKLNYKVFETTRAILT.HYDFVLTADNVDLNG.ILDFEDYKLKK.STIAHDVKSQLR.IMQ.PYYHALYS   7925-8518
CASV             LFKIKQ.HTPSPQHTCCMLCRRFLAEFGLLLHKLNFKVYETTTNIYA.HYDFVLTADNIDLNG.ILDFEDYILREEVLVNIDVKSQLR.IMQ.PYYHTLYS   7815-8411
DKNV             LIKIKS.HTPSPQHTCCMLCRRFLVEFGLLLHKLNYKVFETTTRLLQ.GYEFVLTADNIDLNG.ILDFEDYIPRA.EYFVCDVKYQLR.IMQ.PYYHTLYS   8098-8691
HanaV            LLKIKQ.HTPSPQHTCCMLCRRFLAEFGLLLHKLNYKVFETTRKMLT.HYDFVLTADNIDLNG.ILDFEDYVPKKYIDINIDVTSQLR.IMQ.PYYHMLYS   7916-8512
NseV             LKKIKE.HTPCPQHTCCLLCRRFLAEFGLLMHKLNFKVYETTTTLLR.SYEFVLTADNVDLNG.ILDFEDYLQLD.EPRNVECVDQLR.KMQ.PYYHSLYS   7864-8457
MenoV            LNNIQQ.HSSAPQHTCCMLCRRFLAEFGLLMHKLNKQVFSTINFMFQ.HYDFVLTSDNVDLNG.ILDFEDYTKTE.YTRTLELKDQLR.KMQ.PYYHTLYS   7777-8373
Coronavirinae
SARS-CoV         ...YFN.KKDWYDFVENPDILRVYANLGERVRQSLLKTVQFCDAMRDAGIVGVLTLDNQDLNGNWYDFGDFVQVA.PGCGVPIVDSYYSLLM.PILTLTRA   13494-14120
RbCoV_HKU14      ...YFT.QKDWYDFVENPDIINVYKKLGPIFNRALVNTAEFADALVEAGLVGVLTLDNQDLNGMWYDFGDYVITA.PGCGVAVADSYYSYMM.PILAMCHA   13618-14244
MHV-2            ...YFQ.KKDWYDFVENSDIINVYKKLGPIFNRALLNTAKFADTLVEAGLVGVLTLDNQDLYGQWYDFGDFVKTV.PGCGVAVADSYYSYMM.PMLTMCHA   13538-14164
HCoV_HKU1        ...YFS.KKDWYDFVENPDIINIYKKLGPIFNRALLNTVSFADTLVKVGLVGVLTLDNQDLYGQWYDFGDFIQTA.PGFGVAVADSYYSYMM.PMLTMCHV   13603-14229
EriCoV           ...YFD.NKLWFDFVENPDVIRVYHKLGELVRRAMLSTVKFCDHMVKSGLVGVLTLDNQDLNGKWYDFGDFVVTQ.PGAGVAIVDSYYSYLM.PVLSMTDA   13689-14315
BtCoV/133/2005   ...YFD.NKLWFDFVENPSVIGVYHKLGERIRQAMLNTVKMCDHMVKSGLVGVLTLDNQDLNGKWYDFGDFVITQ.PGAGVAIVDSYYSYLM.PVLSMTNC   13669-14295
BtCoV_HKU5       ...YFD.NKLWFDFVENPNVISVYHKLGERIRQAVLNTVKFCDQMVKSGLVGVLTLDNQDLNGKWYDFGDFVITQ.PGAGVAIVDSYYSYLM.PVLSMTNC   13790-14416
MERS-CoV         ...YFE.NKLWFDFVENPSVIGVYHKLGERVRQAILNTVKFCDHMVKAGLVGVLTLDNQDLNGKWYDFGDFVITQ.PGSGVAIVDSYYSYLM.PVLSMTDC   13538-14161
BtCoV_HKU9       ...YFD.RKDWYDPVENPDIIRVYHKLGETVRKAVLSAVEMADAMVEQGLIGVLTLDNQDLNGQWYDFGDFIEGP.AGAGVAVMDTYYSLAM.PIYTMTDM   12974-13600
BtCoV_CDPHE15    ...YFE.NKLWFDPVENEDIHRVYAKLGVVVARAMLNCVKLCDAMVKAGIVGVLTLDNQDLNGKFYDFGDFVPSL.EGMGVPLCTSYYSYMM.PIMGMTNC   12625-13239
HCoV-NL63        ...YFD.SKGWYDPVENEDIHRVYASLGKIVARAMLKCVALCDAMVAKGVVGVLTLDNQDLNGNFYDFGDFVVSL.PNMGVPCCTSYYSYMM.PIMGLTNC   12532-13146
BtCoV_HKU8       ...YFD.NKNWYDPVENEDIHRVYAKLGCVVANAMLKCVALCDAMVAKGVVGVLTLDNQDLNGNFYDFGDFTIGI.PGVGVPLATSYYSYLM.PVMGMTNC   13028-13642
BtCoV_HKU2       ...YFD.NKVWYDPVENEDLHRVYALLGQRVANAMLKCVKLCDEMVTKGVVGVLTLDNQDLNGNFYDFGDFVDVM.PGMGIPCCTSYYSYMM.PIMTMTNC   12539-13153
BtCoV_1A         ...YFD.NKSWYDPVENEDIHRVYAKLGDVIANAMLKCVALCDAMTEKGIVGVITLDNQDLNGNFYDFGDFVTSI.PGVGVPVCTSYYSYMM.PAMGMANC   13141-13755
ACoV             ...YFE.MKNWFDPVENEDIHRVYAALGTVVANAMLKCVALCDEMVLRGVVGVLTLDNQDLNGNFYDFGDFVLCP.PGMGIPYCTSYYSYMM.PVMGMTNC   12625-13239
BtCoV/512/2005   ...YFD.NKLWFDPVENEDVHRVYAKLGTIVARAMLKCVKYCDAMVEQGIVGVITLDNQDLNGDFYDFGDFVTSV.KGMGVPICTSYYSYMM.PVMGMTNC   12743-13357
PEDV             ...YFN.NKVWFDPVENEDIHRVYALLGTIVSRAMLKCVKFCDAMVEQGIVGVVTLDNQDLNGDFYDFGDFTCSI.KGMGIPICTSYYSYMM.PVMGMTNC   12709-13323
BtCoV_HKU10      ...YFD.NKFWFDPVENEDLHRVYAILGKIVANAMLKCVRLGDAMVKHGVVGVITLDNQDLNGNFYDFGDFAKTL.PGMGVPLCTSYYSYMM.PVMGMTNC   12713-13327
MCoV             ...FFD.NPDWYDPVENEAIHVVYAKLGHIVANAMLKCVALCDAMVEKGYVGIITLDNQDLNGNFYDFGDFVSTI.GGCGCACVTSYYSYMM.PIMGMTSC   12392-13006
FCoV_UU2         ...FFE.NKDWFDPVENEAIHEVYARLGPIVANAMLKCVAFCDAIVEKGYIGIITLDNQDLNGNFYDFGDFVKTA.PGLGCACVTSYYSYMM.PLMGMTSC   12440-13054
IBV              HPKWFEENKDWYDPIENPKYYAMLAKMGPIVRRALLNAVEFGNLMVEKGYVGVITLDNQDLNGKIYDFGDFQKTV.PGAGVPVFDTYYSYMM.PIIAMTDA   12445-13083
BdCoV_HKU22      ...YFD.QPNWYDPVENPDWFSLISRLGPIFQRALIKVAEFCDLMVEKGYIGVVTLDNQDLNGNFYDFGDFKKVL.PGCGVPVTTSYYSYMM.PCLTACDA   12480-13091
SpCoV_HKU17      ...WFG..ENWFDPIENPSFYKEFHKLGDILNRCVLNANKFASACIDAGLVGILTPDNQDLLGQIYDFGDFIITQ.PGNGCVDLASYYSYLM.PIMSMTHM   11520-12167
MuCoV_HKU13      ...WFG..ELWYDPIENPTFYREFHKLGGVLNRCVLNANKFAEACQQAGLVGILTPDNQDLLGQIYDFGDFISTQ.PGNGCCDMSSYYSYLM.PIMSMTHM   11688-12335
CMCoV_HKU21      ...WFD..DQWFDPIENPTFYNEIHKLGVILNRCILNANKFASICRDVGLVGILTPDNQDLLGQIYDFGDFIVTQ.PGNGCVDLESYYSYLM.PLMSMTHM   11157-11804
BuCoV_HKU11      ...WFG..DSWFDPIENPTFYREFHKLGSVLNRCVLNANAFAKACSELGIVGILTPDNQDLLGQIYDFGDFIITQ.PGNGCVDLSSYYSYLM.PIMSMTHM   11448-12095
ThCoV_HKU12      ...WFD..EQWYDPIENQTFYREFHKLGSILNRCVINANTFAKACADAGLVGILTPDNQDLLGQIYDFGDFIITQ.QGNGCVDLASYYSYLM.PIMSMTHM   11499-12146
WECoV_HKU16      ...WFG..DLWYDPIENPTFYREFHKLGSILNRCVLNANAFAKAAADSGVVGILTPDNQDLLGQIYDFGDCILTE.PGNGCIDLSSYYSYLM.PIMSMTHM   11445-12092
NHCoV_HKU19      ...WFV..DGWYDPIENPTFYDEFHKLGSLINNCVVMANKFADTCKTVGLVGILTADNQDLGGQIYDFGDFVVTQ.PGNGCIEMDAYLSYIM.PSMSMTHM   11407-12057
WiCoV_HKU20      ...FFE..GVWYDPIENVHFYTEFHKLGEIINKCVLKANTFVSTVNKLGFLGVLTPDNQDLEGKIYDFGDYIQSK.S.VGCVDMESYYSYLM.PAMSLTHM   11018-11617
Torovirinae
PToV_SH1         ............EFFEDAKQRSH...LAFLLEPAILSFCDIFSYIKKFGLPYKLTLDNMDLCGNLYDFGDYPCP.....NVVDNQSALFVLA.EVWSMTRK   14139-14663
BRV-1            ............EFLDDANQRSY...LANLLEPAILSFCEIFHCVKGCQVPYKITLDNLDLKGQLYDFGDYPCP.....NKVDNQSALFVLA.EVWSMTRK   14292-14816
WBV              ............TCLDPGNYSSYY.NFKEQLRNKLASVIPNVNKILAAELPLAITLDNIDLNGFLYDFGDYPTN.....GKVTNYHVVSCMR.QIATFCSL   14666-15193
FHMNV            ............RVLDPGFYSEYH.FFKSEIRKVLTKCIPNVNIILKANLPMAITLDNIDCNGMLYDFGDYPQK.....EIPSNRHVIEAIR.QLAVFCAL   15435-15962
BPNV             ............GKPEASEQLAN..ELAQQLEPAFDNLDKLRDWCYKYDVQLPITLDNIDLDGQLYDFGDMGTS.....SH.NIDIALSDLM.RLWSLTNR   18564-19085
Arteriviridae
WPDV             ........................................GSCYVTPTVPVILKNPGDTDGCGHEWDFEELTVPR....SRVFAQDIATALRNEAGLMTIG   2889-3233
SHFV-LVR         ............................................AT..CREPLHSPGDQGIDGYLWDFEAPHSKE....AIWLSNQIISACAARRGDAPGC   6587-6937
SHFV-krtg2       ............................................AQ..YQVAQHGPGDQGIDGYLWDFEAPHSKD....LVKFSAEIIAACSARRGDAPRY   6412-6759
SHFV-krtg1       ............................................AE..HIVAQHGPGDQGIDGYLWDFEAPHSKD....LVKFSAEIIAACSARRGDAPAY   6405-6752
SHFV-krc1        ............................................AE..WQPALHGPGNTGIDGYLWDFEEPASKE....ELFLTKQIVDACALRRGDAPAC   6412-6762
PRRSV-2          ............................................ASP.KLLACHGPGNTGIDGTLWDFESEATKE....EVTLSAQIIQACDMRRGDAPEI   7350-7694
PRRSV-1          ............................................IAP.GIQPGHGAGNMGVDGSVWDFETAPTKA....ELELSKQIIQACEVRRGDAPNL   7388-7732
LDV-C            ............................................AD..LISPTHGPGNTGVHGFTWDFEAPPTDL....ELELSEQIITACSIRRGDAPSL   6902-7243
LDV-P            ............................................AE..FFPRTHGPGNTGIHRFVWDFESPPVDL....ELELSEQIITACSMRRGDAPAL   6837-7178
EAV              ........................................CVYALP.TISDFDVSPGDVAVTGERWDFESPGGGR....AKRLTADLVHAFQGFHGASYSY   5468-5839
motifs                                                             ★★★★★★★★★★★★★★★★★★★★★
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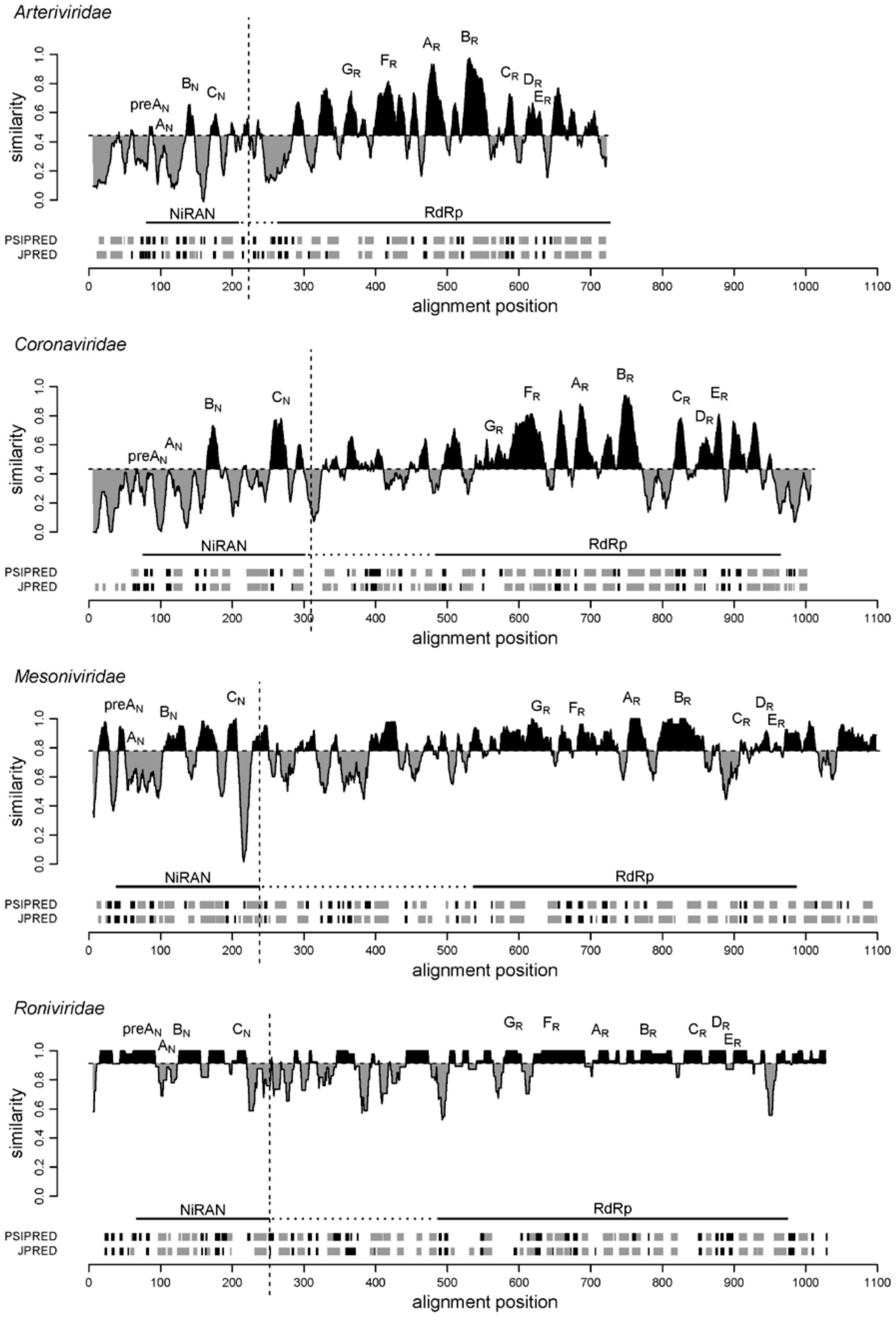
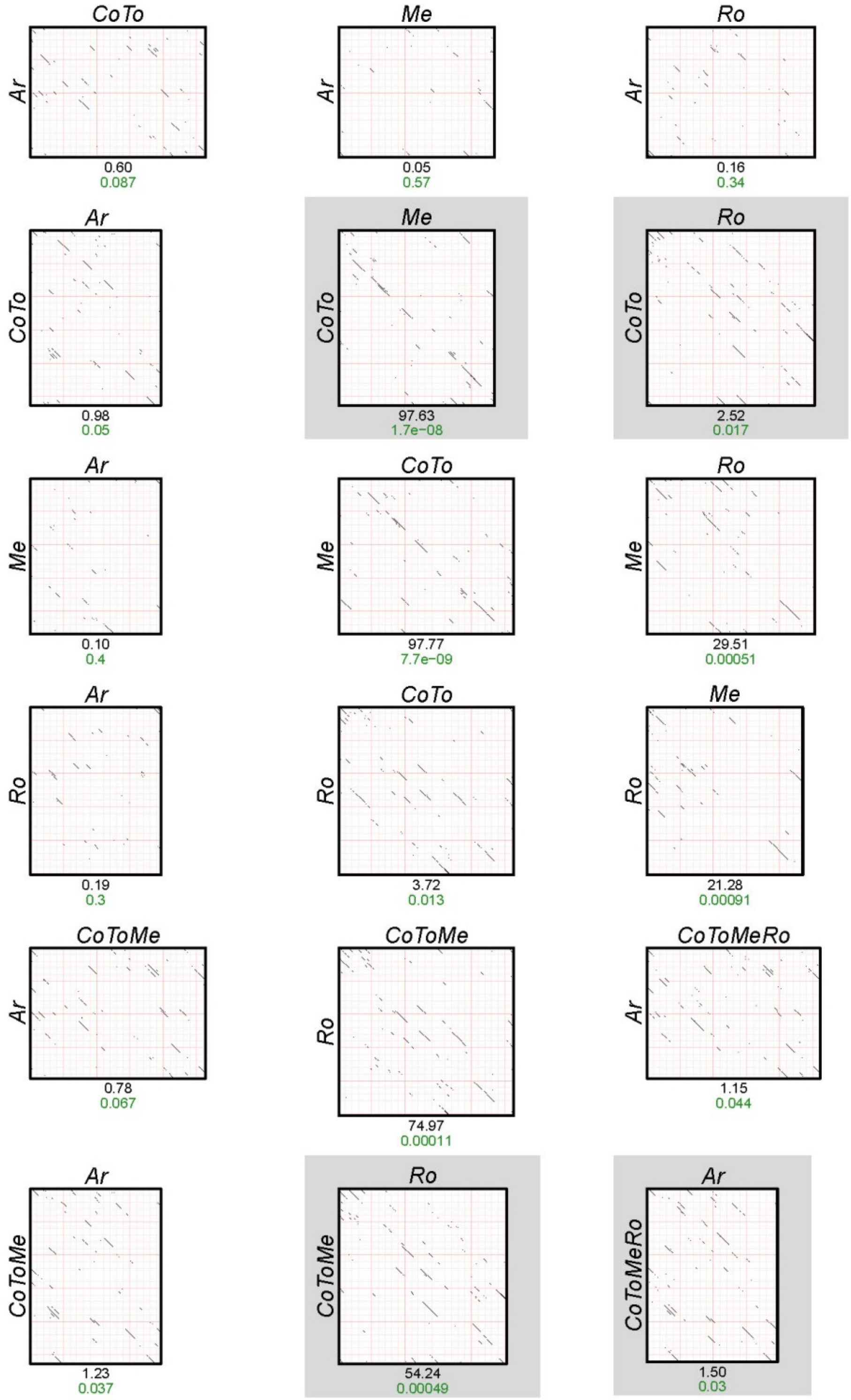
C_N

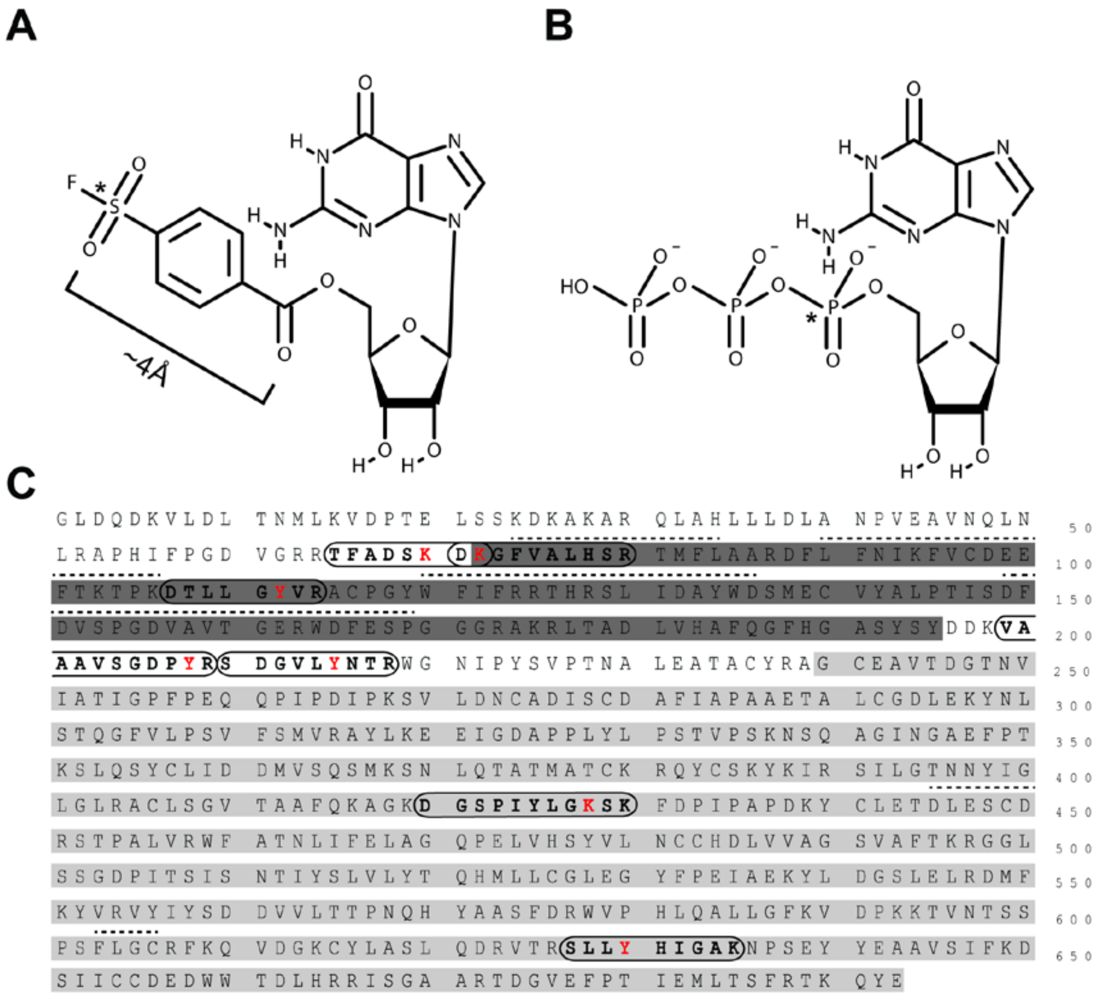
Supplementary Figure 1: The nidovirus-wide NiRAN MSA encompassing conserved motifs. Virus names and accession numbers are listed in Table S1. Fully and partially conserved residues are depicted in white font with red background and red font, respectively. Sequence motifs are indicated by stars. Secondary structure predictions are shown on the top of the MSA. The name of each prediction indicates software used (Jpred 3 or PSIPRED) and which family-specific NiRAN MSA (R, *Roniviridae*; M, *Mesoniviridae*; C, *Coronaviridae*; A, *Arteriviridae*) were used to produce it. The plot was generated with ESPript.



Supplementary Figure 2: Sequence variation, domain organization, and secondary structure of NiRAN-RdRp-containing proteins of nidovirus families. For each family, the similarity density plot obtained for the MSA of proteins including the NiRAN and RdRp domains is shown. To highlight the regional deviation of conservation from that of the MSA average, areas above and below the mean similarity are shaded in black and gray, respectively. Sequence motifs of NiRAN and RdRp are labelled. Uncertainty in respect to the domain boundary between NiRAN and RdRp is indicated by dashed horizontal lines. Domain boundaries used for all bioinformatics analyses are indicated by dashed vertical lines. Below each similarity density plot predicted secondary structure elements are presented in gray for α-helices and black for β-strands.



Supplementary Figure 3: Pairwise MSA-based HMM-HMM comparison of NiRANs of different origins. Each MSA of NiRAN was converted into an HMM profile, all possible pairs of different HMMs were aligned using HH-align. The label at the left and top of each plot specifies the group of viruses used as query and target in HMM-HMM comparison, respectively. Below each dot-plot the confidence (%) of the target being homologous to the query and the E value of the top local hit are shown in black and green, respectively. The four plots highlighted with grey background are also presented in Fig. 3.



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GLDQDKVLDL TNMLKVDPTE LSSKDKAKAR QLAHLLLDLA NPVEAVNQLN   50
LRAPHIFPGD VGRRTFADSK DKGFVALHSR TMFLAARDFL FNIKFVCDEE  100
FTKTPKDTLL GYVRACPGYW FIFRRTHRSL IDAYWDSMEC VYALPTISDF  150
DVSPGDVAVT GERWDFESPG GGRAKRLTAD LVHAFQGFHG ASYSYDDKVA  200
AAVSGDPYRS DGVLYNTRWG NIPYSVPTNA LEATACYRAG CEAVTDGTNV  250
IATIGPFPEQ QPIPDIPKSV LDNCADISCD AFIAPAAETA LCGDLEKYNL  300
STQGFVLPSV FSMVRAYLKE EIGDAPPLYL PSTVPSKNSQ AGINGAEFPT  350
KSLQSYCLID DMVSQSMKSN LQTATMATCK RQYCSKYKIR SILGTNNYIG  400
LGLRACLSGV TAAFQKAGKD GSPIYLGKSK FDPIPAPDKY CLETDLESCD  450
RSTPALVRWF ATNLIFELAG QPELVHSYVL NCCHDLVVAG SVAFTKRGGL  500
SSGDPITSIS NTIYSLVLYT QHMLLCGLEG YFPEIAEKYL DGSLELRDMF  550
KYVRVYIYSD DVVLTTPNQH YAASFDRWVP HLQALLGFKV DPKKTVNTSS  600
PSFLGCRFKQ VDGKCYLASL QDRVTRSLLY HIGAKNPSEY YEAAVSIFKD  650
SIICCDEDWW TDLHRRISGA ARTDGVEFPT IEMLTSFRTK QYE
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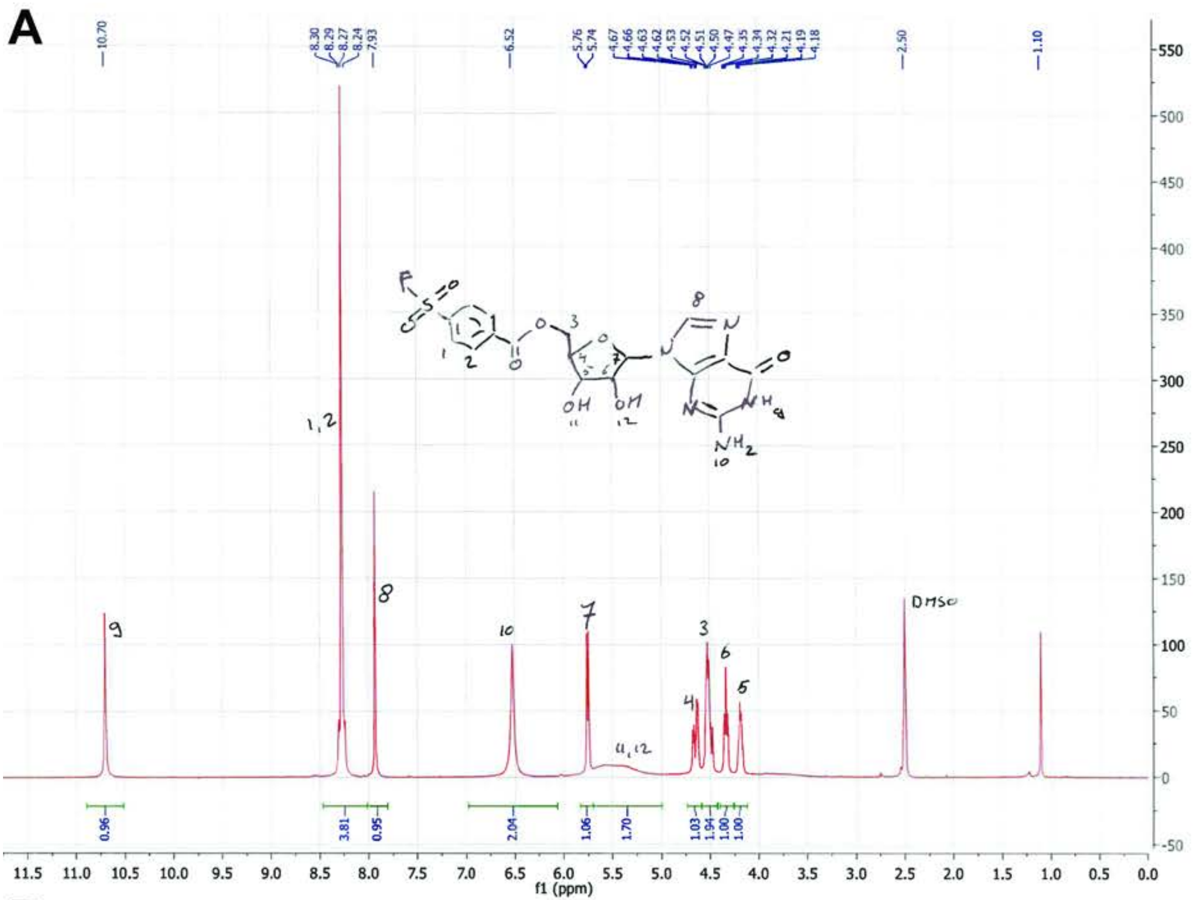
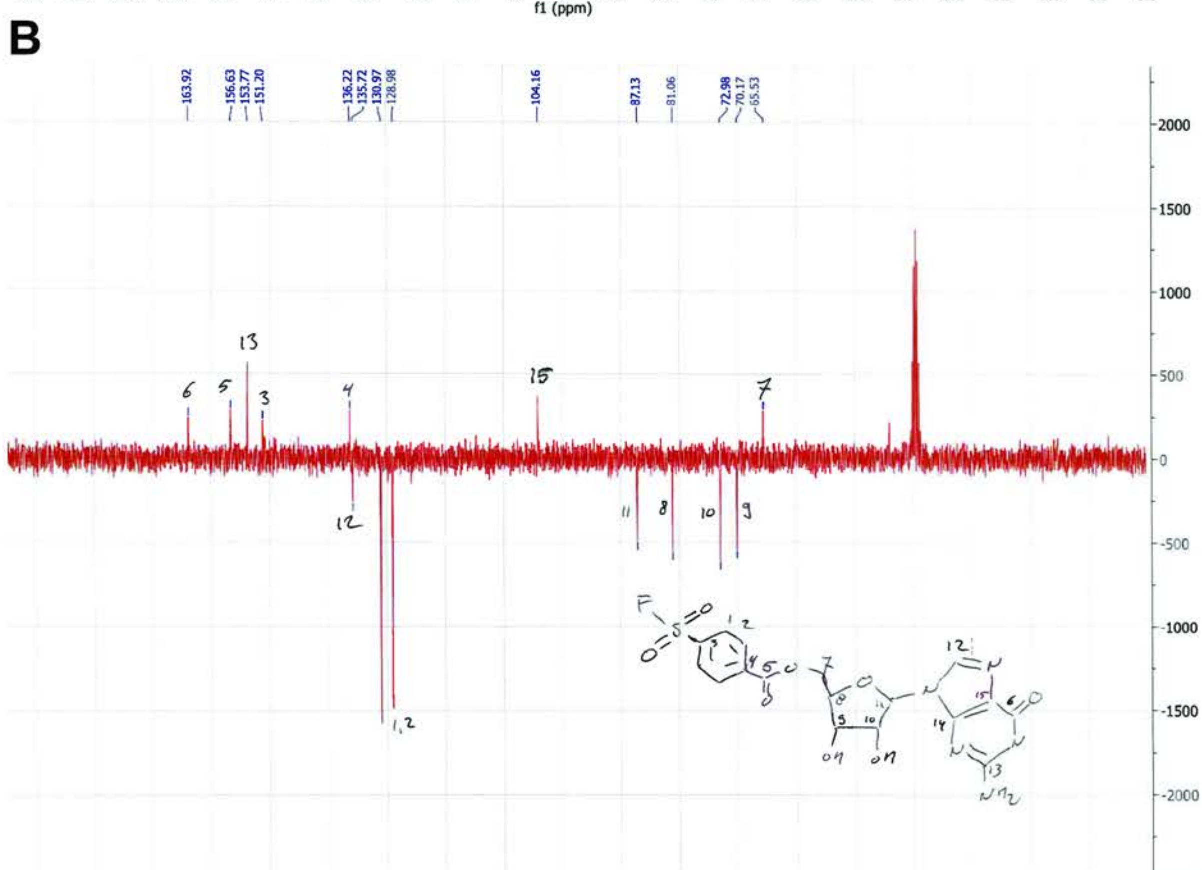
Supplementary Figure 4: (**A**) FSBG and (**B**) GTP structures indicating the spatial separation of the points of attack in FSBG and GTP. Asterisks mark the positions of the nucleophilic attack. (**C**) Mass spectrometry analysis of FSBG-linked EAV nsp9 identified seven unique, modified peptides (outlined) located either in vicinity of the NiRAN (dark gray background) or within the C-terminal RdRp domain (light gray background). Residues carrying the sulfonylbenzoyl modification are colored in red. Sequence or structural motifs are indicated by dashed lines above the sequence in the order preA$_N$, A$_N$, B$_N$, C$_N$, A$_R$, and E$_R$. See also Fig. 2A.


A
10.70
8.30
8.29
8.27
8.24
7.93
6.52
5.76
5.74
4.67
4.66
4.63
4.62
4.53
4.52
4.51
4.50
4.47
4.35
4.34
4.32
4.21
4.19
4.18
2.50
1.10
9
1,2
8
10
7
11,12
4
3
6
5
DMSO
0.96
3.81
0.95
2.04
1.06
1.70
1.03
1.94
1.00
1.00
f1 (ppm)


B
163.92
156.63
153.77
151.20
136.22
135.72
130.97
128.98
104.16
87.13
81.06
72.98
70.17
65.53
6
5
13
3
4
15
7
12
11
8
10
9
1,2
f1 (ppm)

Supplementary Figure 5: NMR analysis of 5’-(4-fluorosulfonylbenzoyl)guanosine. (**A**) ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.70 (s, 1H), 8.38 – 8.12 (m, 4H), 7.93 (s, 1H), 6.52 (broad s, 2H), 5.75 (d, *J* = 4.8 Hz, 1H), 5.75 (broad s, 2H), 4.65 (dd, *J* = 11.9, 3.6 Hz, 1H), 4.59 – 4.42 (m, 2H), 4.34 (t, *J* = 5.1 Hz, 1H), 4.25 – 4.12 (m, 1H). (**B**) ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.92, 156.63, 153.77, 151.20, 136.22, 135.72, 130.97, 128.98, 104.16, 87.13, 81.06, 72.98, 70.17, 65.53. Corresponding peaks and atoms are indicated by numbers.