Anti-Influenza Virus Agents: Synthesis and Mode of Action

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Abstract: Annual epidemics of influenza virus infection are responsible for considerable morbidity and mortality, and pandemics are much more devastating. Considerable knowledge of viral infectivity and replication has been acquired, but many details still have to be elucidated and the virus remains a challenging target for drug design and development. This review provides an overview of the antiviral drugs targeting the influenza viral replicative cycle. Included are a brief description of their chemical syntheses and biological activities. For other reviews, see References.[1–9] Med Res Rev, 28, No. 1, 1–38, 2008

Key words: influenza; synthesis; antiviral drugs; replicative cycle

1. THE VIRAL REPLICATIVE CYCLE

The replicative cycle of influenza virus offers several virus-specific events which could be considered as targets for chemotherapeutic intervention (Fig. 1).[10] From Figure 1 it is apparent that the replicative cycle of influenza virus could be interrupted at several stages, that is (i) virus attachment to the cells mediated by the interaction of hemagglutinin (HA) with its receptor at the cell surface, (ii) endocytosis and fusion of the viral envelope with the cell membrane, (iii) uncoating of the viral particles within the endosomes following penetration of H^+ ions, through the M2 matrix ion channel, into the interior of the virions, (iv) transcription of the viral (−)RNA genome to messenger (+)RNA, through the polymerase complex (PA, PB1, and PB2), (v) translation of mRNA to viral proteins, (vi) packaging and budding of progeny virus particles from the cell surface resulting in (vii) the release of these progeny virions, and allowing further spread of the virus infection. In Figure 1, the sites for chemotherapeutic intervention are highlighted for amantadine, siRNAs (short interfering RNAs) and

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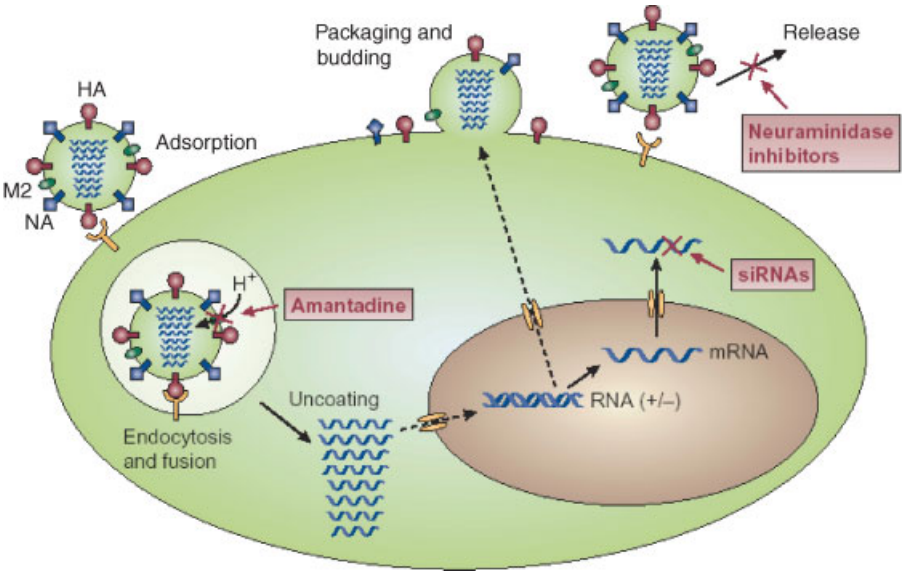


Figure 1. Inhibition of influenza virus replication cycle by antivirals. Figure taken from Ref. 10.

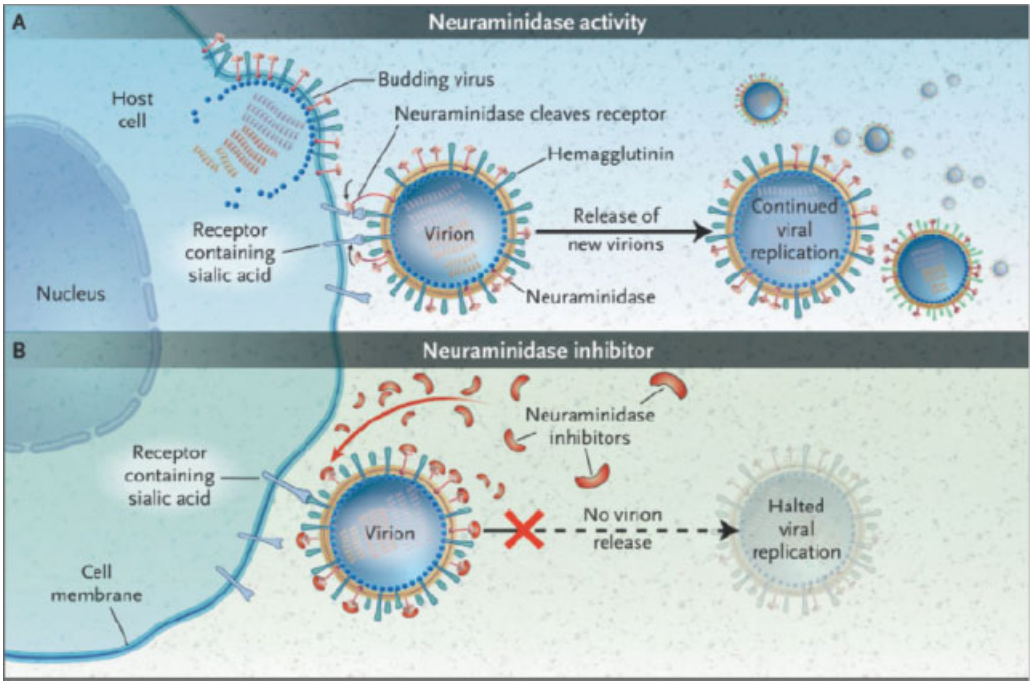


Figure 2. Mechanism of action of neuraminidase inhibitors. Figure taken from ref. 106.

neuraminidase inhibitors. Here we will review the different anti-influenza virus agents in function of their interaction with the different targets within the viral replication cycle.

2. INHIBITION OF THE HEMAGGLUTININ (HA)

Inhibition of binding HA to the epithelial cells could be an efficient means of blocking infection. The crystal structure of HA complexes with various derivatives of sialic acid has been determined.[11] Such knowledge has led to a rational design of carbohydrate-based inhibitors of HA.[12] Collectins also bind to the viral HA and probably prevent infection by interfering with the cellular binding activity of HA.[13] The most effective strategy has been to take advantage of the nature of the interaction of the virus with its receptor, which depends on the thermodynamic advantage offered by engaging in multiple simultaneous binding interactions. Whilst bivalent sialic acid ligands provide some advantage, the most potent and effective sialic acid receptor antagonists that have shown activity *in vitro* incorporate sialic acid moieties onto polymeric templates,[14–16] a strategy used advantageously by nature.[17] The structure and physical properties of the template employed to project the sialic acid residues are of importance, and hydrophobic components appear to be necessary for high affinity binding. For example, the heptasaccharide derivative **5**, a rather complex bivalent ligand, inhibits influenza virus adsorption to erythrocytes (hemagglutination) with an IC_{50} of 0.18 mM,[18] whereas the structurally simpler dimeric ligand **2** inhibits hemagglutination with an IC_{50} of 3 μM.[12]

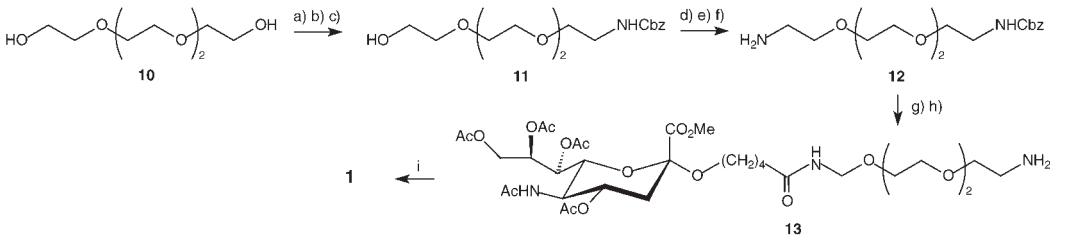
Branched, dendrite like amino acid backbones capped with sialic acid moieties afforded molecules that cluster 4, 8, or 16 residues and are effective inhibitors of hemagglutination of erythrocytes.[19] An alternative approach has focused on the incorporation of differing concentrations of the sialoside lipid **4** into liposomes, followed by irradiation-induced polymerization.[20]

Following synthetically convergent methodology, three different families of compounds of varying linker span length were generated. Peracetylation of methyl-*N*-acetyl-β-D-neuraminate[21] afforded **8**, which in turn was converted into Sialoside **7** by treatment with 5-hexen-1-ol and $Hg(CN)_2$ followed by oxidation with $KMnO_4$ (Scheme 1).

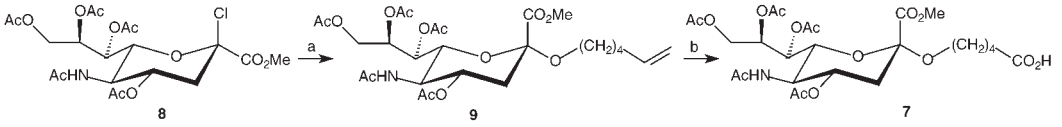
Molecules were made, for example, from ethylene glycol (Scheme 2). The synthesis of the side-chain of the **1** series is exemplified for m = 2.

Synthesis of the side-chain of the **2** series, bearing peptidic linkers-derived from glycine, is exemplified for n = 4 (Scheme 3).

Finally, also urea-based linkers containing piperazine units were investigated, the synthesis of the side-chain of the **3** series is exemplified for $P = 3$, q = 1 (Scheme 4).



Scheme 2. Synthesis of **1** (m = 2): (**a**) NaH, p-toluenesulfonyl chloride, THF (60%); (**b**) NaN_3, DMF (60%); (**c**) PPh_3, THF/H_2O; Et_3N, benzylchloroformate (60%); (**d**) Et_3N, p-toluolsulfonyl chloride, DMF (87%), (**e**) NaN_3, DMF (91%); (**f**) PPh_3, THF/H_2O (80%); (**g**) **7**, 1,1-hydroxybenzotriazole, dicyclohexylcarbodiimide, CH_2Cl_2 (66%); (**h**) Pd $(OH)_2$, H_2, EtOH, (84%); (**i**) Phenylene-1,3-diacetic acid, diphenylphosphoryl azide, DMF, $NaOH_{(aq)}$, MeOH (24%).



Scheme 1. Synthesis of Sialoside 7: (a) 5-hexen-1-ol, $Hg(CN)_2$, 3 Å sieves (70%); (b) $KMnO_4$, $AcOH_{(aq)}$ (93%).

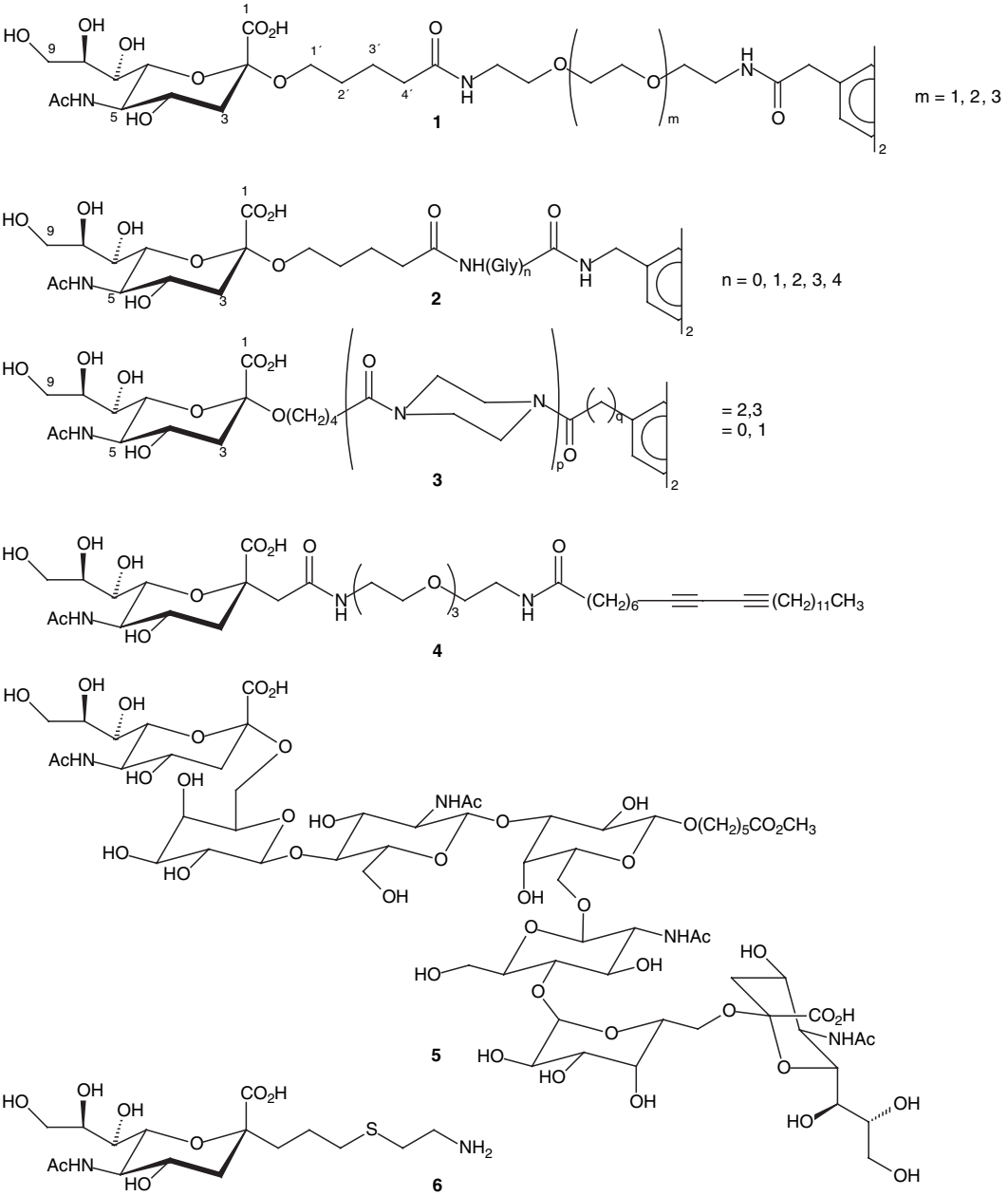
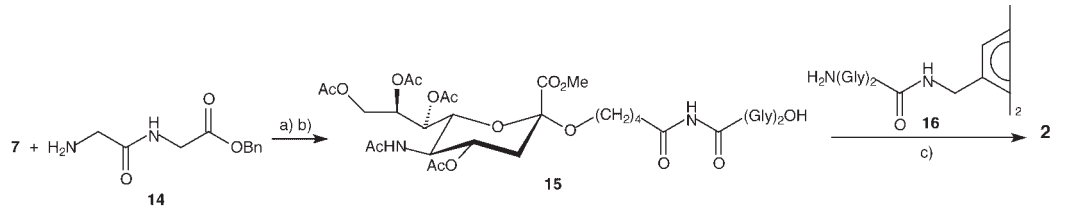
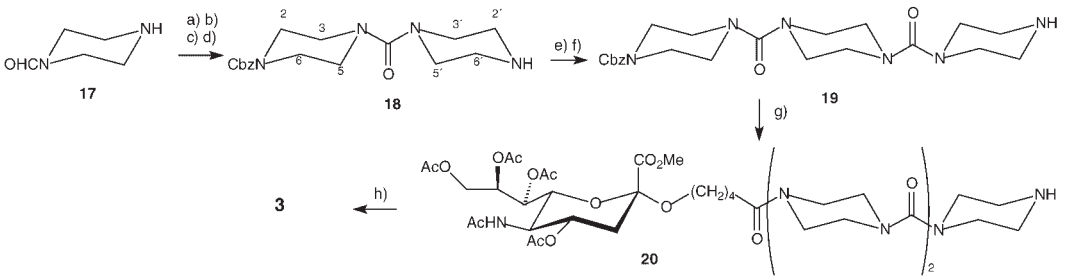


Chart 1. Inhibitors of influenza virus hemagglutinin binding to sialylated proteins.



Scheme 3. Synthesis of 2 (m = 4): (**a**) 1-Hydroxybenzotriazole, dicyclohexylcarbodiimide, *i*—Pr_2EtN (75%); (**b**) $Pd(OH)_2$, H_2, EtOH (88%); (**c**) Diphenylphosphoryl azide, Et_3N, DMF, $NaOH_{(aq)}$, MeOH (33%).



Scheme 4. Synthesis of 3 (P = 3, q = 1): (**a**) Benzylchloroformate (Et_3N, 4-dimethylaminopyridine, CH_2Cl_2 (99%); (**b**) 12M $HCl_{(aq)}$: MeOH (1: 20) (99%); (**c**) Piperazine-1-carboxaldehyde, phosgene, Et_3N (67%); (**d**) 12M $HCl_{(aq)}$: MeOH (1: 20) (87%); (e) Triphosgene, Et_3N, 1-piperazine carboxaldehyde, CH_2Cl_2 (64%); (f) 12M $HCl_{(aq)}$: MeOH (1: 20) (95%); (**g**) 1-benzotriazol-1-oxytris(dimethylamino)-phosphonium hexafluorophosphate, Et_3N, CH_3CN, $Pd(OH)_2$, H_2, MeOH (88%); (**h**) Phenylene 1,3-diacetic acid, benzotriazol-1-yl-*N*-oxytris(dimethylamino)phosphonium hexafluorophosphate, *i*-Pr_2EtN, CH_3CN, $NaOH_{(aq)}$, MeOH (3%).

3. PROTEOLYTIC CLEAVAGE OF HA

The influenza virus HA is synthesized as a single polypeptide precursor ($HA_{()}$) and is cleaved into HA_1 and HA_2 subunits by host proteases.

Proteolytic cleavage of HA at the virion surface is essential for the infectivity of all three influenza types (A, B, C)[22] and, therefore, probably vulnerable to antiviral intervention.[23,24] Proteolytic inhibitors have been shown to possess anti-influenza activity in mice.[25] In addition, pulmonary surfactant may act as an endogenous inhibitor of proteolytic activation of the influenza-A HA.[26] The protease inhibitor ϵ-aminocaproic acid (E-ACA) decreases virus replication in the lungs of influenza-challenged mice.[27] Mechanistic studies with E-ACA on proteases in mice infected with influenza virus suggest that this compound may act at least in part by inhibition of HA cleavage.[28] Influenza virus is believed to enter mammalian cells by endocytosis in clathrin-coated pits. The virus fuses with endosomal membrane through an irreversible conformational change in the HA molecule caused by a lowering of the vesicle pH by action of an ATP-dependent H^+ pump.

BMY-27709 **23** inhibits fusion of H1 and H2 influenza viruses by specifically docking in a hydrophobic pocket around the fusion peptide.[29] An initial survey of the SAR associated with the salicyclic acid element of BMY-27709 **23** indicated an essential role for the 2-hydroxyl (or 2-MeO) and revealed limited tolerance for additional substituents, except the 5-position, where lipophilic substituents such as CH_3, Cl, F, or CF_3, afforded potent antiviral agents with EC_{50}s correlating with inhibition of hemolysis. However, more polar functional groups at the 3-position, specifically CN, NO_2, and CO_2Me afforded analogs considered to be inactive.

The salicylamide derivatives **23–25** can be obtained by heating the cyclic acetonide **32**,[30] simultaneously protecting as well the acid as the phenol moiety, with the corresponding amine in toluene.[31] Alternatively, derivatives **23–25** can be prepared by condensation of the corresponding acid chloride **33** with an amine **34** with Et_3N/DMAP.[32] Further treatment with Lawesson's reagent yields the corresponding thioamides **24c** and **25c** (Scheme 5).

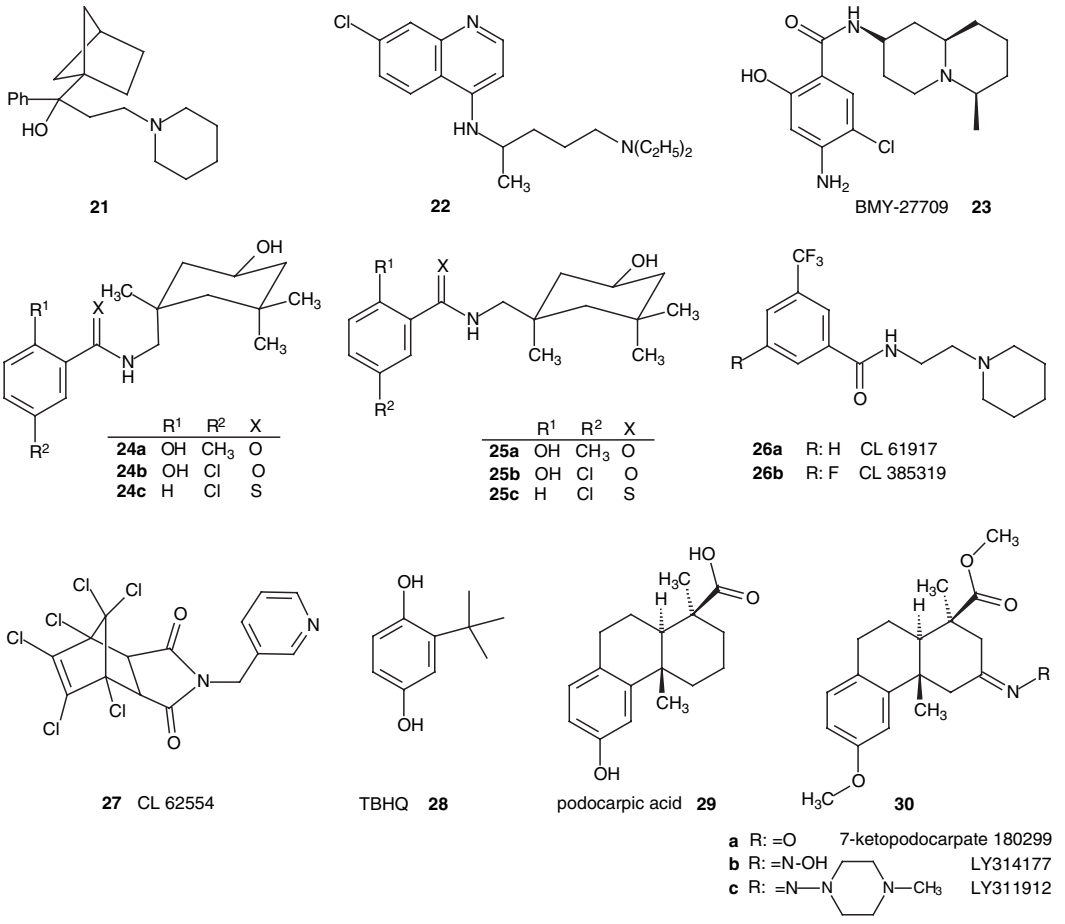
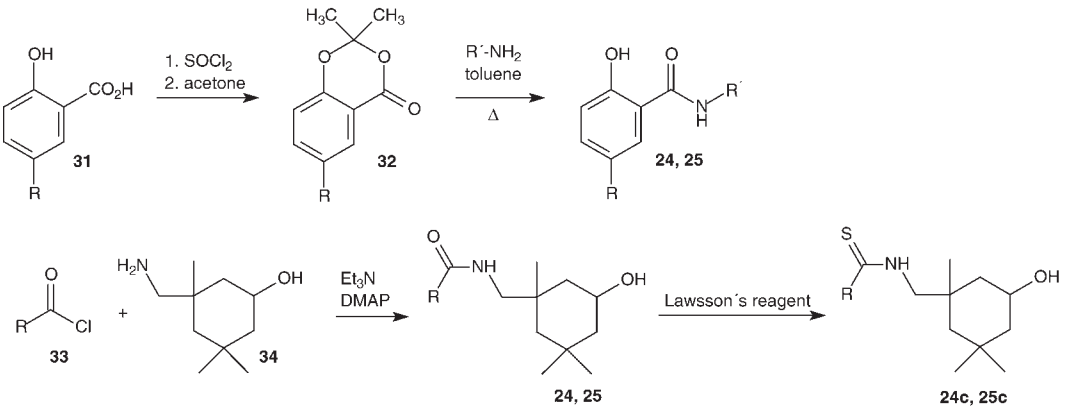


Chart 2. Structures of influenza virus fusion inhibitors.



Scheme 5. Synthesis of Compounds 24 and 25.

The mixtures of the amide diastereomers **24a/25a** and the analogous chloro derivatives **24b/25b** both exhibited EC_{50}s of 0.08 μg/mL for the inhibition of influenza infectivity in cell culture. The most potent inhibitor of these series proved to be the axial thioamide **24c**, EC_{50} 0.02 μg/mL, whilst the equatorial isomer **25c** was inactive at concentrations below the half maximally cytotoxic concentration (CC_{50} of 10 μg/mL).

Structurally similar compounds such as CL61917, CL385319 (**26**), and CL62554 (**27**) (Wyeth–Ayerst)[33] have also been found to be fusion inhibitors of the influenza A virus. These compounds were more active against H1 (IC_{50}s 0.1–12 μg/mL) and H2 (IC_{50}s 0.4–1 μg/mL) than against H3 virus (IC_{50} 24 μg/mL). Tert-butylhydroquinone **28** and its congeners were designed to block the conformational change of HA by a molecular docking programme.[34] However, the HA protein was shown to become resistant to the proteolytic actions of trypsin, thermolysin and proteinase-K after treatment with fusion inhibitors **23**, **26b**, and **28**.

Endosomal acidification can be prevented in a relatively unsophisticated fashion by agents that act as general bases to buffer the gradually declining pH. These are typically basic amine derivatives, which are only effective at concentrations too high to be considered of physiological relevance. For example, millimolar concentrations of the anti-parkinson agent norakin **21**[35] and chloroquine **22**[36] are thought to function in this fashion.

In contrast to stabilizing the HA at low pH, some compounds are able to destabilize it at neutral pH, causing inactivation by a conformational change before endosomal entry. A series of podocarpic acids interact with HA at neutral pH and prevent the low pH-induced change to the fusogenic conformation. These compounds are similarly effective against H1 and H2 influenza viruses but are less active against H3 or influenza B viruses. In mice infected with a lethal challenge of influenza A compounds LY313177 **30b** and LY311912 **30c** gave survival rates of 90% and 80%, respectively, with intraperitoneal doses of up to 200 mg/kg bid for 8 days.[37]

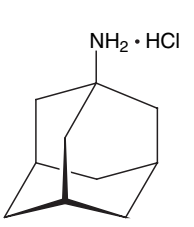
The novel antiviral protein cyanovirin-N (CV-N) was initially discovered based on its potent activity against the human immunodeficiency virus (HIV). Remarkably, however, CV-N and related homologs showed highly potent antiviral activity against almost all strains of influenza A and B virus, including clinical isolates and a neuraminidase inhibitor-resistant strain (EC_{50} = 0.004–0.04 μg/mL). When influenza virus particles were pretreated with CV-N, viral titers were lowered significantly (>1,000-fold). Further studies identified influenza virus hemagglutinin as a target for CV-N, showed that antiviral activity and hemagglutinin binding were correlated, and indicated that CV-N's interactions with hemagglutinin involved oligosaccharides.[38]

However, all of the fusion inhibitors appear to have a narrow spectrum of activity against H1, H2, or H3 influenza A viruses.

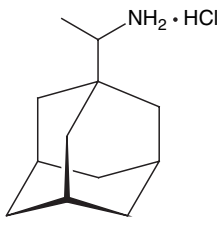
4. VIRAL UNCOATING: M2 INHIBITORS (ION CHANNEL BLOCKERS)

Determination of the molecular mechanism of action of the adamantanes has allowed rational development of additional inhibitors of the M2 ion channel.[39]

Amantadine **35** (1-adamantanamine) has been established as effective in the prophylaxis and treatment of influenza A virus infections.[40–43] Although initially licensed in 1966, the clinical use of adamantines as been limited by central nervous system (CNS) side effects.[44,45]



Amantadine **35**
(Symmetrel ® / PK-Merz ®)



Rimantadine **36**
(Flumadine ®)

Chart 3. Structures of Amantadine **35** and Rimantadine **36**.

Current interest in M2 inhibitors not only led to the development of new, highly potent compounds, but also prompted the optimization of the synthesis of amantadine **35** and rimantadine **36**. Koch-carbonylation[46] is known as the transformation from carbon monoxide with alcohols or olefins and water to carboxylic acids. However, the reaction usually has to be carried out under severe conditions such as high CO pressure, high temperature, and strong acidic conditions; therefore much attention has been focused on a convenient and environmentally benign process for this synthetic route.[47] The preparation of non-symmetrical ketons by the reaction of acyl chlorides and the corresponding Grignard reagents in the presence of catalytic amounts of metal halides has been previously described in the literature. The reaction conditions have been optimized to an efficient, cheap and fast method for the preparation of adamantyl ketons.[48]

Novel amantadine and rimantadine derivatives have been synthesized and evaluated by Kolocouris et al.[49–55]

These new adamantane derivatives were found to inhibit the replication of influenza A virus at an EC_{50} (50% antivirally effective concentration) of 1.2 μg/mL (spiro[cyclopropane-1,2′-adamantan]-2-amine, **37**),[49] 0.56 μg/mL (spiro[pyrrolidine-2,2′-adamantane, **38**),[49] 0.24 μg/mL (spiro[piperidin-2,2′-adamantane, **43**),[52] 0.6 μg/mL (2-1-adamantanyl)pyrrolidine, **47**),[52] 7.8 μM (2-(2-adamantyl)-

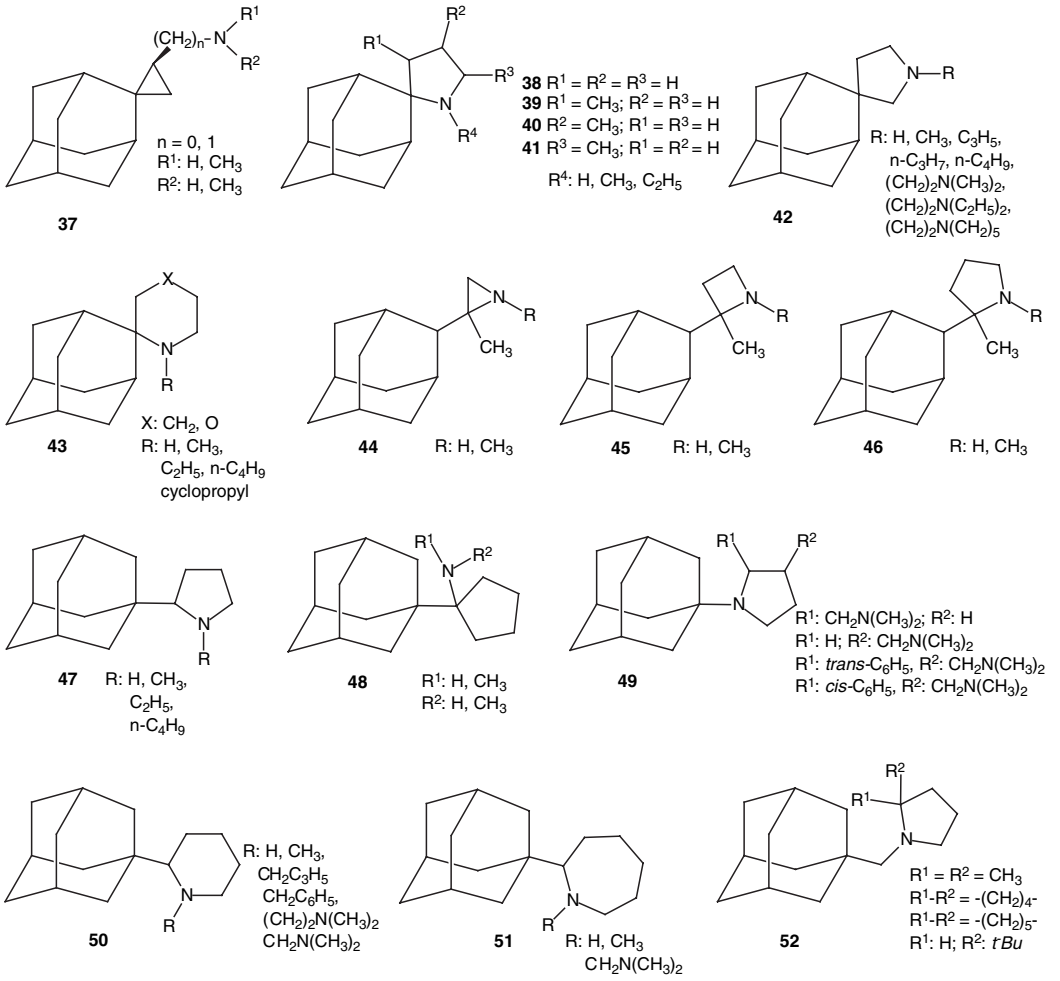


Chart 4. Overview of novel Adamantanamine Derivatives: Compounds **37**, **38**[49], **39**, **40**, **41**[50], **42**[51], **43**[52], **43**, **47**, **48**, **49, 52**[53], **44**, **45**, **46**[54], **47**, **50**, **51**[55].

piperidine),[51] 0.60 μM (3-(2-adamantyl)pyrrolidine, **46**),[50] 3.3 μM (2-(1-adamantyl)piperidine, **50**),[49] and 1.56 μM (2-(1-adamantyl)-2-methylpyrrolidine).[53] In all cases, these EC_{50} values compared favorably to that of amantadine **35** when evaluated under the same experimental conditions.

Bananins[56] (Chart 4) show a close structure relationship to the previously discussed adamantane derivatives. They represent a class of antiviral compounds with a unique structural signature incorporating a trioxa-adamantane moiety covalently bound to a pyridoxal derivative. The bananins were synthesized by the reaction of phloroglucinol (most likely in its triketo tautomeric form) with aromatic aldehydes, catalyzed by hydrochloric acid or sodium hydroxide in aqueous solution.[57] Generally, acidic catalysis was used due to the degradation of pyridoxal under highly basic conditions. Alkaline catalysis was used for reaction with aromatic aldehydes such as vanillin to obtain Vanillinbananin (VBN) **55**. Bananin synthesis is driven by the creation of the highly symmetric trioxa-adamantane-triol (TAT) cage system. The prototypical compound of the TAT series, the vitamin B6-derived bananin (BAN) **53** can be iodinated with subsequent oxidation to iodobananin (IBN) **54**. The iodine in IBN can be replaced by various substituents as exemplified by the synthesis of adeninobananin (ADN) **56**, using an activated adenine nucleobase derivative. Interestingly, BAN **53** is susceptible to Michael addition with the natural product eugenol. This NaOH-catalyzed addition leads to eubananin (EUB) **57**, which can be transformed by cyclic hemiketal condensation into the *ansa*-compound ansabananin (ABN) **58**, inspired by ansamycins such as rifamycin and geldanamycin.[58]

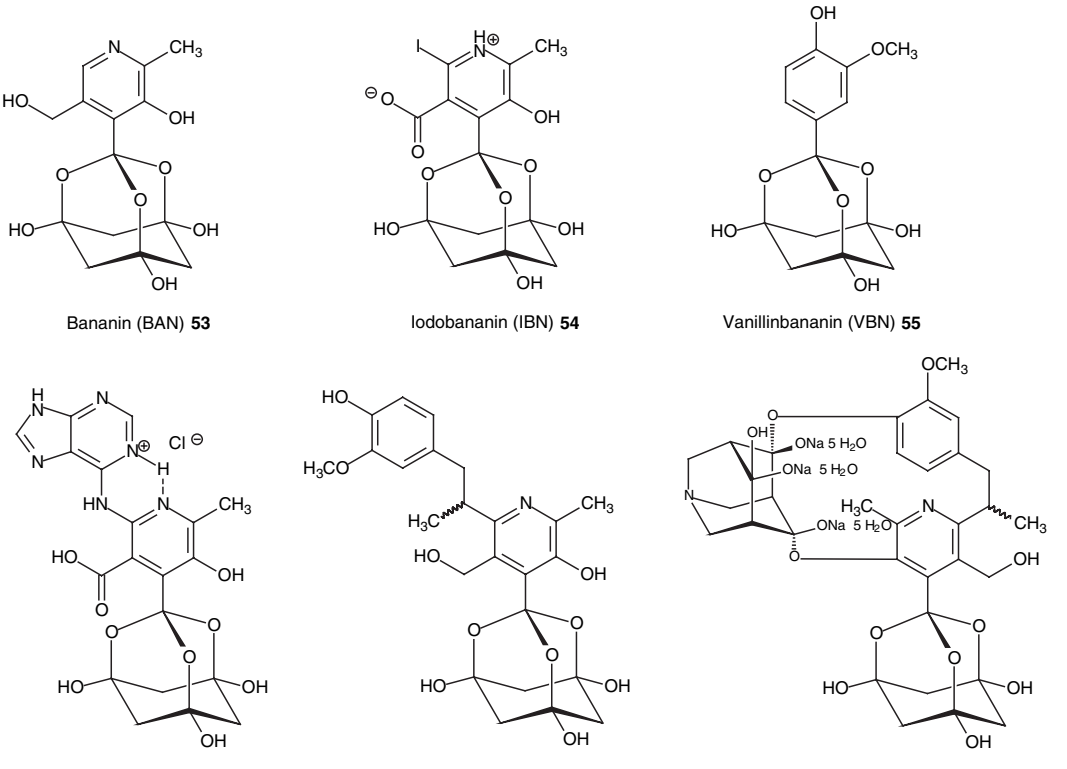


Chart 5. Adamantane-derived Inhibitors of the Helicase Activities and Replication of SARS Coronavirus: Bananin (BAN) **53**, Iodobananin (IBN) **54**, Vanillinbananin (VBN) **55**, Adeninobananin (ADN) **56**, Eubananin (EUB) **57**, Ansabananin (ABN) **58**.

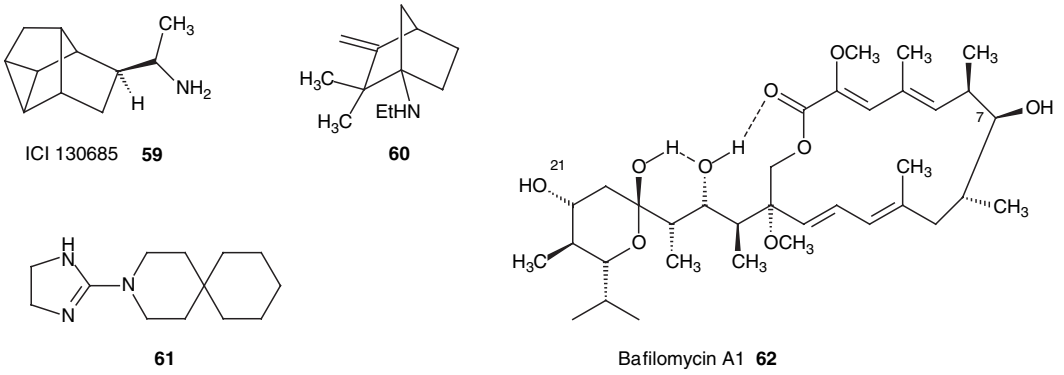


Chart 6. Non-Adamantane M2 channel inhibitors.

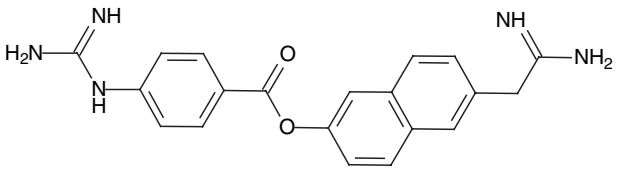
The parent compound bananin **53** inhibited the ATPase activity of the SARS coronavirus helicase with an $^{ATPase}IC_{50}$ value of 2.3 μM. Iodobananin **54** and vanillinbananin **55** exhibited the strongest inhibition, with $^{ATPase}IC_{50}$ values of 0.54 and 0.68 μM, respectively. Inhibition by vanillinbananin **55** indicates that the presence of a six-membered nitrogen heterocycle is not absolutely essential for inhibitory activity. Eubananin **57** showed similar inhibitory activity as bananin itself with an $^{ATPase}IC_{50}$ of 2.8 μM. Interestingly, ansabananin **58** was a weak inhibitor, with an $^{ATPase}IC_{50}$ of 51 μM, while adeninobananin **56** did not show any inhibitory activity at all.

Newer agents related to M2 function (Chart 6) include norbornylamine **60**[59], a novel spiro-compound—BL-1743 (**61**) and bafilomycin A1 (**62**), a macrolide antibiotic. ICI 130685 **59**[60], has advanced into clinical trials but was not approved for clinical use Chart 6.

BL-1743 (**61**)[61] was found to block the M2 channel when expressed in *Xenopus oocytes*.[62] However, cross-resistance with amantadine occurs with this compound. Bafilomycin A1 (**62**) is a specific inhibitor of vacuolar-type H^+ ATPase which completely abolishes the acidified cell components (endosomes and lysosomes) in influenza A and B virus-infected, and uninfected, Madin–Darby canine kidney (MDCK) cells.[63] The action of both of these compounds, unlike amantadin **35**, is reversible.[64]

5. INHIBITORS OF VIRUS-CELL FUSION

There are several opportunities for therapeutic intervention to disrupt the carefully orchestrated role of HA in influenza infectivity. One strategy that has been explored is inhibition of the cellular enzymes responsible for the essential proteolytic cleavage of HA, an important determinant of pathogenicity and cell tropism.[65] However, progress in this area has been confounded by the multitude of cellular and extracellular trypsin-like enzymes capable of performing this step,[66–68] including bacterial proteases, which as co-infectants can potentiate



Futhan **63**

Chart 7. Inhibitor of influenza hemagglutinin processing.

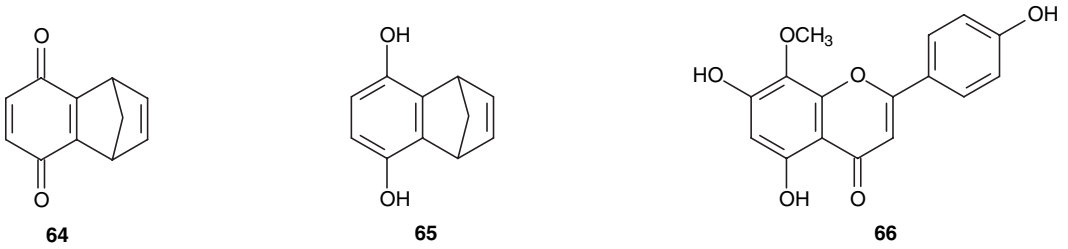


Chart 8. Inhibitors of influenza virus fusion with the cells.

influenza virus infections. Nevertheless, both the trypsin inhibitor futhan **63** and an anti-cathepsin B IgG antibody have been shown to possess influenza-inhibitory activity in an *in vitro* setting.[69,70]

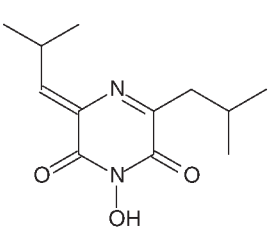
The quinone **64** was identified as a specific inhibitor of the fusion-inducing conformational change ($IC_{50} = 250$ μM); the hydroquinone form **65**[71] proved to be more potent, with an IC_{50} of 25 μM. Although the chemical properties of **64** and **65** make them unlikely drug candidates, these compounds represent the first of a new mechanistic class of influenza virus inhibitors that demonstrate the feasibility of preventing the conformational change of HA with small molecules of limited complexity. More recently, the flavone **66**, which contains some structural elements in common with **64** and **65**, has also been postulated to interfere with the fusion of influenza viral and endosomal membrane.[72] Furthermore, **66** has been reported to be a non-competitive inhibitor of influenza virus sialidase ($IC_{50} = 55$ μM).[73]

6. INHIBITORS OF VIRAL RNA TRANSCRIPTION (VIRAL RNA POLYMERASE)

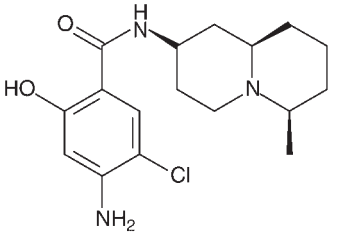
The host cell transcriptional machinery is used by the influenza virus polymerase complex during viral RNA transcription for capping, cap-methylation and splicing of viral mRNAs. The polymerase transcription complex of influenza viruses consists of a trimeric structure. This complex consists of PB1 (transcriptase), PB2 (cap-binding protein and possibly endonuclease) and PA (essential cofactor of unknown function). Association of the polymerase with RNA template, which harbors essential and influenza virus-specific sequence motifs, and with the nucleoprotein (NP, RNA binding protein) is required for full transcriptase activity.[74] The polymerase of influenza viruses contains an endonuclease activity that cuts the host cell mRNA at approximately 10–16 nucleotides from the 5′-cap. It then uses the capped oligonucleotides as primers for the generation of capped, viral mRNA. The endonuclease is a unique and highly conserved target present in all influenza viruses.

Divalent metal ions are required for enzymatic catalysis of the endonuclease reaction[75] and a number of potential metal ion chelating compounds have been described for this target. Flutimide **67** (isolated from *Delitschia confertaspora*) selectively inhibits the cap-dependent transcriptase of influenza A and B viruses. In MDCK cells, flutimide **67** inhibited influenza virus replication with an IC_{50} of 5.1 μM and exhibited no cytotoxictiy at concentrations up to 100 μM.[76]

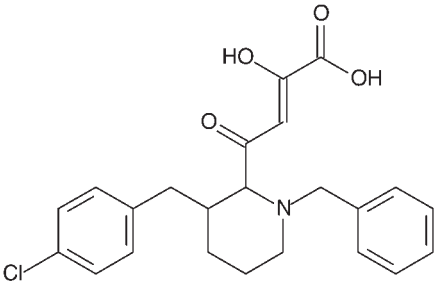
Flutimide **67** is a fully substituted 1-hydroxy-3*H*-pyrazin-2,6-dione containing the following crucial groups: an *N*-hydroxyimide, an imine and an exocyclic enamine with Z-geometry.[77] The endocyclic imine may help in the stabilization of the exocyclic enamine via extended conjugation. The synthetic pathway to obtain this compound with these unusual structural features is outlined in Scheme 6. The *N*-alkylation of (*S*)-Leu-OMe.HCl **71** at 60°C with equimolar amounts of α-bromo-*tert*-butyl acetate in CH_3-CN in the presence of diisopropylethylamine (DIPEA) gave the corresponding *N*-alkylated derivative **72**. After protection of the amino function with



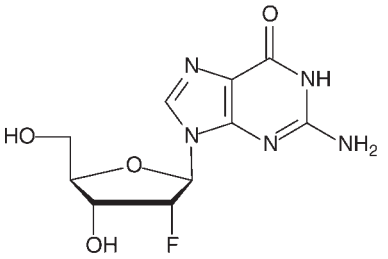
Flutimide **67**



BMY-27709 **68**

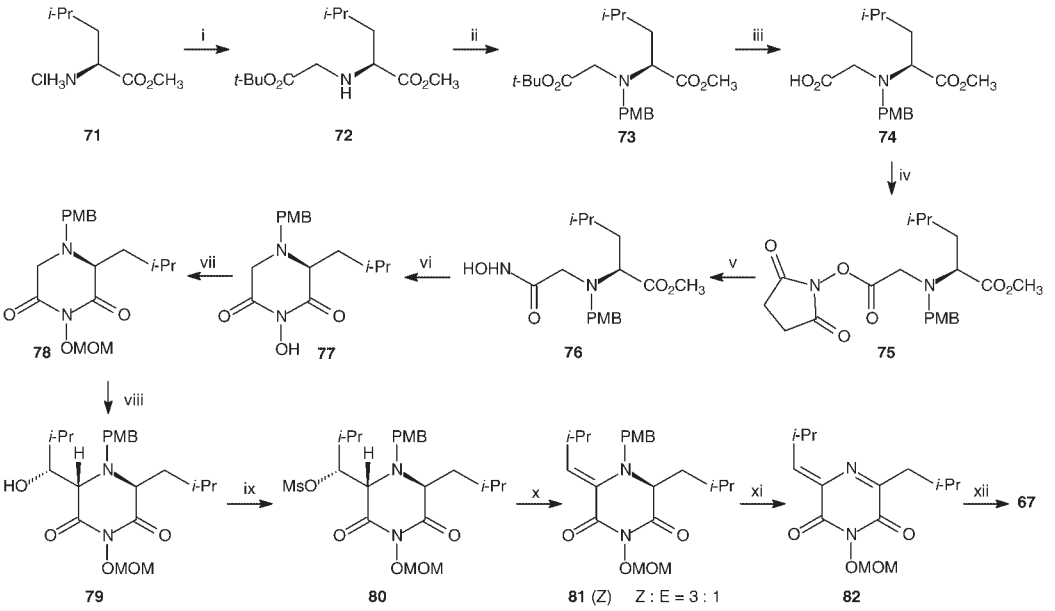


L-735,882 **69**



2-FDG **70**

Chart 9. Structures of inhibitors of influenza virus replication: Flutimide (**67**), BMY-26270 (**68**), L-735,882 (**69**), 2′-deoxy-2′-fluoro-guanosine (2-FDG) (**70**).



Scheme 6. Synthesis Flutimide **67**: i. CH_3CN, DIPEA, t-BuO_2CCH_2Br; ii. CH_3CN, DIPEA, PMB-Cl 90–97% both steps; iii. TFA, CH_2Cl_2, 94%; iv. CH_2Cl_2, TEA, DCC, *N*-hydroxysuccimide, v. EtOH, NH_2OH, vi. EtOH, H_2O, 80–100°C; vii. MOMCl, DIPEA 80% over all steps. viii. LHMDS, THF-HMPA, i-Pr-CHO, 63%; ix. CH_2Cl_2, MsCl, TEA, quant. x. DBU, toluene, 0°C to r.t. 74% Z:E = 3:1; xi. CH_2Cl_2-H_2O, DDQ, 30%; xii. CH_2Cl_2, TFA 12.5%.

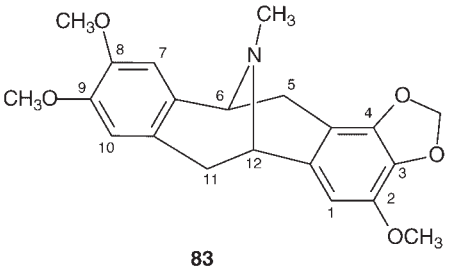
p-methoxybenzyl (PMB) chloride, the *tert*-butyl protecting group of the corresponding ester **73** was selectively hydrolyzed with TFA. The acid group of **74** was activated with *N*-hydroxysuccinimide to produce the active ester **75**, which was then reacted with neutralized hydroxylamine to give compound **76**, which subsequently could be cyclized to **77**. The *N*-hydroxy group of **77** was conveniently protected with MOM chloride to afford MOM ether **78.**

Aldol condensation of **68** with isobutyraldehyde and lithium hexamethyldisilazide (LHMDS) at −78°C exclusively produced the 3*S*,5*S*,11*R*-stereo-isomer of flutimide advanced hydroxy intermediate **79**. The aldol product **79** was reacted with methanesulfonyl chloride (MsCl), DIPEA, and DMAP at −23°C to give in quantitative yield the corresponding mesylate **80**. The β-elimination reactions turned out to be challenging; finally, reaction of mesylate **80** at 0°C with 3 equiv of DBU in toluene gave 74% combined yield of a 3:1 *Z*/*E* mixture of olefins **81a** and **81b**, respectively. The oxidation of **81a** with DDQ in a mixture of CH_2Cl_2–H_2O (2:1) gave the expected oxidized product **82** in moderate yield, due to decomposition of the product, presumably caused by hydration and a subsequent cascade of rearrangement reactions. Finally deprotection with TFA/CH_2Cl_2 gave Flutimide **67**.

BMY-26270 (**68**) selectively inhibits the endonuclease activity of influenza A and B viruses.[78] L735,882 (**69**) is an example of a series of 4-substituted 2,4-dioxobutanoic acid endonuclease inhibitors. This compound showed antiviral activity in cell culture against influenza A and B viruses with IC_{50}s of 6 and 2 μM respectively, and no apparent cytotoxic effects in MDCK cells at concentrations up to 100 μM.[79] Other members of this series showed antiviral activity with IC_{50}s of 0.18 μM and higher.[80]

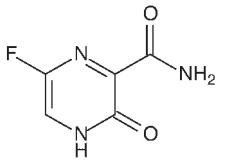
The nucleoside analog 2′-deoxyfluoroguanosine (2-FDG) **70** appears to be a specific inhibitor of influenza transcriptase activity that targets the active site of the polymerase subunit PB1. The triphosphate of 2-FDG **70** showed competitive inhibition with GTP *in vitro*, and incorporation of this nucleoside analog in place of GTP by the influenza virus polymerase leads to chain termination during transcription initiation.[81] The series of various substituted 2′-deoxy-2′-fluororibosides have been synthesized by enzymic pentosyl transfer from 2′-deoxy-2′-fluorouridine.[81] Different strains of influenza A and B viruses were sensitive to 2-FDG with IC_{50}'s ranging from 0.2–1 μM in chicken embryo fibroblast cells and from 2 to 22 μM in MDCK cells.[82] The mean pulmonary viral titer of influenza A and B in mice was significantly reduced after intraperitoneal treatment with 2-FDG (120 mg/kg up to 24 hr post-infection) as compared with untreated controls. This compound also appeared to be more effective than amantadine **35** (against influenza A) and ribavirin **36** (against influenza B).[83] However, both 2-FDG **70** and a pro-drug (2,6-diaminopurine-2′-fluororibonucleoside) have not progressed beyond preclinical development.

Extracts and products of plant origin provide an alternative source for substances with virus-inhibitory activity.[84,85] The pavine alkaloid (−)thalimonine (THI) **83**, isolated from from the Mongolean plant *Thalictrum simplex L.* inhibited markedly the replication of influenza virus A/Germany/27, strain Weybridge (H7N7) and A/Germany/34/strain Rostock (H7N1) in cell cultures of



83

Chart 10. Structure of (−)Thalimonine (3,4-methylene-deoxy-2,8,9-trimethoxypavinan THI) **83**, an alkaloid showing anti-influenza activity.



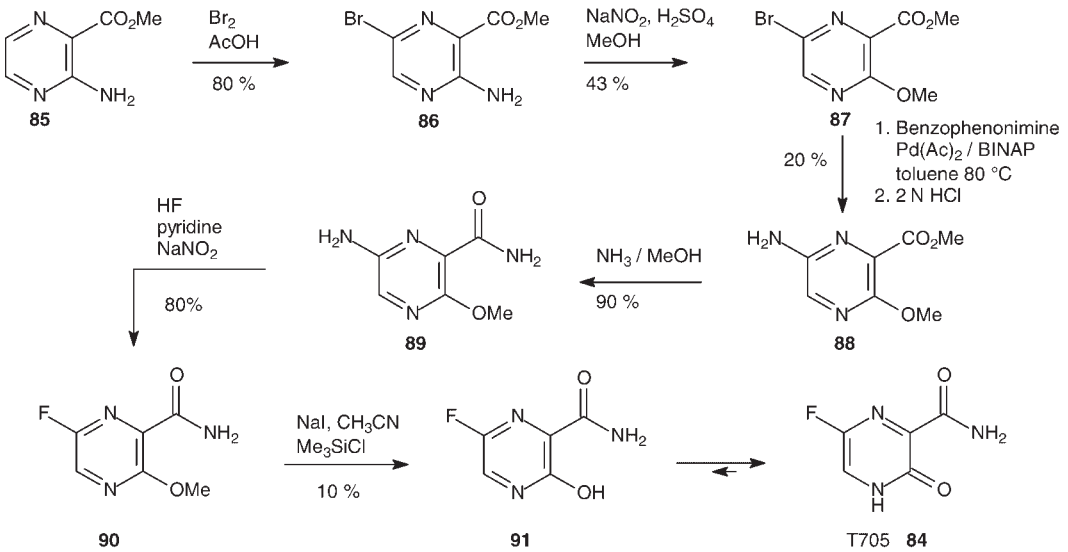
T705 **84**

Chart 11. Structure of T-705 (**84**).

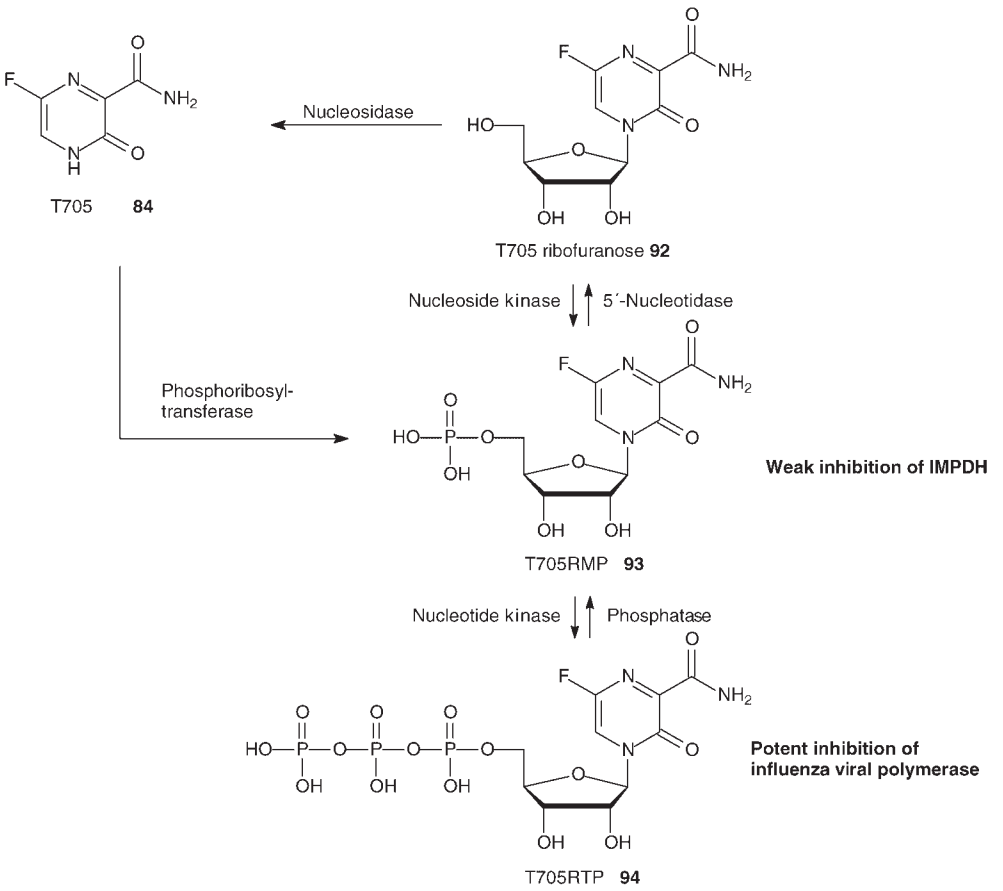
chicken embryo fibroblasts.[86] 0.1–0.64 μM of the alkaloid inhibited the viral replication in a selective and specific way (SI = 640 and 106.6, respectively). Expression of viral glycoproteins hemagglutinen (HA), neuraminidase (NA), and nucleoprotein (NP) on the surface of infected cells, as well as virus-induced cytopathic effects, infectious virus yields, HA production and virus-specific protein synthesis were all reduced. The inhibition was dose-related and dependent on the virus inoculum. Time of addition experiments indicated that viral replication was markedly inhibited when **83** was added 4–5 hr post infection. No inactivating effect on extracellular virus was observed.

T-705 (**84**),[87] a substituted pyrazine compound, has been found to exhibit potent anti-influenza virus activity *in vitro* (IC_{50} = 1.0 μM (CC_{50} >6,370 μM) against influenza A PR/8/34 virus) and *in vivo*.[88]

Synthesis[89–91] of T-705 (**84**) starts from 3-amino-2-pyrazinecarboxamide (**85**). Following bromination to **86**, the 3-amino function is exchanged to a methoxy function. Compound **87** is suitable to undergo a Palladium catalyzed amination reaction (Hartwig–Buchwald reaction[92,93]) with benzophenonimine yielding **88** after subsequent deprotection. Amide **89** is obtained by treatment with MeOH/NH_3 and can be transformed into the fluoro derivative **90** by reaction with sodium nitrite/hydrogen fluoride in pyridine. Deprotection of the methoxy group with sodium iodine/trimethylsilyl chloride in acetonitrile derives **91**, which immediately tautomerizes to the blue-fluorescent (364 nm) compound T705 (**84**).



Scheme 7. Synthesis of T-705 (**84**).



Scheme 8. Hypothetic mechanism of action of T-705 (**84**). T-705 (**84**) may be converted to T-705 ribofuranosyl phosphates **93** (monophosphate) and **94** (diphosphate) by host cell enzymes.

Although T705 (**84**) can hardly be considered a purine nucleoside base, it has been purported that cellular enzymes recognize T705 (**84**) as nucleoside base and convert it to the phosphorylated metabolites **93** and **94**.[94]

Structural similarity with known IMPDH inhibitors (discussed in Section 7) suggested the transformation into the monophosphate **93**, leading to a reduction of the GTP pool size in infected cells. However, inhibition of IMPDH by T-705RMP (**93**) was about 150-fold lower than that by ribavirin (**100**) monophosphate and it became apparent that the antiviral effect of T-705 (**84**) is not a result of the inhibition of IMPDH, indicating that the mode of action of T-705 (**84**) is not the same as that of ribavirin (**100**). Moreover, T-705 (**84**) is converted into T-705RMP (**93**) and T-705RTP (**94**) by cellular kinases. T-705RTP (**94**) is recognized as a natural purine nucleotide by influenza virus polymerase whereas the host cell enzymes can discriminate T-705 (**84**), T-705RMP (**93**), and T-705RTP (**94**) from the natural nucleosides/nucleotides.

The 4,2-bisheterocycle tandem derivatives **95** show modest activity against influenza A virus H3N2 (A3 China/15/90) with IC_{50} values in the range of 29–37 μg/mL.[95] In Chart 12 the most active compounds are depicted. Very recently poly-substituted acylthiourea **96** and its fused heterocycle **97** have been reported, showing IC_{50}s < 0.1 μM against influenza A3/Beijing/30/95/(H1N1) and therefore representing a novel class of highly potent and selective inhibitors of influenza virus,[96] the mechanism of action still remaining to be elucidated.

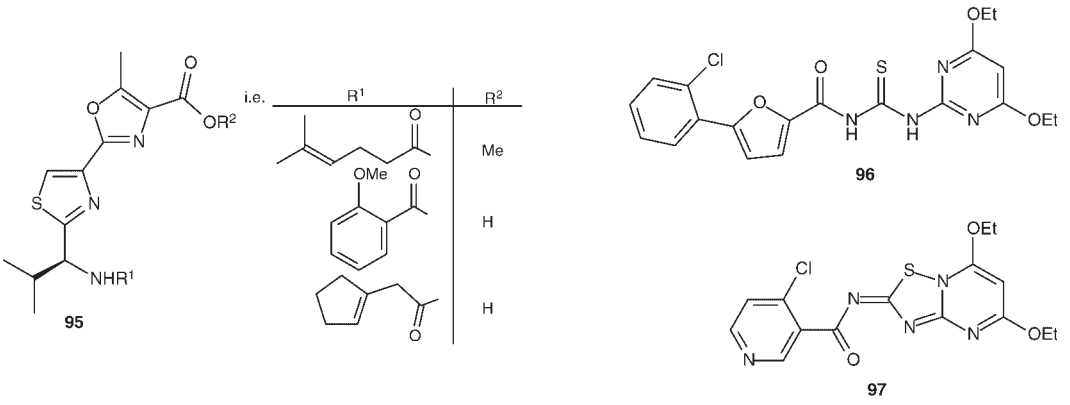
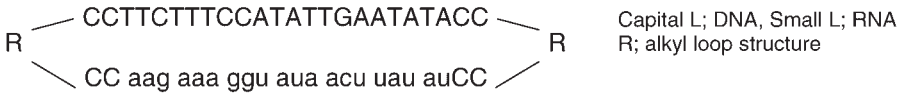


Chart 12. New heterocyclic compounds **95**, **96**, and **97** showing anti-influenza activity.



A phosphorothioate antisense DNA for PB2 of the influenza virus is paired with a sense RNA and on both edges of the double strand are connected with CC-R-CCs, which form the loop structure

Chart 13. A dumbbell structure of antisense oligonucleotide **98** showing anti influenza activity.

Another interesting report of transcription inhibitors is of antisense oligonucleotides targeted at the PB2 genome. An antisense nucleotide that was stabilized by chimera formation between DNA and RNA and had a dumbbell structure on both ends of the nucleotide with cytosine and alkyl loops was synthesized.

This antisense oligonucleotide complementary to influenza viral RNA polymerase components were administered intravenously, in liposome-encapsulated form, to mice, and were shown to significantly prolong survival after infection with the influenza A virus.[97,98]

DNA enzymes targeting the PB2 mRNA translation initiation (AUG) region of the influenza A virus (A/PR/8/34) have been designed. The modified DNA enzymes have one or two N3P-P5P phosphoramidate bonds at both the 3′- and 5′termini of the oligonucleotides, which significantly enhanced their nuclease-resistance. These modified DNA enzymes had the same cleavage activity as the unmodified DNA enzymes, determined by kinetic analyses, and reduced influenza A virus replication by more than 99%, determined by plaque formation. These DNA enzymes are highly specific since their protective effect was not observed in influenza B virus (B/Ibaraki)-infected MDCK cells.[99]

7. IMPDH INHIBITORS

Inhibition of inosine monophosphate dehydrogenase (IMPDH; E.C.1.1.1.250) results in decrease in the intracellular proof of GTP, required for the synthesis of nucleic acids. This mechanism of action accounts for the anti-influenza activity of LY217896 (**99**). Compound **99** is active against several influenza A and B viruses in MDCK cells with IC_{50} values of 0.37–1.19 and 0.75–1.54 μg/mL.[100] In addition, this compound protected mice against influenza A and B infection when it was administered orally immediately, or several days, after the experimental infection. However, the development of **99** has been discontinued because of a lack of clinical efficacy and increased patient serum uric acid levels.[101]

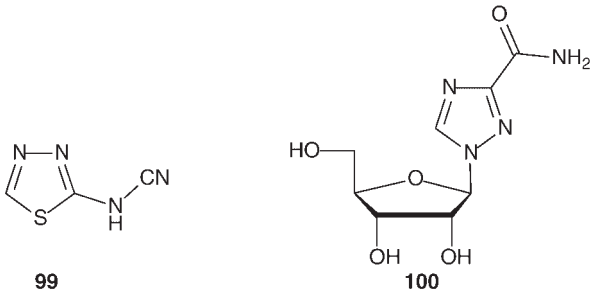


Chart 14. Structures of anti-influenza virus IMPH inhibitors: LY217896 **99** (Amitivir), Ribavirin **100**.

Ribavirin (Virazole) **100**, a synthetic guanosine derivative, is a broad spectrum antiviral that is active against several RNA virus families. Several mechanisms of action have been proposed for the anti-influenza virus activity[102–104] of ribavirin **100** among which IMPH inhibition (see above), inhibition of the mRNA 5′-cap formation and inhibition of the virus-coded RNA polymerases that are necessary to initiate and elongate viral mRNAs.[105]

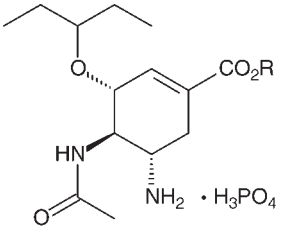
8. NEURAMINIDASE (NA) INHIBITORS

Neuraminidase inhibitors interact with a unique (albeit very late) target in the viral replicative cycle, that is the release of the progeny virus particles from the cells (Fig. 2).[106] Release of the virus particles from the cells requires the action of the virus-associated neuraminidase which cleaves off the terminal *N*-acetylneuraminic acid (sialic acid) (linked through an $\alpha 2>6$ or $\alpha 2>3$ bond with galactose in the influenza A H3N2 and H5N1 receptor, respectively). This cleavage is needed for the virus particles to be released from the infected cells and allows the virus to spread to other cells. Neuraminidase inhibitors prevent this release and thus ''trap'' the newly formed virus particles at the cell surface, thereby inhibiting further virus spread.

A. Oseltamivir

Oseltamivir **101**, the highly water soluble phosphate salt of the trisubstituted cyclohexene ethylcarboxylate, has been claimed as ''molecule of the month'' in December 2005.[107] However, chemistry still remains a challenge. Since the synthesis has been excessively reviewed before,[108–111] only the latest developments will be documented.

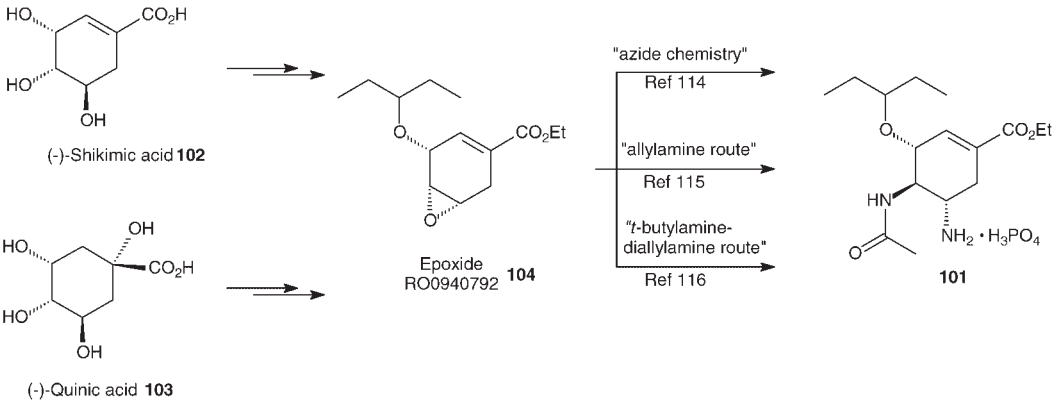
Originally the synthesis started from (−)-shikimic acid **102**, isolated from *Illicium verum* (chinese star anise) or (−)-quininic acid **103**, isolated from the African Cinchona Tree.[112,113] Epoxide RO0640792 **104** can be seen as key intermediate, which can be converted to oseltamivir phosphate using azide-chemistry,[114] an allylamine route[115] or a *t*-butylamine-diallylamine route.[116] The latter would allow a safe scale-up for industrial purposes.



101 R = Et
Oseltamivir
GS4104
Tamiflu ®

101a R = H
Oseltamivir carboxylate
GS4071

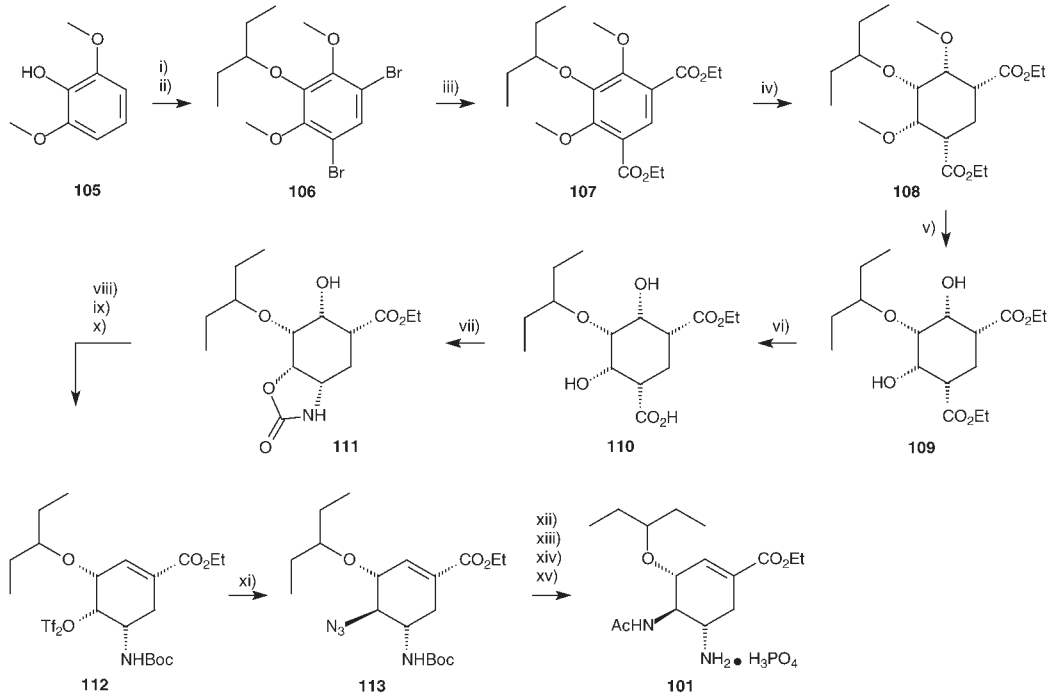
Chart 15. Structure of the NA inhibitor Oseltamivir **101** and Oseltamivir carboxylate **101a**.



Scheme 9. Synthesis of Oseltamivir **101** starting from natural sources.

Due to availability problems of the natural sources in case of a pandemic much effort was paid to cheaper, easier available syntheses.[117]

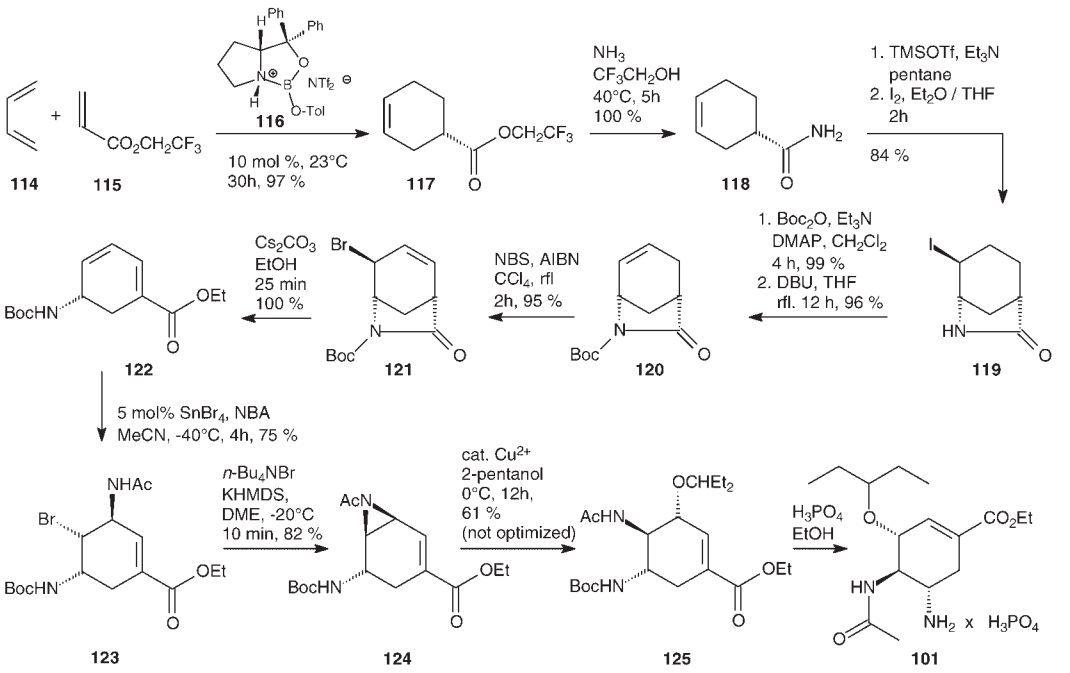
The desymmetrization protocol[118] outlined in Scheme 10 is based on an enzymatic monohydrolysis of an all-*cis meso* diester **109** to the optically active mono-acid **110.** Starting from inexpensive 1,6-dimethoxy phenol **105** the 3-pentyl ether is introduced followed by bromination to the dibromide **106**. The Pd-catalyzed double ethoxycarbonylation furnished the isophalic diester **107**, which was hydrogenated over Ru-Alox to the all-*cis meso*-diester **108** with high selectivity and



Scheme 10. Synthesis of oseltamivir **101** using a desymmetrization concept. (i) $MsOCHEt_2$, KO*t*-Bu, dmso, 50°C, (ii) NBS, DMF, 0°C (90% both steps); (iii) CO (10 bar) 0.5% $Pd(OAc)_2$, dppp, KOAc, EtOH, 110°C, 20 hr, (95%); (iv) H_2 (100 bar) 5% Ru-Alox, EtOAc, 60°C, 24 hr (82%); (v) TMSCl, NaI, MeCN, 40°C (97%); (vi) desymmetrisation PLE, H_2O, pH 8.0 (96%, ee96–98%); (vii) DPPA, NEt_3, CH_2Cl_2, 40°C, (81%); (viii) $(Boc)_2O$, DMAP, cat. rt. (ix) NaH cat. toluene, rfl., (x) Tf_2O, pyridine, CH_2Cl_2, –10°C (83% three steps); (xi) NaN_3, rt. acetone, H_2O, (78%); (xii) H_2, Ra-Co (or Bu_3P/H_2O); (xiii) Ac_2O, Et_3N, (xiv) HBr-AcOH; (xv) H_3PO_4, EtOH (83% 4 steps).

yield. Nearly quantitative and highly selective cleavage of the methyl ether groups of **108** using *in situ* generated TMS-iodide provided the *meso*-dihydroxy intermediate **109**, ready for the enzymatic desymmetrization. Using pig liver esterase (PLE) at slightly elevated temperature (35°C) afforded the desired (S)-(+)-monoacid **110** in high yield and selectivity. Using Yamada–Curtius degradation[119] of **110**, the 5-amino-functionality is introduced, resulting in the formation of the oxazolidinone **111**. Following Boc-protection compound **112** is obtained by heating the mixture in toluene with traces of sodium hydride. By this way an effective and selective formation of the 1,2-double bond with simultaneous cleavage of the oxazolidinone system is achieved. TfO is used to introduce a good leaving group. The 4-amino functionality was finally introduced via S_N2-substitution of **113**, using sodium azide with concomitant inversion of configuration under mild alkaline conditions. Azide reduction, *N*-acetylation, Boc-deprotection and the phosphate salt formation gave the final product **101** in 30% overall yield.

Very recently, a new concept to obtain Oseltamivir **101** by way of a Diels-Alder approach has been reported (Scheme 11).[120] Starting from butadiene **114** and trifluoroethyl acrylate **115** in the presence of the S-proline-derived catalyst **116**, Diels-Alder reaction was carried out to obtain **117** in excellent yield.[121] Ammonolysis of **117** produced amide **118** quantitatively. Iodolactamization of **118** using the Knapp protocol[122] generated lactam **119** in high yield. Following *N*-protection with Boc_2O dehydroiodination occurred cleanly to give **120**, which was allylically brominated using *N*-bromosuccinimide to generate **121**. Treatment of **121** with cesium carbonate quantitatively afforded the diene ethyl ester **122**. A novel $SnBr_4$ catalyzed bromoacetimidation reaction, which was completely regioselective and stereoselective, converted the diene **122** into the bromodiamide **123**. Treatment with *in situ* generated tetra-*n*-butyl-ammonium hexamethyldisilazane furnished the *N*-acetylaziridine **124**, which could be regioselctively converted into the ether **125** by reaction with 3-pentanol and cupric triflate catalysis (not optimized). Finally, removal of the protecting groups afforded oseltamivir **101**.



Scheme 11. Synthesis of oseltamivir **101** by Diels-Alder reaction.

B. Zanamivir

The synthetic strategy[123] to prepare Zanamivir **126**[124,125] originally started from *N*-acetylneuraminic acid **127** (Scheme 12).[126,127] Following protection of the acid function peracetylation gave **128**[128]. Lewis acid-catalyzed elimination and an intramolecular cyclization reaction yielded the 3a,7a-dihydro-4*H*-pyrano[3,4-*d*][1,3]oxazole derivative **129** which, in turn, achieved upon treatment with trimethylsilylazide the azide **130**. Deprotection, followed by reduction of the azide function yielded 4-Amino-Neu5Ac2en **131**, which could be converted into the desired guanidine derivative **126**.[129]

A more recent approach to zanamivir **126** is reported starting from D-glucono-δ-lactone **132** (Scheme 13).[130] Following reaction of D-glucono-δ-lactone **132** with 2,2-diethoxypropane in acetone and methanol with a catalytic amount of p-TsOH the free alcohol function was benzylated to obtain the ester **133**.[131] The corresponding alcohol **134** was obtained by reduction of ester **133** with $LiAlH_4$. Subsequent treatment with Dess–Martin periodinane gave aldehyde **135** which was further converted into the imine **136**. With the help of a highly selective *syn* addition of allylmagnesium bromide the amine **137** was obtained which further was *N*-acetylated and simultaneously debenzylated. Finally, a good leaving group at the alcohol function was introduced to obtain **138**. **138** could be converted into the aziridine key intermediate **139** by reaction with NaH and THF. Using

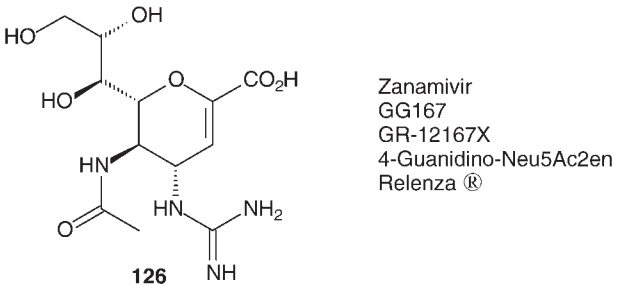
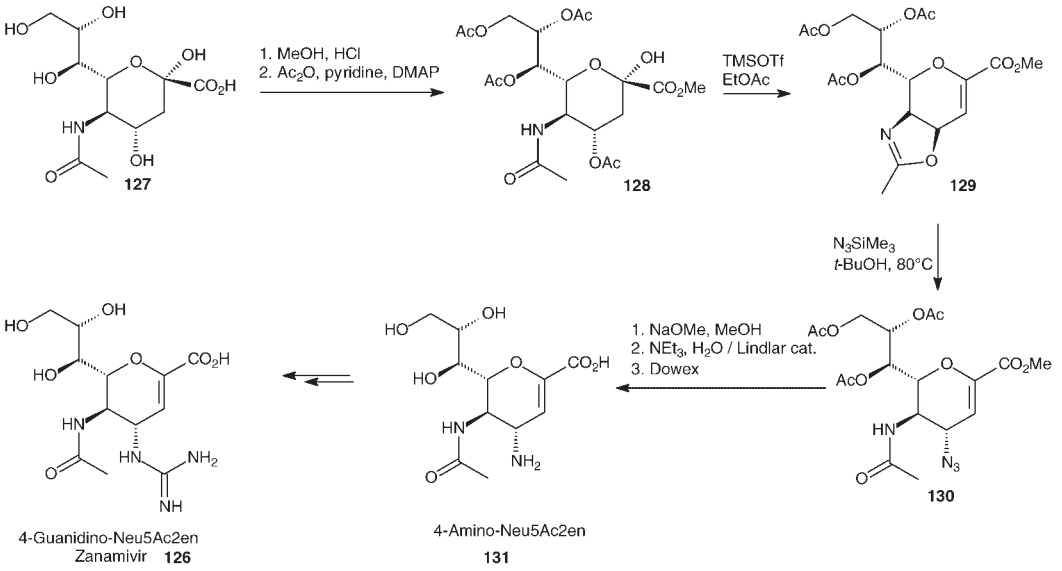
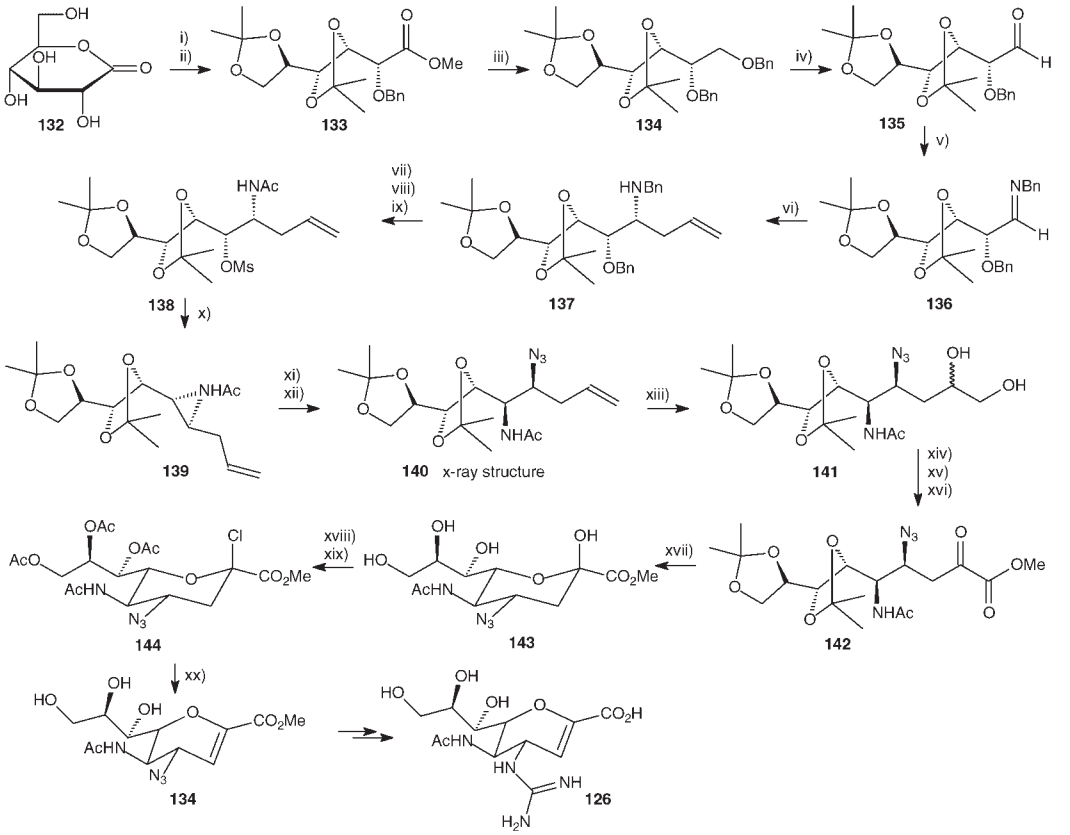


Chart 16. Structure of the NA inhibitor Zanamivir **126**.



Scheme 12. Synthesis of zanamivir **126** starting from *N*-acetylneuraminic acid (NANA) **127**.

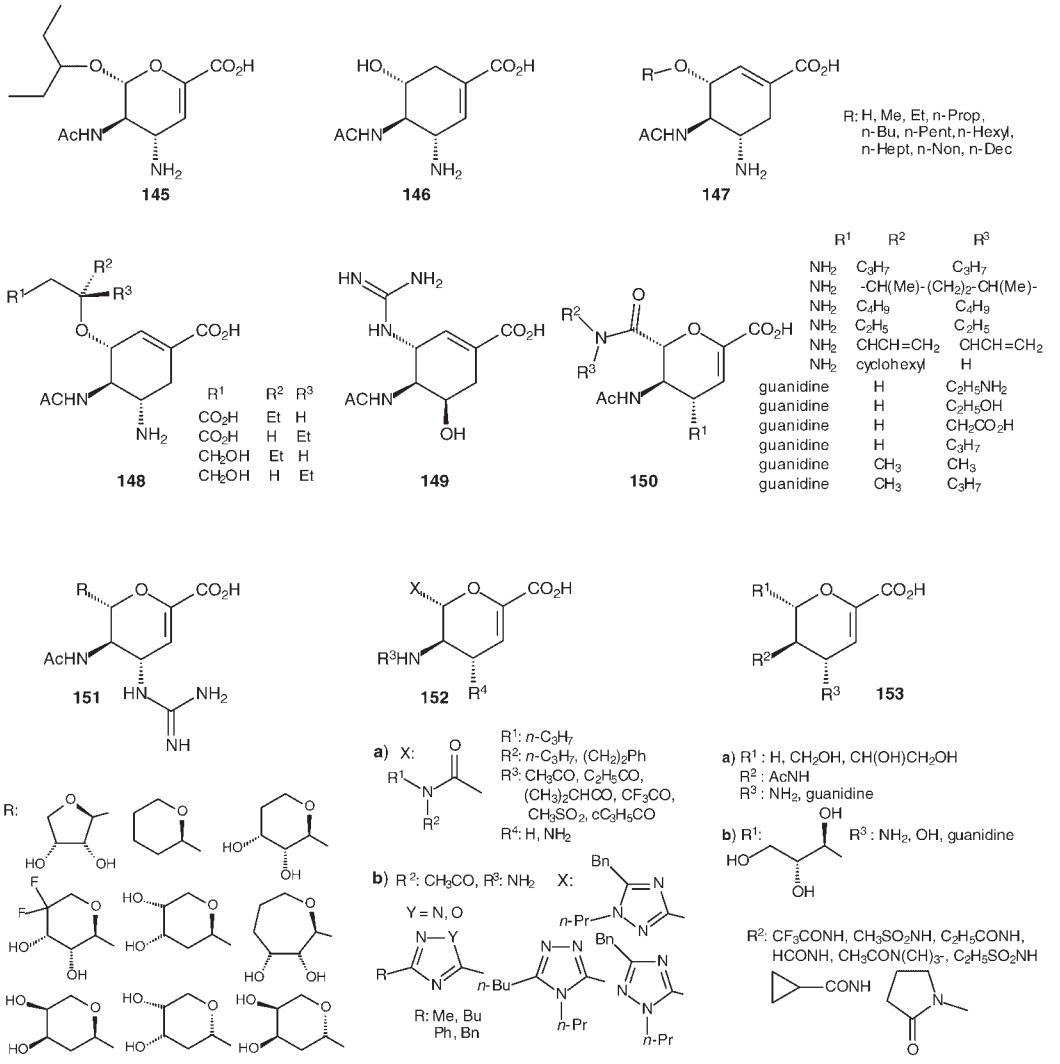


Scheme 13. Synthesis of zanamivir **126** from D-glucono-δ-lactone **132.** (i) TsOH, acetone, DMOP, MeOH 83%; (ii) Ag_2O, BnBr, CH_2Cl_2, 74%; (iii) LAH, THF, 87%; (iv) Dess–Martin periodinan CH_2Cl_2 83%; (v) $MgSO_4$, 4A mol. sieves, $BnNH_2$, THF, 100%; (vi) allylMgBr, Et_2O, 0–25°C, 56%; (vii) Ac_2O, Et_3N, CH_2Cl_2, 88%; (viii) Li, NH_3, THF, 82%; (ix) MsCl, Et_3N, CH_2Cl_2, 84%; (x) NaH, THF, 87%; (xi) NaN_3, NH_4Cl, EtOH, H_2O rfl. 62%; (xii) Ac_2O, Et_3N, DMAP, CH_2Cl_2, 88%; (xiii) cat. OsO_4, NMO, acetone, H_2O, 96%; (xiv) KBr, TEMPO, TBAB, $Ca(ClO)_2$; (xv) MeI, K_2CO_3, DMF, 80% both steps; (xvi) Dess–Martin periodinane CH_2Cl_2; (xvii) 40% HF in MeCN (v/v = 1:19) 52% (both steps); (xviii) Ac_2O, pyridine 65%; xix) HCl (g), CH_2Cl_2, 76%; (xx) DBU, CH_2Cl_2, 97%.

NaN_3 in EtOH-water in the presence of NH_4Cl allowed to open the aziridine ring regioselectively at the less hindered position, and the free amino function was acetylated to obtain **140** (structure confirmed by X-ray). The terminal olefin in **140** was efficiently dihydroxylated by catalytic OsO_4 in the presence of NMO in acetone-water to obtain the diol **141**. The primary hydroxyl group of diol **141** was selectively oxidized under TEMPO base conditions[132], the resulting acid was immediately converted into the corresponding ester, whereas the remaining secondary alcohol was oxidized to the α-ketocarboxylic acid methyl ester **142**. Treatment with HF in MeCN[133] afforded the sugar derivative **143**, which, in turn, was fully acetylated followed by replacement of the α-acetoxyl group with chlorine to obtain **144**.[134] DBU-induced elimination reaction afforded **134**, the known azide intermediate in the previously described synthesis of zanamivir **126**.

C. Sialic Acid-Based NA Inhibitors Other than Zanamivir and Oseltamivir

The family of ulosonic acids has provided potential therapeutic leads in developing inhibitors of corresponding enzymes. Zanamivir (**126**) is a transition state analog of 2,3-didehydro-2-deoxy-*N*-acetylneuraminic acid (Neu5Ac2en), which exhibits high inhibitory activity to influenza neuraminidase (NA) and has been approved for the treatment of influenza. The syntheses of these



R1	R2	R3
NH_2	C_3H_7	C_3H_7
NH_2	-CH(Me)-$(CH_2)_2$-CH(Me)-	
NH_2	C_4H_9	C_4H_9
NH_2	C_2H_5	C_2H_5
NH_2	CHCH=CH_2	CHCH=CH_2
NH_2	cyclohexyl	H
guanidine	H	$C_2H_5NH_2$
guanidine	H	C_2H_5OH
guanidine	H	CH_2CO_2H
guanidine	H	C_3H_7
guanidine	CH_3	CH_3
guanidine	CH_3	C_3H_7

R1	R2	R3
CO_2H	Et	H
CO_2H	H	Et
CH_2OH	Et	H
CH_2OH	H	Et

Chart 17. Modifications of oseltamivir **101** and zanamivir **126**.

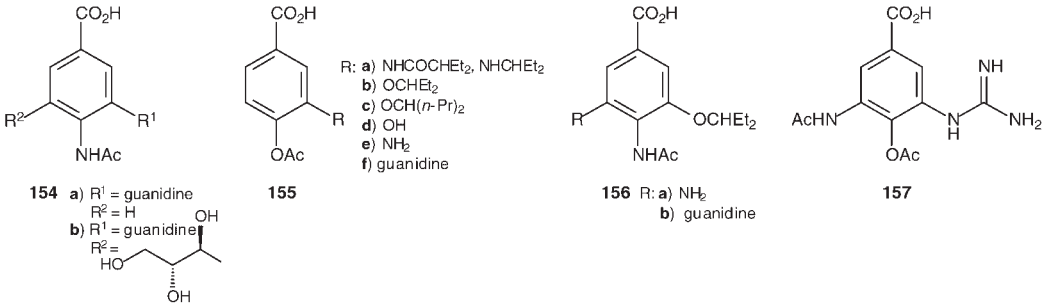


Chart 18. Aromatic sialic acid analogs **154–157**.

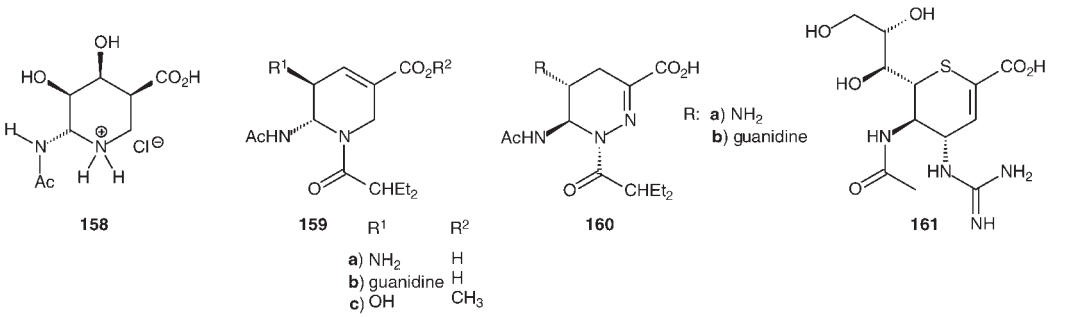


Chart 19. *N* and *S* containing sialic acid analogs **158–161**.

ulosonic acids themselves and their analogs have attracted considerable interest in recent years, and become a current research topic. The synthesis of these compounds has previously been reviewed.[111,135,136]

Compound **145** is a kind of hybride compound between oseltamivir **101** and zanamivir **126** with the heterocyclic core of **126** showing the substitution pattern of **101**. Also, **145** shows good inhibitory activity with marked selectivity for influenza A sialidase.[137] Compounds **146**,[138] **147**,[139] **148**[140] and **149**[141] were designed as carbocyclic sialic acid analogs closely related to oseltamivir **101**. Compounds **150**,[142] **151**,[143] **152**,[144,145] and **153**[146,147] can be seen as analogs of zanamivir **126**.

Compounds **155** and **156** were synthesized and evaluated for their properties as influenza neuraminidase inhibitors.[148] Benzoic acid derivatives **154** and **157**[149] were designed as aromatic sialic acid analogs.[150] The most potent compound out of a large series of tetra substituted benzoic acid derivatives **156**[151] tested *in vitro* were **154a** and **154b**, showing an IC_{50} of 2.5×10^{-6} and $>10^{-4}$ M, respectively, against N9 neuraminidase.

Compound **156b** was highly selective for type A (H2N2) influenza NA (IC_{50}: 1 μM) over type B (B/Lee/40) influenza NA (IC_{50} 500 μM). The X-ray structure of 4-(*N*-acetylamino)-5-guanidino-3-(3-pentyloxy)benzoic acid **156b** in complex with NA revealed that the lipophilic side chain binds to a newly created hydrophobic pocket formed by the movement of Glu 278 to interact with Arg 226, whereas the guanidine of **156b** interacts with a negatively charged pocket created by Asp 152, Glu 120 and Glu 229.

Siastatin B **158** is a broad spectrum sialidase inhibitor isolated from a *Streptomyces* culture and characterized as an unusual 6-acetamido-3-piperidinecarboxylate. The charge distribution in the zwitterion resembles that in the *N*-acetylneuraminate oxocarbenium ion, the putative intermediate in the enzyme-catalyzed reaction, and this may account for its effectiveness in binding sialidases.[152] Starting from Siastatin B **158** 3,4-dehydro-*N*-(2-ethylbutyryl)-3-piperidinecarboxylic acids **159**[153] have been prepared. The compound **159** exhibits strong inhibition particularly against influenza virus A neuraminidase and confers both *in vitro* and *in vivo* antiviral efficacy.[154] 1,4,5,6-Tetrahydropyridazine derivatives **160** possess side chains similar to that of oseltamivir **101**. They show influenza virus neuraminidase-inhibitory activity in a μM range and were synthesized via a hetero Diels-Alder reaction.[155] Compound **161** is the thioisoster of zanamivir **126**. It could be shown, that these thioisosters are as bioactive as their oxygen counterparts as inhibitors of influenza virus sialidase.[156]

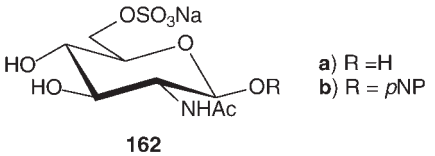


Chart 20. *p*-Nitrophenyl (pNP)-*N*-acetyl-6-sulfo-D-glucosamines **162**.

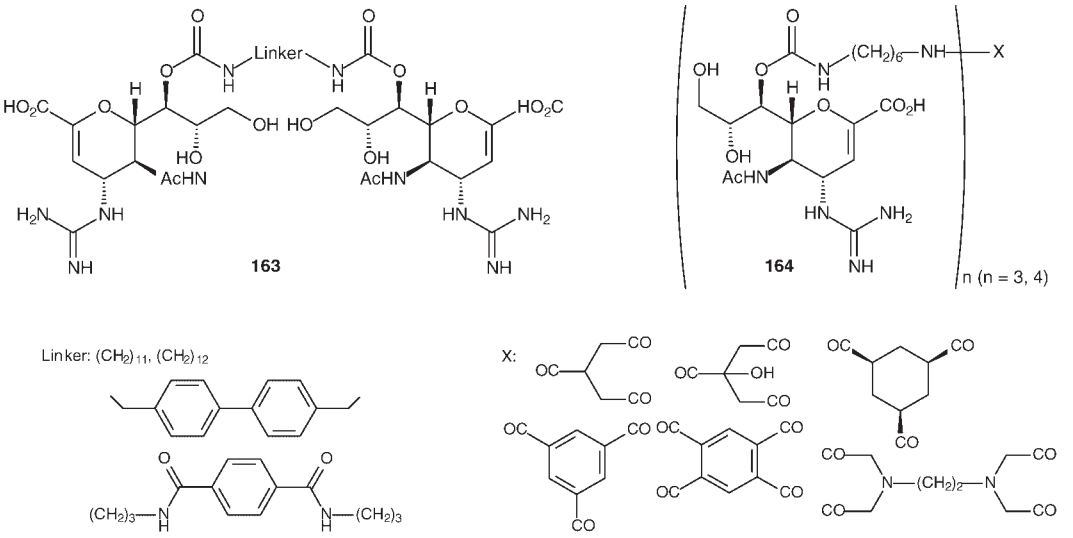


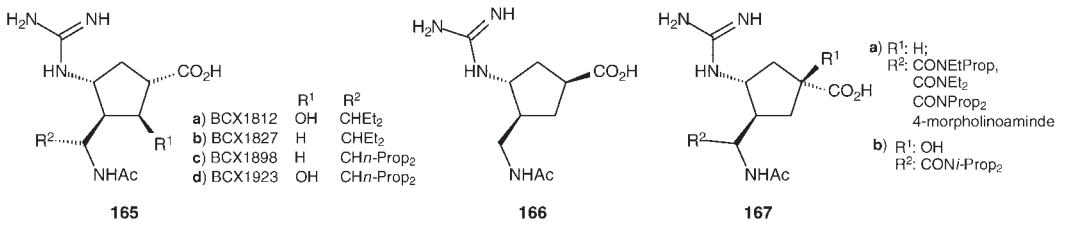
Chart 21. Dimeric, trimeric and tetrameric inhibitors **163** and **164** of influenza neuraminidase.

6-Sulfo-d-GlcNAc **162a** with a molecular geometry close to that of *N*-acetylneuraminic acid (Neu5Ac) was hypothesized to serve as a simple Neu5Ac mimic possessing high potential in biochemical and medicinal applications. The hypothesis was evidenced with a neuraminidase inhibition assay using *p*-nitrophenyl (pNP) 3-, 4-, and 6-sulfo-β-d-GlcNAc and 6-sulfo-β-d-Glc **162a**, in which only pNP 6-sulfo-β-d-GlcNAc **162b** was found to show substantial activity.[157]

The multimeric compounds **163**[158] and **164**[159] have been developed as potential second generation compounds intended for inhalation in both therapy and prophylaxis of influenza virus infections. They are significantly more antivirally active than the monomer zanamivir **126** and also showed long-lasting protective activity when tested in mouse influenza virus infectivity experiments. Furthermore, polyvalent sialidase inhibitors bearing zanamivir **126** on a poly-L-glutamine backbone have been described.[160]

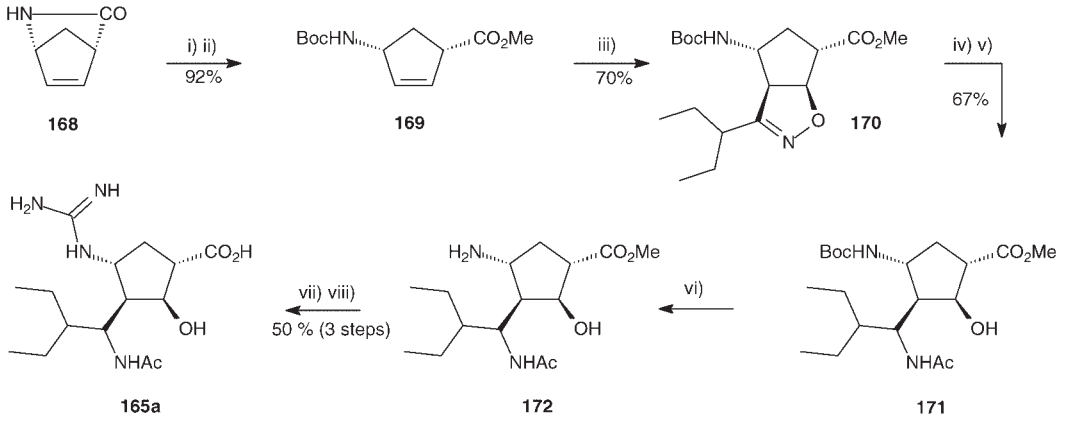
D. Five-Membered Ring-Based NA Inhibitors

Peramivir BCX-1812 **165a** potently inhibits the neuraminidase enzyme N9 from H1N9 virus *in vitro* with a 50% inhibitory concentration (IC_{50}) of 1.3 ± 0.4 nM. On-site dissociation studies indicate that peramivir **165a** remains tightly bound to N9 NA ($t1/2$ >24 hr), whereas, zanamivir **126** and oseltamivir carboxylate **101** dissociated rapidly from the enzyme ($t_{1/2}$ = 1.25 hr). Additional efficacy studies indicate that a single injection of peramivir **165a** (2–20 mg/kg)[161] was comparable to a q.d. × 5 day course of orally administered oseltamivir **101** (2–20 mg/kg/day) in preventing lethality in H3N2 and H1N1 influenza models.[162,163] A single intramuscular injection of peramivir **165a** was found effective in the treatment of influenza virus infections, and this may provide an interesting lead to be used in case of an influenza outbreak.[164,165] Compound **166** was found to have an IC_{50} value of 28 μM against neuraminidase N2 and 115 μM against N9, which is superior to the DANA series compound having the same functional groups.[166] This mode of binding for the guanidino group is analogous to that observed in the crystal structure of zanamivir (**3**) with influenza A neuraminidase.[167] The 1-ethylpropylamide, diethylamide, dipropylamide, and 4-morpholinylamide of the **167a** group showed very good inhibitory activity (IC_{50} = 0.015–0.080 μM) against the neuraminidase A form, but modest activity (IC_{50} = 3.0–9.2 μM) against the neuraminidase B. Of the 1-carboxy-1-hydroxy derivatives **167b**, the diethylamide and the dipropylamide, were also investigated; however, they were less active than the compounds without the 1-hydroxy group.[168]



165 166 167

Chart 22. Cyclopentane derivatives **165**, **166** and **167** as NA Inhibitors.



168 169 170 165a 172 171

Scheme 14. Synthesis of peramivir **165a**. (i) HCl, MeOH; (ii) $(Boc)_2O$, Et_3N; (iii) PhNCO, Et_3N, 2-ethyl-1-nitrobutane; (iv) H_2, PtO_2, MeOH, HCl (100 psi); (v) Ac_2O, Et_3N; (vi) HCl, ether; (vii) pyrazolecarboxamide × HCl, $(i\text{-Pr})_2EtN$; (viii) NaOH.

Synthesis[169] of peramivir **165a** (Scheme 14) starts from (−)-lactam (2-azabicyclo[2.2.1]hept-5-en-3-one **168**, which was hydrolyzed with methanolic HCl; the resultant amino ester on reaction with Boc anhydride produced compound **169**. Cycloaddition (3+2) of compound **169** with the nitrile oxide produced from 2-ethyl-1-nitrobutane, phenyl isocyanate, and triethylamine, gave cycloadduct **170** and other isomers (< 10%). Cycloadduct **170** was isolated from the mixture of isomers and was hydrogenated in methanol with an equivalent amount of aqueous HCl in the presence of PtO_2 at 100 psi to give an amine hydrochloride, which was reacted with acetic anhydride to give the corresponding *N*-acetyl derivative **171**. Compound **171** on reaction with ethereal HCl gave deblocked amine **172**. Compound **172** on guanylation with pyrazole carboxamidine hydrochloride in DMF in the presence of diisopropylethylamine gave the corresponding guanidino ester, which on hydrolysis with NaOH gave the desired compound **165a**.

A-315675 **173** is a novel, pyrrolidine-based compound that was evaluated in this study for its ability to inhibit A and B strain influenza virus neuraminidases in enzyme assays and influenza virus replication in cell culture. A-315675 **173** effectively inhibited influenza A N1, N2, N9, and B strain neuraminidases with inhibitor constant (K_i) values between 0.024 and 0.31 nM. These values were comparable to or lower than the K_i values measured for oseltamivir carboxylate (GS4071) **101**, zanamivir **126**.[170]

Performing the coupling reaction of *N-tert*-butoxycarbonyl-2-(*tert*-butyldimethylsiloxy)pyrrole **178**[171] and *S*-trityl sulfenimine **179**[172] with triflic acid (0.8 equiv) in THF/heptane at −40°C yielded 95% of desired isomer **180**.[173] Introduction of the *cis*-propenyl group at C-4 to obtain **181** required a stereoselective *trans*-addition of the *in situ* generated propenyl cuprate. Partial reduction of **181** to the hemiaminal with DIBALH was followed by conversion to the α-methoxycarbamate through treatment with PPTS in methanol. Reaction of this compound with TMSCN in the presence of

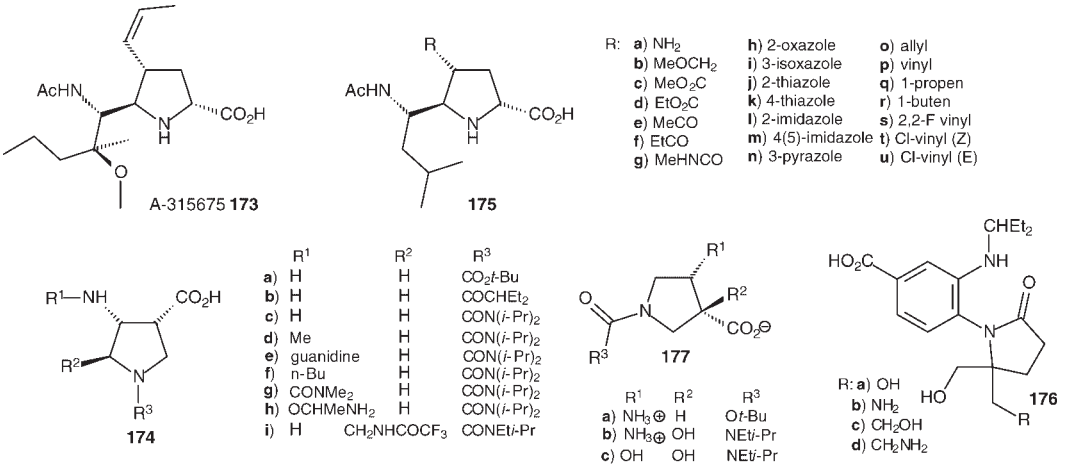
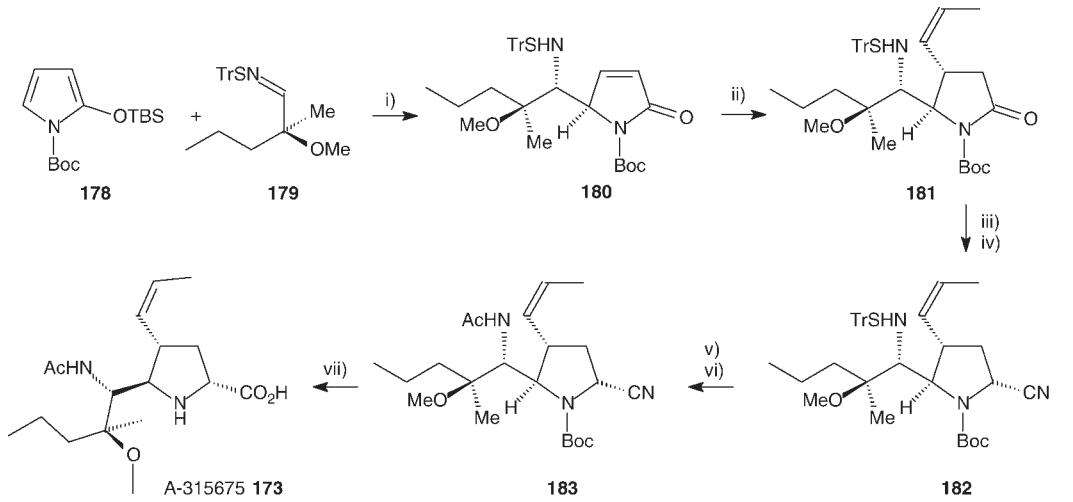


Chart 23. Pyrrolidin-derivatives **173–176** as NA inhibitors.

$BF_3 \times Et_2O$ gave compound **182**. The tritylsulfenyl protecting group in **182** proved to be susceptible to acidic hydrolysis and was removed by refluxing in methanol with PPTS. Acetylation of the amine gave crystalline intermediate **183** (X-ray crystallographic analysis was obtained), and treatment with 6N HCl at 60°C gave **173** in high yield.[174,175]

Compound A-192558 **174i**, is the most potent NA inhibitor in this series ($IC_{50} = 0.2$ μM against NA A and 8 μM against NA B). The X-ray crystallographic structure of A-192558 **174i** bound to NA revealed the predicted interaction of the carboxylic group with the positively charged pocket (Arg118, Arg292, Arg371) and interaction of the trifluoroacetamino residue with the hydrophobic pocket (Ile222, Trp178) of the enzyme active site. Surprisingly, the ethyl and isopropyl groups of the urea functionality-induced a conformational change of Glu276, turning the Glu276/Glu277 hydrophilic pocket, which normally accommodates the triglycerol side chain of substrate sialic acid, into an induced hydrophobic pocket.[176] Of the **175** series, *Z*-propenyl-analog **175q** was found to be



Scheme 15. Synthesis of A-315675 **173**. (i) TfOH (0.8 equ.) THF/heptane, −40°C, 18:1, 95%; (ii) *cis*-1-propenylmagnesium bromide, Cu(I)Br × DMS, TMSCl, 89%; (iii) DIBAL (87%) PPTS, MeOH, TMSCN, $BF_3 \times Et_2O$ 77%; (iv) DIBALH, TMSCN, TMSOTf 97%; (v) PPTS, MeOH, reflux, Ac_2O, Et_3N 69%; (vi) TFA, MeOH, Ac_2O, Et_3N 76%; (vii) 6N HCl, 60C, 99%.

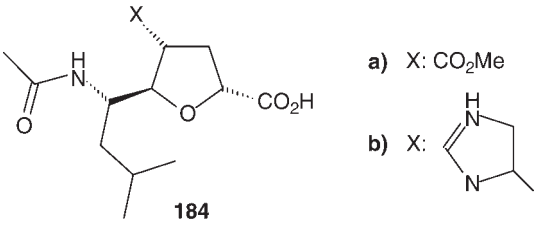
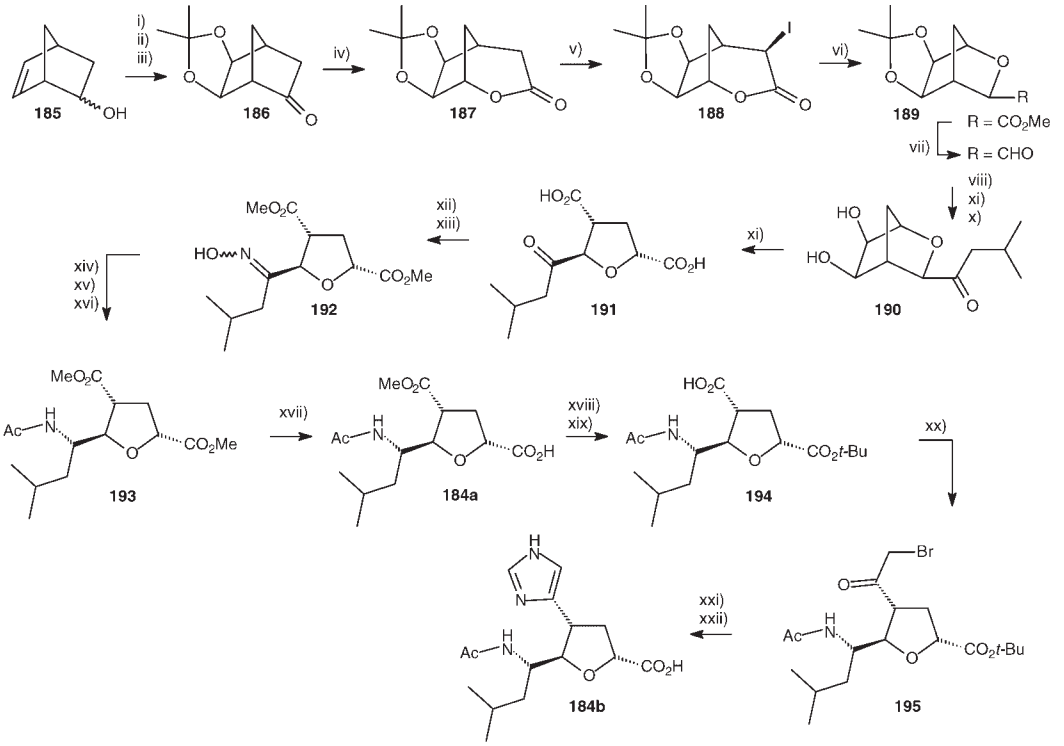


Chart 24. Tetrahydrofuran-derivatives **184** as NA inhibitors.

the most potent inhibitor of both A and B neuraminidases (IC_{50} = 0.020 and 0.030 μM, respectively), whereas the IC_{50}s of the other derivatives were found in a range of 0.045–15 μM.[177] Compound **176b** was found the most potent of the benzoic acid analog series **176** (IC_{50} = 0.52 μM for influenza A and IC_{50} 26 μM for influenza B neuraminidases).[178] Novel α- and β-amino acid inhibitors of influenza neuraminidase bearing a pyrrolidine moiety, exhibited K_i values in the 50 μM range against influenza virus A/N2/Tokyo/3/67 neuraminidase but exhibited weaker activity against influenza virus B/Memphis/3/89 neuraminidase. Limited optimization of the pyrrolidine series **177** resulted in compound **177a**, which was about 24-fold more potent than 2-deoxy-2,3-dehydro-*N*-acetylneuraminic acid in an anti-influenza cell culture assay using A/N2/Victoria/3/75 virus. Pyrrolidine



Scheme 16. Synthesis of tetrahydrofuran derivatives **184**: (i) DMSO, $(COCl)_2$, DCM −78°C to rt. 85%; (ii) OsO_4, NMMO, rt., 3 hr, 90%; (iii) 2,2-dimethoxypropane, TsOH, rt, 1 hr, 90%; (iv) MCPBA, $CHCl_3$, then aluminium oxide, 50%; (v) LHMDS, I_2, −78°C, 0.5 hr, 81%; (vi) K_2CO_3, MeOH, rt., 0.5 hr, 80–90%; (vii) DIBALH, DCM, −78°C, 1 hr, 80%; (viii) isobutylmagnesium bromide, 0°C, 1 hr; (ix) DMSO, $(COCl)_2$, DCM, −78°C—rt. 51% three steps. (x) 2N HCl, MeOH, 40°C, 2 hr, 98%; (xi) $RuCl_3 \times H_2O$, $NaIO_4$, rt., 4 hr; (xii) MeI, CH_2Cl_2, DIEA, 68% 2 steps; (xiii) $H_2NOH \times HCl$, DIEA, MeOH, $(nBu)_4NI$, 1 hr, 90%; (xiv) H_2, Ac_2O, Ra-Ni, iso-propanol, 39%; (xv) TFA, 2 hr; (xvi) Ac_2O, DCM, 2 hr, 100%; (xvii) LiOH (1 equ), THF, 71%; (xviii) TBTA, $BF_3 \times Et_2O$ (xix) LiOH, THF, 40%, 2 steps; (xx) i-BuOCOCl, CH_2N_2, then HBr, 60% (xxi) formamidine, NH_3, 45°C, O.N. 20%; (xxii) 6N HCl, 4 hr, 96%.

analogs in which the α- or β-amino groups were replaced with hydroxyl groups were 365- and 2,600-fold weaker inhibitors, respectively. These results underscore the importance of the amino group interactions with the Asp 152 and Tyr 406 side chains and have implications for anti-influenza drug design.[179]

Commercially available 5-norbornen-2-ol **185** was converted to the protected dihydroxy ketone **186** via standard procedure Swern-Oxidation, dihydroxylation and protection.[180] A Bayer–Villiger rearrangement was carried out so as to obtain **187**. The undesired, methylene lactone decomposed completely by treatment with aluminum oxide. Following the preparation of the iodolactone **188**, treatment with K_2CO_3 afforded the key intermediate **189**, which was deprotected to **190** and subjected to oxidative ring-opening. This provided the desired THF derivative **191** which was converted into the oxime **192**. Reduction by catalytic hydrogenation in the presence of Boc-anhydride gave a mixture of two separable acetyl-amino derivatives **193**. The more base-labile 5-methylester could selectively be hydrolyzed yielding the monoacid **184a**.[181] After protection of the 5-carboxylate group, the 3-carbomethoxyl group of the obtained **184a** was hydrolyzed to get **194** and converted into the bromomethyl ketone moiety of **195** via diazomethyl ketone. Condensation of **195** with formamidine in liquid ammonia introduced the imidazole moiety, and upon deprotection the desired compound **184b** was afforded.

The IC_{50} values of compounds **184a** and **184b** against NA A (A/Tokyo/3/67) were 410 and 580 nM, respectively. They were 10-fold less potent than the pyrrolidine analog **175**c. Against NA B (B/Memphis/3/89), compounds **184a** and **184b** were about 20-fold less potent than **175**c (IC_{50} = 960 and 1,000 nM, respectively).

9. DISRUPTION OF THE VIRAL ENVELOPE

Defensins are low molecular weight antimicrobial peptides produced by phagocytes and in various epithelial locations, including lung and trachea,[182] having a broad spectrum activity against a variety of pathogens including bacteria, fungi, and viruses. Defensins have activity against various enveloped viruses, including influenza.[183] These peptides bind to microbial surfaces and induce formation of membrane pores.[184] Defensins could, therefore, inhibit infectivity of influenza through disrupting the envelope of extracellular viral particles. Defensins present in the airway (Human β defensins 1 and 2) are also chemotactic for dendritic cells and memory T cells, and may, therefore, stimulate adaptive immune responses.[185] Recombinant production of defensins and other low molecular weight antimicrobial peptides is an attractive area for antiviral research because of the broad spectrum activity of these agents and their potential to modulate host defense functions.

10. SMALL INTERFERING (si)RNAs

Short interfering RNAs (siRNAs) specific for conserved regions of influenza virus genes were found to reduce virus production in the lungs of infected mice, when the siRNAs were given intravenously (i.v.) in complexes with a polycation carrier either before or after initiating virus infection.[186] Delivery of siRNAs specific for highly conserved regions of the nucleoprotein or acidic polymerase significantly reduced lung virus titers in influenza A virus-infected mice and protected the animals from lethal challenge. This protection was specific and not mediated by an antiviral interferon response. The influenza-specific siRNA treatment was broadly effective and protected animals against lethal challenge with highly pathogenic avian influenza A viruses of the H5 and H7 subtypes.[187] That specific siRNA would be effective against influenza could be readily predicted from equally effective results obtained with other specific siRNAs, that is, against the SARS (severe acute respiratory syndrome) coronavirus in comparable situations.[188]

Phosphorothioate oligonucleotides (PS-ONs) (i.e., REP, a 40-mer PS-ON) offer potential, when administered as aerosol in the prophylaxis and therapy of influenza infection.[189] Similarly, antisense phosphorodiamidate morpholino oligomers (ARP-PMOs) could be further pursued for their potential in the treatment of H5N1 influenza A virus infections.[190]

11. CONCLUSION

Several drugs are available that could be used, either alone or in combination, in the treatment (prophylaxis or therapy) of a pandemic influenza, whether avian or human, virus infection. These include adamantan(amin)e derivatives (i.e., amantadine), neuraminidase inhibitors (i.e., oseltamivir), ribavirin and interferon. Combinations of different antivirals acting against influenza at different stages of viral replication could be an important area of research in the future should such combination strategy prove clinically successful in the treatment of HIV infection.

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