CARBOHYDRATE-DERIVED MOLECULAR DIVERSITY: SYNTHESIS AND APPLICATION IN CELL BIOLOGY

MA JIMEI

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CARBOHYDRATE-DERIVED MOLECULAR DIVERSITY: SYNTHESIS AND APPLICATION IN CELL BIOLOGY

MA JIMEI

School of Physical and Mathematical Sciences

A thesis submitted to the Nanyang Technological University in partial fulfillment of the requirement for the degree of Doctor of Philosophy

2010
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SUMMARY

Part 1

Tamiflu is currently the most effective drug for the treatment of influenza. In this part, a practical synthesis of Tamiflu was developed using novel synthetic route, cheap reagents, and abundantly available starting material D-glucal. This strategy features Claisen rearrangement of hexose to obtain the cyclohexene backbone and introduction of diamino groups through tandem intramolecular aziridination and ring opening. In addition, our synthetic protocol allows late-stage functionalization for facile and flexible synthesis of Tamiflu analogues.

Using the synthesized Tamiflu and its active metabolite (oseltamivir carboxylate), we investigated their influence on morphology, proliferation, differentiation and vesicular exocytosis (regulated secretion) of neuroendocrine PC12 cells. It was found that oseltamivir carboxylate significantly inhibited the vesicular exocytosis of PC12 cells, postulating a mechanism underlying the Tamiflu side-effects, particularly, its possible adverse influences on neurotransmitter release in the central nervous system.
Part 2

Carbohydrate-carbohydrate or carbohydrate-lectin interactions play a vital role in biological processes such as cell-cell recognition, immunological response, metastasis, and fertilization. These interactions and consequent cellular events could be studied by interfacing carbohydrate-coated carbon nanotube network devices with living cells. In this work, we first fabricated thin-film networks of single-walled carbon nanotubes by interacting with glycoside-coupled pyrene. This network device interfaced biocompatibly with living cells, improving PC12 cell adhesion and growth. As a biosensor, the device aided to electrochemically detect the dynamic secretion of biomolecules. This unique approach provides real-time and noninvasive measurements from living cells with high sensitivity, high temporal resolution, high throughput and ease of detection. This study is one of the examples which shows the combination of glycobiology and nanotechnology could lead to a new strategy for probing the cell machinery and elucidating molecular level life process.

Part 3

1,2-\textit{cis}-Mannosides play important roles in cellular biology and therefore their synthesis is necessary for biological studies. However, efficient formation of 1,2-\textit{cis}-
mannosides is still a huge challenge in current oligosaccharide chemistry. The most efficient and elegant method thus far is intramolecular aglycon delivery (IAD) where PMB, NAP, allyl, vinyl and allenyl groups have been introduced to tether the aglycon to the glycosyl donor. The propargyl group is found to be capable of forming an insoluble silver acetylide complex, which could be easily separated via simple filtration and recovered through acidification. Besides the potential for easy purification, propargyl groups can also act as protecting groups. Thus, we chose to investigate the feasibility of employing propargyl as a tethering media, delivering aglycon to the glycosyl donor to synthesize 1,2-cis-mannosides.

![Diagram of IAD process](image)

**Part 4**

A sulfonated amorphous carbon-based solid acid derived from D-glucose was synthesized. It was explored for its catalytic efficiency in Friedel-Crafts reactions of indoles with various α,β-unsaturated carbonyl compounds in H₂O or H₂O/THF at room temperature. Moderate to excellent yields were afforded. The catalyst could be recycled up to 5 times without any significant loss of catalytic activity.

![Diagram of Friedel-Crafts reaction](image)
<table>
<thead>
<tr>
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<td>δ</td>
<td>chemical shift</td>
</tr>
<tr>
<td>Ac</td>
<td>acetyl</td>
</tr>
<tr>
<td>acac</td>
<td>acetylacetone</td>
</tr>
<tr>
<td>AIBN</td>
<td>azobisisobutyronitrile</td>
</tr>
<tr>
<td>Alloc</td>
<td>allyloxycarbonyl</td>
</tr>
<tr>
<td>aq.</td>
<td>aqueous</td>
</tr>
<tr>
<td>ATRP</td>
<td>atom transfer radical polymerization</td>
</tr>
<tr>
<td>BBB</td>
<td>blood brain barrier</td>
</tr>
<tr>
<td>BIEMA</td>
<td>2-(2-bromoisobutyloxy)ethyl methacrylate</td>
</tr>
<tr>
<td>BIPHEPHOS</td>
<td>6,6'--{[3,3'-bis(1,1-dimethylethyl)-5,5'-dimethoxy[1,1'-bipheyl-2,2'-diyl]bis(oxy)}\bis{dibenzo[d,j][1,3,2]dioxaphosphepine}</td>
</tr>
<tr>
<td>Bn</td>
<td>benzyl</td>
</tr>
<tr>
<td>Boc</td>
<td>tert-butoxycarbonyl</td>
</tr>
<tr>
<td>br s</td>
<td>broad singlet</td>
</tr>
<tr>
<td>Bu</td>
<td>butyl</td>
</tr>
<tr>
<td>Bz</td>
<td>benzyoyl</td>
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<tr>
<td>calcd.</td>
<td>calculated</td>
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<td>CAN</td>
<td>ceric ammonium nitrate</td>
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<tr>
<td>cat.</td>
<td>catalytic</td>
</tr>
<tr>
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</tr>
<tr>
<td>CDI</td>
<td>1,1’-Carbonyldiimidazole</td>
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<tr>
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<td>central nervous system</td>
</tr>
<tr>
<td>CNTs</td>
<td>carbon nanotubes</td>
</tr>
<tr>
<td>COD</td>
<td>cyclooctadiene</td>
</tr>
<tr>
<td>CSA</td>
<td>camphorsulfonic acid</td>
</tr>
<tr>
<td>cm⁻¹</td>
<td>inverse centimeter</td>
</tr>
<tr>
<td>Cy</td>
<td>cyclohexane; cyclohexyl</td>
</tr>
<tr>
<td>d</td>
<td>doublet</td>
</tr>
<tr>
<td>D-A</td>
<td>Diels-Alder</td>
</tr>
<tr>
<td>dba</td>
<td>dibenzylideneacetone</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>DBU</td>
<td>1,8-diazabicyclo[5.4.0]undec-7-ene</td>
</tr>
<tr>
<td>DCE</td>
<td>dichloroethane</td>
</tr>
<tr>
<td>dd</td>
<td>doublets of doublet</td>
</tr>
<tr>
<td>ddd</td>
<td>doublets of doublets of doublet</td>
</tr>
<tr>
<td>DEAD</td>
<td>diethyl azodicarboxylate</td>
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<tr>
<td>DDQ</td>
<td>2,3-dichloro-5,6-dicyano-1,4-benzoquinone</td>
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<td>2,3-dihydropyran</td>
</tr>
<tr>
<td>DIAD</td>
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<td>DIBAL-H</td>
<td>diisobutylaluminium hydride</td>
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<tr>
<td>DMA</td>
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<tr>
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<td>Dess-Martin periodinane</td>
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<tr>
<td>DPPA</td>
<td>diphenylphosphoryl azide</td>
</tr>
<tr>
<td>DPPDA</td>
<td>diphenyl phosphorazidate</td>
</tr>
<tr>
<td>dpdp</td>
<td>1,3-bis(diphenylphosphino)propane</td>
</tr>
<tr>
<td>dq</td>
<td>doublets of quartet</td>
</tr>
<tr>
<td>dt</td>
<td>doublets of triplet</td>
</tr>
<tr>
<td>DTBMP</td>
<td>2,6-di-tert-butyl-4-methylpyridine</td>
</tr>
<tr>
<td>EDCI</td>
<td>1-ethyl-3-(3-dimethylaminopropyl) carbodiimide</td>
</tr>
<tr>
<td>ee</td>
<td>enantiomeric excess</td>
</tr>
<tr>
<td>EI</td>
<td>electron ionization</td>
</tr>
<tr>
<td>eq.</td>
<td>equivalent</td>
</tr>
<tr>
<td>ESI</td>
<td>electronspray ionization</td>
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<tr>
<td>Et</td>
<td>ethyl</td>
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<tr>
<td>F-C</td>
<td>Friedel-Crafts</td>
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<tr>
<td>FITC</td>
<td>fluorescein isothiocyanate</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier transform infrared spectroscopy</td>
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<tr>
<td>GalNAc</td>
<td>galactosamine</td>
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<td>GlcNAc</td>
<td>glucosamine</td>
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h  

hour

HA  

hemagglutinin

HEPES  

4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

HPA  

*Helix Pomatia* agglutinin

HPLC  

high performance liquid chromatography

HRMS  

high resolution mass spectroscopy

HSVM  

high-speed vibration milling

IAD  

intramolecular aglycon delivery

IBX  

2-iodoxybenzoic acid

IR  

infrared

*i*Pr  

isopropyl

*J*  

coupling constants

KHMD  

potassium hexamethyldisilazide

LiHMDS  

lithium hexamethyldisilazide

MAIG  

3-methacryloyl-1,2:5,6-di-isopropylidene-d-glucofuranose

M  

concentration (mol/L)

M*+*  

parent ion peak (mass spectrum)

m  

multiplet

*m*CPBA  

*meta*-chloroperoxybenzoic acid

Me  

methyl

MOM  

methoxymethyl

min  

minute

MS  

mass spectrum

M.S.  

molecular sieves

Ms  

methanesulfonyl

MTBE  

methyl tert-butyl ether

MVK  

methyl vinyl ketone

MWNTs  

multi-walled carbon nanotubes

NA  

neuraminidase

NaHMDS  

sodium hexamethyl disilazide

NAP  

2-naphthylmethyl

NBA  

*N*-bromoacetamide

NBS  

*N*-bromosuccinimide

*n*Bu  

*n*-butyl
<table>
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<th>Definition</th>
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<td>N-chlorosuccinimide</td>
</tr>
<tr>
<td>NIS</td>
<td>N-iodosuccinimide</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>NMO</td>
<td>4-methylmorpholine N-oxide</td>
</tr>
<tr>
<td>NOESY</td>
<td>nuclear overhauser enhancement spectroscopy</td>
</tr>
<tr>
<td>Ns</td>
<td>4-nitrobenzenesulfonyl</td>
</tr>
<tr>
<td>OTf</td>
<td>trifluoromethanesulfonate</td>
</tr>
<tr>
<td>p</td>
<td>para</td>
</tr>
<tr>
<td>PCC</td>
<td>pyridinium chlorochromate</td>
</tr>
<tr>
<td>PDC</td>
<td>pyridinium dichromate</td>
</tr>
<tr>
<td>Pd/C</td>
<td>palladium on carbon</td>
</tr>
<tr>
<td>Ph</td>
<td>phenyl</td>
</tr>
<tr>
<td>Phth</td>
<td>phthalate</td>
</tr>
<tr>
<td>Piv</td>
<td>pivaloyl; trimethylacetyl</td>
</tr>
<tr>
<td>PLE</td>
<td>pig liver esterase</td>
</tr>
<tr>
<td>PMB</td>
<td>p-methoxybenzyl</td>
</tr>
<tr>
<td>PMP</td>
<td>p-methoxyphenyl</td>
</tr>
<tr>
<td>pN</td>
<td>piconewton (10^{-12} newtons)</td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
</tr>
<tr>
<td>PPTS</td>
<td>pyridinium p-toluenesulfonate</td>
</tr>
<tr>
<td>q</td>
<td>quartet</td>
</tr>
<tr>
<td>RAFT</td>
<td>reversible addition-chain transfer fragmentation</td>
</tr>
<tr>
<td>rt</td>
<td>room temperature</td>
</tr>
<tr>
<td>s</td>
<td>singlet</td>
</tr>
<tr>
<td>SCVCP</td>
<td>self condensing vinyl copolymerization</td>
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<tr>
<td>SES</td>
<td>2-(trimethylsilyl)ethanesulfonyl</td>
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<td>SPG</td>
<td>Schizophyllan</td>
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<td>SWNTs</td>
<td>single-walled carbon nanotubes</td>
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<td>t</td>
<td>triplet</td>
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<td>TBAI</td>
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<td>TBAF</td>
<td>tetrabutylammonium fluoride</td>
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<td>Acronym</td>
<td>Full Form</td>
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<td>-----------</td>
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<tr>
<td>TEMPO</td>
<td>2,2,6,6-tetramethylpiperidine-1-oxyl</td>
</tr>
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<td>triethylsilyl</td>
</tr>
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<td>trifluoroacetic acid</td>
</tr>
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<td>trifluoroacetic anhydride</td>
</tr>
<tr>
<td>Tf</td>
<td>trifluoromethanesulfonyl</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>THP</td>
<td>tetrahydropyran</td>
</tr>
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<td>TLC</td>
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<td>TPAP</td>
<td>tetrapropylammonium perruthenate</td>
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<tr>
<td>Tr</td>
<td>triphenylmethyl</td>
</tr>
<tr>
<td>Ts</td>
<td>p-toluenesulfonyl</td>
</tr>
<tr>
<td>v</td>
<td>volume</td>
</tr>
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</table>
PART 1

Sugar-based Synthesis of Tamiflu and Its Inhibitory Effects on Cell Secretion

1. INTRODUCTION

Influenza, a common infectious disease, resulted in heavy casualty which amounted to thousands annually and millions during its outbreaks. The influenza pandemic in 1918 (the Spanish flu) was estimated to have killed 20 to 50 million people worldwide.\cite{1} Thereafter, two serious pandemics in 1957 (the Asian flu) and 1968 (the Hong Kong flu) killed millions of people.\cite{2} In 2005, officials of the World Health Organization (WHO) estimated the death toll from the avian H5N1 influenza (bird flu) could amount to between 2 to 7.4 million. Subsequently, the 2009 outbreak of influenza A H1N1 swept across the whole world and resulted in thousands of casualties. These epidemics consume a significant portion of the world’s expenditure as billions of dollars are allocated for healthcare expenses and productivity losses each year.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{neuraminidase_inhibitors.png}
\caption{The working mechanism of neuraminidase inhibitors. Neuraminidase of influenza virus cleaves the binding of hemagglutinin with the cellular receptor at sialic acid residue to replicate new viruses. Neuraminidase inhibitors block this replication to prevent infection.}
\end{figure}
Although immunization is traditionally an effective approach to fight against flu epidemics, the vaccine has limited availability and cannot be stockpiled for more than 18 months. In addition, the vaccine is easily inactivated when influenza variants occur.\textsuperscript{[3]} Thus, it is necessary to search for a more efficient alternative. The emergence of small-molecule antiviral agents represents a novel opportunity for effective prevention and therapy of influenza.

The schematic presentation for the pathogenesis of influenza is shown in Figure 1. The inflection of influenza begins by attaching the hemagglutinin (HA) to sialidase of the host cell surface protein. Thereafter, neuraminidase (NA) cleaves the $O$-glycosidic bonds that connect the sialidase to the host cell membrane to release a new virion. Scheme 1 showed the cleaving of HA in molecular term.

\begin{center}
\includegraphics[width=0.8\textwidth]{Scheme1.png}
\end{center}

**Scheme 1.** Proposed mechanism for the hydrolysis of sialic acid by neuraminidase.
To effectively prevent new virions from duplicating effectively, low molecular weight drug molecules should be sialic acid (N-acetylneuraminic acid, 1) mimic to inhibit the NA.\cite{4} The X-ray crystal structures of NA in influenza A and B were determined in the 1980s, which further helped in understanding NA and designing of more potent inhibitors. With the aid of computational chemistry, two inhibitors, zanamivir (GSK’s Relenza, 2) and oseltamivir phosphate (3·H$_3$PO$_4$, Gilead’s Tamiflu, 4) were designed (Figure 2) and eventually marketed in 1999. These two NA inhibitors had been tested to be active *in vitro* against all known influenza strains, including the recent H5N1 avian strain and H1N1 swine flu strain.\cite{5} However, due to low bioavailability, zanamivir has been administered by inhalation, which can cause problems in patients with underlying respiratory disease. On the other hand, Tamiflu is a prodrug, which is hydrolyzed hepatically to the active metabolite, the free carboxylate of oseltamivir (oseltamivir acid, 5). The presence of an ester group in Tamiflu greatly increases its bioavailability. Tamiflu became the first orally active neuraminidase inhibitor commercially developed and administered as capsules.

**Figure 2.** Chemical structures of sialic acid (1), zanamivir (2), oseltamivir (3) Tamiflu (4) and oseltamivir acid (5).
It is well known that Tamiflu is a potent inhibitor of neuraminidases for both influenza A and B viruses and is currently used for treatment of influenza viral infection including avian flu (H5N1) and swine flu (H1N1). Although oseltamivir’s efficacy is quite powerful, adverse effects have been reported. According to a report of US Food and Drug Administration (FDA) in 2005, 1184 cases of side effects out of 11.1 million patients had been reported.[6] These adverse effects were exhibited not only in the digestive system, but also in the central nervous system (CNS). FDA adverse events reporting system data showed that there were 596 cases of neuropsychiatric events with oseltamivir out of 7.3 million patients following the 2006-2007 flu season.[7] It is most dismaying that the mysterious psychiatric consequences have led to 54 adolescent suicides in Japan as of 2007.[8] The molecular mechanisms that underlie Tamiflu’s side-effects have yet to be unveiled.

Oseltamivir may affect the central nervous system because it acts as a neuraminidase inhibitor.[9] Neuraminidase is not only the key enzyme for the replication of the influenza virus, it also plays an important role in mammalian physiological processes including CNS development, autoimmune responses, cell apoptosis and functions. Once Tamiflu is absorbed in vivo, other normal actions of neuraminidase except cleaving of sialic acid residues might be inhibited.

Zhang et al. measured the biodistribution of [11C]oseltamivir and [11C]oseltamivir carboxylate in mice using the dissection method and micro-positron emission tomography.[10] In vivo experiments elucidated the presence of [11C]oseltamivir and [11C]oseltamivir carboxylate in the mice plasma as well as the brain. This demonstrated that both oseltamivir and oseltamivir carboxylate could pass the blood brain barrier (BBB) and enter the brain. It was also found that the dosage of
oseltamivir in the brain was significantly higher in newborn mice than those in adult mice.\cite{11} Tamiflu and oseltamivir could not readily pass the BBB, but they may penetrate into CNS when the BBB is impaired or immature.\cite{10,12}

Furthermore, some solvents such as alcohol in media could increase the BBB permeability of Tamiflu as investigated by Izumi et al.. They reported the neuropsychiatric effects of oseltamivir on CNS using juvenile rats and their hippocampal slices.\cite{12} Although unitary injection of oseltamivir did not cause any changes in the rats’ behavior within 2 h, injection of oseltamivir followed by ethanol dramatically altered the duration of loss of lightning reflex. Administration of 100 μM oseltamivir induced paired-pulse facilitation in population spikes and oseltamivir carboxylate facilitated neuronal firing. These studies showed that Tamiflu had clear effects on mice brain neuronal excitability. Moreover, administration of oseltamivir carboxylate together with 60 mM ethanol elicited further facilitation. The speculation was that abnormal behavior after administration of Tamiflu was due to the simultaneous influence of ethanol or CNS stimulants in medication.

The Usami group also discovered that oseltamivir could enhance rat hippocampal network synchronization.\cite{13} They investigated the effect of oseltamivir on the excitability of hippocampal networks using eletrophysiological recording and functional multineuron calcium imaging techniques. The eletrophysiological recording showed that the neuron pairs emitted intermittent bursts of action potentials when 100 μM oseltamivir was applied while there were no correlation between action potentials and neuron pairs in control conditions. The functional multineuron calcium images also showed that the hippocampal CA3 neurons increased gradually in activity rates and synchronized globally when 100 μM oseltamivir was involved. Another NA
inhibitor, zanamivir, showed similar results as oseltamivir. Since sialic acid modulates neurite adhesion between hippocampal neurons,\textsuperscript{[14]} it is possible that oseltamivir regulates sialylation-mediated neurite adhesion and enhances interneurons network synchronicity.

Yoshino \textit{et al.} investigated the changes in dopamine and its metabolism in the medial prefrontal cortex of rats after systemic administration of Tamiflu by using microdialysis.\textsuperscript{[15]} Their research results exhibited that extracellular dopamine in the medial prefrontal cortex was significantly increased after administration of Tamiflu as compared to the control values. Similarly, there were remarkable increments for the metabolites of dopamine (3,4-dihydroxyphenylacetic acid and homovanillic acid) as well. These findings suggest that Tamiflu promotes dopamine release in the medial prefrontal cortex. Since dopamine neurotransmission in CNS influences animal behavior, there is possibility that the abnormal behavior of humans could be associated with the Tamiflu treatment.

It is noteworthy that most cases of severe adverse effects were reported from Japan. The possible reason could be attributed to the fact that Japan constituted 70\% of the worldwide demand of Tamiflu each year.\textsuperscript{[16]} Wei \textit{et al.} found that a nonsynonymous single nucleotide polymorphism in human cytosolic sialidase is the target of Tamiflu.\textsuperscript{[17]} The single nucleotide polymorphism, which could increase the unintended binding affinity of mammalian sialidase to Tamiflu, exists in 9.29\% of Asians and none in Europeans and African Americans. Treatment of people who have this polymorphism with Tamiflu might further their in \textit{vivo} sialidase activity. They also noticed that the reported neuropsychiatric adverse effects of Tamiflu were similar to the symptoms of human sialidase-related disorders. Their detailed analysis provided a
testable hypothesis for the bewildering Tamiflu side effects cases in Japan. However, the adverse effects of Tamiflu are complicated and still remain unclear. It is therefore necessary to execute deeper and more careful investigation on these effects.

Tamiflu was co-developed by Gilead Sciences and Hoffmann-La Roche (Roche) and is currently marketed by Roche. The first commercial synthetic route of Tamiflu which involves a 10-step process uses naturally occurring (−)-shikimic acid (6) as a starting material, which was limited in availability. Since Roche announced a production shortage in 2005, various research groups have devoted themselves to developing new methodologies to synthesize Tamiflu. For example, asymmetric Diels-Alder chemistry and Michael reaction have been exploited to generate the cyclohexene backbone. Commercially available compounds such as (−)-quinic acid, L-serine, xylose, mesoaziridine, substituted cyclohexanediene, lactone, pyridine, 2,6-dimethoxyphenol, D-mannitol, L-methionine, ethyl benzoate and ribose have been utilized as the alternative starting materials for robust syntheses of Tamiflu. These synthetic strategies were reviewed here based on different starting material.

1. (−)-Shikimic acid

Oseltamivir acid 5 was the most effective drug among the sialic acid mimics investigated by Gilead Inc. on influenza A and B. It was first synthesized by Kim et al. in 1997 (Scheme 2). Epoxide 7 was prepared though esterification and epoxidation from 6 according to the literature procedure. Upon protection of the free hydroxyl group with a MOM group, the epoxide was ring-opened by NaN₃ in the presence of
NH₄Cl to give 8. Aziridine 9 was formed after mesylation and reduction of azide. Ring-opening of 9 with sodium azide was applied again to give compound 10. Exposing 10 to HCl in methanol afforded compound 11, which was treated with TrCl and MsCl successively to generate aziridine 12. The aziridine ring was opened by 3-pentanol in the presence of BF₃·Et₂O. The free amine of 13 was then protected with acetyl group to give 14. Lastly, reduction of azide and hydrolysis of the ester group provided oseltamivir acid 5.

Scheme 2. First synthetic route to oseltamivir acid developed by Gilead Science Inc. Reagents and conditions: (a) SOCl₂, MeOH, then Ph₃P, DEAD, THF, 77%; (b) MeOCH₂Cl, DIPEA, CH₂Cl₂, reflux, 3.5 h, 97%. (c) NaN₃, NH₄Cl, MeOH/H₂O, reflux, 15 h, 86%; (d) MeSO₂Cl, Et₃N, CH₂Cl₂, 0 °C to rt, 15 min, 99%; (e) Ph₃P, THF, 0 °C to rt, 3 h, then Et₃N/H₂O, 0 °C to rt, 3 h, 78%; (f) NaN₃, NH₄Cl, DMF, 65-70 °C, 21 h, 77%; (g) HCl, CH₃OH, rt, 4 h, 99%; (h) TrCl, Et₃N, CH₂Cl₂, 0 °C to rt, 3 h; (i) MeSO₂Cl, Et₃N, CH₂Cl₂, 0 °C to rt, 22 h, 86% for 2 steps; (j) BF₃·Et₂O, 3-pentanol, 70-75 °C, 2 h; (k) Ac₂O, DMAP, pyridine, rt, 18 h, 69% for 2 steps; (l) Ph₃P, THF then Et₃N/H₂O, 50 °C, 10 h, 90%; (m) KOH, THF/H₂O, rt, 40 min then Dowex 50WX8, 75%.
In this route, the regio- and stereospecific nucleophilic ring-opening of the epoxide has been achieved possibly due to the steric effect and vicinal electronegative inductive of the alkyloxy group. This method was extensively used by various groups in later synthetic strategy to introduce two amino groups. However, this synthetic route is limited to milligram scale, solely for the purpose of medicinal chemistry study.

After the establishment of oseltamivir acid’s high efficacy, oseltamivir phosphate was finally identified to be a new drug for treatment of influenza in order to improve the oral bioavailability. Therefore, Gilead Inc. and Roche Ltd. were the pioneers in the synthesis of Tamiflu.

(−)-Shikimic acid 6 is mainly isolated from star anise fruit, which is usually cultivated in China. 1 Kg of 6 could be extracted from 30 kg of dry anise star. It can also be generated by the fermentation of 

\[ E. coli \]

Shikimic acid is undoubtedly an excellent starting material for the synthesis of Tamiflu. Shikimic acid has a similar backbone structure as target molecule and only a few functional group interconversions were needed to achieve oseltamivir phosphate. The first pilot synthetic route started from 6 was developed by Rohloff et al. in Gilead (Scheme 3).[22] Shikimic acid was first esterified by refluxing with SOCl₂ in EtOH to give ethyl shikimic acetate 19, which was converted to 17 with 2,2-dimethoxypropane ketalization and mesylation. Regioselective reduction of the ketal and subsequent epoxide formation proceeded by the treatment of 17 with TMSOTf and BH₃·Me₂S \[^{34}\] followed by heating with KHCO₃ in EtOH/H₂O. Epoxide 18 was ring-opened by NaN₃ to generate a 10:1 mixture of 19 and 20, which was intramolecularly cyclized with Me₃P in MeCN to form aziridine 21. Aziridine opening by NaN₃ in DMF furnished 22, which was subsequently acetylated with Ac₂O. Reduction of azide 23 was achieved by hydrogenation with Raney nickel.
in EtOH and the catalyst was removed by filtration. With addition of 85% H$_3$PO$_4$ to
the filtrate, 4 would be obtained in 71-75% yield. During the whole process, no
chromatographic purification was needed and two intermediates, 18 and 23, were
isolated by crystallization. This process is relatively concise and could be carried out
in kilogram-scale.

Scheme 3. Gilead first synthesis of Tamiflu started from (−)-shikimic acid. Reagents
and conditions: (a) SOCl$_2$, EtOH, reflux; (b) 3-pentanone, TsOH; (c) MsCl, Et$_3$N,
CH$_2$Cl$_2$, 0-5 °C, 80% for 3 steps; (d) TMSOTf, BH$_3$·Me$_2$S, and then KHCO$_3$,
H$_2$O/EtOH, 65 °C, 1 h, 60-72%; (e) NaN$_3$, NH$_4$Cl, H$_2$O/EtOH, 70-75 °C, 86%; (f)
Me$_3$P, MeCN, 2 h, 97%; (g) NaN$_3$, NH$_4$Cl, DMF, 70-80 °C; (h) Ac$_2$O, aq. NaCO$_3$,
CH$_2$Cl$_2$, 44% for 2 steps; (i) i) Ra-Ni, H$_2$, EtOH, 16 h; ii) 85% H$_3$PO$_4$, EtOH, 55-65 °C,
then 0 °C, 71-75% for 2 steps.

Karpf et al. in Roche Ltd. successfully improved the process route which utilized 6 as
the starting material (Scheme 4) in 2001.$^{[35]}$ Shikimic acid was transformed to the
corresponding ethyl ester 16 by refluxing with SOCl$_2$ in EtOH. After removal of the

Part 1 Sugar-based synthesis of Tamiflu and its inhibitory effects on cell secretion
solvent, crude 16 was treated with 2,2-dimethoxypropane and TsOH in EtOAc to yield ketal 24. The generated methanol was removed under reduced pressure. Mesylation of 24 followed by transketalization with 3-pentanone furnished ketal 25, which is a highly crystalline compound and could be easily purified.

Scheme 4. Roche’s first generation process for Tamiflu synthesis started from (−)-shikimic acid. Reagents and conditions: (a) SOCl₂, EtOH, reflux, 3.5 h, 97%; (b) Me₂C(OMe)₂, TsOH, EtOAc, 35 °C, 15-200 mbar, 95%; (c) MsCl, Et₃N, EtOAc, 30 min, 89%; (d) 3-pentanone, CF₃SO₂H, 98%; (e) Et₂SiH, TiCl₄, CH₂Cl₂, −34 °C, 2 h; (f) NaHCO₃, H₂O/EtOH, 65 °C, 2.5 h, 80% for 2 steps; (g) allyl amine, MgBr₂·OEt₂, MTBE/MeCN, 55 °C, 16 h, and then (NH₄)₂SO₄, H₂O, 97%; (h) Pd/C, EtOH, H₂NCH₂CH₂OH, reflux, 3 h, and then H₂SO₄/H₂O, 77%; (i) i) PhCHO, tBuOMe; ii) MsCl, Et₃N, filtration; iii) allyl amine, 112 °C, 15 h; iv) HCl/H₂O, 80%; (j) Ac₂O, AcOH, MsOH, tBuOMe, 15 h, 20 °C, 83%; (k) i) 10% Pd/C, EtOH, H₂NCH₂CH₂OH, reflux, 3 h; ii) H₃PO₄, EtOH, 70% for 2 steps.

Reduction of 25 with Et₂SiH/TiCl₄ in CH₂Cl₂ at −34 °C afforded the major product 26, which was converted to epoxide 18 upon heating with NaHCO₃ solution in EtOH/H₂O. Epoxide 18 was attacked by allyl amine in the presence of MgBr₂·OEt₂ in...
tBuOMe/MeCN to furnish 27 as the major product. Deallylation of 27 by Pd/C in ethanol with the aid of ethanolamine resulted in the formation of 28. The successive treatment of 28 with benzaldehyde, MsCl/Et3N, allyl amine and aqueous HCl afforded 29. Only one filtration and simple workup are required for this step. The amino group at C-4 was introduced and the configuration of C-5 was reversed simultaneously in this step. Acetylation of 29 led to 30, which was subjected to deallylation and phosphorylation to furnish salt 4. This synthetic approach is suitable for industrial process and it is well characterized by (1) azide-free condition, (2) easy purification of intermediates and (3) concise synthetic route with high total yields (22%-24%).

Scheme 5. Roche second generation process for Tamiflu originating from epoxide 18. Reagents and conditions: (a) tBuNH₂, MgCl₂, toluene, 25 °C, 6 h, 50 °C, 8 h, 96%; (b) MsCl, Et₃N, toluene, 5 °C, 20 min, 70 °C, 3 h, 93%; (c) PhSO₃H, (allyl)₂NH, 120 °C, 5.5 h, 93%; (d) Ac₂O, NaOAc, 110 °C, 4 h, 94%; (e) HCl, EtOH, < 20 °C, 92%; (f) TFA, 50 °C, 1.5 h, 97%; (g) NDMBA, Pd(OAc)₂, PPh₃, then H₃PO₄, EtOH, 88%.

Based on the previous two processes, Harrington et al. in Roche Ltd. amended the route to Tamiflu again in 2004 (Scheme 5).[^18c] Epoxide 18 was regioselectively ring-opened by tBuNH₂-MgCl₂ complex to 31, which was subsequently mesylated and cyclized to form aziridine 32. Then (allyl)₂NH was used as a nucleophile in the
regioselective opening of the aziridine ring which was catalyzed by PhSO$_3$H. The secondary amine was efficiently acetylated by heating 33 with Ac$_2$O in the presence of NaOAc at 110 °C for 4 h. During the screening of acid catalyzed cleavage of the t-butyl group, it was found that a precipitate of 35 could be produced when 34 was treated with HCl in ethanol at room temperature. Thereby, it is a great chance to purify the intermediate by simple filtration. Finally, removal of the t-butyl group by TFA afforded 36. Elimination of diallyl amine in the presence of 1,3-dimethylbarbituric acid/Pd(OAc)$_2$/PPh$_3$ and subsequent phosphorylation of the resulting mixture yielded 4. This process features a regiospecific epoxide opening, a selective O-mesylation of t-butylamino alcohol and an efficient cleavage of t-butyl group from an aliphatic t-butylamide. Compared to the first Roche process, the second process significantly simplified the manipulation procedure (mesylation without protection and deprotection) and improved the total yield from 35-38% to 61%.

![Scheme 6. Shi’s first generation synthesis of Tamiflu started from (-)-shikimic acid. Reagents and conditions: (a) i) SOCl$_2$, EtOH, reflux, 3.5 h, 97%; ii) Me$_2$C(OMe)$_2$, TsOH, EtOAc, 35 °C, 95%; (b) BzCl, Et$_3$N, DMAP, CH$_2$Cl$_2$, 0 °C to rt, 5 h, 98%; (c)](image-url)
HCl, EtOAc/H2O, rt, 6 h, 94%; (d) MsCl, Et3N, DMAP, EtOAc, 0 °C, 1 h, 97%; (e) NaN3, DMF/H2O, −5 to 0 °C, 4 h, 95%; (f) Ph3P, THF, rt, 2 h, then Et3N, THF/H2O, rt, overnight, 88%; (g) Ac2O, Et3N, EtOAc, 0 °C, 0.5 h, 98%; (h) BF3·OEt2, 3-pentanol, −5 to 0 °C, 0.5 h, 92%; (i) K2CO3, EtOH, rt, 6 h, 90%; (j) MsCl, Et3N, CH2Cl2, 0 °C, 1 h, 95%; (k) NaN3, DMF/H2O, 90 °C, 3 h, 84%; (l) Lindlar catalyst, H2, EtOH, rt, 16 h; (m) H3PO4, EtOAc/EtOH, 50 °C, 0.5 h, 91% for 2 steps.

In early 2009, Shi et al. reported a 13-step synthetic route to Tamiflu starting from (−)-shikimic acid (Scheme 6).[36] Compound 24 was synthesized according to the literature procedure reported.[18d] Reaction of 24 with BzCl and Et3N in the presence of DMAP furnished 37. Treatment of 37 with HCl in EtOAc/H2O resulted in diol 38, which was then mesylated to 39. The mesylate group at allylic position was stereoselectively substituted by the azido group. Treatment of 40 with Ph3P and Et3N successively provided aziridine 41. Acetylation of 41 and subsequent ring-opening by 3-pentanol in the presence of BF3·Et2O resulted in 42. Removal of benzoyl group with K2CO3 and subsequent mesylation furnished 43. Substitution of MsO group at C-5 with azide afforded 23. Azide 23 was reduced by hydrogenation in the presence of Lindlar’s catalyst. Tamiflu was then obtained by treating 3 with H3PO4 in EtOAc/EtOH.

Although the overall yield is good (40%) and the reagents are relatively cheap, this route is still not efficient for large-scale production. Soon after, they managed to improve and a further reduction of the synthetic route to 9 steps (Scheme 7).[18f] Compound 16 was synthesized according to the literature procedure.[18d] Treatment of 16 with MsCl and Et3N furnished trimesylate 44, which was stereoselectively substituted at C-3 by azido group in aq. acetone. The high stereoselectivity could be attributed to the axial bond of MsO group at allylic C-3. Moreover, this substitution
was conducted at low temperature to avoid dehydrogenation. Aziridine 46 was formed after the successive treatment of 51 with Ph3P and Et3N. Acetylation of 46 with Ac2O and Et3N resulted in 47, which was selectively ring-opened by 3-pentanol in the presence of BF3·Et2O. Compound 43 was converted to 4 following the same procedure as demonstrated above in scheme 5.

Scheme 7. Improved synthetic route to Tamiflu by Shi’s group. Reagents and conditions: (a) NaOEt, EtOH, rt, 97%; (b) MsCl, Et3N, DMAP, EtOAc, 0 °C, 1 h, 93%; (c) NaN3, Me2CO/H2O, 0 °C, 4 h, 92%; (d) Ph3P, THF, rt, 0.5 h, then Et3N, H2O, rt, 24 h, 84%; (e) Ac2O, Et3N, EtOAc, 0 °C, 0.5 h, 98%; (f) BF3·OEt2, 3-pentanol, −8 to 0 °C, 1 h, 86%.

Almost simultaneously, Karpf and Trussardi from Roche Ltd. published a synthetic route which is similar to that of Shi’s synthetic strategy, with the exception of the aziridination step (Scheme 8).[37] In Shi’s tactic, the azide was reduced by Ph3P and the aziridine was subsequently formed under basic conditions. However, Karpf and Trussardi employed diethyl phosphite to generate an iminophosphite, followed by aziridination and Arbusov-type cleavage to obtain 50.

The most challenging task in these two routes was the formation of trimesylate 44, which was prone to elimination to form an aromatic compound. The solution to this...
problem was the proper addition sequence (triethyl amine was added after MsCl) and the lowering of the reaction temperature (0 °C). Although NaN₃ was used as the nitrogen resource in both routes, both strategies are short and efficient and the yields are 47% and 20% respectively. In addition, the authors tried to purify the compounds using crystallization to avoid chromatography. These two tactics were characterized with cheap reagents, easy manipulation and purification, short synthetic steps and higher yields.

Scheme 8. Partial improved route to Tamiflu exploited by Karpf and Trussardi. Reagents and conditions: (a) P(OEt)₃, toluene, reflux, 5 h; (b) BF₃·OEt₂, 3-pentanol, rt, 16 h, MTBE crystallization, 55% for 2 steps; (c) H₂SO₄, EtOH, reflux, 16 h; and then Ac₂O, EtOAc, rt, 1 h, 73%.

2. (−)-Quinic acid

Due to limited resources of (−)-shikimic acid in the late 20th century, Gilead Inc. presented an alternative synthetic route which utilized (−)-quinic acid 52 as the starting material to synthesize ketal 17 (Scheme 9).[18d] (−)-Quinic acid 52 was reacted with 2,2-dimethoxypropane and TsOH in EtOAc to give lactone 53, which was treated with sodium ethoxide in ethanol and subsequently mesylated to give compound 55.
The free hydroxyl group in $55$ was eliminated by the addition of $\text{SO}_2\text{Cl}_2$/pyridine to give a mixture of $25$, $56$ and $57$. The undesired $56$ was removed by treatment with pyrrolidine and $(\text{Ph}_3\text{P})_4\text{Pd}$ followed by acid extraction. Further crystallization provided pure compound $25$, which was transketalized to $17$ by 3-pentanone in the presence of HClO$_4$. The yield of $17$ starting from $52$ was not ideal to produce in large-scale production. Later, Roche further modified these reaction conditions to improve the yield from 20% to 44%. However, as $(-)$-quinic acid is a natural product which is also in limited availability, this route is not widely encouraged for industrial production.

**Scheme 9.** First synthesis of Tamiflu starting from $(-)$-quinic acid. Reagents and conditions: (a) 2,2-dimethoxypropane, TsOH, acetone, reflux, 2 h; (b) NaOEt, EtOH, rt, 2 h; (c) MsCl, Et$_3$N, CH$_2$Cl$_2$, 0-5 °C, 1.5 h, 69% for 3 steps; (d) $\text{SO}_2\text{Cl}_2$, pyridine, CH$_2$Cl$_2$, −30 to −20 °C, then pyrrolidine, $(\text{Ph}_3\text{P})_4\text{Pd}$, EtOAc, 35 °C, 3.5 h, 30%; (e) 3-pentanone, HClO$_4$, 40 °C, 25 mmHg, 95%.

3. Diene and dienophile

Shikimic acid and quinic acid are natural products found in low quantity. Roche publicly announced the shortage of Tamiflu in 2005 due to the limited starting material.
Thereafter, many researchers in academia and pharmaceutical industry dedicated themselves to exploit new methods to synthesize Tamiflu.

Scheme 10. First Diels-Alder synthesis of precursor to Tamiflu. Reagents and conditions: (a) ZnCl₂, neat, 50 °C, 72 h, 77%; (b) Chirazyme L-2, methylcyclohexane, aq. pH 8 buffer, 1 °C, 97% ee, 20%; (c) DPPA, toluene, 70 °C, 18 h; (d) NaOEt, EtOH, rt, 1 h, 53% for 2 steps; (e) NaHMDS, THF, −60 °C, 15 h, 94%; (f) MsCl, Et₃N, CH₂Cl₂, rt; (g) 3-pentanol, BF₃·OEt₂, CH₂Cl₂, 62% for 2 steps; (h) 20% H₂SO₄, EtOH, 70 °C, 22 h, 68%.

The first approach, which is independent of shikimic acid and quinic acid, commenced with a ZnCl₂ catalyzed Diels-Alder (D-A) reaction between furan and ethyl acrylate (Scheme 10).[38] The exo-endo products were formed in a 9:1 ratio. The desired exo product was obtained in 97% ee and 20% yield at 75% conversion rate by enzyme resolution. Treatment of 60 with DPPA in toluene furnished endo-aziridine 61, which was transesterified with EtOH to afford 62. The biscyclic 62 was treated with NaHMDS in THF to give the alcohol 63. Mesylation of 63 with MsCl and Et₃N was carried out before the aziridine ring was regioselectively opened by 3-pentanol. After removal of the diethylphosphoryl group, 64 was isolated in its hydrochloride form. Compound 64 could be easily converted to 4 by known procedures.[35] This approach
may not be adopted in manufacturing due to the low overall yield. Nonetheless, with the new synthetic design such as the inclusion of D-A reaction and enzyme resolution, many groups were inspired to come up with better and efficient strategies to synthesize Tamiflu.

Scheme 11. Synthetic route to Tamiflu developed by Corey’s group. Reagents and conditions: (a) 10 mol% of 67, neat, 23 °C, 30 h, 97%; (b) NH₃, CF₃CH₂OH, 40 °C, 5 h, 100%; (c) i) TMSOTf, Et₃N, pentane; ii) I₂, Et₂O/THF, 2 h, 84%; (d) Boc₂O, Et₃N, DMAP, CH₂Cl₂, 4 h, 99%; (e) DBU, THF, reflux, 12 h, 96%; (f) NBS, cat. AIBN, CCl₄, reflux, 2 h, 95%; (g) Cs₂CO₃, EtOH, 25 min, 100%; (h) 5 mol% of SnBr₂, N-bromoacetamide, MeCN, −40 °C, 4 h, 75%; (i) nBu₄NBr, KHMDS, DME, −20 °C, 10 min; (j) cat. Cu(OTf)₂, 3-pentanol, 0 °C, 12 h, 61%; (k) H₃PO₄, EtOH.

Corey’s group⁴⁰ commenced their synthesis with an asymmetric D-A reaction between butadiene with trifluoroethyl acrylate, catalyzed by S-proline-derived catalyst 67 (Scheme 11).⁴⁰ The first step could be performed on a multigram scale. The adduct 68, which was formed in 97% yield with > 97% ee, was ammonolyzed to amide 69 in quantitative yield. Then iodolactam 70 was prepared according to the
Knapp method.[40] The amide of 70 was protected as a Boc derivative and HI was eliminated by refluxing 71 in THF in the presence of DBU. Lactam 73 was formed after allylic bromination. The lactam 73 was treated with Cs₂CO₃ in EtOH to give ester 74. Bromoacetamidation occurred regio- and stereoselectively by treating 74 with N-bromoacetamide in MeCN at −40 °C. Aziridine 76 was easily formed by using in situ generated tetra-ₙ-butylammonium hexamethyldisilazane and subsequently, ring-opening by 3-pentanol in the presence of Cu(OTf)₂ furnished 77. Tamiflu was obtained by the treatment of 77 with H₃PO₄ in EtOH. This synthesis was carried out in milligram scale and some steps could be further optimized. Corey did not patent this process, allowing its application in industry.

Although potential hazardous azide-containing reagents are not used, Corey’s group used some other reagents such as trifluoroethyl acrylate and CCl₄ which are environmentally unfriendly. Okamura et al. accomplished the intermediate 74 in a green manner (Scheme 12).[20d] The diene 79 was prepared from pyridone 78 with reference to the literature procedure.[41] Base-catalyzed D-A reaction between 79 and ethyl acrylate afforded the bicyclic 80, which was reduced to 81 by NaBH₄. The Ns group was replaced with the Boc group by treating 81 with PhSH and K₂CO₃ in MeCN, followed by treatment with Boc₂O. Reaction of 82 with NaIO₄ in aq. THF provided the isomers 83 and 84. This mixture was converted to 74 by treating with NaBH₄/CeCl₃ in MeOH and then MsCl/Et₃N in THF.

The cyclohexene ring was constructed by the D-A reaction, but the starting materials diene 79 and 78 were not commercially available. The reaction conditions are indeed greener but the yields are much lower when compared with Corey’s methodology. In addition, the purification process was quite difficult because racemic 74 was obtained.
Scheme 12. Synthesis of Corey’s Tamiflu intermediate 74. Reagents and conditions: (a) i) nBuLi, NsCl, THF, 0 °C, 24 h, 89%; ii) AcOH, cat. H2SO4, rt, 30 h, 81%; (b) ethyl acrylate, aq. NaOH, rt, 24 h, 83%; (c) NaBH4, THF, 0 °C, 2 h, 77%; (d) PhSH, K2CO3, MeCN, rt, 3 h, then Boc2O, 24 h, 55%; (e) NaIO4, THF/H2O, 0 °C, 3 h; (f) i) NaBH4, CeCl3, MeOH, 0 °C, 2 h; ii) MsCl, Et3N, DMAP, THF, 0 °C to rt, 4 h, 33% from 82.

Shibasaki et al. reported four routes to synthesize Tamiflu. The first two routes were lengthy and shuttled between protection-deprotection steps. To improve the synthetic efficacy, the third generation synthesis had been proposed which commenced with Diels-Alder reaction between 85 and 86 (Scheme 13).[20b] The resulting diastereoisomers 87 were treated with TMSN3 and DMAP in one pot to produce isomers 88, which were treated with HCl to give pure 89. The undesired exo isomer was decomposed during acidic cleavage. The Curtius rearrangement was conducted under heating at 80 °C in tBuOH. Subsequently an intramolecular addition of 3-hydroxyl group to the C-4 isocyanate group and an intermolecular addition of tBuOH to the C-5 isocyanate group afforded carbamate 90. Hydrolysis of oxazolidinone with aqueous LiOH and successive N-acetylation generated 92, which was further oxidized by isobutyric anhydride/DMSO.[42] Subsequent chiral HPLC purification afforded pure enone 93. Michael addition of TMSCN to enone 93, α-bromination and consequent
HBr elimination with Et$_3$N produced β-cyanoenone 94. Enone 94 was selectively reduced by LiAlH(OtBu)$_3$ to give alcohol 95. 3-Pentanol ether was introduced through aziridine formation and subsequent ring-opening. Hydrolysis of cyanide and cleavage of Boc group with HCl/EtOH produced oseltamivir 3.

Scheme 13. Shibasaki’s group third generation synthesis of Tamiflu. Reagents and conditions: (a) THF, rt, 2 h; (b) TMSN$_3$, DMAP, rt, 2 h; (c) 1 N HCl, 4 °C, 10 min, 55% for 3 steps; (d) tBuOH, reflux, 13 h; (e) LiOH; (f) Ac$_2$O, Et$_3$N, DMAP, CH$_2$Cl$_2$, rt, 2.5 h; (g) Isobutyric anhydride, DMSO, then chiral HPLC, 53% for 4 steps; (h) i) TMSCN, Ni(COD)$_2$, COD; ii) NBS, Et$_3$N; (i) LiAlH(OtBu)$_3$, 44% for 2 steps; (j) DEAD, PPh$_3$, 66%; (k) BF$_3$·OEt$_2$, 3-pentanol, −20 °C, 15 min, 56%; (l) HCl, EtOH, 60%.

The third generation route is more concise than the previous two. However, it is not safe to carry out the Curtius rearrangement under refluxing conditions. Chiral HPLC separation of enone 93 and the low yield of cyanoenone 94 are the main constraints to scaling up.
The latest fourth generation synthesis developed by Shibasaki’s group was reported in 2009.[20c] Since the starting material \( \text{85} \) is not stable under acidic conditions, they developed a D-A reaction catalyzed by \( \text{Ba(OrPr)}_2/\text{F2-FujiCAPO (106)} \) and CsF to give the adducts in 99% yield (d.r. 5:1) with 91% ee of \( \text{99} \) (Scheme 14). The mixture was hydrolyzed to acid, which was treated with DPPA and Et\(_3\)N to afford diacyl azide \( \text{89} \). During this transformation, the desired product \( \text{89} \) derived from \( \text{99} \) could be purified by column chromatography. Carbamate \( \text{90} \) was prepared from \( \text{89} \) according to the same procedure as described above. Then carbamate amine was protected by acetyl group. Compound \( \text{100} \) was allylic substituted by dicyanomethyl acetate \( \text{101} \) catalyzed by [Pd\(_2\)(dba)\(_3\)]CHCl\(_3\) and dppf to afford \( \text{102} \). \( \alpha \)-Epoxide was exclusively formed by the treatment of \( \text{102} \) with in situ generated trifluoroperacetic acid. Acetoxydicyanomethyl group was converted to ethoxycarbonyl and subsequently, epoxide opening occurred in a one pot reaction under K\(_2\)CO\(_3\) in EtOH. Later, the configuration of 3-hydroxy group of \( \text{105} \) was inverted by Mitsunobu esterification and one-pot ethanolysis. Aziridine \( \text{76} \) was produced under Mitsunobu conditions using Me\(_2\)PPh and DIAD. The aziridine \( \text{76} \) was ring-opened by 3-pentanol in the presence of BF\(_3\)·Et\(_2\)O. Tamiflu was obtained after cleavage of the Boc group with TFA and treatment with H\(_3\)PO\(_4\). The key steps in the fourth generation synthesis are an asymmetric D-A reaction to build the cyclohexene framework, a Curtuis rearrangement to introduce two amino groups, an allylic substitution to obtain ester and aziridine opening with 3-pentanol. The authors claimed that some steps could be carried out on a multigram scale and most of the intermediates could be isolated by crystallization.
Scheme 14. Catalytic asymmetric synthesis of Tamiflu by Shibasaki’s group. 
Reagents and conditions: (a) Ba(O_{i}Pr)_{2}, F2-FujiCAPO, CsF, THF, \(-20^\circ\text{C}\), 36-96 h, then aq. 1 M HCl, 91%; (b) aq. 2 M NaOH, MeOH, 60 °C, 10 h; (c) DPPA, Et_{3}N, THF, 0 °C, 21 h, 95% for 2 steps; (d) tBuOH, 80 °C 13 h; (e) Ac_{2}O, Et_{3}N, DMAP (10 mol%), CH_{2}Cl_{2}, rt, 2.5 h, 80% for 2 steps; (f) 101, [Pd_{2}(dba)_{3}]·CHCl_{3}, dppf, toluene, 60 °C, 30 min, 85%; (g) TFAA, urea/H_{2}O_{2}, Na_{2}HPO_{4}, CH_{2}Cl_{2}, 4 °C, 2 h; (h) K_{2}CO_{3}, EtOH, rt, 5 h; (i) DEAD, PPh_{3}, p-nitrobenzoic acid, THF, \(-20^\circ\text{C}\), 1.5 h; LiOH, EtOH, \(-20^\circ\text{C}\), 15 min, 65% for 3 steps; (j) DIAD, Me_{2}PPh, Et_{3}N, CH_{2}Cl_{2}, 4 °C, 10 min, 76%; (k) BF_{3}·OEt_{2}, 3-pentanol, \(-20^\circ\text{C}\), 15 min, 75%; (l) TFA; H_{3}PO_{4}, 73%.

4. Cyclohexene compound

Shibasaki is a pioneer in introducing desymmetry concept in the synthesis of Tamiflu. His group embarked on their first generation synthesis of Tamiflu with catalytic enantioselective desymmetrization of a meso-aziridine.\(^{[25a]}\) After screening a few metal catalysts, a combination of 2 mol% of Y(O{Pr})_{3} and 4 mol% of chiral ligand 106 in propionitrile was found to be an optimized condition for aziridine ring-opening with TMSN_{3} (Scheme 15). Chiral 108 was obtained in 99% ee after recrystallization.
Scheme 15. Shibasaki group’s first generation synthesis of Tamiflu. Reagents and conditions: (a) i) 2 mol% Y(OtPr)_3, 106, TMSN_3, CH_3CH_2CN, rt, 48 h, 96%, 91% ee; ii) recrystallization from iPrOH, 72%, 99% ee; (b) Boc_2O, DMAP, MeCN, rt, 3 h; (c) 4 M NaOH, rt, 2 h, 98% for 2 steps; (d) i) Ph_3P, MeCN, 50 °C, 3 h; ii) H_2O, 40 °C, 2 h; (e) Boc_2O, Et_3N, CH_2Cl_2, rt, 2 h, 90% for 2 steps; (f) SeO_2, Dess-Martin periodinane, dioxane, 80 °C, 12 h; (g) i) Dess-Martin periodinane, CH_2Cl_2, 4 °C; ii) recrystallization form iPrOH/hexane, 62% for 2 steps, >99% ee; (h) Ni(COD)_2, COD, TMSCN, THF, 60 °C, 65 h; (i) i) NBS, THF, 20 min; ii) Et_3N, 4 °C, 40 min; (j) LiAlH(OtBu)_3, THF, 4 °C, 30 min, 60% for 3 steps; (k) DEAD, Ph_3P, THF, 4 °C, 1 h, 87%; (l) 3-pentanol, BF_3·OEt_2, 4 °C, 1 h, 52%; (m) TFA, CH_2Cl_2, 4 °C to rt, 3 h; (n) Boc_2O, Et_3N, CH_2Cl_2, 4 °C, 30 min, 63% for 2 steps; (o) Ac_2O, DMAP, pyridine, rt, 1 h, 84%; (p) i) 4.2 M HCl in EtOH, 60 °C, 4 h; ii) H_2O, 4 °C, 3 h, 53%; (q) 85% H_3PO_4, EtOH, 50%.

Amide was protected by Boc group before the 3,5-dinitrobenzoyl group was cleaved by NaOH. Azide was reduced to amine by PPh_3 in MeCN and then protected by Boc.
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group to give 110. Treatment of diamide 110 with SeO$_2$[43] and DMP resulting in a mixture of 111 and 112. Pure 112 was obtained by treating the mixture with DMP again followed by recrystallization. 1,4-Addition of TMSCN to 112 catalyzed by Ni(COD)$_2$ gave 113, which was converted to $\gamma$-keto nitrile 114 by treating with NBS and Et$_3$N. The ketone was selectively reduced to alcohol 115 with LiAlH(OtBu)$_3$.

Applying Mitsunobu conditions to 115 generated aziridine 116, which was then ring-opened by 3-pentanol in the presence of BF$_3$$\cdot$OEt$_2$. Treatment of 117 with TFA afforded free diamine 118. Then, amine at C-5 was selectively protected by Boc group due to the less steric hindrance. Another amine at C-4 was acetylated with Ac$_2$O in pyridine. Hydrolysis of nitrile with HCl/EtOH and H$_2$O and concomitant cleavage of the Boc group provided oseltamivir 3, which was finally converted to Tamiflu with H$_3$PO$_4$ salt formation.

Scheme 16. Shibasaki group’s second generation synthesis of Tamiflu. Reagents and conditions: (a) i) I$_2$, K$_2$CO$_3$, KI, CHCl$_3$, 60 °C, 19 h, 83%; ii) DBU, CH$_2$Cl$_2$, rt, 4 h, 85%; (b) Boc$_2$O, Et$_3$N, DMAP, CH$_2$Cl$_2$, rt, 2 h, 99%; (c) AcSH, 2,6-lutidine, CHCl$_3$, 60 °C, 19 h, 83%; (d) Cs$_2$CO$_3$, nBuOH, 4 °C to rt, 16 h, 86%; (e) Dess-Martin periodinane, CH$_2$Cl$_2$, 4 °C to rt, 15 min, 90%; (f) (EtO)$_2$P(O)CN, LiCN, THF, −20 °C, 4 h; (g) i) toluene, sealed tube, 140 °C, 40 min; ii) aq. NH$_4$Cl, rt, 3 h, 78% for 3 steps; (h) DEAD, Ph$_3$P, pNO$_2$C$_6$H$_4$CO$_2$H, THF, 4 °C, 15 min; ii) 1 M LiOH, H$_2$O/THF, −20 °C, 2.5 h, 80%.
Although Shibasaki’s group used a starting material other than shikimic acid and quinic acid, the aziridine 107 had to be prepared from 1,4-cyclohexene in 5 steps according to the literature method. Stoichiometric amount of toxic SeO$_2$ was used to promote the allylic oxidation. Later, the authors made some improvements based on the first generation synthesis (Scheme 16).[25b]

Firstly, aziridine ring-opening condition was further optimized. When 2,6-dimethylphenol was used as an additive[44] and the amount of catalyst and chiral ligand was reduced to half, the reaction went faster, giving similar yield and ee. The chiral ligand could be recycled through extraction with base and regeneration without decreasing the catalytic activity. Preparation of azido amide 109 was similar to that of the first generation procedure. Subjecting 109 to I$_2$/K$_2$CO$_3$/KI in CHCl$_3$ followed by DBU provided oxazolidinone 120, which was protected as a Boc derivative. Then, the azide was reduced to acetylamide by AcSH. The resulting oxazolidinone 122 was hydrolyzed by Cs$_2$CO$_3$ in nBuOH to give alcohol 123, which was oxidized to enone 124 with DMP. 1,2-Addition of diethoxy cyanophosphonate to enone 124 produced 125, which was transformed to 126 undergoing an allylic rearrangement under heating at 140 °C in sealed tube and subsequent elimination carried out by the addition of aqueous NH$_4$Cl solution. The chirality of the hydroxyl group at C-3 was reversed by a Mitsunobu reaction of 126 with p-nitrobenzoic acid, followed by hydrolysis in one pot to give alcohol 95.

In the second generation synthesis, usage of toxic SeO$_2$ was avoided and some cumbersome protection-deprotection procedures were reduced under optimized conditions. However, an azide reagent was used as the nitrogen source and the overall
yield was still low. The low efficacy of these two routes motivated the authors to develop alternative synthetic strategies.

Scheme 17. Synthesis of Tamiflu exploited by Kann’s group. Reagents and conditions: (a) Fe$_2$(CO)$_6$, toluene, 55 °C, 86%; (b) Ph$_3$CPF$_6$, CH$_2$Cl$_2$, rt, 94%; (c) 129, DIPEA, CH$_2$Cl$_2$, 0 °C, 75%; (d) i) preparative HPLC, 47% for (−)-130; ii) HPF$_6$, Et$_2$O, 0 °C, 94%; (e) i) BocNH$_2$, DIPEA, CH$_2$Cl$_2$, 0 °C, 86%; ii) H$_2$O$_2$, NaOH, EtOH, 0 °C, 95%; (f) mCPBA, CH$_2$Cl$_2$, −70 °C to rt, 95%; (g) NaN$_3$, NH$_4$Cl, DME/EtOH/H$_2$O, 0 °C, 95%; (h) MsCl, Et$_3$N, CH$_2$Cl$_2$, 0 °C, 65%; (i) 3-pentanol, Cu(OTf)$_2$, 0 °C, 48%. (l) H$_3$PO$_4$, EtOH.

Kann et al. applied the cationic iron carbonyl chemistry$^{[45]}$ to synthesize Tamiflu (Scheme 17).$^{[26a]}$ The starting material could be synthesized from acrolein and the phosphonium salt of ethyl 4-bromobut-2-enoate according to the literature method.$^{[46]}$ The diene was heated with diiron nonacarbonyl in toluene and subsequently treated with Ph$_3$CPF$_6$ to generate racemic iron carbonyl complex salt 128, which was then purified by chiral resolution. Reaction of 128 with chiral alcohol 129 produced
diastereomers (+)-130 and (−)-130, which were separated by preparative HPLC. The desired (−)-128 was obtained by treating (−)-130 with HPF₆. Reaction of (−)-128 with BocNH₂ and decomplexation with H₂O₂/NaOH afforded 131. The more electron-rich alkene of 131 was selectively epoxidized by mCPBA to furnish epoxide 132, which was ring-opened by sodium azide. Treatment of 133 with MsCl followed by Ph₃P/Et₃N resulted in aziridine 134. Acetylation of 134 with Ac₂O afforded 76, which was subsequently ring-opened with 3-pentanol and treated with phosphoric acid to produce Tamiflu 4.

**Scheme 18.** Synthesis of Tamiflu started from cis-1,2-dihydrocatechol 136 exploited by Fang et al. Reagents and conditions: (a) Dimethoxypropane, TsOH·H₂O, acetone, 0 °C to rt, 0.5 h; (b) cat. SnBr₄, NBA, H₂O, CH₃CN, 0 °C, 8 h, 75% for 2 steps; (c) LiHMDS, THF, −10 to 0 °C, 0.5 h; (d) 3-pentanol, BF₃·OEt₂, −10 to 0 °C, 6 h, 73% for 2 steps; (e) conc. HCl, MeOH, 50 °C, 6 h, 94%; (f) AcOCMe₂COBr, THF, 0 °C to rt, 3.5 h; (g) LiBH₂Et₃, THF, 0 °C to rt, 2 h, 82% for 2 steps; (h) DPPA, DIAD, PPh₃, THF, 40 °C, 24 h, 84%; (i) [Ni(CO)₂(PPh₃)₂], DIPEA, EtOH, THF, 80 °C, 24 h, 81%; (j) i) H₂, Lindlar catalyst; ii) H₃PO₄, EtOH, 50 °C, 6 h, 91%.

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Iron carbonyl chemistry and chiral resolution were applied in Kann’s strategy. The highlight of their work is the potential to synthesize various analogues of oseltamivir. It is due to the fact that various nucleophiles can attack the iron carbonyl cation. However, the chiral HPLC purification and the usage of NaN₃ and mCPBA make this methodology difficult to scale up. This strategy is suitable to prepare oseltamivir and different analogues, which might be used to investigate bioactivity in medicinal chemistry.

Fang et al. explored a new method to access Tamiflu (Scheme 18).[26b] This strategy started from bromoarene cis-1,2-dihydrodiol, which is a commodity chemical and can be prepared by microbial oxidation of bromobenzene.[47] Two hydroxy groups were protected by 2,2-dimethoxypropane to give 137. Reaction of 137 with NBA, catalyzed by SnBr₄, resulted in bromoamide 138,[20a] which was converted to aziridine 139 under LiHMDS. Aziridine ring-opening with 3-pentanol was promoted by BF₃·OEt₂ to afford compound 140. Treatment of 140 with concentrated HCl in MeOH yielded diol 141, which was reacted with α-acetoxyisobutyryl bromide to generate compound 142. The bromine at the allylic position and the acetyl group of the ester were simultaneously reduced by LiBHEt₃ to give alcohol 143. Mitsunobu reaction of alcohol 143 with DPPA furnished azide 144. Treatment of 144 with stoichiometric amount of Ni(CO)₂(PPh₃)₂ and EtOH provided ester 23.[48] The synthesis of Tamiflu was completed with the reduction of the azide and phosphate formation.

This synthetic route was further improved to avoid the use of hazardous azide reagents. Bu₄NOCN was used as a nitrogen source instead of DPPA to install amino group be introduced on C-5 (Scheme 19). Treatment of 143 with Bu₄NOCN/PPh₃/ DDQ followed by tBuOH produced compound 145. Since direct Pd-catalyzed carbonylation
of 145 failed, the bromide was converted to iodide to improve the reaction activity. Then, carboxylation of 146 proceeded successfully with Pd(OAc)$_2$ as the catalyst in EtOH to give carboxylate 147. Concurrent removal of the Boc group and salt formation with H$_3$PO$_4$ furnished Tamiflu 4.

Scheme 19. Alternative synthetic route to Tamiflu from 143 exploited by Fang et al..

Reagents and conditions: (a) DDQ, PPh$_3$, $n$Bu$_4$NOCN, CH$_3$CN, rt, 18 h, then $t$BuOH, reflux, 24 h, 78%; (b) CuI, KI, DMEDA, $n$BuOH, 120 °C, 24 h; (c) cat. [Pd(OAc)$_2$], CO, NaOAc, EtOH, rt, 24 h, 82% for 2 steps; (d) H$_3$PO$_4$, EtOH, 50 °C, 6 h, 81%.

The highlight of this work is that the carboxylate could be replaced by phosphonate by the phosphorylation in the late stage to give tamiphosphor, which was found to be an effective NA inhibitor as well as Tamiflu. This strategy was concise and furnished Tamiflu in 21% overall yield. The synthesis was demonstrated on gram-scale.

Banwell reported a formal synthetic route of Tamiflu 4 starting from diol 136 (Scheme 20).[26c] The monoprotected 148 was obtained by treatment of 136 with 4-methoxybenzaldehyde dimethyl acetal and subsequent reduction with DIBAL-H. The alcohol 148 was converted to $N$-hydroxycarbamate by subjecting it to CDI and hydroxyamine successively. Reaction of 149 with TsCl and Et$_3$N provided $N$-tosyloxy carbamate 150, which was readily transformed to acylaziridine 151 in the presence of Cu(MeCN)$_2$PF$_6$ and K$_2$CO$_3$.[49] Regioselective ring-opening of 151 with 3-pentanol
afforded carbamate 152, which was subsequently hydrolyzed with LiOH to give compound 153. Acetylation of the amine group and removal of the PMB group resulted in diol 154, which is an intermediate of Fang’s protocol.

Scheme 20. Synthesis of Tamiflu starting from cis-1,2-dihydrodiol developed by Banwell et al.. Reagents and conditions: (a) 4-Methoxybenzaldehyde dimethyl acetal, (+)-CSA, toluene, 0 °C, 1.5 h; (b) DIBAL-H, Et$_3$N, toluene, −78 to −30 °C, 5 h, 85% for 2 steps; (c) i) CDI, MeCN, 0 °C, 1 h; ii) NH$_2$OH·HCl, imidazole, 0 to 18 °C, 16 h, 56% at 88% conversion; (d) TsCl, Et$_3$N, Et$_2$O, 0 to 18 °C, 16 h, 79%; (e) Cu(MeCN)$_4$PF$_6$, K$_2$CO$_3$, MeCN, 3-pentanol, 0 to 18 °C, 16 h, 43%; (f) LiOH, 1,4-dioxane/water, 100 °C, 48 h, 85%; (h) AcCl, Et$_3$N, 0 to 18 °C, 1 h, 99%; (i) HCl, CH$_3$OH, 35 °C, 16 h, 90%.

The synthetic route to intermediate 141 developed by Banwell et al. is lengthier than Fang’s method. The highlight of Banwell’s strategy is the application of an intramolecular aziridination to install an amino group at C-4.
5. Aromatic compound

Fukuyama’s group\(^{28b}\) synthesized Tamiflu starting with pyridine, which was reduced to dihydropyridine in the presence of CbzCl and NaBH\(_4\) in methanol (Scheme 21).\(^{50}\) Diels-Alder reaction between Cbz protected dihydropyridine and acrolein, catalyzed by MacMillan catalyst 157, afforded a mixture of aldehydes.\(^{51}\) Kraus oxidation\(^{52}\) of this mixture furnished the corresponding carboxylic acid including 159. During the workup, the carboxylic acids were basified into an aqueous solution with sodium bicarbonate, to which bromine was added. Then, the desired lactone 160 was obtained through extraction followed by crystallization. Hydrogenolysis of 160 in the presence of Boc\(_2\)O afforded 161, which was oxidized with NaIO\(_4\) and a catalytic amount of RuO\(_2\cdot n\)H\(_2\)O to give 162. Compound 162 was ammonolysed in \(t\)BuOH to 163, which was in turn mesylated to 164 under MsCl and Et\(_3\)N. Hoffmann rearrangement then proceeded when 164 was treated with PhI(OAc)\(_2\) and allyl alcohol.\(^{53}\) After alcoholysis and dehydrobromination in the presence of NaOEt in ethanol, 165 was transformed to aziridine 166. 3-Pentanoyl was then introduced stereoselectively with the aid of BF\(_3\)·OEt\(_2\). Deprotection and acetylation of 167 furnished 168. Removal of the alloc group under Pd/C, Ph\(_3\)P and 1,3-dimethylbarbituric acid in ethanol and treatment with phosphoric acid afforded crystalline 4.

The advantages of this strategy are: (1) the starting material is cheap and is available in large quantities, (2) most reagents are common and inexpensive. Expensive RuO\(_2\cdot n\)H\(_2\)O was used but it could be recycled. Most intermediates are crystalline compounds and easy to purify. However, the total yield reported is 5.6%, which can still be improved.
**Scheme 21.** Synthetic route of Tamiflu starting from pyridine. Reagents and conditions: (a) CbzCl, NaBH₄, MeOH, −50 to −35 °C, 1 h; (b) 157, acrolein, CH₃CN/H₂O, rt, 12 h; (c) NaClO₂, NaH₂PO₄, 2-methyl-2-butene, tBuOH/H₂O, 0 °C to rt, 1 h; (d) Br₂, aq. NaHCO₃, CH₂Cl₂, rt, 26% for 4 steps; (e) H₂, Pd/C, Boc₂O, EtOH/THF, rt, 2 h, 92%; (f) RuO₂, NaIO₄, DCE/H₂O, 80 °C, 1.5 h, 86%; (g) NH₃, tBuOH, THF, 0 °C, 95%; (h) MsCl, Et₃N, CH₂Cl₂, rt, 1 h, 91%; (i) allyl alcohol, Phl(OAc)₂, 4 Å M.S., toluene, 60 °C, 10 h, 88%; (j) NaOEt, EtOH, 0 °C, 87%; (k) BF₃·Et₂O, 3-pentanol, −20 °C, 62%; (l) TFA, CH₂Cl₂, 0 °C to rt, then Ac₂O, pyridine, 88%; (m) Pd/C, Ph₃P, 1,3-dimethylbarbituric acid, EtOH, reflux, 40 min, then H₃PO₄, 76%.

In 2008, Zutter *et al.* from Roche published another route to synthesize Tamiflu. Instead of shikimic acid and quinic acid, this strategy used cheap and readily available 2,6-dimethoxyphenol as starting material (Scheme 22). The 3-pentyl group was installed at the beginning by reacting 169 with 3-pentyl mesylate in the presence of KOtBu in DMSO. Treatment of 170 with NBS in DMF afforded crystalline dibromide 171, which was converted to dicarboxylate 172 by Pd-catalyzed ethoxycarbonylation. Hydrogenation of 172 catalyzed by Ru-Al₂O₃ in EtOAc provided pure cis meso-
dicarboxylate 173, which was treated with TMSCl and NaI to cleave the two methyl
groups. The symmetrical diester 174 was hydrolyzed by pig liver esterase (PLE)^{54} to
give monoacid 175. Combination of 175 with DPPA and Et$_3$N resulted in
oxazolidinone 176 through Curtius rearrangement and subsequently intramolecular
esterification. Cyclohexenol 177 was generated by treating 176 with Boc$_2$O and
DMAP in toluene followed by catalytic amount of NaH. The hydroxyl group at C-4
was triflated with Tf$_2$O and then substituted by NaN$_3$ to furnish the required
configuration. The synthesis ended up with reduction of azide with catalytic
hydrogenation followed by acetylation, cleavage of Boc group with HBr/AcOH and
finally salt formation with H$_3$PO$_4$.

Zutter et al. used a cheap starting material and accomplished the synthesis of Tamiflu
with 30% of overall yield. It is good idea to introduce the 3-pentanyl ether at initial
steps because it is difficult to directly install in later stages. Although azide compound
is detrimental in industrial process, the formation of oxazolidinone 176 is very
ingenious. Moreover, this is an excellent example of application of desymmetrization
in synthesis of Tamiflu.
Scheme 22. Synthesis of Tamiflu exploited by Zutter. Reagents and conditions: (a) 3-pentanylmethanesulfonate, KOrBu, DMSO, 50 °C, quant.; (b) NBS, DMF, 0 °C to rt, 90%; (c) CO (10 bar), 0.5% Pd(OAc)$_2$, dppp, KOAc, EtOH, 110 °C, 20 h, 95%; (d) H$_2$ (100 bar), 5% Ru-Al$_2$O$_3$, EtOAc, 60 °C, 82%; (e) TMSCl, NaI, MeCN, cat. H$_2$O, 97%; (f) PLE pH 8 buffer, 98%; (g) DPPA, Et$_3$N, CH$_2$Cl$_2$, 40 °C, 81%; (h) Boc$_2$O, cat. DMAP, toluene, rt, then cat. NaH, toluene, reflux; (i) Tf$_2$O, pyridine, −10 °C, CH$_2$Cl$_2$, 85%; (j) NaN$_3$, acetone/H$_2$O, rt, 78%; (k) H$_2$, Ra-Co; (l) Ac$_2$O, Et$_3$N, then HBr-AcOH, EtOAc; (m) H$_3$PO$_4$, EtOH, 83%.

Hudlicky considered oseltamivir as a latent symmetry molecule.$^{[18a]}$ Based on this consideration, ethyl benzoate was used as starting material (Scheme 23). Desymmetrization concept was applied to the first step. Ethyl benzoate was fermented with *E. Coli* JM 109 (pDTG601A)$^{[55]}$ to give diol 181. After protection with isopropylidiene, diol was subsequently reacted with $N$-hydroxy acetamide with the aid of NaIO$_4$ to furnish 183.$^{[56]}$ Cleavage of the N-O bond in the presence of [Mo(CO)$_6$]
afforded 184, which was treated with MsCl/Et3N to form 185. After the hydrolysis of oxazoline and hydrogenation of double bond, 185 was transformed to 187. Hydroxy group of 187 was mesylated before substitution with NaN3. Treatment of 189 with DBU in CH2Cl2 afforded 190, which could be converted to Tamiflu in 3 steps by Fang’s method.

This formal synthesis utilized a commercially available and cheap staring material, but the fermentation is not so efficient since the yield was only 1 gL⁻¹. However, the symmetry-based design leads to diversity of substituents onto the cyclic diene diol backbone.

Scheme 23. Formal synthesis of oseltamivir exploited by Hudlicky group. Reagents and conditions: (a) E. Coli JM 109 (pDTG601A); (b) dimethoxypropane, TsOH, rt; (c) CH3CONHOH, NaIO4, MeOH, rt, 70% for 2 steps; (d) [Mo(CO)6], MeCN/H2O (15:1), 75%; (e) MsCl, Et3N, DMAP, CH2Cl2, rt, 54%; (f) CaCO3, EtOH/H2O (1:1), 72%; (g) Rh/Al2O3 (5 mol%), 60 psi H2, 85% aq. EtOH, 95%; (h) Ms2O, Et3N, CH2Cl2, rt, 73%; (i) NaN3, acetone/H2O, rt, 86%; (j) DBU, CH2Cl2, rt, 85%.
An alternative route to synthesize intermediate 77 was reported by Hudlicky et al. recently (Scheme 24). Treatment of 184 with CrO₃/Ac₂O in CH₂Cl₂ resulted in cyclohexenone 191 through a 3,3-oxidation rearrangement. This reaction should be performed below 4 °C to avoid aromatization. Conversion of the ketone to oxime incorporated the nitrogen source, which was then hydrogenated and protected by Boc group in one pot. Elimination of 193 promoted by NaOEt generated the desired allylic alcohol 105. Attempts to incorporate of 3-pentyl ether with direct alkylation gave low yields. Following Shibasaki and Corey’s protocol,[20a, 20c] 77 could be attained in reasonable yield by aziridine formation under Mitsunobu conditions and subsequent ring-opening catalyzed by Lewis acid. Three steps were reduced based on the first generation. Regrettably, alkylation of allylic alcohol with 3-pentanol directly was not successful. The medium scale production of cis-diol is practical. But the efficiency needed to be improved and purification conditions of intermediates had to be optimized to be scaled up.

**Scheme 24.** Alternative synthesis of intermediate 77 exploited by Hudlicky group. Reagents and conditions: (a) CrO₃, Ac₂O, CH₂Cl₂, 4 °C, 5 min; (b) NH₂OH·HCl, EtOH, pyridine, 75-82% for 2 steps; (c) 5% Rh/Al₂O₃, H₂, aq. EtOH, Boc₂O, 93%; (d) NaOEt, EtOH, 94%; (e) 3-Iodopentane, Ag₂CO₃.

*Part 1 Sugar-based synthesis of Tamiflu and its inhibitory effects on cell secretion*
6. Amino acid

Earlier, Yao et al. synthesized not only zanamivir intermediate but also Tamiflu precursor.[23, 58] Cheap amino acid L-serine was chosen as starting material to access the Tamiflu precursor 210 (Scheme 25). Olefin 195 was prepared from L-serine according to the literature procedures.[58-59] OsO₄ catalyzed dihydroxylation of 195 with NMO provided diol 196, which was hydrogenated in the presence of Pd(OH)₂/C to give 197. The free amine and the primary alcohol of 198 was protected by Cbz and TBDPS group respectively. Swern oxidation followed by Wittig reaction resulted in olefin 200. The N,O-acetal was selectively cleaved by catalytic BiBr₃ to give 201.[60] Swern oxidation and subsequent Grignard reaction with vinylmagnesium bromide afforded a major product 202, which was bearing MOMCl to furnish 203. Ring closing metathesis promoted by the second generation Grubbs catalyst 211, led to the corresponding cyclohexene backbone which possessed the exact configurations as that of Tamiflu. After the deprotection of TBDPS group, alcohol was converted to acid by PCC oxidation and Kraus oxidation. Acid 206 was esterified in ethanol with the aid of EDCI. Reaction of 207 with HCl/EtOH resulted in cleavage of both MOM and Boc group. Acetylation of amine at C-4 and removal of Cbz group at C-5 afforded the precursor 210. The characteristics of Yao’s protocol comprises of readily available and inexpensive staring material and construction of cyclohexene framework by ring closing metathesis. However too many protection-deprotections made this strategy to be cumbersome.
Scheme 25. Synthesis of precursor to oseltamivir developed by Yao’s group. Reagents and conditions: (a) i) HCl/MeOH; ii) Boc₂O, Et₃N, THF, 90%; iii) Dimethoxypropane, acetone, BF₃·OEt₂, 91%; iv) DIBAL-H, toluene, 70%; (b) i) N-(4-methoxybenzyl)-hydroxylamine, MgSO₄, CH₂Cl₂, 74%; ii) ZnBr₂, allylmagnesium bromide, THF/Et₂O, −70 °C, 4 h; 87%; iii) Zn-Cu(OAc)₂, AcOH/H₂O, 70 °C; iv) CbzCl, aq. NaHCO₃, EtOAc; c) OsO₄, NMO, acetone/H₂O, 89%; (d) Pd(OH)₂/H₂/CH₃OH, 35 °C; (e) CbzCl, NaHCO₃, H₂O/EtOAc, 86% for 2 steps; (f) TBDPSCl, imidazole, CH₂Cl₂, rt, 96%; (g) i) (COCl)₂, DMSO, CH₂Cl₂, Et₃N, −78 °C; ii) Ph₃PCH₃Br, nBuLi, THF, −78 °C to rt, 86% for 2 steps; (h) BiBr₃, MeCN, rt, 89%; (i) (COCl)₂, DMSO, CH₂Cl₂, Et₃N, −78 °C; ii) vinylMgBr, ZnBr₂, THF, −78 to −30 °C, 56%; (j) MOMCl, DIPEA, CH₂Cl₂, rt, 98%; (k) cat. 211, CH₂Cl₂, rt, 98%; (l) TBAF, THF, rt, 96%; (m) i) PCC, 4 Å M.S., CH₂Cl₂, rt; ii) NaClO₂, K₂HPO₄, 2,3-dimethylbuta-1,3-diene, tBuOH/THF/H₂O, 10 °C to rt, 88% for 2 steps; (n) EtOH, HOBT, EDCI, DIPEA, CH₂Cl₂, rt, 85%; (o) 5% HCl/EtOH, 0 °C to rt; (p) AcCl, Na₂CO₃, EtOH, 0 °C to rt, 83% for 2 steps; (q) Pd(OAc)₂, Et₃SiH, Et₃N, CH₂Cl₂, 0 °C to rt, 92%.
Mandai and Oshitari published two successive papers on the synthesis of oseltamivir in the same journal. One of the methods started from L-methionine derivatives (Scheme 26). Ester 212 was reduced to aldehyde by DIBAL-H and it is in turn protected by PMP group. Meanwhile, convergent starting material 225 was prepared by reacting 3-pentanol with bromoacetic acid and subsequent conversion to corresponding acyl chloride. Combination of 213 with 225 under basic condition resulted in cis-β-lactam 214 with 99% ee. Oxidation of 214 with NCS afforded sulfoxide, which was thermally eliminated to give 215. Acid-sensitive Boc group was removed by TFA and then replaced by phthaloyl group. Cleavage of PMP group by CAN-promoted oxidation furnished lactam 216 without affecting other functional groups. After protecting amide with acetyl group, the lactam was opened by EtSH/Et3N to produce thioester 217. Alkene 217 underwent a Rh(acac)(CO)2 and BIPHEPHOS 222 catalyzed hydroformylation to form 218. Reduction of thioester to aldehyde was carried out under the condition of 10% Pd/C and Et3SiH. The cyclohexene ring of 219 was then formed through an intramolecular aldol reaction catalyzed by Bn2NH·TFA. Oxidation of 220 and subsequent reduction with NaBH4 afforded 221. Esterification and selective deprotection furnished oseltamivir 3.

Natural abundant amino acid was used as starting material here. Two amino groups were introduced before construction of cyclohexene frame without the usage of azide reagents. But reiterative protection-deprotection decreased efficiency of the synthesis.
Scheme 26. Synthetic route to Tamiflu started from L-methionine. Reagents and conditions: (a) DIBAL-H, toluene, −70 °C, 2.5 h, 91%; (b) p-anisidine, MgSO₄, CH₂Cl₂, 0 °C to rt, overnight, quantitative; (c) DIPEA, 225, CH₂Cl₂, −15 °C to rt, overnight, 55%; (d) NCS, MeCN/EtOH/H₂O, rt, 40 min, quantitative; (e) α-pinene-decalins, NaHCO₃, 155 °C, 6 h, 81%; (f) TFA, CH₂Cl₂, 0 °C to rt, 1.5 h; then PhthNCO₂Et, Et₃N, THF, 50 °C, 7.5 h, 86%; (g) CAN, MeCN/H₂O, 0 °C, 1 h, 80%; (h) LiHMDS, THF, −78 °C, 15 min, AcCl, −78 °C, 1 h, then EtSH, Et₃N, −78 to −20 °C, 3.5 h, 94%; (i) Rh(acac)(CO)₂, BIPHEPHOS, CO/H₂, THF, 65 °C, 7 h, 88%; (j) 10% Pd/C, Et₃SiH, CH₂Cl₂, rt, 1.5 h; (k) Bn₂NH·TFA, toluene, 50 °C, 10 h, 62% for 2 steps; (l) NaClO₂, NaHPO₄, 2-methyl-2-butene, tBuOH/THF/H₂O, 0 °C, 1 h, rt, overnight, 86%; (m) NaBH₄, iPrOH/H₂O, rt, overnight, 93%; (n) K₂CO₃, EtOH/H₂O, rt, 0.5 h; removal of the solvent; then EtI, DMSO, rt, 40 h, 85%; (o) 4 M HCl in 1,4-dioxane, EtOH, rt, 24 h, 83%; (p) KH, bromoacetic acid, 60 °C, 20 h, quantitative; (q) SOCl₂, 80 °C, 10 h, 94% for 2 steps.
7. Carbohydrate

Fang et al. were the first to use a carbohydrate as starting material to synthesize Tamiflu (Scheme 27).[24] The synthesis started from 1,2-O-isopropylidene-α-D-xylofuranose 226, which is easily prepared from D-xylose according to the literature.[62] The primary alcohol was selectively protected by PivCl to give 227. PCC oxidation and imination with hydroxyamine afforded 228, which was in turn reduced by LiAlH₄ to give 229. Acetylation of amine, cleavage of the ketal under acidic conditions and reaction with BnOH generated a mixture of anomers 230. The mixture was treated with 2,2-dimethoxypropane to form 231. The primary alcohol was converted to triflate and subsequently replaced by triethyl phosphonoacetate. Catalytic hydrogenation and treatment of 232 with NaH in THF provided carboxylate 233, which underwent intramolecular Horner-Wadsworth-Emmons reaction. Azide was incorporated under Mitsunobu reaction conditions to give 234.[63] The ketal was removed by refluxing 234 in HCl/EtOH. The stereochemistry of hydroxy group at C-3 was reversed by treating with Tf₂O/pyridine and KNO₂ in DMF successively. Then 3-pentanyl ether was directly introduced by reacting 190 with 3-pentanyl trichloroacetimidate. Culmination of 4 was realized by reduction of azide and salt formation with H₃PO₄.

Fang et al. accomplished their synthesis in milligram scale with 15% overall yield. They provided a new way to introduce 3-pentyl ether and incorporate amino group with hydroxyamine without aziridine ring-opening. Notably, they also synthesized some Tamiflu analogues with the same strategy. These analogues were tested and proved to be potential NA inhibitors.
**Scheme 27.** Synthesis of Tamiflu developed by Fang group. Reagents and reaction conditions: (a) Me$_3$CCOCI, pyridine, 0 °C, 8 h, 89%; (b) i) PDC, Ac$_2$O, reflux, 1.5 h; ii) NH$_2$OH·HCl, pyridine, 60 °C, 24 h, 82%; (c) LiAlH$_4$, THF, reflux, 1.5 h, 88%; (d) i) Ac$_2$O, pyridine, 25 °C, 3 h; ii) 4 M HCl in 1,4-dioxane, BnOH, toluene, 0 to 25 °C, 24 h, 85%; (e) 2,2’-dimethoxypropane, TsOH, toluene, 80 °C, 4 h, 90%; (f) i) Tf$_2$O, pyridine, CH$_2$Cl$_2$, −15 °C, 2 h; ii) EtO$_2$CCH$_2$PO(OEt)$_2$, NaH, 15-crown-5, DMF, 25 °C, 24 h, 80%; (g) i) H$_2$, Pd/C, EtOH, 25 °C, 24 h; ii) NaH, THF, 25 °C, 1 h, 83%; (h) DPPDA, DIAD, PPh$_3$, THF, 25 °C, 48 h; (i) HCl, EtOH, reflux, 1 h, 83%; (j) i) Tf$_2$O, pyridine, CH$_2$Cl$_2$, −15 to −10 °C, 2 h; ii) KNO$_2$, 18-crown-6, DMF, 40 °C, 24 h, 70%; (k) Cl$_3$CC(=NH)OCH$_2$H, CF$_3$SO$_3$H, CH$_2$Cl$_2$, 25 °C, 24 h, 78%; (l) H$_2$, Lindlar catalyst, EtOH, rt, 16 h, 85%; (m) H$_3$PO$_4$, EtOH, 40 °C, 1 h, 91%.

Mandai and Oshitari synthesized Tamiflu not only from amino acid but also carbohydrate.$^{[30]}$ Aldehyde 239 was prepared from d-mannitol according to the literature method in 2 steps (Scheme 28)$^{[64]}$. Grignard reaction between vinylmagnesium bromide and 239 afforded 240, which was treated with triethyl orthoacetate and catalytic propionic acid to form 241, which underwent a facile orthoester Claisen rearrangement. DIBAL-H reduction of 241 led to the transformation of ketal to 3-pentyl ether and ester to alcohol. The free alcohols were protected with
THP groups before the asymmetric dihydroxylation of the double bond. The newly produced hydroxy groups were mesylated with MsCl/pyridine and subsequently substituted by azide. Azide 244 was reduced by LiAlH₄ to generate diamine 245. Due to the difference of steric hindrance, reaction of diamine 245 with PhthNCO₂Et and Ac₂O/pyridine successively furnish 246. Cleavage of THP group by CSA afforded diol 247. Oxidation of 247 with TEMPO and subsequently intramolecular aldol reaction provided 219. Synthesis of Tamiflu from 219 was completed following the preceding protocol developed by same researchers.^[31]

![Scheme 28. Synthetic route to Tamiflu started from d-mannitol.](image-url)

**Scheme 28.** Synthetic route to Tamiflu started from d-mannitol. Reagents and conditions: (a) 3,3-dimethoxypentane, CSA, DMF, 40 °C, 2 h, then KIO₄, KHCO₃, H₂O/THF, rt, 61%; (b) vinylmagnesium bromide, THF, 0 °C, 1 h, 88%; (c) MeC(OEt)₃, 2% propionic acid, 132 °C, 14 h, 95%; (d) DIBAL-H, toluene, 0 °C, 2 h, rt, 3 h; (e) DHP, PPTS, CH₂Cl₂, rt, 24 h; (f) MsNH₂, AD-mix-β, tBuOH/H₂O, 0 °C, 8 h, rt, 13 h; (g) MsCl, pyridine, 0 °C, 2 h, rt, 8 h; (h) NaN₃, DMSO, 80 °C, 48 h; (i) LiAlH₄, THF, rt, overnight; (j) Et₃N, DMAP, PhthNCO₂Et, THF, 0 °C, 1.5 h, then Ac₂O, pyridine, rt, 14 h; (k) CSA, MeOH, rt, 1 h, 32% from 261; (l) TEMPO, KBr, aq. NaOCl, NaHCO₃, CH₂Cl₂/H₂O, 5 °C, 15 min; (m) Bn₂NH-TFA, toluene, 50 °C, 11 h, 82% for 2 steps.
This synthesis followed the same strategy as the preceding one, to construct the
cyclohexene skeleton using different starting material. Sodium azide was used to
introduce both amino groups. The entire synthetic route is not efficient enough to scale
up.

A formal synthesis of Tamiflu started from inexpensive and commercially available D-
ribose, had been achieved by Chen et al. (Scheme 29).\textsuperscript{[32]} 1-Hydroxy and 2,3-hydroxy
groups were protected by methyl group and 3-pentylidene respectively. After
iodization under I\textsubscript{2}/Ph\textsubscript{3}P/imidazole, alcohol was converted to iodoribose 250. A
domino Bernet-Vasella reaction and Reformatsky-type allylation were proceeded by
refluxing 250 with Zn in THF/H\textsubscript{2}O and then with 2-(bromoethyl)acrylate. Ring-
closing olefin metathesis of 251 with the 2nd generation Hoveyda-Grubbs catalyst
afforded 252. The 3-pentylidene ketal was transformed to 3-pentyl ethyl by sonication
in the presence of AlCl\textsubscript{3}/Et\textsubscript{3}SiH in CHCl\textsubscript{3}. 5-Hydroxy group of 253 was mesylated by
MsCl/Et\textsubscript{3}N. 4-Hydroxy group of 254 was converted to triflate as a good leaving group,
which was substituted by NaN\textsubscript{3} in acetone/H\textsubscript{2}O. Aziridine 21 was generated from 256
through Staudinger reaction and cyclization. Finally, aziridine could be converted to
Tamiflu by reported procedures in 3 steps.\textsuperscript{[22]}

Chen et al. constructed the cyclohexene backbone from a natural sugar D-ribose, which
is cheap and readily available. Tedious protection procedures are avoided in this
conversion. They took the advantage of intrinsic chirality on sugar moiety to maintain
the configurations in the target compound.
Scheme 29. Synthetic route of Tamiflu developed by Chen et al. Reagents and conditions: (a) 3-pentanone, MeOH/HCl, HC(OMe)₃, reflux, 89%; (b) I₂, imidazole, PPh₃, CH₃CN/PhMe, reflux, 90%; (c) Zn, THF/H₂O, reflux, 3h, then 2-(bromoethyl)acrylate, reflux, 78%; (d) 257, DCE, reflux, 99%; (e) AlCl₃, CHCl₃, sonication, Et₃SiH, 67%; (f) MsCl, Et₃N, −20 °C, 92%; (g) Tf₂O, pyridine, −10 to 0 °C, (h) NaN₃, acetone/H₂O, 86% for 2 steps; (i) nBu₃P, THF, then Et₃N, H₂O, 84%.

8. Others

Barry Trost and Ting Zhang chose 6-oxabicyclo[3.2.1]oct-3-en-7-one 258 as staring material because it has the six-membered carbon skeleton (Scheme 30). They first attempted to use protected secondary amines as nucleophiles to open the lactone. Unfortunately this reaction was failed. It could be attributed to the fact that the nucleophilicity of NHBoc₂, NHCbz₂, NH(CHO)₂, phthalimide were not strong enough to open the lactone. To increase the nucleophilicity, TMS-phthalimide was used to attack the lactone in the presence of (η³-C₃H₅PdCl)₂ and Trost ligand. The resulting TMS ester was ethanolyzed in one pot to give 259, which was further sulfonylated by
PhSSO\textsubscript{2}Ph and KHMDS. The obtained diastereomers were oxidized by \textit{m}CPBA which underwent thermal elimination in the presence of DBU to generate 261. To obtain desired \(\gamma,\delta\)-aziridine, various metal catalysts were applied in the aziridination reaction such as Cu, Ag and Au catalysts. But either bad selectivities or low reactivities were observed. Based on many attempts, single isomer 262 was achieved in good yield under PhI(O\textsubscript{2}CCMe\textsubscript{3})\textsubscript{2}, SESNH\textsubscript{2}, \([\text{Rh}_2(\text{esp})_2]\) (bis-[rhodium(\(\alpha,\alpha,\alpha',\alpha'\)-tetramethyl-1,3-benzenedipropionic acid)]) and MgO in chlorobenzene.\textsuperscript{65} Subsequently, aziridine was ring-opened by 3-pentanol in the presence of BF\textsubscript{3}·OEt\textsubscript{2}. After acetylation with Ac\textsubscript{2}O in pyridine by microwave irradiation, 265 was treated with TBAF to remove SES group. Finally, removal of phth-protecting group by hydrazine in ethanol afforded 3.

\[
\begin{align*}
\text{Scheme 30.} \quad \text{Synthetic route to oseltamivir developed by Trost. Reagents and conditions: (a) 2.5 mol\% } & \quad [(\eta^3\text{-C}_3\text{H}_5\text{PdCl})_2], \quad 7.5 \text{ mol\% } (R,R)-266, \quad \text{trimethylsilyl phthalimide, THF, 40 °C, then TsOH·H}_2\text{O, EtOH, reflux, 84\%, 98\% ee; (b) KHMDS, PhSSO}_2\text{Ph, THF, } & \quad -78 \text{ °C to rt, 94\%; (c) mCPBA, NaHCO}_3, \quad 0 \text{ °C, then DBU, 60 °C, toluene, 85\%; (d) 2 mol\% } & \quad 267, \quad \text{SESNH}_2, \quad \text{PhI(O}_2\text{CCMe}_3)_2, \quad \text{MgO, PhCl, 0 °C to rt, 86%;}
\end{align*}
\]
(e) BF$_3$·Et$_2$O, 3-pentanol, 75 °C, 65%; (f) DMAP, pyridine, Ac$_2$O, Microwave, 150 °C, 1 h, 84%; (g) TBAF, THF, rt, 95%; (h) NH$_2$NH$_2$, EtOH, 68 °C, quantitative.

This synthetic route is concise and efficient and the overall yield is 30%. It features palladium-catalyzed asymmetric allylic alkylation and Rh-catalyzed regio- and stereoselective aziridination on an electron-deficient diene system. But there still exist some drawbacks such as high expensive metal catalysts were utilized. Moreover, the author mentioned that the starting material is commercially available, but it is not widely provided. It could be prepared from 3-cyclohexene-1-carboxylic acid in 2 steps.

Scheme 31. Three one-pot synthetic route to oseltamivir. Reagents and conditions: (a) 278, ClCH$_2$CO$_2$H, CH$_2$Cl$_2$, rt, 40 min; (b) i) 279, Cs$_2$CO$_3$, 0 °C, 3 h; ii) Cs$_2$CO$_3$, EtOH, rt, 15 min; (c) CH$_3$C$_6$H$_4$SH, EtOH, −15 °C, 36 h, 70% for 3 steps; (d) TFA, CH$_2$Cl$_2$, 2 h; (e) i) (COCl)$_2$, cat. DMF, CH$_2$Cl$_2$, 1 h; ii) NaN$_3$, acetone/H$_2$O, 0 °C, 20 min; (f) AcOH, Ac$_2$O, rt, 49 h; (g) Zn, TMSCl, EtOH, 70 °C, 2 h; (h) NH$_3$, K$_2$CO$_3$, EtOH, 6 h, 82% from 273.
Hayashi group prepared Tamiflu in three one-pot operations (Scheme 31).\[^{[21]}\] Firstly, Michael reaction of pentanyloxy acetaldehyde and nitroalkene was catalyzed by diphenylprolinol silyl ether 278.\[^{[66]}\] The Michael adduct 270 was \textit{in situ} reacted with vinylphosphonate 279 and transformed to the core ring, which underwent an intramolecular Horner-Wadsworth-Emmons reaction. Although the diastereoselectivity is not good, the single diastereomer 273 was obtained after treating the mixture of 271 and 272 with thiocresol and base in one pot. Removal of tert-butoxycarbonyl group by TFA afforded carboxylic acid 274. Reaction of 274 with (COCl)$_2$ and NaN$_3$ successively resulted in acyl azide 275. Crude 275 was treated with AcOH/Ac$_2$O to form amine 276, which underwent a Curtius rearrangement and subsequent amide formation.\[^{[67]}\] It is noteworthy that this Curtius rearrangement proceeded at room temperature and decreased potential hazards. The nitro group was reduced to free amine in the presence of Zn/HCl in EtOH. Finally oseltamivir was generated after bubbling ammonia and addition of K$_2$CO$_3$ to compound 277. Till now, Hayashi’s methodology is the shortest to be reported. The total yield is rather high (57%). Two starting materials needed another two steps respectively. This synthetic route was performed on milligram scale.

Tamiflu is a small but fantastic molecule. It has attracted so many chemists to pursue new strategy to access it. In 2009, John Andraos in York university published a paper on evaluation of material efficiency of synthesis of Tamiflu.\[^{[68]}\] The algorithm of evaluation is based on reaction mass efficiencies, oxidation level index, atom economy and overall yield. Almost all the synthetic routes published before 2009 were included
in the evaluation. Roche’s second generation synthesis is the most efficient among those protocols referred to the combined indexes.

The strategies for synthesis of Tamiflu have been summarized here. The chemical structure of Tamiflu is an ethyl 1-cyclohexenecarboxylate with 3 chiral functionalities. Common methods used for basic construction of cyclohexene skeleton are: 1) Taking advantage of starting material containing cyclohexene ring, such as shikimic acid; 2) D-A reaction between diene and dieneophile; 3) Ring closing metasis. The aporia in the synthesis of Tamiflu are installment of three chiral functionalities. The main strategies for installment of the 3-pentanyl ether are: 1) Reduction of pentylidene ketal; 2) Aziridine ring opening with 3-pentanol; 3) $S_N2$ substitution. Routinely incorporation of two amino groups: 1) Epoxide or aziridine ring-opening with nitrogen-containing nucleophiles; 2) Curtius rearrangement.

A few synthetic routes could be accessed to Tamiflu analogues, which may be explored to develop new drugs. Some novel and versatile methods could be also applied in the synthesis of similar drug like compounds. In the future, global atom economy and greenness should be considered more in design of the new synthesis. We hope the supply of Tamiflu would meet the global need with all efforts. Meanwhile, the adverse effect of Tamiflu should draw more attention and deeper responding researches have to be done.

Tamiflu is undoubtedly a popular molecule of high importance, and intensive efforts have been made to develop alternative routes to Tamiflu with various strategies for cyclohexenyl ring formation and from readily available and less expensive starting materials. However, some of the starting materials are still expensive or need several-
steps preparation. In our synthetic route, we used a cheap and abundant carbohydrate as the starting material to synthesize Tamiflu. Meanwhile, we investigated the effects of Tamiflu and its metabolite OC on the morphology, differentiation, cytoskeleton organization and vesicular exocytosis of neuroendocrine PC12 cells to understand the adverse effects of Tamiflu.
2. RESULTS AND DISCUSSION

2.1 Proposed synthesis of Tamiflu started from D-galactal

2.1.1 Retrosynthetic analysis

Carbohydrates are structurally diverse molecules and contain a wide variety of stereochemical properties. They have been commonly served as a chiral pool for the total synthesis of numerous bioactive natural products over the decades.\(^{[69]}\) As such, we decided to use a carbohydrate as the starting material in our synthesis towards Tamiflu. Initially, D-galactal was chosen as the starting material because the stereochemistry at the C-4 of galactal matches the stereochemistry at the C-3 of oseltamivir 3. The retrosynthetic analysis is depicted in Scheme 32.

![Scheme 32](image)

**Scheme 32.** First retrosynthetic analysis of oseltamivir.

We proposed that the 3-pentanyl ether of 3 could be installed in the final stage. Two *trans* amino groups could be introduced through tandem intermolecular aziridination of 281 and ring-opening by *N*-containing nucleophiles (Scheme 32). We envisioned that the stereochemistry at C-4, C-5 could be configured by taking advantage of the
regioselectivity and stereoselectivity during the formation and ring-opening of the aziridine. The aldehyde 281 could be generated from galactal 282, employing a 3,3-sigmatropic rearrangement as a critical step to form a six-membered carbocycle with the desired chiral configurations at C-2, C-3. Galactal 282 is commercially available and can also be synthesized from D-galactose.

2.1.2 Synthesis of intermediate 281

In order to reduce the number of synthetic steps required and facilitate easy removal of protecting groups, p-methoxylbenzyl (PMB) group was used on R1 and R2. The synthetic route was preliminary designed as shown in Scheme 33.

![Scheme 33. Proposed synthetic route to oseltamivir 3 started from D-galactal.](image)

Compound 283 could be synthesized from galactal 282 through a three steps of protection, deprotection and functional group interchange reactions. Carbocycle 281
could be formed through Claisen rearrangement from 283. The aldehyde functionality on 281 could be converted to ester 284 through oxidative esterification. The alkene 284 could be transformed to 280 through intermolecular aziridination and ring-opening with NaN₃. Subsequently, deprotection and dehydroxylation of compound 285 would furnish compound 287. Aziridine could then be ring-opened by 3-pentanol and azide could be reduced to amine to give oseltamivir 3.

Focusing on the initial conversion of 282 to 283, treatment of d-galactal with TBSCl and imidazole afforded 6-TBS galactal 288, which was reacted with 2 equivalents of PMBBr and NaH in DMF to furnish 289 (Scheme 34). Garcia-Alles et al. reported that the primary trimethylsilylated alcohol of glucal could be oxidized by PCC to give an aldehyde in the presence of secondary alcohol.[70] Therefore, we decided to oxidize C-6 of 289 to aldehyde with PCC in one step. However, the reaction did not proceed. CrO₃/pyridine also failed to oxidize 289. Alternatively, the TBS group was removed to give a free primary alcohol 290, which could then be oxidized to aldehyde 291. Cleavage of TBS group with TBAF proceeded easily at room temperature.

Scheme 34. Synthesis of compound 291. Reagents and conditions: (a) TBSCl, imidazole, DMF, overnight, 79%; (b) PMBBr, NaH, TBAI, DMF/THF, 0 °C, 1 h, rt, 2 h, 90%; (c) TBAF, THF, rt, 3 h, 75%.
The oxidation of primary alcohol to aldehyde was initially carried out with PCC in CH₂Cl₂ in the presence of 4 Å M.S. which is usually used as water scavenger to give aldehyde 291 in 52% yield (Table 1). Alternatively, reaction of 290 with TPAP and NMO under the same condition resulted in a complex mixture and no desired product was observed. However, treatment of 290 with IBX in DMSO afforded aldehyde 291 in 80% yield. Finally, a good 86% yield was observed from Dess-Martin periodinane oxidation on alcohol 290. Thus, DMP was deemed to be the optimal oxidant for oxidation of alcohol 290.

**Table 1.** Optimization of conditions for oxidation of 290 to aldehyde 291.

<table>
<thead>
<tr>
<th>Oxidant</th>
<th>Solvent</th>
<th>Isolated yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCC</td>
<td>CH₂Cl₂</td>
<td>52%</td>
</tr>
<tr>
<td>TPAP/NMO</td>
<td>CH₂Cl₂</td>
<td>-</td>
</tr>
<tr>
<td>IBX</td>
<td>DMSO</td>
<td>80%</td>
</tr>
<tr>
<td>DMP</td>
<td>CH₂Cl₂</td>
<td>86%</td>
</tr>
</tbody>
</table>

Heating aldehyde 291 with Cp₂TiMe₂ in toluene at 80 °C afforded alkene 283 in 17% yield. Alternatively, alkene 283 could also be prepared from aldehyde 291 through a Wittig reaction, whereby ylide reagent was generated from ylide salt and base, followed by *in situ* reaction with aldehyde. Treatment of aldehyde 291 with...
Ph$_3$PCH$_3$I/NaNH$_2$ afforded the alkene 283 in 10% yield while ylide reagent produced from Ph$_3$PCH$_3$Br/\textit{n}BuLi provided alkene 283 in 30% yield (Table 2). In hope of improving the yield, different bases such as \textit{t}BuOK, NaH and LiHMDS were used, but proved to be inefficient. With \textit{n}BuLi, TLC showed that the starting material was completely consumed with only one major product was formed. The thought that the product was unstable on the silica gel column promoted us to use neutral Al$_2$O$_3$ instead of silica gel for column chromatography. With that, the isolated yield of alkene 283 was improved to 62%.

\begin{table}[h]
\centering
\caption{Optimization of Wittig reaction of 291.}
\begin{tabular}{cccc}
\hline
Ylide reagent & Base & solvent & Temperature & Yield \\
\hline
Ph$_3$PCH$_3$I & NaNH$_2$ & THF & 0 °C to rt & 10%$^{[a]}$ \\
Ph$_3$PCH$_3$Br & \textit{n}BuLi & THF & −78 °C to rt & 30%$^{[a]}$, 62%$^{[b]}$ \\
Ph$_3$PCH$_3$Br & \textit{t}BuOK & Et$_2$O & 0 °C to rt & 22%$^{[a]}$ \\
Ph$_3$PCH$_3$Br & NaH & THF & 0 °C to rt & 20%$^{[a]}$ \\
Ph$_3$PCH$_3$Br & LiHMDS & THF & −20 °C to rt & 24%$^{[a]}$ \\
\hline
\end{tabular}
\end{table}

$^{[a]}$ Isolated yield after column chromatography on silica gel; $^{[b]}$ isolated yield after column chromatography on neutral Al$_2$O$_3$. 

Part 1 Sugar-based synthesis of Tamiflu and its inhibitory effects on cell secretion
Table 3. Optimization of Claisen rearrangement of functionalized galactal 283.

<table>
<thead>
<tr>
<th>Reagent/solvent</th>
<th>Temp. (°C)</th>
<th>Time (h)</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bu₃N</td>
<td>180</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>xylene</td>
<td>180</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>Ph₂O</td>
<td>210</td>
<td>12</td>
<td>23%</td>
</tr>
<tr>
<td>silica gel/Ph₂O</td>
<td>200</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>dichlorobenzene</td>
<td>230</td>
<td>18</td>
<td>-</td>
</tr>
<tr>
<td>dichlorobenzene (microwave)</td>
<td>240</td>
<td>0.7</td>
<td>10%</td>
</tr>
<tr>
<td>PdCl₂/toluene</td>
<td>110</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>Me₃Al/toluene</td>
<td>50</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>Me₃Al-PPh₃/DCM</td>
<td>25</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Me₃Al-H₂O/DCM</td>
<td>−20</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Me₃Al/2,4,6-tribromophenol/DCM</td>
<td>−78</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>BF₃/Et₂O</td>
<td>−60</td>
<td>2</td>
<td>-</td>
</tr>
</tbody>
</table>

Unfortunately, transformation of the sugar ring backbone of 283 to a cyclohexene skeleton did not proceed as expected. [3,3]-Sigmatropic rearrangement reaction hardly proceeded even at high temperatures in different solvents (Table 3). Low
yields were obtained when the reaction was carried out in dichlorobenzene (microwave) or Ph$_2$O. In addition, deprotected product was observed when Lewis acids such as AlMe$_3$ and BF$_3$·OEt$_2$ were used. The synthesis could not proceed since the yield could not be improved.

As postulated by Büchi,$^{[71]}$ this rearrangement is controlled by a facial preference via a boat-like transition state (Figure 3). This rearrangement reaction could possibly be unsuccessful with this scaffold due to the strong steric hindrance of the configuration itself. The dipole-dipole interaction of two oxygens on C-3 and C-4 is also energetic unfavoraled.

\[ \Delta E = 32.7 \text{ kcal/mol} \]
\[ \Delta E = 29.9 \text{ cal/mol} \]

**Figure 3.** Claisen rearrangement transition state of mimic of 283 and 297.

To confirm this speculation, we synthesized 297 following similar procedures to that of 283 (Scheme 35). To our delight, Claisen rearrangement of 297 proceeded smoothly and gave 298 in good yield when 297 was heated at 210 ºC in Ph$_2$O in a sealed tube.
Scheme 35. Synthesis of compound 298. Reagents and conditions: (a) TBSCl, imidazole, DMF, 0 °C to rt, overnight, 81%; (b) PMBBr, NaH, TBAI, THF/DMF, 0 °C, 1 h, rt, 2 h, 72%; (c) TBAF, THF, rt, 3 h, 85%; (d) DMP, CH₂Cl₂, rt, 3 h, 80%; (e) Ph₃PCH₃Br, nBuLi, THF, −78 °C to rt, 2 h, 60%; (f) Ph₂O, 210 °C, 8 h, 77%.

2.2 Synthesis of Tamiflu started from D-glucal

2.2.1 Retrosynthetic analysis

Thus, we decided to use D-glucal as an alternative starting material and had to revise our retrosynthetic route. We planned to utilize intramolecular aziridination to introduce two trans-amine groups with the aid of the C-4 configuration on glucal. As shown in Scheme 36, compound 3 is the prototype of the target molecule. It could be easily generated from 190 by etherification and reduction. Compound 190 could be produced from 299 through olefin formation, hydrolysis and acetylation. Compound 300 in our retrosynthetic analysis was identified as the pivotal intermediate, which could be used to synthesize 299 through tandem intramolecular aziridination, ring-opening by N-nucleophiles. Compound 300 could be derived from aldehyde 301 through selective deprotection and sulfamate formation or carbamate formation. Aldehyde 301 could be transformed from functionalized glucal 302, employing a
[3,3]-sigmatropic rearrangement as a critical step. Glucal 292 is commercially available or can be readily synthesized from D-glucose.

Scheme 36. Second retrosynthetic analysis of oseltamivir.

2.2.2 Construction of cyclohexene core 307 (a precursor to 300)

The alternative synthesis commenced with the functionalization of D-glucal. Scheme 37 shows the construction of a six-membered carbon backbone, starting from commercially available D-glucal. Installation of 4,6-benzylidene acetal and silylation of 3-hydroxyl furnished fully protected D-glucal 304, which underwent selective opening of the benzylidene acetal with DIBAL-H in CH₂Cl₂ at −15 °C to provide free primary alcohol 305 (65% yield, starting from D-glucal). The primary hydroxyl group in 305 was oxidized using Dess-Martin periodinane or Swern reagents to aldehyde, which was subjected to Wittig methylenation to give the terminal olefin 302 in 67% yield. The next step was the critical Claisen rearrangement reaction, which allowed ready access to the carbocycle 301 from sugar ring 302 while retaining the desired configuration. This reaction was conducted in a sealed reaction vessel at 210 °C in
diphenyl ether and the aldehyde 301 was diastereoselectively obtained in an excellent yield of 88%.

**Scheme 37.** Synthesis of compound 301. Regents and conditions: (a) p-anisaldehyde diethyl acetal, PPTS, DMF, 3 h, 78%; (b) TBSCl, imidazole, DMAP, DMF, rt, 2 h, 90%; (c) DIBAL-H, CH₂Cl₂, −20 °C to rt, 2 h, 84%; (d) i) DMP, CH₂Cl₂, rt, 2 h; ii) Ph₃PCH₃Br, nBuLi, −78 °C to rt, 1 h, 60%; (e) Ph₂O, 210 °C, 88%.

Oxidation of aldehyde 301 to ethyl ester 306 was initially carried out with oxone in ethanol, which gave a 43% yield (Table 4). I₂/KOH in ethanol also gave an unsatisfactory yield of 42%. However, the oxidation of 301 to ethyl ester 306 gave the best yield (87%) when it was conducted with NaClO₂/NaH₂PO₄ in the presence of 2-methyl-2-butene, followed by esterification with EtI.

**Table 4.** Optimization of ethyl esterification.
Next, the PMB group was successively removed with DDQ to form alcohol 307 in 92% yield (Scheme 38).

2.2.3 Introduction of two amino groups

With 307 in hand, we attempted to prepare compound 308 through sulfonamide, which is a common precursor for intermolecular aziridination. However, a mixture of CISO₂NCO, formic acid and acetonitrile with 307 in DMA failed to give the desired sulfonamide. Alternatively, carbamate 300 was easily prepared by treating alcohol with trichloroacetyl isocyanate followed by K₂CO₃/MeOH (Scheme 39).
Then conditions for intramolecular aziridination of 300 were optimized (Table 5). Conventional conditions, such as Rh₂(OAc)₄, PhI(OAc)₂, MgO, CH₂Cl₂ was applied at room temperature, but no new product was produced. Conducting the same reaction at 50 °C in a sealed tube afforded compound 309 in 12% yield. Other catalysts such as Rh₂(tfacam)₄ and Cu(MeCN)₄PF₆ could not promote the aziridination.

Table 5. Optimization of aziridination of carbamate 300.

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Auxiliary and solvent</th>
<th>Temp.</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>PhIO, CH₂Cl₂</td>
<td>50 °C</td>
<td>-</td>
</tr>
<tr>
<td>Rh₂(OAc)₄</td>
<td>PhI(OAc)₂, MgO, CH₂Cl₂</td>
<td>rt</td>
<td>-</td>
</tr>
<tr>
<td>Rh₂(OAc)₄</td>
<td>PhI(OAc)₂, MgO, CH₂Cl₂</td>
<td>50 °C</td>
<td>309 (12%)</td>
</tr>
<tr>
<td>Rh₂(OAc)₄</td>
<td>PhIO, MgO, CH₂Cl₂</td>
<td>50 °C</td>
<td>-</td>
</tr>
<tr>
<td>Rh₂(tfacam)₄</td>
<td>PhI(OAc)₂, MgO, CH₂Cl₂</td>
<td>rt</td>
<td>-</td>
</tr>
<tr>
<td>Rh₂(tfacam)₄</td>
<td>PhIO, MgO, CH₂Cl₂</td>
<td>rt</td>
<td>-</td>
</tr>
<tr>
<td>Rh₂(tfacam)₄</td>
<td>PhIO, MgO, CH₂Cl₂</td>
<td>50 °C</td>
<td>-</td>
</tr>
<tr>
<td>Cu(MeCN)₄PF₆</td>
<td>PhIO, MgO, MeCN</td>
<td>rt</td>
<td>-</td>
</tr>
<tr>
<td>Cu(MeCN)₄PF₆</td>
<td>PhIO, MgO, MeCN</td>
<td>50 °C</td>
<td>-</td>
</tr>
<tr>
<td>Cu(MeCN)₄PF₆</td>
<td>PhI(OAc)₂, MgO, MeCN</td>
<td>50 °C</td>
<td>-</td>
</tr>
</tbody>
</table>
The thought that the aziridine 308 maybe was unstable promoted us to carry out the aziridination and ring-opening in one pot. However, no desired product was formed when TMSN₃/TBAF was used as nucleophile. Alternative nucleophile such as NaN₃ furnished only trace amounts of the desired product (Table 6). Even by changing the catalysts, the yield of the desired product could not be increased.

Table 6. Optimization of aziridination and ring-opening with azide from carbamate.

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Reagents/solvent</th>
<th>Temp.</th>
<th>Yields</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rh₂(OAc)₄</td>
<td>PhI(OAc)₂, MgO, NaN₃/CH₂Cl₂</td>
<td>50 °C</td>
<td>trace 299</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>+10% 309</td>
</tr>
<tr>
<td>Rh₂(OAc)₄</td>
<td>PhI(OAc)₂, MgO, TMSN₃/CH₂Cl₂</td>
<td>50 °C</td>
<td>-</td>
</tr>
</tbody>
</table>

Inspired by Fleming’s work, we considered enhancing the feasibility of accessing the nitrene species. As such, the substituted carbamate 311 was prepared in 77% yield (75% conversion) by treating alcohol 307 with CDI/NH₂OH followed by TsCl/Et₃N (Scheme 40).
Scheme 40. Preparation of carbamate 311 from alcohol 307. Reagents and conditions: (a) CDI, CH₂Cl₂, rt, 2 h, then NH₂OH·HCl, pyridine, rt, 3 h; (b) TsCl, Et₃N, Et₂O, 0 °C to rt, overnight, 71% (2 steps).

The Rh₂(OAc)₄ catalyzed aziridination in dichloromethane at room temperature gave moderate yield (63%). Further optimization showed that (CuOTf)₂·toluene was an ideal catalyst for this transformation, furnishing the highest yield (94%) as determined by crude ¹H NMR (Table 7).

Table 7. Optimization of aziridination of tosyl-carbamate 311.

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Solvent</th>
<th>Temp. (°C)</th>
<th>Yield(¹H NMR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rh₂(OAc)₄</td>
<td>CH₂Cl₂</td>
<td>25</td>
<td>63%</td>
</tr>
<tr>
<td>Cu(OTf)₂</td>
<td>CH₃CN</td>
<td>25</td>
<td>78%</td>
</tr>
<tr>
<td>IPrCu</td>
<td>CH₃CN</td>
<td>25</td>
<td>83%</td>
</tr>
<tr>
<td>Cu(MeCN)₄PF₆</td>
<td>CH₃CN</td>
<td>25</td>
<td>86%</td>
</tr>
<tr>
<td>(CuOTf)₂·toluene</td>
<td>CH₃CN</td>
<td>25</td>
<td>91%</td>
</tr>
</tbody>
</table>
With this optimized aziridination conditions, ring opening with \( N \)-containing nucleophiles was sequentially conducted in a one-pot manner. Unsatisfactorily, \( p \)-methoxybenzylamine was regioselectively introduced to compound 308 in a one pot process which delivered two diastereomers (\( 312/313 = 3:1 \)). Allyl amine gave similar result.

The product structures were determined by 2D NMR. For compound 312, a distinct spot was observed on the NOESY spectra, due to correlation between H-1 and H-2, where as the similar observation with compound 313 was not found (Figure 4). A similar phenomenon was observed in the NOESY spectra of compound 314 and 315. The formation of 312 and 314 was due to the epimerization of 313 and 315 respectively through deprotonation/protonation process under basic conditions. The
configuration of 312 and 314 was relatively stable than that of 313 and 315, where the steric hindrance is strong between OTBS and CO₂Et group.

Figure 4. The comparison of NOESY spectra between compound 312 and 313.
Fortunately, when the ring-opening reaction was conducted without any base using TMSN₃ as a nucleophile, the desired compound 299 was formed stereoselectively and regioselectively in 82% yield (Scheme 41). Notably, this aziridine intermediate has the provision to synthesize Tamiflu analogues by using different N-, O- or S-containing nucleophiles.

Table 8. Attempts for hydrolysis of oxazolidinone

<table>
<thead>
<tr>
<th>Base</th>
<th>Temperature</th>
<th>Time (h)</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cs₂CO₃</td>
<td>rt</td>
<td>3</td>
<td>trace 317+recovered 299</td>
</tr>
<tr>
<td>Cs₂CO₃</td>
<td>80 °C</td>
<td>2</td>
<td>318+ recovered 299</td>
</tr>
<tr>
<td>LiOH</td>
<td>rt</td>
<td>3</td>
<td>80% 317+ recovered 299</td>
</tr>
<tr>
<td>NaOH</td>
<td>rt</td>
<td>4</td>
<td>30% 317+318+ recovered 299</td>
</tr>
</tbody>
</table>

The next step could be either opening the oxazolidinone or olefin formation. Hydrolysis of the oxazolidinone 299 by bases was first tried (Table 8). However, treatment of 299 with Cs₂CO₃/EtOH at room temperature failed to form 316.
Conducting the same reaction at reflux provided compound 318. Diastereomer 317 was obtained as the major product when treating 299 with LiOH/EtOH. Treatment of 299 with NaOH afforded a mixture of 317 and 318.

We envisioned that attachment of an electron-withdrawing group to the amide may facilitate the hydrolysis of oxalidinone. Reaction of 299 with Ac₂O and pyridine in the presence of DMAP failed to provide 319. Acetylation was accomplished by treating 299 with AcCl and NaH in THF. However, hydrolysis of 319 with Cs₂CO₃ in EtOH at room temperature formed 299 (Table 9). It was observed that an increase of temperature to 80 °C resulted in 321. The desired product was not formed even when stronger bases such as LiOH and NaOMe were used. On the other hand, strong acid such as 2 M HCl in EtOH removed the TBS and acetyl group instead of hydrolyzing the oxazolidinone.

**Table 9.** Screening conditions for hydrolysis of N-acetyl oxazolidinone
### Table

<table>
<thead>
<tr>
<th>Base</th>
<th>Temperature</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cs$_2$CO$_3$</td>
<td>rt</td>
<td>15% $^{299}$ + recovered $^{319}$</td>
</tr>
<tr>
<td>Cs$_2$CO$_3$</td>
<td>80 °C</td>
<td>$^{321}$</td>
</tr>
<tr>
<td>LiOH/LiCl</td>
<td>rt</td>
<td>$^{299}$ + recovered $^{319}$</td>
</tr>
<tr>
<td>NaOMe</td>
<td>rt</td>
<td>$^{299}$</td>
</tr>
</tbody>
</table>

However, it was easy to hydrolyze the oxazolidinone selectively when acetyl group was changed to Boc group. This was verified by successful hydrolysis of $^{324}$ (Scheme 42). Unfortunately, treatment of $^{25}$ with DBU at room temperature failed to form olefin $^{52}$. We also envisioned to install 3-pentanyl group by S$_2$N$_2$ reaction before the olefin formation. However, reaction of $^{325}$ with Tf$_2$O could not produce the desired triflate product, in which triflate moiety could be used as a good leaving group. In order to perform the elimination by changing OTBS into good leaving group, cumbersome protections and deprotections were required. Therefore, we considered to perform the elimination of the OTBS group prior to the hydrolysis of oxazolidinone.

**Scheme 42.** Hydrolysis of oxazolidinone. Reagents and conditions: (a) Boc$_2$O, Et$_3$N, DMAP, CH$_2$Cl$_2$, rt, 2 h, 90%; (b) Cs$_2$CO$_3$, EtOH, 2 h, 74%.
Treatment of 299 with DBU in acetonitrile at room temperature for 24 h failed to generate the desired α,β-unsaturated carboxylate. Chirality on C-1 position was partially epimerized to give a pair of diastereoisomers through deprotonation/protonation process. The structure was confirmed by X-ray analysis. Interestingly, aromatization occurred when 299 was treated with DBU at reflux (Table 10).

**Table 10.** Screening conditions for elimination of OTBS group.

<table>
<thead>
<tr>
<th>Base</th>
<th>Temperature</th>
<th>Solvent</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>DBU</td>
<td>rt</td>
<td>CH₃CN</td>
<td>317+recovered 299</td>
</tr>
<tr>
<td>DBU</td>
<td>80 °C</td>
<td>CH₃CN</td>
<td>327</td>
</tr>
<tr>
<td>NaH</td>
<td>rt</td>
<td>THF</td>
<td>317</td>
</tr>
<tr>
<td>NaOH</td>
<td>rt</td>
<td>EtOH</td>
<td>317+318</td>
</tr>
</tbody>
</table>
Since a one step reaction was unable to access 326, we had to achieve it in a stepwise manner (Scheme 43). TBS group was removed by heating 299 at reflux in HCl/EtOH for 2 h (65% yield). Treatment of 299 with TBAF in THF provided 322 in higher yield (75%). Mesylation of 322 and subsequent treatment with DBU furnished 326 in 77% yield. Reaction of 326 with Boc2O and Et3N in the presence of DMAP in CH2Cl2 resulted 328 in 80% yield.

Scheme 43. Synthesis of compound 328. Reagents and conditions: (a) TBAF, THF, rt, overnight, 75%; (b) MsCl, Et3N, CH2Cl2, 0 °C, 1 h, rt, 2 h, then DBU, rt, 1 h, 77%; (c) Boc2O, Et3N, DMAP, CH2Cl2, rt, 2 h, 80%.

Alternatively, compound 328 could also be achieved by protecting 299 with Boc followed by subsequent elimination of OTBS (Scheme 44). Reaction of 324 with TBAF in THF afforded 329 and a trace amount of 328. Treatment of 329 with MsCl and Et3N was able to generate 328 in 86% yield.
Scheme 44. Hydrolysis of carbamate. Reagents and conditions: (a) TBAF, THF, rt, 3 h, 30% 328, 58% 329; (b) MsCl, Et3N, CH2Cl2, rt, 3 h, 86%.

The formation of 328 during TBAF processing, which is not observed during reaction 299 with TBAF, could be due to the incorporation of the electron-withdrawing Boc group. Inspired by this phenomenon, reaction of 324 with DBU was conducted to form the olefin in a one step manner. To our delight, the reaction went smoothly to afford 328 in 62% yield. Then oxazolidinone was successfully ring-opened by a catalytic amount of Cs2CO3 in EtOH (Scheme 45).

Scheme 45. Hydrolysis of N-Boc-oxazolidinone. Reagents and conditions: (a) DBU, MeCN, rt, 3 h, 62%; (b) Cs2CO3, EtOH, rt, h, 82%.

Finally, we tried to install the 3-pentanyl group on the allylic alcohol. Reaction of alcohol with TfCl failed to give the desired triflate. Mesylation of 330 with MsCl was
successful. However, treatment of mesylate with 3-pentanol and NaH in THF failed to form the desired product. Aromatization of 331 occurred under these conditions instead to yield 332 (Scheme 46).

\[
\begin{align*}
\text{BOCHN} & \quad \text{N}_3 \\
\text{HO} & \quad \text{CO}_2\text{Et} \\
\text{C} & \quad \text{C} \\
\text{MsO} & \quad \text{BOCHN} \\
\text{HO} & \quad \text{CO}_2\text{Et} \\
\text{C} & \quad \text{C} \\
\text{BOCHN} & \quad \text{N}_3 \\
\text{CO}_2\text{Et} & \quad \text{C} \\
\text{C} & \quad \text{C} \\
\end{align*}
\]

**Scheme 46.** Attempt to install 3-pentanyl group on C-3 at 330.

### 2.2.4 Completion of synthesis of Tamiflu

The configuration of alcohol 330 was reversed by using Shibasaki’s strategy.\textsuperscript{[25b]} Oxidation of alcohol with Dess-Martin periodinane and subsequent selective reduction by LiAl(OtBu)\textsubscript{3} resulted in alcohol 334 in 78% yield. Subjecting 334 to Mitsunobu condition led to aziridine 335, which was regioselectively ring-opened by 3-pentanol in the presence of BF\textsubscript{3}·Et\textsubscript{2}O to furnish 336. The Boc group was replaced by acetyl through treating 336 with TFA and subsequent Ac\textsubscript{2}O/Et\textsubscript{3}N (Scheme 47). The stereochemistry of 23 was confirmed using x-ray crystallography (Figure 5).
Scheme 47. Synthesis of compound 23 from 330. Reagents and conditions: (a) i) DMP, CH₂Cl₂, rt, 2 h; ii) LiAl(OtBu)₃, THF, −20 °C, 87%; (b) PPh₃, DEAD, THF, h, 83%; (c) 3-pentanol, BF₃·Et₂O, −20 °C, 2 h, 80%; (d) i) TFA, CH₂Cl₂; ii) Ac₂O, Et₃N, CH₂Cl₂, 81%.

Since the OTBS group of 324 was successfully eliminated in one step, it should be possible to apply it on 319 as well. Indeed, treatment of 319 with DBU in CH₃CN afforded 337 in 67% yield. Although the yield was modest, this step could avoid the
redundant Boc protection and deprotection. Substituted carbamate 337 was then hydrolyzed using Cs$_2$CO$_3$ in EtOH to provide alcohol 235 in 63% yield and 24% of 326 (Scheme 48). Compound 326 could be acetylated with AcCl to provide 337.

Scheme 48. Synthesis of compound 256. Reagents and conditions: (a) DBU, MeCN, rt, overnight, 67%; (b) Cs$_2$CO$_3$, EtOH, rt, 2 h, 72%, (c) AcCl, NaH, THF, 90%.

The chirality of C-3 was reversed via Dess-Martin oxidation followed by LiAlH(O$\text{t}$Bu)$_3$ reduction, stereospecifically yielding intermediate 190 in 70% yield. It was then treated with MsCl/Et$_3$N to produce an aziridine intermediate, which was subjected to regioselective ring-opening with 3-pentanol/BF$_3$·Et$_2$O to generate ether 23 in 52% yield (Scheme 49).

Scheme 49. Installation of 3-pentanyl group. Reagents and conditions: (a) i) DMP, CH$_2$Cl$_2$, rt, 2 h; ii) LiAl(O$\text{t}$Bu)$_3$, THF, −20 °C, 70%; (b) i) MsCl, Et$_3$N, CH$_2$Cl$_2$; ii) 3-pentanol, BF$_3$·Et$_2$O, 4 Å M.S., 52%.
The azido group in 23 was reduced to amine with PPh$_3$ in THF/H$_2$O (90% yield). Finally, oseltamivir phosphate salt (Tamiflu) was obtained by treatment of 3 with H$_3$PO$_4$ in EtOH (85% yield). At the same time, oseltamivir was also prepared to test for bioactivities. Hydrolysis of oseltamivir 3 with LiOH in water afforded oseltamivir acid 5 (Scheme 50).

**Scheme 50.** Synthesis of 4 and 5 from 23. Reagents and conditions: (a) PPh$_3$, THF/H$_2$O, reflux, 2 h, 90%; (b) H$_3$PO$_4$, EtOH, 80%; (c) LiOH, THF/H$_2$O, rt, 3 h, 57%.

### 2.3 Effects of Tamiflu and OC on PC 12 cells

With Tamiflu and oseltamivir acid in hand, we investigated the effects of Tamiflu and OC on neuroendocrine PC12 cells which are a widely used cell model to study vesicular neural differentiation and exocytosis of neurotransmitters/ hormones.

The morphology changes of PC12 cells treated with/without OC were first examined. PC12 cells were pretreated with 0.5 mM OC. Time-lapse images were taken after 20 h by using total internal reflection fluorescence microscopy (TIRFM). Compared to the
controlled experiment, the morphology of PC12 cells treated with OC did not change apparently (Figure 6).

Figure 6. The morphology of PC12 cells with (left) or without (right) oseltamivir carboxylate treatment (0.5 mM for 20 h).

The neurite outgrowth of PC12 was induced by application of 100 ng/mL nerve growth factor to the medium with or without 0.5 mM OC. The images were taken under microscope after 48 h. Figure 7 showed that PC12 cells differentiate similarly in both cases.

Figure 7. PC12 cells differentiation with (left) or without (right) oseltamivir carboxylate treatment.

Similarly, no obvious difference of the organization of actin network in PC12 cells with or without OC treatment was observed (Figure 8).
The above results showed that OC had no obvious influences on cell morphology, proliferation, differentiation induced by nerve growth factor, and organization of cytoskeleton networks. These observations may explain why Tamiflu is generally well tolerated.

On the other hand, we investigated the effects of OC on vesicular exocytosis of PC12 cells. The large dense core secretory vesicles (LDCVs) in PC12 cells were selectively labelled by overexpression of enhanced green fluorescence protein (EGFP)
conjugated neuropeptide-Y (NPY-EGFP). Typical TIRFM images of fluorescently labelled vesicles in an OC treated and an untreated PC12 were presented in Figure 9. Each bright fluorescent dot indicates the footprint of a subplasmalemmal vesicle. There was no appreciable difference in the total number of visible vesicles between the treated and untreated cells. All the secretory vesicles in neuroendocrine or neuron cells move constantly.

Interestingly, the velocity of vesicle movement in OC treated cells is dramatically reduced (587 vesicles in 11 treated cells: 55.1 ± 1.7 nm/s vs. 657 vesicles from 14 untreated cells: 183.1 ± 5.5 nm/s) (Figure 10a). In the OC-treated cells, the vesicle movements were more severely confined as compared to the untreated control (0.27 ± 0.02 μm² vs. 0.58 ± 0.07 μm²) (Figure 10b). The reduction in the motion area cannot be attributed to decrease in the vesicle dwell time. On the contrary, vesicles stay in the subplasmalemmal region for much longer time in the treated cells compared to control (63.8 ± 1.8 s vs. 41.2 ± 1.5 s) (Figure 10c). In addition to its inhibitory influences on vesicle lateral trafficking, OC also caused large decrease in the rate of vesicle arrival from the inner cytosol (23.3 ± 3.1 vesicles/cell arrived in 2 minutes vs. 52.1 ± 3.6 vesicles/cell) (Figure 10d). The rate of vesicle retrieval back into the inner cytosol, which is in balance with vesicle arrival to keep the total number of subplasmalemmal vesicles remain steady, was similarly affected. Taken together, OC severely impaired both lateral and vertical trafficking of LDCV vesicles in PC12 cells. In comparison to OC, Tamiflu also similarly suppressed vesicle trafficking, but to a less extent (Figure 10). Sufficient mobility is believed to be important to ensure vesicle fusion competence and fast replenishment of readily releasable vesicles during a continuous
stimulation. We therefore reasoned that oseltamivir carboxylate may inhibit vesicular exocytosis.

**Figure 10.** The statistics of the vesicle average velocity, motion area, dwell time, and total number of vesicles arrived from the inner cytosol during 2 min imaging, respectively. The statistics shown as mean ± SEM is from 587 vesicles in 11 OC treated cells or 564 vesicles in 10 Tamiflu treated cells or 657 vesicles from 14 untreated control cells.

Vesicular exocytosis was assayed by carbon fiber microelectrode (CFM) based amperometry measurement which is able to detect exocytosis with single vesicle sensitivity and millisecond resolution.\[^{75}\] Figure 11a shows a typical amperometric trace from a PC12 cell recorded by a voltage-biased (700 mV) CFM positioned on the cell surface, in response to local perfusion of high-K\(^+\) solution which evokes voltage-activated Ca\(^{2+}\) current, and in turn, triggers Ca\(^{2+}\)-dependent exocytosis. Each current spike corresponds to single vesicular release of catecholamine molecules including dopamine, epinephrine, and norepinephrine. As compared to the control, the average number of amperometric spikes in response to a 2-minute high K\(^+\) stimulation was
significantly inhibited by OC treatment (19 OC-treated cells: 22.8 ± 3.8 spikes/cell vs. 40 control cells: 45.4 ± 4.1 spikes/cell) (Figure 11a, bottom), whereas Tamiflu did not appreciably affect the extent of exocytosis (21 Tamiflu treated cells: 41.9 ± 4.7 spikes/cell).

**Figure 11.** Oseltamivir carboxylate inhibits vesicular exocytosis in PC12 cells. (a) A representative amperometric recording from an untreated PC12 cell in response to a two-minute high K⁺ stimulation and the statistics (bottom) of the average total spike number from the control cells (40 cells: 45.4 ± 4.1 spikes/cell), tamiflu-treated cells (21 cells: 41.9 ± 4.7 spikes/cell), and OC-treated cells (19 cells: 22.8 ± 3.8 spikes/cell). (b) The average amperometric spikes averaged from 1,814 fusion events from the control cells (left, gray curve), 880 fusion events recorded from tamiflu-treated cells (middle, dotted curve), and 434 fusion events recorded from OC-treated cells (right, dashed curve).

The characteristic waveform of amperometric spike reveals the kinetics of quantal vesicle fusion catalyzed by secretory proteins such as SNAREs. Individual amperometric spikes were extracted and analyzed to investigate the effects of Tamiflu and OC on vesicle fusion kinetics. The mean amperometric spikes averaged from all the recorded signals from the control cells (1,814 spikes from 40 cells), Tamiflu treated cells (880 spikes from 21 cells), and OC treated cells (434 spikes from 19 cells) are displayed in Figure 11b. The mean amperometric amplitude from the OC
Part 1 Chapter 2 Results and discussion

treated cells was much smaller than that from the control cells (8.83 ± 0.49 pA vs. 15.91 ± 0.47 pA). In comparison, the amplitude reduction caused by Tamiflu was less (10.53 ± 0.33 pA).

It is not unexpected that Tamiflu, particularly after being hydrolyzed to OC, could have profound influences in cell functions because its target, neuraminidases, are implicated in many essential cellular processes by removing the terminal sialic acid from glycoproteins and glycolipids.[76] The influences of Tamiflu and OC on the central nervous system have been investigated using rat brain synaptosomes. It was found that neither Tamiflu nor OC affected release of monoamine neurotransmitters.[77] In another study, it was reported that Tamiflu increased dopamine levels in the rat medial prefrontal cortex.[15] As for this, further investigations are still needed to find out the real caused of this situation. These observations obtained from different experimental preparations using biochemical assays are seemingly discrepant to our findings on PC12 cells obtained by single cell recording or imaging with single vesicle sensitivity and millisecond resolution. Our experiments demonstrate that OC significantly inhibited exocytosis which is a fundamental process occurring in neurons and many other secretory cells. Therefore it is possible that, once the hydrolyzed Tamiflu (OC) passes the blood-brain barrier, it might inhibit the release of neurotransmitters from presynaptic neurons and consequently modulates neurotransmission in the central nervous system. Excitation or depression effects in the central nervous system may be resulted depending on the types of neurons and neurotransmitters being affected.
3 CONCLUSION

Our synthetic route to Tamiflu is based on the cheap and easily available starting material D-glucal (Scheme 51). Taking advantage of naturally occurring chiral centres and innate stereochemistry on the D-glucal scaffold, most of reactions in our synthesis occur in a regio- and stereoselective manner to readily produce the desired products in high yields. Other highlights of this work include the 3,3-sigmatropic rearrangement reaction, which allows the construction of the cyclohexene core with conjugated carboxylate moiety. Similar strategy can also be employed to form other functionalized six-membered carbocycles from widely available O-containing sugar scaffolds. In our method, vicinal diamino groups on C-4 and C-5 was introduced by stereoselectively intramolecular nitrogen delivery with a tethered carbamate on C-3 position, followed by regio- and stereoselective ring opening of aziridine. Elegantly, this strategy fixes the stereochemistry at both C-4 and C-5 successively. The third key step is the installation of pentyloxy group at C-3, realized through regio- and stereo-selective ring opening of the aziridine intermediate. Furthermore, the functionalizations on the three contiguous chiral centers, C-3, C-4, and C-5 occurring at the late stage of our synthesis allow efficient synthesis of Tamiflu analogues by varying nucleophiles and alkyloxy groups. From this, new Tamiflu analogues may be able to overcome the virus resistance against Tamiflu and to avoid the side-effect problems of Tamiflu. The practical synthesis strategy presented here may provide not only an alternative to synthesis of Tamiflu but also the opportunities of designing Tamiflu-like molecular tools to study the cellular functions mediated by neuraminidases.
Scheme 51. Final synthetic routes to Tamiflu started from D-glucal. Reagents and conditions: (a) p-anisaldehyde diethyl acetal, PPTS, DMF, rt, 2 h, 78%; (b) TBSCl, imidazole, DMAP, DMF, rt, 3 h, 90%; (c) DIBAL-H, CH₂Cl₂, −20 °C, 2 h, 84%; (d) i) DMP, CH₂Cl₂, rt, 2 h, ii) Ph₃PCH₃Br, nBuLi, THF, −78 °C, 1 h, rt, 1 h, 71%; (e) Ph₂O, 210 °C, 88%; (f) i) NaClO₂, NaH₂PO₄, 2-methyl-2-butene, tBuOH/H₂O, rt, 2 h, ii) EtI, K₂CO₃, DMF, 3 h, 87%; (g) DDQ, CH₂Cl₂/H₂O, rt, 6 h, 92%; (h) i) CDI, CH₂Cl₂, rt, 2 h, then NH₂OH·HCl, pyridine, rt, 4 h, ii) TsCl, Et₃N, Et₂O, rt, 12 h, 71%; (i) (CuOTf)₂toluene, K₂CO₃, MeCN, rt, overnight, then TMSN₃, TBAF, THF, 0 °C, 3 h, 82%; (j) i) AcCl, NaH, THF, 0 °C to rt, 2 h, ii) DBU, MeCN, rt, overnight, 67%; (k) Cs₂CO₃, EtOH, rt, 2 h, 72%; (l) i) DMP, CH₂Cl₂, rt, 2 h, ii) LiAl(OtBu)₃, THF, −20 °C, 2 h, 78%; (m) i) MsCl, Et₃N, CH₂Cl₂, 0 °C to rt, 3 h, ii) 3-pentanol, BF₃·Et₂O, 4 Å M.S., −20 °C, 3 h, 52%; (n) i) Ph₃P, THF/H₂O, reflux, 2 h, then H₃PO₄, EtOH, 85%.
In the present study, we found that the active Tamiflu metabolic (OC) significantly inhibited the vesicular exocytosis in neuroendocrine PC12 cells. Such inhibition on the ubiquitous exocytotic process may explain why Tamiflu causes a variety of problems including nausea, vomiting, diarrhea, headache, vertigo, insomnia, somnolence, and behavioural excitement, etc. Further investigations is needed to be carried out to reveal the detailed molecular mechanisms of how Tamiflu interferes with the exocytotic pathways and possibly other cell functions involving neuraminidases. [78]
4. EXPERIMENTAL SECTION

General: All reagents and solvents were obtained from commercial suppliers (Sigma-Aldrich, Fluka and Alfa Aesar) and used without further purification unless stated. Evaporation of organic solutions was achieved by rotary evaporation with a water bath temperature below 40 °C. Product purification by flash column chromatography was accomplished using silica gel 60 (0.010-0.063 mm). Chromatograms were visualized by fluorescence quenching with UV light at 254 nm or by staining using a basic solution of potassium permanganate. Technical grade solvents were used for chromatography and were distilled prior to use. Optical rotations were measured in CHCl₃ or H₂O on a Schmidt + Haensdch polarimeter with a 1 cm cell (c given in g/100 mL). IR spectra were recorded using FTIR Restige-21 (Shimadzu). NMR spectra were recorded at room temperature on 300 MHz Bruker ACF 300, 400 MHz Bruker DPX 400 NMR spectrometers. The residual solvent signals were taken as the reference (7.26 ppm for ¹H NMR spectra and 77.0 ppm for ¹³C NMR spectra in CDCl₃, 4.70 ppm for ¹H NMR spectra in D₂O). Chemical shift (δ) is reported in ppm, coupling constants (J) are given in Hz. The following abbreviations classify the multiplicity: s = singlet, d = doublet, t = triplet, m = multiplet or unresolved, br = broad signal. LCMS (ESI) spectra were recorded on Finnigan LCQ Deca XP MAX. HRMS (ESI) spectra were recorded on a Waters Q-Tof premier™ mass spectrometer. HRMS (EI) spectra were recorded on a Thermo mass spectrometer. X-ray crystallographic data was collected by using a Bruker X8Apex diffractometer with Mo K/α radiation (graphite monochromator). Cell culture, TIRFM imaging and amperometric measurements were conducted by Dr. Chen Peng group from SCBE.
**Part 1 Sugar-based synthesis of Tamiflu and its inhibitory effects on cell secretion**

**Cell culture:** PC12 cells (ATCC, Manassas, VA) were cultured in advanced RPMI 1640 (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum, 5% horse serum, 1% penicillin-streptomycin (Gibco) at 37 °C. Cells were seeded on poly-L-lysine coated coverslips 4-6 days prior to experiments. In some experiments, PC12 cells were pretreated with 0.5 mM Tamiflu or OC for 20 hours.

**TIRFM imaging:** PC12 cells were transfected with NPY-EGFP plasmids (a kindly gift from Dr. Wolf Almers, Oregon Health Sciences University) using FuGENE-6 Transfection Kit (Roche Diagnostics GmbH, Germany), 1-2 days prior to the experiments. TIRFM imaging was conducted at room temperature in a bath solution contains (in mM, titrated to pH 7.2): 150 NaCl, 2.4 KCl, 2 MgCl$_2$, 2 CaCl$_2$, 10 glucose, and 10 HEPES. Time-lapse TIRFM images were acquired, at 2Hz, from a Zeiss Axiovert 200 inverted microscope (Carl Zeiss Inc., Germany) equipped with an oil immersion 100 × TIRF objective (1.45 NA) and a CCD camera with pixel size of 0.248 μm.

**Amperometric measurements:** Amperometric signals were recorded at room temperature from a 5-μm carbon fiber microelectrode (ALA Scientific Instruments, Westbury, NY), using an EPC-10 double patch-clamp amplifier (HEKA Electronik, Lambrecht, Germany) with sampling frequency of 4 kHz and low-pass filtering at 1 kHz. The bath solution is the same as described above. Exocytosis was triggered by local perfusion of high-K$^+$ solution which contains (in mM, titrated to pH 7.2): 40 NaCl, 105 KCl, 6 CaCl$_2$, 1 MgCl$_2$, and 10 HEPES (titrated to pH 7.2). Amperometric signals were individually analyzed using an Igor (WaveMetrics, Lake Oswego, OR) program, Amperometric Spike Analysis 8.15.
Synthetic procedures and products characterization

6-tert-Butyldimethylsilyl-D-galactal (288):

\[
\text{tert-Butyldimethylsilyl chloride (11.6 g, 0.08 mol) was added portionwise to a}
\text{solution of D-galactal (9.4 g, 0.06 mol) and imidazole (10.5 g, 0.15 mol) in anhydrous}
\text{DMF (100 mL) at 0 °C. The mixture was allowed to warm to room temperature and}
\text{stirred overnight. The mixture was diluted with ether (100 mL) and washed with}
\text{water and brine. The organic layer was dried over Na}_2\text{SO}_4\text{, filtered, and concentrated}
\text{in vacuo. The residue was purified by column chromatography on silica gel}
\text{(EtOAc/hexane = 1: 1) to give 288 (13.2 g, 79%) as a colorless oil. [α]_D^{23} = +9.8 (c =}
\text{0.5 in CHCl}_3\text{); (lit. [79] [α]_D^{20} = +17.7 (c = 1.26 in CHCl}_3\text{)); ¹H NMR (400 MHz,}
\text{CDCl}_3\text{: δ = 6.38 (dd, J = 6.2, 1.4 Hz, 1H), 4.72 (dd, J = 6.2, 1.9 Hz, 1H), 4.33-4.30}
\text{(m, 1H), 4.14-4.09 (m, 1H), 3.97 (dd, J = 10.8, 5.0 Hz, 1H), 3.92 (dd, J = 10.8, 3.7 Hz,}
\text{1H), 3.89-3.21 (m, 1H), 3.22 (d, J = 5.1 Hz, 1H), 2.76 (d, J = 10.2 Hz, 1H), 0.91 (s,}
\text{9H), 0.11 (s, 6H) ppm; ¹³C NMR (100 MHz, CDCl}_3\text{: δ = 144.5, 103.1, 75.7, 66.1,}
\text{64.2, 63.4, 25.8, 18.2, −5.5, −5.6 ppm; IR (neat): ν = 3385, 3053, 2926, 2855, 1458,}
\text{1261, 1107, 837 cm}^{-1}\text{; MS (m/z) 283 [M+Na]^+; HRMS (ESI): calcd. for}
\text{C}_{12}\text{H}_{24}\text{O}_5\text{SiNa [M+Na]^+, 283.1342; found, 283.1333.}
\]

6-tert-Butyldimethylsilyl-3,4-di(4-methoxybenzyl)-D-galactal (289):
NaH (4.3 g, 0.11 mol, 60% in mineral oil) was added in small portions to a solution of 288 (11.6 g, 0.045 mol), TBAI (1.6 g, 4.45 mmol) and PMBBr (14.1 mL, 0.10 mol) in anhydrous THF/DMF (30 mL/70 mL) under N₂ at 0 °C. After 1 h at 0 °C, the mixture was stirred at room temperature for another 2 h. The suspension was quenched with NH₄Cl solution and extracted with EtOAc (2 × 60 mL). The combined organic layers were washed with brine, dried over Na₂SO₄, filtered, concentrated and purified by column chromatography on silica gel (EtOAc/hexane = 1: 5) to give 289 (20.3 g, 90%) as a colorless oil. [α]D³³ = +12.2 (c = 1.0 in CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ = 7.30-7.26 (m, 4H), 6.90-6.84 (m, 4H), 6.30 (dd, J = 6.2, 1.3 Hz, 1H), 4.84-4.81 (m, 2H), 4.63-4.56 (m, 3H), 4.47 (s, 1H), 4.17 (s, 1H), 3.98-3.93 (m, 2H), 3.87-3.73 (m containing s, 7H), 0.89 (s, 9H), 0.05 (s, 6H) ppm; ¹³C NMR (100 MHz, CDCl₃): δ = 159.1 (2C), 144.1, 130.7, 130.6, 129.6, 129.4, 129.0, 113.7(2C), 113.6, 100.1, 77.6, 73.1, 71.4, 70.8, 70.5, 70.4, 66.5, 55.2 (2C), 25.9, 18.3, −5.3, −5.4 ppm; IR (neat): ν = 2930, 2854, 1612, 1514, 1248, 1083, 837, 779 cm⁻¹; MS (m/z) 524 [M+Na]⁺; HRMS (ESI): calcd. for C₂₈H₄₀O₆SiNa [M+Na]⁺, 523.2492; found, 523.2496.

3,4-Di(4-methoxybenzyl)-D-galactal (290):
TBAF (60 mL, 0.06 mol, 1 M solution in THF) was added dropwise to a solution of 289 (20.0 g, 0.04 mol) in dry THF (20 mL) at room temperature. The mixture was stirred for 3 h before concentration. The residue was flashed by column chromatography (EtOAc/hexane = 1:1) to give 290 as a white solid (11.58 g, 75%).

mp 85-86 °C; [α]_D^{22} = −97.2 (c = 1.0 in CHCl₃); \(^1\)H NMR (400 MHz, CDCl₃): δ = 7.30-7.26 (m, 4H), 6.90-6.87 (m, 4H), 6.39 (d, J = 6.2 Hz, 1H), 4.83 (dd, J = 6.1, 3.8 Hz, 1H), 4.75 (d, J = 11.6 Hz, 1H), 4.65 (d, J = 11.6 Hz, 1H), 4.57 (dd, J = 11.6, 3.8 Hz, 2H), 4.14 (t, J = 3.8 Hz, 1H), 4.09-4.05 (m, 1H), 3.97-3.92 (m, 2H), 3.81 (s, 6H), 3.73-3.67 (m, 1H), 2.41-2.39 (m, 1H) ppm; \(^1^3\)C NMR (100 MHz, CDCl₃): δ = 159.4, 159.2, 144.9, 130.2, 130.0, 129.7, 129.2, 113.8 (2C), 98.8, 75.6, 72.1, 71.4, 70.7, 69.0, 61.3, 55.2 ppm; IR (neat): ν = 3447, 2934, 1645, 1612, 1514, 1248, 1034, 822 cm⁻¹; MS (m/z) 410 [M+Na]⁺; HRMS (ESI): calcd. for C₂₂H₂₆O₆Na [M+Na]⁺, 409.1627; found, 409.1626.

\((2S,3R,4R)-3,4\text{-Di(4-methoxybenzyloxy)}-3,4\text{-dihydro-2H-pyran-2-carbaldehyde}\) (291):

![Image](image_url)

To a solution of 290 (11.2 g, 0.03 mol) dry CH₂Cl₂ (100 mL) was added Dess-Martin periodinane (24.6 g, 0.06 mol) at room temperature. The suspension was stirred for 3 h and then quenched with Na₂S₂O₅/NaHCO₃ (80 mL, v/v=1:1). The resulting solution was partitioned. The organic layer was washed with sat. NaHCO₃ and brine, dried over Na₂SO₄, filtered and concentrated. The residue was purified by column...
chromatography on silica gel (EtOAc/hexane = 1: 2) to give \(\text{291}\) (9.58 g, 86%) as a colorless oil. \(\alpha\)\(^{22}\)D = +28.8 (c = 0.55 in CHCl\(_3\)); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta = 9.77\) (s, 1H), 7.28-7.23 (m, 4H), 6.89-6.85 (m, 4H), 6.46 (d, \(J = 6.1\) Hz, 1H), 4.88 (dd, \(J = 6.1, 5.0\) Hz, 1H), 4.72 (d, \(J = 11.6\) Hz, 1H), 4.60-4.50 (m, 3H), 4.42 (d, \(J = 4.7\) Hz, 1H), 4.18 (dd, \(J = 4.7, 3.4\) Hz, 1H), 4.05 (t, \(J = 4.1\) Hz, 1H), 3.81 (s, 3H), 3.80 (s, 3H) ppm; \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta = 197.9, 159.4, 159.2, 144.8, 130.2, 130.0, 129.5, 129.2, 113.8, 113.7, 99.3, 78.3, 74.5, 71.7, 70.3, 66.7, 55.2\) ppm; IR (neat): \(\nu = 2934, 2837, 1732, 1604, 1514, 1249, 1033, 822, 756\) cm\(^{-1}\); MS (m/z) 384 [M]\(^+\); HRMS (EI): calcld. for C\(_{22}\)H\(_{24}\)O\(_6\) [M]\(^+\), 384.1573; found, 384.1501.

\((2R,3R,4R)-3,4\)-Di(4-methoxybenzyloxy)-2-vinyl-2,3-dihydro-2\(H\)-pyran (283):

\[
\text{OMBOPM} \\
\text{PMBO} \\
\]

To a suspension of methyltriphenylphosphonium bromide (14.5 g, 40.6 mmol) in anhydrous THF (60 mL) was added \(n\)BuLi (1.6 M in hexane, 22.8 mL, 36.5 mmol). After the addition was complete, the mixture was stirred at room temperature for 2 h and then cooled to \(-78^\circ\)C. Then a solution of aldehyde \(\text{291}\) (7.8 g, 20.3 mmol) in anhydrous THF (40 mL) was added dropwise. The suspension was stirred for 30 min at \(-78^\circ\)C and 1 h at room temperature. The mixture was then quenched with saturated aqueous NH\(_4\)Cl solution and diluted with EtOAc. The solution was separated and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with water and dried over Na\(_2\)SO\(_4\). After removing the solvent, the residue was purified by flash chromatography on neutral Al\(_2\)O\(_3\) (EtOAc/ hexane = 1: 10 to 1: 2) to
afford the alkene 283 (4.81 g, 62%) as a colorless oil. \([\alpha]_D^{23} = -42.4 \, (c = 0.63 \text{ in } \text{CHCl}_3); \) 
\(^1\text{H NMR (400 MHz, CDCl}_3\): } \delta = 7.30-7.26 \, (m, 4H), 6.89-6.86 \, (m, 4H), 6.40 \, (dd, J = 6.3, 1.5 \text{ Hz, 1H}), 6.07 \, (ddd, J = 17.2, 10.5, 6.6 \text{ Hz, 1H}), 5.33 \, (dt, J = 17.2, 1.3 \text{ Hz, 1H}), 5.23 \, (dt, J = 11.7, 1.3 \text{ Hz, 1H}), 4.84-4.79 \, (m, 2H), 4.65 \, (d, J = 11.7 \text{ Hz, 1H}), 4.56 \, (dd, J = 14.9, 11.9 \text{ Hz, 1H}), 4.37 \, (d, J = 6.4 \text{ Hz, 1H}), 4.21-4.20 \, (m, 1H), 3.81-3.80 \, (m \text{ containing } 2s, 7H) \text{ ppm; } \)^{13}\text{C NMR (100 MHz, CDCl}_3\): } \delta = 159.2, 159.1, 144.3, 134.4, 130.5, 130.4, 129.7, 129.1, 117.6, 113.7, 113.6, 99.9, 77.5, 72.9, 72.8, 70.6, 70.3, 55.2 \, (2C) \text{ ppm; IR (neat): } \nu = 2999, 2904, 2835, 1645, 1612, 1514, 1248, 1034, 822 \, \text{cm}^{-1}; \) 
\(\text{MS (m/z) 406 [M+Na]}^+; \) 
\(\text{HRMS (ESI): calcd. for } C_{23}H_{26}O_5Na [M+Na]^+; \) 
found, 405.1680.

\((1S,5S,6R)-5,6\text{-Di(4-methoxybenzyl)-3-cyclohexene-1-carbaldehyde (281):}\)

![Chemical structure](attachment:image.png)

A solution of alkene 283 (3.0 g, 7.8 mmol) in diphenyl ether (3 mL) was heated to 210 °C and stirred in a sealed tube filled with nitrogen. After 12 h, the mixture was cooled to room temperature and purified by column chromatography on silica gel (hexane to EtOAc/ hexane =1: 5) to give aldehyde 281 (0.69 g, 23%) as a colorless oil.

\(^1\text{H NMR (400 MHz, CDCl}_3\): } \delta = 9.85 \, (d, J = 2.0 \text{ Hz, 1H}), 7.31 \, (d, J = 8.5 \text{ Hz, 2H}), 7.21 \, (d, J = 8.5 \text{ Hz, 2H}), 6.87 \, (d, J = 9.3 \text{ Hz, 4H}), 5.87-5.78 \, (m, 2H), 4.71-4.59 \, (m, 3H), 4.39 \, (d, J = 11.5 \text{ Hz, 1H}), 4.05 \, (d, J = 4.0 \text{ Hz, 1H}), 3.81 \, (s, 3H), 3.80 \, (s, 3H), 3.76 \, (dd, J = 11.0, 3.4 \text{ Hz, 1H}), 3.23-3.17 \, (m, 1H), 2.28-2.20 \, (m, 1H) \text{ ppm; } \)^{13}\text{C NMR (100 MHz, CDCl}_3\): } \delta = 204.4, 159.2, 130.7, 129.8, 129.6, 129.5, 124.9, 113.8 \,(2C), \)
77.3, 71.1, 70.7, 68.0, 55.3 (2C), 46.4, 25.3 ppm; IR (neat): ν = 3005, 2934, 2837, 1721, 1612, 1514, 1250, 1034, 822, 756 cm⁻¹; MS (m/z) 405 [M+Na]⁺; HRMS (EI): calcd. for C₂₃H₂₆O₅ [M]⁺, 382.1780; found, 382.1760.

**Ethyl (1S,5S,6R)-5,6-di(4-methoxybenzyloxy)-3-cyclohexene-1-carboxylate (284):**

![Structure of Ethyl (1S,5S,6R)-5,6-di(4-methoxybenzyloxy)-3-cyclohexene-1-carboxylate (284)](image)

To a solution of 281 (80.0 mg, 0.21 mmol) and 2-methyl-2-butene (0.3 mL) in a tBuOH/H₂O mixture (6 mL, 5:1 v/v) was added NaH₂PO₄ (75.6 mg, 0.63 mmol) and sodium chlorite (57.0 mg, 80 wt %, 0.63 mmol). The mixture was stirred at room temperature for 2 h. The resulting solution was separated between EtOAc and brine. The aqueous layer was extracted with EtOAc. The combined organic layers were dried over Na₂SO₄, filtered, and concentrated *in vacuo* to give colorless oil. To a solution of compound obtained above in DMF (3 mL) were added K₂CO₃ (58.0 mg, 0.42 mmol) and EtI (50.4 μL, 0.63 mmol). The mixture was stirred at room temperature for 3 h. The mixture was partitioned between Et₂O and H₂O. The aqueous layer was extracted with Et₂O. The combined organic layers were dried over Na₂SO₄, filtered, and concentrated. The residue was purified by column chromatography on silica gel (EtOAc/ hexane = 1: 5) give 284 (71.6 mg, 80%) as a colorless oil. ¹H NMR (500 MHz, CDCl₃): δ = 7.28 (d, J = 8.6 Hz, 2H), 7.23 (d, J = 8.6 Hz, 2H), 6.87 (d, J = 8.6 Hz, 2H), 6.85 (d, J = 8.6 Hz, 2H), 5.82-5.79 (m, 2H), 4.66 (d, J = 11.9 Hz, 1H), 4.58 (d, J = 11.9 Hz, 1H), 4.55 (d, J = 11.3 Hz, 1H), 4.45 (d, J = 11.3 Hz, 1H), 4.19-4.12 (m, 2H), 3.94 (t, J = 4.2 Hz, 1H), 3.81 (s, 3H) 3.80 (s, 3H), 3.80-3.77 (m, 1H),
Part 1 Chapter 4 Experimental section

3.15 (dt, $J = 10.6, 6.0$ Hz, 1H), 2.45-2.41 (m, 1H), 2.36-2.30 (m, 1H), 1.24 (t, $J = 7.2$ Hz, 3H) ppm; $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta = 174.9, 159.2, 131.0, 130.5, 129.5, 129.4$ (2C), 125.1, 113.7 (2C), 78.2, 71.6, 71.3, 68.3, 60.6, 55.3, 41.5, 29.3, 14.3 ppm; IR (neat): $\nu = 2928, 2851, 1728, 1510, 1247, 1098, 851$ cm$^{-1}$; MS (m/z) 443 [M+Na]$^+$; HRMS (ESI): calcd. for C$_{23}$H$_{36}$O$_5$SiNa [M+Na]$^+$, 443.2230; found, 443.2214.

6-tert-Butyldimethylsilyl-D-glucal (293):

![6-tert-Butyldimethylsilyl-D-glucal (293)](image)

Prepared according to the procedure of 288 and purified by column chromatography on silica gel (EtOAc/hexane = 1: 2) to give 293 (81% yield) as a colorless oil. $^1$H NMR (400 MHz, CDCl$_3$): $\delta = 6.31$ (d, $J = 6.0$ Hz, 1H), 4.73 (dd, $J = 6.0, 2.2$ Hz, 1H), 4.25 (s, 1H), 4.00-3.97 (m, 1H), 4.14-4.09 (m, 1H), 3.91-3.89 (m, 1H), 3.80-3.79 (m, 2H), 3.25 (s, 1H), 2.58 (d, $J = 6.0$ Hz, 1H), 0.90 (s, 9H), 0.10 (s, 6H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta = 144.2, 102.4, 76.6, 72.3, 69.2, 63.8, 25.8, 18.2, -5.4, -5.2$ ppm; IR (neat): $\nu = 3405, 2953, 2855, 1459, 1262, 1108, 837$ cm$^{-1}$; MS (m/z) 261 [M+H]$^+$; HRMS (ESI): calcd. for C$_{12}$H$_{25}$O$_4$Si [M+H]$^+$, 261.1522; found, 261.1519.

6-tert-Butyldimethylsilyl-3,4-di(4-methoxybenzyl)-D-glucal (294):

![6-tert-Butyldimethylsilyl-3,4-di(4-methoxybenzyl)-D-glucal (294)](image)
Prepared according to the procedure of 289 and purified by column chromatography on silica gel (EtOAc/hexane = 1: 8) to give 294 (72% yield) as a colorless oil. ¹H NMR (400 MHz, CDCl₃): δ = 7.27 (dd, J = 8.8, 3.4 Hz, 4H), 6.87 (d, J = 8.8 Hz, 4H), 6.37 (d, J = 6.0 Hz, 1H), 4.82-4.80 (m, 1H), 4.78 (d, J = 11.2 Hz, 1H), 4.66 (d, J = 11.2 Hz, 1H), 4.58 (d, J = 11.2 Hz, 1H), 4.52 (d, J = 11.2 Hz, 1H), 4.45 (d, J = 8.8 Hz, 1H), 4.17 (s, 1H), 3.94-3.78 (m containing s, 9H), 0.91 (s, 9H), 0.08 (s, 6H) ppm; ¹³C NMR (100 MHz, CDCl₃): δ = 159.2 (2C), 144.6, 130.6, 130.5, 129.6, 129.4, 129.3, 113.8(2C), 99.9, 78.1, 75.6, 73.9, 73.5, 70.4, 61.7, 55.2, 25.9, 18.3, −5.2, −5.4 ppm; IR (neat): ν = 2930, 2857, 1647, 1612, 1514, 1248, 1098, 835, 779 cm⁻¹; MS (m/z) 524 [M+Na]⁺; HRMS (ESI): calcd. for C₂₈H₄₀O₆SiNa [M+Na]⁺, 523.2492; found, 523.2494.

3,4-di(4-methoxybenzyl)-D-glucal (295):

Prepared according to same procedure as 290 and purified by column chromatography (EtOAc/hexane = 1: 1) to give 295 as a white solid (85% yield). ¹H NMR (400 MHz, CDCl₃): δ = 7.28-7.24 (m, 4H), 6.88 (dd, J = 8.8, 2.8 Hz, 4H), 6.39 (d, J = 6.0 Hz, 1H), 4.86 (dd, J = 6.0, 2.8 Hz, 1H), 4.78 (d, J = 10.8 Hz, 1H), 4.66-4.59 (m, 2H), 4.50 (d, J = 11.2 Hz, 1H), 4.19 (d, J = 5.2 Hz, 1H), 3.94-3.89 (m, 1H), 3.84-3.74 (m containing s, 9H), 2.03 (s, 1H) ppm; ¹³C NMR (100 MHz, CDCl₃): δ = 159.4, 159.3, 144.5, 130.2, 130.1, 129.7, 129.4, 113.9 (2C), 100.2, 77.3, 75.2, 74.2, 73.3, 70.3, 61.8,
55.3 ppm; IR (neat): $\nu = 3422, 2933, 1650, 1514, 1248, 1034, 818, 760 \text{ cm}^{-1}$; MS (m/z) 409 [M+Na]$^+$; HRMS (ESI): calcd. for C$_{22}$H$_{26}$O$_6$Na [M+Na]$^+$, 409.1627; found, 409.1630.

(2S,3S,4R)-3,4-Di(4-methoxybenzyloxy)-3,4-dihydro-2$H$-pyran-2-carbaldehyde (296):

\[
\begin{align*}
&\text{O} \\
&\text{OHC} \\
&\text{PMBO} \\
&\text{OPMB}
\end{align*}
\]

Prepared according to the same procedure as 291 and purified by column chromatography on silica gel (EtOAc/hexane = 1: 3) to give 296 (80% yield) as a colorless oil. $^1$H NMR (400 MHz, CDCl$_3$): $\delta = 9.55$ (s, 1H), 7.31-7.12 (m, 4H), 6.91-6.84 (m, 4H), 6.65 (d, $J = 6.2$ Hz, 1H), 5.07-5.03 (m, 1H), 4.58-4.51 (m, 3H), 4.46 (s, 2H), 4.04 (dd, $J = 4.2$, 2.4 Hz, 1H), 3.81 (s, 3H), 3.80 (s, 3H), 3.76-3.75 (m, 1H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta = 198.6, 162.2, 159.5, 159.2, 144.8, 130.0, 129.6, 129.5, 129.4, 129.1, 114.0, 113.8, 113.7, 100.3, 79.2, 71.9, 71.4, 69.2, 66.6, 55.2 (2C) ppm; IR (neat): $\nu = 2934, 2837, 1732, 1612, 1514, 1248, 1034, 820, 758 \text{ cm}^{-1}$; MS (m/z) 407 [M+Na]$^+$; HRMS (ESI): calcd. for C$_{22}$H$_{24}$O$_6$ [M+Na]$^+$, 407.1471; found, 406.1478.

(2R,3S,4R)-3,4-Di(4-methoxybenzyloxy)-2-vinyl-2,3-dihydro-2$H$-pyran (297):
Prepared according to the same procedure as 283 and purified by column chromatography (EtOAc/hexane = 1: 10 to 1: 5) to give 297 (55 mg, 60%) as a colorless oil. $^1$H NMR (400 MHz, CDCl$_3$): $\delta = 7.27-7.23$ (m, 4H), 6.88-6.85 (m, 4H), 6.40 (d, $J = 6.1$ Hz, 1H), 6.08-5.99 (m, 1H), 5.42 (d, $J = 17.2$ Hz, 1H), 5.30 (d, $J = 10.6$ Hz, 1H), 4.85 (dd, $J = 6.1$, 2.7 Hz, 1H), 4.70 (d, $J = 10.9$ Hz, 1H), 4.62 (d, $J = 10.9$ Hz, 1H), 4.54 (dd, $J = 21.2$, 11.3 Hz, 2H), 4.30 (t, $J = 7.5$ Hz, 1H), 4.17-4.16 (m, 1H), 3.81 (s, 3H), 3.80 (s, 3H), 3.56 (dd, $J = 8.5$, 6.1 Hz, 1H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta = 159.3$, 159.2, 144.4, 134.4, 130.5, 130.2, 129.6, 129.3, 118.2, 113.8 (2C), 100.5, 78.0, 77.9, 75.1, 73.4, 70.4, 55.3 ppm; IR (neat): $\nu = 3007$, 2901, 1645, 1614, 1514, 1248, 1036, 820, 750 cm$^{-1}$; MS (m/z) 406 [M+Na]+; HRMS (ESI): calcd. for C$_{23}$H$_{26}$O$_5$Na [M+Na]+, 405.1678; found, 405.1683.

(1S,5R,6R)-5,6-Di(4-methoxybenzyloxy)-3-cyclohexene-1-carbaldehyde (298):

A solution of alkene 297 (39.0 mg, 0.1 mmol) in diphenyl ether (0.4 mL) was heated to 210 °C and stirred in a sealed tube filled with nitrogen. After 2 h, the mixture was cooled to room temperature and purified by column chromatography on silica gel (hexane to EtOAc/hexane = 1: 5) to give aldehyde 298 (30.0 mg, 77%) as a colorless oil. $^1$H NMR (400 MHz, CDCl$_3$): $\delta = 9.65$ (d, $J = 2.2$ Hz, 1H), 7.28-7.22 (m, 4H),
6.89-6.86 (m, 4H), 5.83-5.72 (m, 2H), 4.76 (d, \( J = 10.7 \) Hz, 1H), 4.63-4.52 (m, 3H), 4.13-4.11 (m, 1H), 3.96 (dd, \( J = 9.0, 6.0 \) Hz, 1H), 3.81 (s, 3H), 3.80 (s, 3H), 2.81-2.75 (m, 1H), 2.38-2.21 (m, 2H) ppm; \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \( \delta = 202.0, 159.3, 159.2, 130.2, 130.1, 129.6, 129.4, 129.3, 127.4, 125.9, 113.8, 77.6, 76.4, 73.0, 70.8, 55.2, 50.1, 23.8 \) ppm; IR (neat): \( \nu = 3005, 2911, 2835, 1724, 1611, 1512, 1248, 1034, 820 \) cm\(^{-1}\); HRMS (EI): calcd. for C\(_{23}\)H\(_{26}\)O\(_5\) [M]\(^+\), 382.1780; found, 382.1763.

**4,6-Di-O-(4-methoxybenzylidene)-D-glucal (303):**

To a solution of \( \alpha \)-(+)-glucal 292 (14.6 g, 0.10 mol) and \( p \)-anisaldehyde diethyl acetal (29.5 mL, 0.15 mol) in dry DMF (150 mL), PPTS (2.51 g, 0.01 mol) was added. The mixture was stirred at 25 °C under reduced pressure to remove ethanol as it was generated for 2 h. Then DMF was removed under reduced pressure. The residue was dissolved in CH\(_2\)Cl\(_2\) and then washed with saturated aqueous NH\(_4\)Cl, H\(_2\)O and brine. The organic layer was dried over Na\(_2\)SO\(_4\), filtered, and then concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (EtOAc/hexane = 1: 2) to afford 303 (22.7 g, 86%) as a white solid. mp 120-122 °C (lit.\(^{[80]}\) mp 104-105 °C); \([\alpha]_D^{22} = -6.7 \) (c = 1.0 in CHCl\(_3\)); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta = 7.42 \) (d, \( J = 8.7 \) Hz, 2H), 6.90 (d, \( J = 8.7 \) Hz, 2H), 6.34 (dd, \( J = 6.1, 1.5 \) Hz, 1H), 5.55 (s, 1H), 4.77 (dd, \( J = 6.1, 1.9 \) Hz, 1H), 4.50-4.47 (m, 1H), 4.36 (dd, \( J = 10.3, 5.0 \) Hz, 1H), 3.80 (s, 3H), 3.93-3.75 (m, 3H), 2.44 (d, \( J = 4.2 \) Hz, 1H) ppm; \(^{13}\)C
NMR (100 MHz, CDCl$_3$): $\delta = 160.2, 144.1, 129.5, 127.5, 113.7, 103.5, 101.7, 80.6, 68.3, 68.2, 66.5, 55.3$ ppm; IR (neat): $\nu = 3292, 2906, 1639, 1518, 1254, 1096, 824, 756$ cm$^{-1}$. MS (m/z) 265 [M+H]$^+$; HRMS (ESI): calcd. for C$_{14}$H$_{16}$O$_5$Na [M+Na]$^+$, 287.0895; found, 287.0888.

3-tert-Butyldimethylsilyl-4,6-Di-O-(4-methoxybenzylidene)-D-glucal (304):

To a solution of 303 (15.9 g, 60.0 mmol) in DMF (100 mL), imidazole (9.8 g, 144.0 mmol), TBSCl (10.9 g, 72.0 mmol) and DMAP (0.73 g, 6.0 mmol) were added at room temperature under nitrogen atmosphere. After 3h at room temperature, the reaction mixture was diluted with Et$_2$O, washed with saturated NH$_4$Cl solution and dried over Na$_2$SO$_4$. Evaporation of the organic solvent under reduced pressure gave a crude product, which was purified by flash column chromatography on silica gel (EtOAc/ hexane = 1: 15) to afford 304 (20.4 g, 90%) as a colorless oil. $[\alpha]_D^{22} = -97.3$ (c = 1.0 in CHCl$_3$); $^1$H NMR (400 MHz, CDCl$_3$): $\delta = 7.42$ (d, $J = 8.7$ Hz, 2H), 6.89 (d, $J = 8.7$ Hz, 2H), 6.29 (dd, $J = 6.1, 1.4$ Hz, 1H), 5.56 (s, 1H), 4.67 (dd, $J = 6.1, 1.9$ Hz, 1H), 4.51-4.49 (m, 1H), 4.35-4.31 (m, 1H), 3.88-3.76 (m, 6H), 0.90 (s, 9H), 0.10-0.08 (d, 6H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta = 159.9, 143.3, 129.9, 127.3, 113.5, 105.4, 101.2, 80.5, 68.8, 68.3, 67.3, 55.2, 25.8, 18.2, -4.4, -4.8$ ppm; IR (neat): $\nu = 2932, 2858, 1640, 1520, 1103, 868$ cm$^{-1}$; MS (m/z) 379 [M+H]$^+$; HRMS (ESI): calcd. for C$_{14}$H$_{16}$O$_5$ [M+H]$^+$, 379.1941; found, 379.1938.
3-tert-Butyldimethylsilyl-4-O-(4-methoxybenzyl)-D-glucal (305):

To a solution of 304 (20.0 g, 52.8 mmol) in freshly distilled CH₂Cl₂ (100 mL), DIBAL-H (1 M in toluene, 68.6 mL, 68.6 mmol) was slowly added at −15 °C under nitrogen. The reaction mixture was stirred at −15 °C for 20 min and at rt for 2 h, then it was cooled to 0 °C and MeOH (1 mL) was added dropwise. EtOAc (60 mL) and sodium potassium tartrate (20%-H₂O, 80 mL) were added at room temperature. The mixture was stirred for 1 h, extracted with EtOAc (2 × 60 mL). The organic layers were washed with brine (2 × 100 mL) and dried over Na₂SO₄. Filtration and evaporation of the solvent under reduced pressure gave a crude product, which was purified by flash column chromatography on silica gel (EtOAc/hexane = 1: 3) to afford 305 (16.9 g, 84%) as a colorless oil. [α]D²² = −20.6 (c = 1.0 in CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ = 7.28 (d, J = 8.6 Hz, 2H), 6.88 (d, J = 8.6 Hz, 2H), 6.33 (d, J = 6.0 Hz, 1H), 4.77 (d, J = 11.0 Hz, 1H), 4.67 (dd, J = 4.9, 2.9 Hz, 1H), 4.63 (d, J = 11.0 Hz, 1H), 4.35-4.33 (m, 1H), 3.96-3.92 (m, 1H), 3.82-3.80 (m, 5H), 3.63-3.60 (m, 1H), 2.10 (t, J = 6.6 Hz, 1H), 0.92 (s, 9H), 0.12 (s, 6H) ppm; ¹³C NMR (100 MHz, CDCl₃): δ = 159.9, 143.3, 130.0, 129.6, 113.9, 103.4, 76.4, 73.5, 68.6, 55.3, 25.8, 18.0, −4.4, −4.7 ppm; IR (neat): ν = 3445, 2932, 2859, 1651, 1516, 1250, 1096, 837 cm⁻¹; MS (m/z) 404 [M+Na]⁺; HRMS(ESI): calcd. for C₂₀H₃₂O₅SiNa [M+Na]⁺, 403.1917; found, 403.1902.
(2R,3R,4R)-4-(tert-Butyldimethylsilyloxy)-3-(4-methoxybenzyl)-2-vinyl-2,3-dihydro-2H-pyran (302):

Dess-Martin periodinane (25.5 g, 60.0 mmol) was added to a solution of 305 (15.2 g, 40.0 mmol) in dry CH₂Cl₂ (200 mL). The suspension was stirred for 2 h at room temperature under nitrogen. Saturated aqueous Na₂S₂O₃ (100 mL) and NaHCO₃ (100 mL) were added slowly to the reaction mixture. The resulting solution was separated. The organic layer was washed with saturated NaHCO₃ solution and brine, dried over Na₂SO₄, filtered through a pad of Celite, evaporated to afford crude aldehyde as a colorless oil. To a suspension of methyltriphenylphosphonium bromide (23.7 g, 66.2 mmol) in anhydrous THF (100 mL) was added a solution of nBuLi (1.6 M in hexane, 34.5 mL, 55.2 mmol). After the addition completed, the resulting orange solution was stirred at room temperature for 2 h and then cooled to −78 °C. Then a solution of aldehyde (13.9 g, 36.8 mmol) in 40 mL anhydrous THF was added via a cannula. The suspension was stirred for 30 min at −78 °C and 1 h at room temperature, after which it was quenched with saturated aqueous NH₄Cl solution and diluted with EtOAc. The solution was separated and the aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with water and dried over Na₂SO₄. After removing the solvent, the residue was purified by flash chromatography on neutral Al₂O₃ (EtOAc/hexane = 1: 40) to afford the alkene 302 (10.1 g, 67%) as a colorless oil. [α]D²² = −44.1 (c = 1.0 in CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ = 7.26 (d, J = 8.6 Hz, 2H), 6.87 (d, J = 8.6 Hz, 2H), 6.33 (d, J = 6.1 Hz, 1H), 6.06-5.97 (m, 1H),
5.42-5.26 (m, 2H), 4.71-4.57 (m, 3H), 4.36-4.34 (m, 1H), 4.28 (t, $J = 7.6$ Hz, 1H), 3.80 (s, 3H), 3.42 (dd, $J = 6.2, 8.6$ Hz, 1H), 0.92 (s, 9H), 0.1 (s, 6H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta =$ 159.3, 143.2, 134.6, 130.2, 129.6, 118.1, 113.7, 103.9, 80.1, 78.1, 73.8, 69.1, 55.3, 25.8, 18.0, -4.4, -4.6 ppm; IR (neat): $\nu =$ 2955, 2856, 1647, 1516, 1250, 1092, 837 cm$^{-1}$; MS (m/z) 399 [M+Na]$^+$; HRMS (ESI): calcd. for C$_{21}$H$_{32}$O$_4$SiNa [M+Na]$^+$, 399.1968; found, 399.1976.

$(1S,5R,6R)-6$(tert-Butyldimethylsilyloxy)-5-(4-methoxybenzyloxy)-3cyclohexene-1-carbaldehyde (301):

\[
\begin{align*}
\text{PMB} & \quad \text{OTBS} \\
\quad & \quad \quad \text{CHO}
\end{align*}
\]

A solution of alkene 302 (7.5 g, 19.9 mmol) in diphenyl ether (7 mL) was heated to 210 °C and stirred in a sealed tube filled with nitrogen. After 2 h, the mixture was cooled to room temperature and purified by column chromatography on silica gel (EtOAc/ hexane =1: 60 to 1: 15) to give aldehyde 301 (6.6 g, 88%) as a colorless oil. [$\alpha$]$_D^{22} =$ -37.8 ($c =$ 1.2 in CHCl$_3$); $^1$H NMR (400 MHz, CDCl$_3$): $\delta =$ 9.72 (s, 1H), 7.24 (d, $J =$ 8.6 Hz, 2H), 6.87 (d, $J =$ 8.6 Hz, 2H), 5.85-5.69 (m, 2H), 4.49 (dd, $J =$ 17.6, 11.3 Hz, 2H), 4.23 (dd, $J =$ 5.2, 7.6 Hz, 1H), 3.83-3.80 (m, 4H), 2.71-2.66 (m, 1H), 2.43-2.20 (m, 2H), 0.86 (s, 9H), 0.07 (d, 6H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta =$ 202.1, 159.1, 130.3, 129.3, 127.9, 125.3, 113.7, 77.3, 70.5, 70.4, 55.2, 52.2, 25.8, 22.2, 18.1, -4.3, -4.9 ppm; IR (neat): $\nu =$ 2926, 1728, 1514, 1248, 1101, 837, 777 cm$^{-1}$; MS (m/z) 400 [M+Na]$^+$; HRMS (ESI): calcd. for C$_{21}$H$_{32}$O$_4$SiNa [M+Na]$^+$, 399.1968; found, 399.1957.
Ethyl (1S,5R,6R)-6-(tert-butyldimethylsilyloxy)-5-(4-methoxybenzyloxy)-3-cyclohexene-1-carboxylate (306):

Prepared according to the same procedure as 284 and purified by column chromatography on silica gel (EtOAc/hexane = 1:15) give 306 (87% yield) as a colorless oil. $[\alpha]_D^{22} = -47.4 \ (c = 1.0 \ in \ CHCl_3)$; $^1$H NMR (400 MHz, CDCl$_3$): $\delta = 7.26$ (d, $J = 8.4$ Hz, 2H), 6.86 (d, $J = 8.4$ Hz, 2H), 5.75-5.65 (m, 2H), 4.51 (q, $J = 11.2$ Hz, 2H), 4.15-4.06 (m, 3H), 3.90 (s, 1H) 3.80 (s, 3H), 2.79-2.72 (m, 1H), 2.41-2.25 (m, 2H), 1.24 (t, $J = 7.1$ Hz, 3H), 0.84 (s, 9H), 0.05 (d, 6H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta = 173.6, 158.9, 130.6, 129.2, 127.3, 125.5, 113.6, 79.9, 72.1, 70.2, 60.5, 55.2, 47.3, 28.0, 25.8, 18.1, 14.0, -4.0, -5.3$ ppm; IR (neat): $\nu = 3449, 2932, 2855, 1736, 1512, 1250, 1103, 837 \ cm^{-1}$; MS (m/z) 443 [M+Na]$^+$; HRMS (ESI): calcd. for C$_{23}$H$_{36}$O$_5$SiNa [M+Na]$^+$, 443.2230; found, 443.2219.

Ethyl (1S,5R,6R)-6-(tert-butyldimethylsilyloxy)-5-hydroxy-3-cyclohexene-1-carboxylate (307):

DDQ (4.7 g, 20.7 mmol) was added to a solution of 306 (5.8 g, 13.8 mmol) in CH$_2$Cl$_2$/H$_2$O (80 mL, v/v = 1:1). The mixture was stirred at room temperature. After 6 h, Na$_2$S$_2$O$_3$ (80 mL) was added to the mixture. The suspension was filtered.
filtrate was extracted with CH₂Cl₂. The organic layer was washed with saturated NaHCO₃ solution and brine successively, dried over Na₂SO₄, filtered, and evaporated. The residue was purified by column chromatography on silica gel (EtOAc/ hexane = 1:8) to give 307 (3.82 g, 92%) as a colorless oil. [α]D²² = −42.5 (c = 1.03 in CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ = 5.71-5.57 (m, 2H), 4.13 (q, J = 7.1 Hz, 2H), 4.06 (s, 1H), 3.93 (dd, J = 9.6, 6.5 Hz, 1H) 2.74 (dd, J = 17.3, 7.8 Hz, 1H), 2.35-2.33 (m, 2H), 2.03 (d, J = 6.5 Hz, 1H), 1.26 (t, J = 7.1 Hz, 3H), 0.86 (s, 9H), 0.14 (s, 3H), 0.07 (s, 3H) ppm; ¹³C NMR (100 MHz, CDCl₃): δ = 174.0, 128.4, 126.5, 74.8, 72.9, 60.6, 46.7, 28.3, 25.8, 18.1, 14.0, −4.2, −5.0 ppm; IR (neat) ν = 3480, 2932, 2855, 1736, 1254, 1126, 837 cm⁻¹; MS (m/z) 323 [M+Na]+; HRMS (ESI): calcd. for C₁₅H₂₈O₄SiNa [M+Na]+, 323.1655; found, 323.1642.

Ethyl (1S,5R,6R)-6-(tert-butyldimethylsilyloxy)-5-(carbamoyloxy)-3-cyclohexene-1-carboxylate (300):

![Chemical Structure](attachment:image.png)

Trichloroacetyl isocyanate (0.32 mL, 2.66 mmol) was added to a solution of 307 (0.40 g, 1.33 mmol) in dry dichloromethane (10 mL). After 3 h, methanol (6.0 mL) and K₂CO₃ (0.92 g, 6.65 mmol) were added. The mixture was stirred overnight at room temperature. The resulting suspension was filtered through a pad of Celite and washed with dichloromethane (6 mL). The filtrate was concentrated and purified by chromatography on silica gel (EtOAc/ hexane = 1:3) to give 300 as a white solid (0.40 g, 87%). mp 71-73 °C; [α]D²² = −50.1 (c = 1.0 in CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ = 5.71-5.57 (m, 2H), 4.13 (q, J = 7.1 Hz, 2H), 4.06 (s, 1H), 3.93 (dd, J = 9.6, 6.5 Hz, 1H) 2.74 (dd, J = 17.3, 7.8 Hz, 1H), 2.35-2.33 (m, 2H), 2.03 (d, J = 6.5 Hz, 1H), 1.26 (t, J = 7.1 Hz, 3H), 0.86 (s, 9H), 0.14 (s, 3H), 0.07 (s, 3H) ppm; ¹³C NMR (100 MHz, CDCl₃): δ = 174.0, 128.4, 126.5, 74.8, 72.9, 60.6, 46.7, 28.3, 25.8, 18.1, 14.0, −4.2, −5.0 ppm; IR (neat) ν = 3480, 2932, 2855, 1736, 1254, 1126, 837 cm⁻¹; MS (m/z) 323 [M+Na]+; HRMS (ESI): calcd. for C₁₅H₂₈O₄SiNa [M+Na]+, 323.1655; found, 323.1642.
CDCl₃): δ = 5.74 (dd, J = 10.0, 5.2 Hz, 1H), 5.56 (dd, J = 10.0, 1.9 Hz, 1H), 5.08 (dd, J = 4.8, 2.2 Hz, 1H), 4.85 (br s, 2H), 4.16-4.08 (m, 3H), 2.79 (dd, J = 17.5, 8.5 Hz, 1H), 2.35-2.33 (m, 2H), 1.25 (t, J = 7.1 Hz, 3H), 0.82 (s, 9H), 0.07 (s, 3H), 0.04(s, 3H) ppm; ¹³C NMR (100 MHz, CDCl₃): δ = 173.4, 156.3, 128.0, 125.2, 76.4, 60.6, 47.2, 28.2, 25.6, 18.0, 14.0, −4.5, −5.1 ppm; IR (neat) ν = 3439, 2930, 2857, 1715, 1643, 1381, 1315 cm⁻¹; MS (m/z) 366 [M+Na]^+; HRMS (ESI): calcd. for C₁₆H₂₉NO₅SiNa [M+Na]^+, 366.1713; found, 366.1715.

Ethyl (1S,5R,6R)-6-(tert-butyldimethylsilyloxy)-5-(tosyloxycarbamoyloxy)-3-cyclohexene-1-carboxylate (311):

![Ethyl (1S,5R,6R)-6-(tert-butyldimethylsilyloxy)-5-(tosyloxycarbamoyloxy)-3-cyclohexene-1-carboxylate (311)](image)

Alcohol 307 (3.55 g, 11.8 mmol) was dissolved in dichloromethane (60 mL) at room temperature. Carbonyl diimidazole (2.87 g, 17.7 mmol) was added in one portion and the solution was stirred for 2 h. The solution was washed with a saturated solution of NH₄Cl (2 × 50 mL). The organic layer was dried over Na₂SO₄, filtered and concentrated. The residue was dissolved in pyridine (40 mL) at room temperature and NH₂OH·HCl (1.64 g, 23.6 mmol) was added in one portion. The solution was stirred for 4 h. Dichloromethane (80 mL) was then added and the resulting solution was washed with 10% solution of sulphuric acid (3 × 60 mL). The organic layer was dried over Na₂SO₄, filtered and concentrated. The N-hydroxycarbamate was purified by flash chromatography on silica gel (EtOAc/ hexane = 1: 2) to give N-hydroxy carbamate 310 as a white solid (2.78 g, 87% conversion yield, 0.88 g of alcohol 307...
was recovered). mp 83-85 °C; \([\alpha]_D^{22} = -62.9 \ (c = 1.03 \ in \ CHCl_3)\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta = 7.30\) (s, 1H), 6.81 (s, 1H), 5.80 (d, \(J = 10.1 \ Hz, 1H\)), 5.56 (d, \(J = 10.1 \ Hz, 1H\)), 5.16-5.15 (m, 1H), 4.20-4.11 (m, 3H), 2.83-2.77 (m, 1H), 2.37-2.36 (m, 1H), 1.26 (t, \(J = 7.1 \ Hz, 3H\)), 0.83 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H) ppm; \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta = 173.3, 158.6, 129.0, 124.3, 71.0, 60.8, 46.9, 27.7, 25.6, 18.0, 14.0, -4.4, -5.2 \ ppm\); IR (neat): \(\nu = 3292, 3019, 2930, 2857, 1734, 1255, 1215, 1111, 756 \ cm^{-1}\); MS (m/z) 383 [M+Na]; HRMS (ESI): calcd. for C\(_{16}\)H\(_{29}\)NO\(_6\)SiNa [M+Na]\(^+\), 382.1662; found, 323.1660.

To a solution of 310 (2.6 g, 7.2 mmol) in Et\(_2\)O (60 mL) at 0 °C, was added p-toluenesulfonyl chloride (1.51 g, 7.92 mmol). Triethylamine (1.05 mL, 7.56 mmol) was then added slowly and the resulting white suspension was stirred for 12 h at room temperature. The mixture was washed with water and brine, dried over Na\(_2\)SO\(_4\). The solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel (EtOAc/hexane = 1: 4) to give compound 311 (3.29 g, 89%) as a white solid. mp 121-123 °C; \([\alpha]_D^{22} = -50.3 \ (c = 0.97 \ in \ CHCl_3)\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta = 8.03\) (s, 1H), 7.87 (d, \(J = 8.2 \ Hz, 2H\)), 7.34 (d, \(J = 8.2 \ Hz, 2H\)), 5.70 (d, \(J = 5.0 \ Hz, 1H\)), 5.18 (d, \(J = 9.6 \ Hz, 1H\)), 5.01 -4.99 (m, 1H), 4.17-4.08 (m, 2H), 4.01 (dd, \(J = 10.3, 7.1 \ Hz, 1H\)), 2.75-2.68 (m, 1H), 2.44 (s, 1H), 2.31-2.28 (m, 2H), 1.26 (t, \(J = 7.1 \ Hz, 3H\)), 0.79 (s, 9H), 0.02 (s, 3H), −0.01 (s, 3H) ppm; \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta = 173.1, 154.9, 146.1, 130.2, 129.7, 129.6, 128.9, 123.7, 78.9, 70.7, 60.8, 47.1, 28.0, 25.6, 21.8, 17.9, 14.0, -4.5, -5.2 \ ppm\); IR (neat): \(\nu = 3366, 3019, 2930, 2857, 1734, 1381, 1215, 1111, 756 \ cm^{-1}\); MS (m/z) 536 [M+Na]; HRMS (ESI): calcd. for C\(_{23}\)H\(_{35}\)NO\(_8\)SSiNa [M+Na]\(^+\), 536.1750; found, 536.1740.
Ethyl (3aR,4S,6S,7R,7aR)-7-(tert-butyldimethylsilyloxy)-4-(4-methoxybenzyl amino)-2-oxooctahydro benzoxazole-6-carboxylate (312):

The N-tosyloxy carbamate 311 (0.11 g, 0.22 mmol), K₂CO₃ (0.91 g, 0.66 mmol) and (CuOTf)₂toluene (5.7 mg, 0.011 mmol) were dissolved in acetonitrile (5 mL) at room temperature under nitrogen. The mixture was stirred vigorously for 12 h. The suspension was filtered through Celite and washed by CH₂Cl₂. The filtrate was concentrated. To the solution of crude product in THF (3 mL), 4-methoxybenzyl amine (20.8 µL, 0.264 mmol) and triethylamine (6.1 µL, 0.044 mmol) were added at room temperature under nitrogen. The mixture was stirred for 4 h and concentrated. The residue was purified by column chromatography on silica gel (EtOAc/hexane = 1: 4 to EtOAc/hexane = 1: 1) to give compound 312 (57 mg, 54%) and 313 (19 mg, 18%) as colorless oils. [α]D²³ = +14.1 (c = 1.0 in CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ = 7.21 (d, J = 8.4 Hz, 2H), 6.86 (d, J = 8.4 Hz, 2H), 6.09 (s, 1H), 4.44 (t, J = 7.1 Hz, 1H), 4.21-4.10 (m, 3H), 3.79 (s, 3H), 3.76-3.73 (m, 2H), 3.63 (d, J = 12.7 Hz, 1H), 3.01 (dd, J = 9.8, 5.1 Hz, 1H), 2.79 (dt, J = 9.7, 5.0 Hz 1H), 2.08-2.02 (m, 1H), 1.77-1.70 (m, 1H), 1.26 (t, J = 7.1 Hz, 3H), 0.84 (s, 3H), 0.15 (s, 3H), 0.06 (s, 3H) ppm; ¹³C NMR (100 MHz, CDCl₃): δ = 173.3, 159.4, 158.8, 131.6, 129.2, 113.9, 81.2, 72.1, 60.9, 57.4, 55.2, 53.3, 50.8, 43.1, 27.3, 25.7, 17.9, 14.0, −4.6, −5.5 ppm; IR (neat) ν = 3310, 2930, 2855, 1575, 1512, 1427, 1111, 837, 756 cm⁻¹; MS (m/z) 480 [M+H]⁺; HRMS (ESI) calcd. for C₂₄H₃₉N₂O₆Si [M+H]⁺, 479.2577; found 479.2570.
Ethyl (3aR,4R,6S,7R,7aR)-7-(tert-butyldimethylsilyloxy)-4-(4-methoxybenzyl amino)-2-oxooctahydro benzoxazole-6-carboxylate (313):

$$\alpha_D^{23} = +13.1 \ (c = 1.0 \ \text{in CHCl}_3); \ ^1H \text{ NMR (400 MHz, CDCl}_3): \ \delta = 7.17 \ (d, \ J = 8.4 \ \text{Hz, 2H}), \ 6.85 \ (d, \ J = 8.4 \ \text{Hz, 2H}), \ 5.76 \ (t, \ J = 5.5 \ \text{Hz, 1H}), \ 4.32-4.22 \ (m, \ 1H), \ 4.15-4.10 \ (m, \ 2H), \ 3.97 \ (dd, \ J = 7.2, 4.8 \ \text{Hz, 1H}), \ 3.79 \ (s, \ 3H), \ 3.74 \ (s, \ 1H), \ 3.22 \ (d, \ J = 8.0 \ \text{Hz, 1H}), \ 2.93-2.91 \ (m, \ 1H), \ 2.80-2.77 \ (m, \ 1H), \ 2.49 \ (q, \ J = 7.0 \ \text{Hz, 1H}), \ 2.22-2.20 \ (m, \ 1H), \ 1.23 \ (t, \ J = 7.2 \ \text{Hz, 3H}), \ 0.85 \ (s, \ 9H), \ 0.11 \ (s, \ 3H), \ 0.03 \ (s, \ 3H) \ \text{ppm}; \ ^{13}C \text{ NMR (100 MHz, CDCl}_3): \ \delta = 174.3, \ 164.0, \ 159.0, \ 130.4, \ 128.9, \ 114.0, \ 71.3, \ 71.1, \ 60.9, \ 55.3, \ 46.0, \ 44.1, \ 42.5, \ 37.2, \ 25.7, \ 24.1, \ 18.0, \ 13.9, \ -4.5, \ -5.1 \ \text{ppm}; \ \text{IR (neat) } \nu = 3306, \ 2930, \ 2855, \ 1732, \ 1514, \ 1248, \ 1115, \ 837, \ 756 \ \text{cm}^{-1}; \ \text{MS (m/z) } 480 \ [\text{M+H}]^+; \ \text{HRMS (ESI) calcd. for C}_{24}\text{H}_{39}\text{N}_2\text{O}_6\text{Si} \ [\text{M+H}]^+, \ 479.2577; \ \text{found } 479.2577.

Ethyl (3aR,4S,6S,7R,7aR)-4-(allylamino)-7-(tert-butyldimethylsilyloxy)-2-oxoocta hydro benzoxazole-6-carboxylate (314):

Prepared according to the same procedure as 37 and purified by column chromatography on silica gel (EtOAc/hexane = 1: 4 to EtOAc/hexane = 2: 1) to give
compound 314 (52%) and 315 (15%) as colorless oils. \([\alpha]_D^{23} = +6.6 \ (c = 0.8 \ \text{in CHCl}_3)\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta = 5.95 \ (s, 1H), 5.89-5.79 \ (m, 1H), 5.21-5.10 \ (m, 1H), 4.45 \ (t, J = 7.1 \ Hz, 1H), 4.22 \ (dd, J = 9.3, 6.7 \ Hz, 1H), 4.18-4.10 \ (m, 2H), 3.73 \ (t, J = 6.1 \ Hz, 1H), 3.33 \ (dd, J = 14.0, 5.8 \ Hz, 1H), 3.18 \ (dd, J = 14.0, 6.1 \ Hz, 1H), 2.98 \ (dd, J = 10.2, 5.9 \ Hz, 1H), 2.75 \ (dt, J = 9.4, 5.2 \ Hz, 1H), 2.09-2.02 \ (m, 1H), 1.72-1.65 \ (m, 1H), 1.27 \ (t, J = 7.2 \ Hz, 3H), 0.84 \ (s, 9H), 0.14 \ (s, 3H), 0.06 \ (s, 3H) \ ppm; \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta = 173.3, 159.2, 136.2, 116.6, 81.1, 71.8, 60.9, 57.3, 53.2, 49.7, 43.2, 27.2, 25.7, 17.9, 14.1, −4.6, −5.4 \ ppm; IR (neat) \(\nu = 3306, 2930, 2857, 1732, 1250, 1111, 839, 756 \ \text{cm}^{-1}\); MS (m/z) 400 [M+H]\(^+\); HRMS (ESI) calcd. for C\(_{19}\)H\(_{35}\)N\(_2\)O\(_5\)Si [M+H]\(^+\), 399.2315; found 399.2312.

**Ethyl (3aR,4R,6S,7R,7aR)-4-(allylamino)-7-(tert-butyldimethylsilyloxy)-2-oxooctahydro benzoazolate-6-carboxylate (315):**

\[\text{OTBS} \quad \overset{\text{CO}_2\text{Et}}{\text{N}} \quad \overset{\text{N}}{\text{H}} \quad \overset{\text{N}}{\text{H}} \quad \overset{\text{CO}_2\text{Et}}{\text{O}} \]

\([\alpha]_D^{23} = 1.45 \ (c = 0.8 \ \text{in CHCl}_3)\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta = 5.85-5.76 \ (m, 1H), 5.55 \ (t, J = 5.6 \ Hz, 1H), 5.19-5.10 \ (m, 1H), 4.19-4.04 \ (m, 2H), 4.22 \ (dd, J = 7.0, 4.5 \ Hz, 1H), 3.80-3.75 \ (m, 3H), 3.26 \ (d, J = 8.6 \ Hz, 1H), 2.92 \ (s, 1H), 2.78 \ (t, J = 5.3 \ Hz, 1H), 2.51 \ (dd, J = 13.6, 6.7 \ Hz, 1H), 2.26-2.18 \ (m, 1H), 1.25 \ (t, J = 7.1 \ Hz, 3H), 0.86 \ (s, 9H), 0.12 \ (s, 3H), 0.04 \ (s, 3H) \ ppm; \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta = 174.4, 163.9, 134.2, 116.2, 71.2, 71.0, 60.9, 45.8, 43.0, 42.4, 37.1, 25.7, 23.9, 18.0, 14.0, −4.5, −5.1 \ ppm; IR (neat) \(\nu = 3308, 2928, 2855, 1736, 1643, 1531, 1252, 1118, 839, \)}
779 cm$^{-1}$; MS (m/z) 400 [M+H]$^+$; HRMS (ESI) calcd. for C$_{19}$H$_{35}$N$_2$O$_5$Si [M+H]$^+$, 399.2315; found 399.2306.

**Ethyl (3aR,4S,6S,7R,7aR)-4-azido-7-(tert-butyldimethylsilyloxy)-2-oxooctahydrobenzoxazole-6-carboxylate (299):**

The N-tosyloxy carbamate 311 (2.98 g, 5.8 mmol), K$_2$CO$_3$ (4.01 g, 29.0 mmol) and (CuOTf)$_2$·toluene (150 mg, 0.29 mmol) were dissolved in acetonitrile (60 mL) at room temperature under nitrogen. The mixture was stirred vigorously for 16 h. The suspension was filtered through Celite and washed by CH$_2$Cl$_2$. The filtrate was concentrated. The crude product was dissolved in THF, to which trimethylsilyl azide (2.02 mL, 11.6 mmol) and TBAF (5.8 mL, 5.8 mmol) were added at 0 °C under nitrogen. The mixture was warmed to room temperature and stirred for 4 h. The mixture was filtered through silica gel and washed with EtOAc. The filtrate was evaporated and purified by column chromatography on silica gel (EtOAc/hexane = 1:2) to give compound 299 (1.83 g, 82%) as a light yellow solid. mp 66-68 °C; [$\alpha$]$_D^{22}$ = +4.8 (c = 1.0 in CHCl$_3$); $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ = 6.45 (s, 1H), 4.41 (t, $J$ = 6.6 Hz, 1H), 4.21 (dd, $J$ = 9.1, 6.6 Hz, 1H), 4.16 (q, $J$ = 7.2 Hz, 2H), 3.96-3.92 (m, 1H), 3.83 (t, $J$ = 6.6 Hz, 1H), 2.73 (dd, $J$ = 9.1, 4.7 Hz, 1H), 2.14 (ddd, $J$ = 14.2, 9.1, 4.0 Hz, 1H), 1.97-1.90 (m, 1H), 1.27 (t, $J$ = 7.2 Hz, 3H), 0.84 (s, 9H), 0.14 (s, 3H), 0.06 (s, 3H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ = 172.3, 159.0, 80.5, 71.3, 61.2,
57.9, 56.3, 43.1, 26.1, 25.6, 17.9, 14.0, −4.6, −5.5 ppm; IR (neat): ν = 3581, 2932, 2859, 2106, 1767, 1384, 1254, 1181, 837, 779 cm⁻¹; MS (m/z) 386 [M+Na]⁺; HRMS (ESI): calcd for C₁₆H₂₉N₄O₅Si [M+H]⁺, 385.1907; found, 385.1904.

Ethyl (3aR,4S,6R,7R,7aR)-4-azido-7-(tert-butyldimethylsilyloxy)-2-oxooctahydrobenzoxazole-6-carboxylate (317):

\[
\text{OTBS} \quad \text{CO}_2\text{Et}
\]

Mp 140-142 °C; [α]D^{22} = +64.8 (c = 1.5 in CHCl₃); \(^1\)H NMR (400 MHz, CDCl₃):
\[\delta = 5.71 (s, 1H), 4.60 (s, 1 H), 4.44 (dd, J = 5.6, 2.8 Hz, 1H), 4.28-4.20 (m, 1H), 4.12-4.04 (m, 1H), 3.46-3.38 (m, 2H), 2.73 (dt, J = 12.1, 2.8 Hz, 1H), 2.19-2.14 (m, 1H), 2.08-1.98 (m, 1H), 1.29 (t, J = 7.1 Hz, 3H), 0.85 (s, 9H), 0.11 (s, 3H), 0.04 (s, 3H) ppm; IR (neat): ν = 3581, 2930, 2857, 2100, 1769, 1254 cm⁻¹; MS (m/z) 385 [M+Na]⁺; HRMS (ESI): calcd for C₁₆H₂₉N₄O₅Si [M+H]⁺, 385.1907; found, 385.1898.

Ethyl (3aR,4S,6S,7R,7aR)-3-tert-butoxycarbonyl-4-azido-7-(tert-butyldimethylsilyloxy)-2-oxohexahydrobenzoxazole-6-carboxylate (324):

\[
\text{OTBS} \quad \text{CO}_2\text{Et}
\]

Part 1 Sugar-based synthesis of Tamiflu and its inhibitory effects on cell secretion
To a solution of compound 299 (0.45 g, 1.17 mmol) was in CH₂Cl₂ (15 mL), Boc₂O (0.41 mL, 1.76 mmol), Et₃N (0.33 mL, 2.34 mmol) and 4-dimethylaminopyridine (DMAP, 14.3 mg, 0.12 mmol) were sequentially added. The resulting mixture was stirred for 2 h at room temperature. The reaction mixture was washed with saturated NH₄Cl solution (2 × 20 mL). The organic layer was dried over Na₂SO₄, filtered, and concentrated. The crude product was used for next step without further purification.

¹H NMR (400 MHz, CDCl₃): δ = 4.38-4.35 (m, 1 H), 4.33-4.30 (m, 1H), 4.29-4.21 (m, 2H), 4.16 (dq, J = 7.1, 1.9 Hz, 2H), 2.78 (dt, J = 8.3, 4.4 Hz, 1H), 2.05-1.93 (m, 2H), 1.56 (s, 9H), 1.27 (t, J = 7.1 Hz, 3H), 0.86 (s, 9H), 0.14 (s, 3H), 0.08 (s, 3H) ppm; ¹³C NMR (100 MHz, CDCl₃): δ = 171.7, 151.1, 149.6, 85.0, 70.3, 61.3, 57.9, 57.1, 43.0, 27.9, 25.7, 25.6, 17.9, 14.0, −4.8, −5.4 ppm; MS (m/z) 507 [M+Na]⁺, 385 [M-Boc+H]⁺; HRMS (ESI): calcd. for C₂₁H₃₆N₄O₇SiNa [M+Na]⁺, 507.2251; found, 507.2251.

Ethyl 2-oxo-2,3-dihydrobenzoxazole-6-carboxylate (327):

\[
\begin{align*}
\text{H NMR (300 MHz, CDCl₃): } & \delta = 8.73 \text{ (br s, 1H), 7.96 (dd, } J = 8.2, 1.4 \text{ Hz, 1H), 7.89} \\
& \text{ (d, } J = 1.4 \text{ Hz, 1H), 7.12 (d, } J = 8.2 \text{ Hz, 1H), 4.39 (q, } J = 7.1 \text{ Hz, 2H), 1.41 (t, } J = 7.1 \\
& \text{ Hz, 3H) ppm; } ¹³C \text{ NMR (75 MHz, CDCl₃): } \delta = 165.7, 154.9, 143.5, 133.0, 126.6, \\
& 125.6, 111.4, 109.2, 61.3, 14.3 \text{ ppm.}
\end{align*}
\]
Ethyl (3aR,4S,6S,7R,7aR)-4-azido-7-hydroxy-2-oxo-octahydrobenzoxazole-6-carboxylate (322):

TBAF (0.9 mL, 1 M in THF) was added dropwise to a solution of 299 (0.23 g, 0.6 mmol) in THF. The mixture was stirred overnight at room temperature. Solvent was removed and the residue was flashed by column chromatography on silica gel (EtOAc/hexane = 1:1) to give 322 (0.12 g, 75%) as a colorless oil. $^1$H NMR (400 MHz, CDCl$_3$): $\delta = 6.59$ (s, 1H), 4.53 (t, $J = 7.5$ Hz, 1H), 4.20 (dd, $J = 14.2, 7.1$ Hz, 2H), 4.12 (dd, $J = 10.6, 8.1$ Hz, 1H), 4.04-3.98 (m, 2H), 2.71 (dt, $J = 10.3, 5.5$ Hz, 1H), 2.12-2.00 (m, 2H), 1.28 (t, $J = 7.1$ Hz, 3H), 0.84 (s, 9H), 0.14 (s, 3H), 0.06 (s, 3H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta = 173.2, 159.4, 80.0, 70.8, 61.5, 57.4, 56.3, 41.5, 26.7, 14.1$ ppm; IR (neat): $\nu = 3320, 2106, 1720, 1643, 1251, 748$ cm$^{-1}$; MS (m/z) 271 [M+H]$^+$; HRMS (ESI): calcd. for C$_{11}$H$_{16}$N$_4$O$_4$Na [M+Na]$^+$, 293.0862; found, 293.0869.

Ethyl (3aR,4S,7aS)-4-azido-2-oxo-2,3,3a,4,5,7a-hexahydrobenzoxazole-6-carboxylate (326):
Et$_3$N (0.54 mL, 3.9 mmol) and MsCl (0.15 mL, 1.95 mmol) were successively added to a solution of 322 (0.36 g, 1.3 mmol) in dry CH$_2$Cl$_2$ (15 mL) at 0 °C under N$_2$. The mixture was allowed to warm to room temperature and stirred for 2 h. The mixture was washed with aqueous NH$_4$Cl solution and brine. The organic phase was dried over Na$_2$SO$_4$, filtered. DBU (0.39 mL, 2.6 mmol) was added to the organic layer. The resulting mixture was stirred overnight at room temperature. Solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel (EtOAc/hexane = 1: 6 to 1:2) to give 326 (0.25 g, 77%) as a colorless oil. 

$[\alpha]_D^{23} = +155.5$ ($c = 0.4$ in CHCl$_3$); $^1$H NMR (400 MHz, CDCl$_3$): $\delta = 6.99-6.97$ (m, 1H), 6.10 (s, 1H), 5.17-5.14 (m, 1H), 4.27 (t, $J = 7.1$ Hz, 2H), 3.70-3.59 (m, 2H), 3.04 (dd, $J = 17.3$, 4.3 Hz, 1H), 2.30-2.23 (m, 1H), 1.33 (t, $J = 7.1$ Hz, 3H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta = 164.9$, 152.6, 138.9, 132.5, 77.2, 61.7, 60.1, 55.0, 26.7, 14.1 ppm; IR (neat) $\nu = 3018$, 2110, 1767, 1719, 1215, 762 cm$^{-1}$; MS (m/z) 253 [M+H]$^+$; HRMS (ESI): calcd. for C$_{10}$H$_{12}$N$_4$O$_4$Na [M+Na]$^+$, 275.0756; found, 275.0751.

**Ethyl (3a$R$,4S,7aS)-3-tert-butoxycarbonyl-6-ethyl-4-azido-2-oxo-3a,4,5,7a-tetrahydrobenzooxazole-6-carboxylate (328):**

![Chemical structure](attachment:image)

Prepared according to similar procedure as 324 and obtained as a white solid (80% yield). mp. 87-88 °C; $[\alpha]_D^{22} = -84.5$ ($c = 1.0$ in CHCl$_3$); $^1$H NMR (400 MHz, CDCl$_3$): $\delta = 6.87-6.86$ (m, 1H), 5.09 (dd, $J = 7.3$, 3.9 Hz, 1H), 4.38 (t, $J = 7.3$ Hz, 1H), 4.25 (q,
Part 1 Chapter 4 Experimental section

\[ J = 7.1 \text{ Hz}, 2\text{H}], 4.09 (dt, J = 7.2, 4.2 \text{ Hz}, 1\text{H}), 2.78 (dd, J = 18.0, 4.2 \text{ Hz}, 1\text{H}), 2.50 (dd, J = 18.0, 7.2 \text{ Hz}, 1\text{H}), 1.31 (t, J = 7.1 \text{ Hz}, 3\text{H}) \text{ ppm}; ^{13}\text{C NMR (100 MHz, CDCl}_3\text{):} \]
\[ \delta = 164.8, 150.9, 149.4, 132.7, 129.7, 85.0, 69.8, 61.6, 57.5, 56.1, 27.8, 26.1, 14.1 \text{ ppm}; \text{ IR (neat):} \nu = 2982, 2106, 1817, 1721, 1253, 1080, 758 \text{ cm}^{-1}; \text{ MS (m/z) 376 [M+Na]^{+}; HRMS (ESI): calcd for C}_{15}\text{H}_{20}\text{N}_{4}\text{O}_{6}\text{Na [M+Na]^{+}, 375.1281; found, 375.1217.}\]

**Ethyl (3S,4R,5S) 5-azido-4-(tert-butoxycarbonylamino)-3-hydroxy-1-cyclohexene-1-carboxylate (330):**

\[
\begin{align*}
\text{HO} & \quad \text{CO}_2\text{Et} \\
\text{Boch} & \quad \text{N} \\
\text{N}_3 & 
\end{align*}
\]

Cs\(_2\)CO\(_3\) (11.1 mg, 0.034 mmol) was added to a solution of compound 328 (0.12 g, 0.34 mmol) in ethanol (5 mL). The mixture was stirred for 3 h at room temperature. Solvent was removed and the residue was purified by column chromatography on silica gel (EtOAc/hexane = 1:6 to 1:2) to give compound 330 (0.09 g, 82\%) as a white solid. [\(\alpha\)]\(_D\)^{22} = +61.7 (c = 0.7 in CHCl\(_3\)); \(^1\text{H NMR (400 MHz, CDCl}_3\text{):} \delta = 6.87 (d, J = 1.4 \text{ Hz}, 1\text{H}), 5.14 (d, J = 7.7 \text{ Hz}, 1\text{H}), 4.47 (s, 1\text{H}), 4.21 (q, J = 7.1 \text{ Hz}, 2\text{H}), 3.79 (s, 2\text{H}), 2.91-2.81 (m, 2\text{H}), 2.35-2.29 (m, 1\text{H}), 1.46 (s, 9\text{H}), 1.30 (t, J = 7.1 \text{ Hz}, 3\text{H}) \text{ ppm}; ^{13}\text{C NMR(100 MHz, CDCl}_3\text{):} \delta = 165.8, 155.9, 136.1, 130.5, 80.3, 65.1, 61.2, 56.8, 53.4, 29.6, 28.3, 14.1 \text{ ppm}; \text{ IR (neat) \nu = 3366, 2978, 2104, 1713, 1699, 1490, 1250, 1163, 756 cm}^{-1}; \text{ MS (m/z) 350 [M+Na]^{+}; HRMS (ESI): calcd for C}_{14}\text{H}_{22}\text{N}_{4}\text{O}_{5}\text{Na [M+Na]^{+}, 349.1488; found, 349.1486.}\]
Part 1 Chapter 4 Experimental section

**Ethyl (3R,4R,5S) 5-azido-4-(tert-butoxycarbonylamino)-3-hydroxy-1-cyclohexene-1-carboxylate (334):**

![Chemical Structure Image]

DMP (0.38 g, 0.90 mmol) was added to a solution of compound 330 (0.20 g, 0.60 mmol) in dry CH₂Cl₂ (15 mL). The mixture was stirred 2 h at room temperature. Aqueous Na₂S₂O₃ and NaHCO₃ solution (30 mL, v/v = 1:1) was added portionwise. The resulting solution was separated. The organic phase was washed with NaHCO₃ solution (15 mL) and brine (15 mL), dried over Na₂SO₄, concentrated. The crude product was dissolved in dry THF (20 mL) and added dropwise to a solution of LiAlH(OtBu)₃ (0.90 mL, 1 M in THF) at −20 °C. The mixture was allowed to warm to room temperature and stirred 2 h. Saturated NH₄Cl solution was added to quench the reaction. The precipitate was removed by filtration through a Celite pad. The filtrate was extracted with EtOAc three times, and the combined organic layer was washed with brine and dried over Na₂SO₄. The solvent was removed under reduced pressure, and the residue was purified by column chromatography on silica gel (EtOAc/hexane = 4:1) to give compound 334 (0.15 g, 78%) as a white solid. mp. 110-111 °C; [α]D²² = +67.5 (c = 1.0 in CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ = 6.81 (t, 1H), 5.08 (d, J = 4.8 Hz, 1H), 4.54 (s, 1H), 4.39 (s, 1H), 4.21 (q, J = 7.1 Hz, 2H), 3.67-3.61 (m, 1H), 3.46-3.40 (m, 1H), 2.90 (dd, J = 17.5, 5.2 Hz, 1H), 2.44-2.36 (m, 1H), 1.46 (s, 9H), 1.29 (t, J = 7.1 Hz, 3H) ppm; ¹³C NMR (100 MHz, CDCl₃): δ = 165.7, 157.1, 138.5, 127.6, 81.1, 71.4, 61.1, 58.1, 57.9, 29.6, 28.2, 14.1 ppm; IR (neat) ν = 3382, 2978, 2105, 1715, 1699, 1493, 1249, 1163, 756 cm⁻¹; MS (m/z) 349
[M+Na]$^+$; HRMS (ESI): calcd. for C$_{14}$H$_{22}$N$_4$O$_5$Na [M+Na]$^+$, 349.1488; found, 349.1492.

(1S,5S,6S)-7-tert-butyl 3-ethyl 5-azido-7-azabicyclo[4.1.0]hept-2-ene-3,7-dicarboxylate (335):

![Structure of 335](image)

To a solution of PPh$_3$ (0.20 g, 0.78 mmol) and 334 (0.10 g, 0.31 mmol) in THF (10 mL), DEAD (0.12 mL, 0.78 mmol) in THF (1 mL) was added and the resulting mixture was stirred at 0 °C. After 3 h, mixture was concentrated, and purified by column chromatography on silica gel (EtOAc/hexane = 1: 7) to give compound 335 (0.079 g, 83%) as a colorless oil. $[\alpha]_D^{22} = -77.1$ (c = 1.0 in CHCl$_3$); $^1$H NMR (400 MHz, CDCl$_3$): δ = 7.19 (dd, $J = 4.6$, 3.3 Hz , 1H), 4.32-4.30 (m, 1H), 4.21 (dq, $J = 7.1$, 2.1 Hz, 2H), 3.13-3.05 (m, 2H), 2.91 (dt, $J = 17.5$, 2.0 Hz, 1H), 2.37 (ddd, $J =17.5$, 5.4, 3.2 Hz, 1H), 1.46 (s, 9H), 1.29 (t, $J = 7.1$ Hz, 3H) ppm; $^{13}$C NMR (400 MHz, CDCl$_3$): δ = 165.5, 160.5, 133.3, 129.7, 82.5, 61.0, 52.7, 40.6, 33.3, 27.8, 25.9, 14.2 ppm; IR (neat): ν = 3420, 3019, 2982, 2911, 1701, 1260, 1215, 1153, 756 cm$^{-1}$; MS (m/z) 331 [M+Na]$^+$; HRMS (ESI): calcd. for C$_{14}$H$_{20}$N$_4$O$_4$Na [M+Na]$^+$, 331.1382; found, 331.1369.

Ethyl (3R,4R,5S)-4-(tert-butoxycarbonylamino)-5-azido-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate (336):
To a solution of 335 (55.5 mg, 0.18 mmol) in 3-pentanol (2.5 mL), BF$_3$·OEt$_2$ (0.5 M in 3-pentanol, 0.72 mL, 0.36 mmol) was added dropwise, and the resulting mixture was stirred at $-20$ °C. After 2 h, saturated aqueous NaHCO$_3$ was added to quench the reaction. The product was extracted with CH$_2$Cl$_2$ (3 × 20 mL) and the combined organic layer was dried over Na$_2$SO$_4$. The solvent was removed under reduced pressure, and the residue was purified by column chromatography (EtOAc/hexane = 1:6) to give compound 336 (57 mg, 80%) as a white solid. $^1$H NMR (400 MHz, CDCl$_3$): $\delta = 6.77$ (s, 1H), 4.92 (d, $J = 5.2$ Hz, 1H), 4.49 (d, $J = 4.3$ Hz, 1H), 4.22 (dq, $J = 5.7$, 1.0 Hz, 2H), 3.34 (t, $J = 4.4$ Hz, 1H), 3.12-3.10 (m, 1H), 2.91 (dd, $J = 14.1$, 5.8 Hz, 1H) 2.22-2.16 (m, 1H), 1.53-1.50 (m, 4H), 1.45 (s, 9H), 1.29 (t, $J = 5.7$ Hz, 3H), 0.91 (t, $J = 5.9$ Hz, 3H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta = 165.8$, 155.2, 138.2, 128.1, 82.3, 79.9, 73.5, 61.0, 58.4, 57.5, 30.7, 28.3, 26.3, 25.7, 14.2, 9.6, 9.3 ppm; MS (m/z) 297 [M-Boc+H]$^+$; HRMS (ESI): calcd. for C$_{19}$H$_{32}$N$_4$O$_5$Na [M+Na]$^+$, 419.2270; found, 419.2267.

**Ethyl (3aR,4S,7aS)-3-acetyl-4-azido-2-oxo-3a,4,5,7a-tetrahydrobenzoxazole-6-carboxylate (337):**

![Structure of 337]
A solution of 319 (1.78 g, 4.63 mmol) in THF (8 mL) was added to a suspension of NaH (60% in mineral oil, 0.28 g, 6.95 mmol) in THF (20 mL) at 0 °C. The mixture was stirred for 30 mins after warming to room temperature. Then the mixture was cooled to 0 °C and acetyl chloride (0.40 mL, 5.56 mmol) was added dropwise. After stirring 1 h at room temperature, the resulting solution was quenched with sat. NH₄Cl. The mixture was diluted with EtOAc and washed with NaHCO₃ and brine, dried over NaSO₄, filtered, concentrated and dried in vacuo. The crude compound was dissolve in MeCN (20 mL), to which DBU (1.38 mL, 9.26 mmol) was added dropwise. The mixture was stirred for 24 h at room temperature. Solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel (EtOAc/hexane = 1: 4) to give compound 337 (0.91 g, 67%) as a white solid. mp 137-139 °C; [α]D²² = −136.7 (c = 0.92 in CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ = 6.90-6.86 (m, 1H), 5.15-5.12 (m, 1H), 4.59 (t, J = 7.1 Hz, 1H), 4.36 (q, J = 7.1 Hz, 2H), 4.22 (dt, J = 6.8, 4.1 Hz, 1H), 2.73 (dt, J = 18.0, 2.0 Hz, 1H), 2.59-2.54 (m, containing s, 4H), 1.33 (t, J = 7.1 Hz, 3H) ppm; ¹³C NMR (100 MHz, CDCl₃): δ = 170.9, 164.9, 152.7, 133.1, 129.5, 70.4, 61.7, 56.7, 55.1, 25.9, 24.0, 14.1 ppm; IR (neat): ν = 3339, 2932, 2857, 2106, 1790, 1715, 1373, 1256, 1213, 1026, 758 cm⁻¹; MS (m/z) 339 [M+Na]⁺; HRMS (ESI): calcd. for C₁₂H₁₄N₄O₅Na [M+Na]⁺, 317.0862; found, 317.0856.

**Ethyl (3S,4R,5S) 4-acetamido-5-azido-3-hydroxy-1-cyclohexene-1-carboxylate (235):**
Prepared according to similar procedure as 330 and purified by column chromatography on silica gel (EtOAc/hexane = 3:1) to give compound 235 (0.53 g, 72%) and 326 (0.11 g, 16%) as a colorless oil (326 could be acetylated following similar procedure as 337). \([\alpha]_D^{22} = +82.0 \) (\(c = 1.5\) in CHCl₃); \(^1\)H NMR (400 MHz, CDCl₃): \(\delta = 6.89\) (d, \(J = 1.2\) Hz, 1H), 6.23 (d, \(J = 8.4\) Hz, 1H), 4.45 (d, \(J = 4.4\) Hz, 1H), 4.22 (q, \(J = 7.1\) Hz, 2H), 4.12 (dt, \(J = 9.7, 4.3\) Hz, 1H), 3.80 (dt, \(J = 9.7, 5.4\) Hz, 1H), 3.43 (s, 1H), 2.89 (dd, \(J = 18.1, 5.3\) Hz, 1H), 2.35 (dd, \(J = 18.1, 5.3\) Hz, 1H), 2.06 (s, 3H), 1.30 (t, \(J = 7.1\) Hz, 3H) ppm; \(^13\)C NMR (100 MHz, CDCl₃): \(\delta = 171.1, 165.8, 136.1, 130.5, 64.8, 61.3, 56.3, 52.1, 29.6, 23.4, 14.1\) ppm; IR (neat): \(\nu = 3340, 2106, 1643, 1252, 750\) cm\(^{-1}\); MS (m/z) 269 \([\text{M}+\text{H}]^+\); HRMS (ESI): calcd. for C₁₁H₁₆N₄O₄Na \([\text{M}+\text{Na}]^+\), 291.1069; found, 291.1065.

**Ethyl (3R,4R,5S)-4-acetamido-5-azido-3-hydroxy-1-cyclohexene-1-carboxylate (190):**

Prepared according to similar procedure as 334 and the residue was purified by column chromatography on silica gel (EtOAc/hexane = 4:1) to give compound 190 (0.29 g, 70%) as a colorless oil. \([\alpha]_D^{22} = +93.4 \) (\(c = 1.8\) in CHCl₃); \(^1\)H NMR (400 MHz, CDCl₃): \(\delta = 6.80\) (s, 1H), 6.37 (s, 1H), 5.15 (br s, 1H), 4.4-4.38 (m, 1H), 4.21...
(q, \( J = 7.1 \) Hz, 2H), 3.69-3.65 (m, 2H), 2.93 (dd, \( J = 19.0, 4.4 \) Hz, 1H), 2.43-2.37 (m, 1H), 2.09 (s, 3H), 1.29 (t, \( J = 7.1 \) Hz, 3H) ppm; \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \( \delta = 172.9, 165.6, 138.8, 127.5, 71.1, 61.2, 57.8, 57.7, 29.6, 23.4, 14.1 \) ppm; IR (neat): \( \nu = 3316, 2100, 1714, 1258 \) cm\(^{-1}\); MS (m/z) 269 [M+H]\(^+\); HRMS (ESI): calcd. for C\(_{11}\)H\(_{17}\)N\(_4\)O\(_4\) [M+H]\(^+\), 269.1250; found, 269.1251.

**Ethyl (3\(R\),4\(R\),5\(S\))-4-acetamido-5-azido-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate (23):**

![Chemical structure](image)

To a solution of \( 190 \) (0.20 g, 0.74 mmol) in CH\(_2\)Cl\(_2\) (15 mL), Et\(_3\)N (0.30 mL, 2.22 mmol) and MsCl (86.0 \( \mu \)L, 1.12 mmol) were added at 0 °C. After stirring for 3 h at room temperature, the mixture was washed by sat. NaHCO\(_3\) solution, sat. NH\(_4\)Cl solution and brine successively. The organic phase was dried over Na\(_2\)SO\(_4\), filtered, concentrated. The crude compound was dried *in vacuo* and dissolved in 3-pentanol (5.0 mL). To which activated 4Å molecular sieves (500 mg) and BF\(_3\)·OEt\(_2\) (0.5 M in 3-pentanol, 1.47 mL, 0.74 mmol) was added dropwise. The resulting mixture was stirred at 0 °C. After 2 h, saturated aqueous NaHCO\(_3\) was added to quench the reaction. The product was extracted with CH\(_2\)Cl\(_2\) (3 \( \times \) 20 mL) and the combined organic layer was dried over Na\(_2\)SO\(_4\). The solvent was removed under reduced pressure, and the residue was purified by column chromatography (EtOAc/hexane = 1:6) to give compound \( 23 \) (0.13 g, 52%) as a white solid. mp 138-140 °C; [\( \alpha \)]\(_D\)\(^{22} = \)
−41.4 (c = 1.02 in CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ = 6.77 (s, 1H), 6.03 (d, J = 7.4 Hz, 1H), 4.54 (d, J = 8.7 Hz, 1H), 4.28-4.17 (m, 3H), 3.37-3.31 (m, 2H), 2.91 (dd, J = 17.6, 5.6 Hz, 1H), 2.26-2.17 (m, 1H), 2.02 (s, 1H), 1.53-1.45 (m, 4H), 1.28 (t, J = 7.1 Hz, 3H), 0.89 (dt, J = 4.2, 7.4 Hz, 3H) ppm; ¹³C NMR (100 MHz, CDCl₃): δ = 171.1, 165.8, 137.9, 128.1, 82.0, 73.4, 61.0, 57.9, 57.2, 30.5, 26.2, 25.6, 23.5, 14.1, 9.5, 9.2 ppm; IR (neat): ν = 3390, 3019, 2102, 1709, 1215, 756 cm⁻¹; MS (m/z) 339 [M+H]⁺; HRMS (ESI): calcd. for C₁₆H₂₆N₄O₄Na [M+Na]⁺, 361.1852; found, 361.1843.

Ethyl (3R,4R,5S)-4-acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylate phosphate (Tamiflu 4):

\[
\text{PPh}_3 \quad \text{was added to a solution of azide 23 (0.12 g, 0.35 mmol) in THF/water (2.5 mL, 4:1 v/v) at room temperature. The mixture was refluxed for 3 h. The reaction mixture was cooled to room temperature. Solvent was removed under reduced pressure. The residue was diluted with CH}_2\text{Cl}_2 \quad \text{and washed with brine (20 mL). The organic layer was dried over Na}_2\text{SO}_4, \text{filtered, and concentrated. The residue was purified by column chromatography on silica gel to give oseltamivir 3 (97.7 mg, 90%) as a colorless oil. [α]D}^{22} = −58.1 (c = 1.0 in CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ=6.76 (s, 1H), 5.95 (d, J = 8.0 Hz, 1H), 4.18 (q, J = 6.8 Hz, 3H), 3.52 (dd, J = 18.4, 8.6 Hz, }\]
Amine 3 (60.1 mg, 0.19 mmol) was dissolved in ethanol (3 mL) and added slowly in portions to a hot (55 °C) solution of phosphoric acid (85%, 21.9 mg, 0.19 mmol) in ethanol (1.2 mL). After cooling to 0 °C, the precipitates were collected by filtration and rinsed with cold acetone (2 × 5 mL) to afford 4 (62.5 mg, 80%) as white crystals. mp. 184-186 °C (lit.\[24\] mp. 189-191 °C); [α]_D\textsuperscript{22} = −35.3 (c = 1.0 in H_2O) (lit.\[24\] [α]_D\textsuperscript{20} = −35.8 (c = 1.0 in H_2O)); \textsuperscript{1}H NMR (400 MHz, D_2O): δ = 6.86 (s, 1H), 4.34 (d, J = 8.9 Hz, 1H), 4.25 (q, J = 7.1 Hz, 2H), 4.07 (dd, J = 11.7, 9.0 Hz, 1H), 3.74 (t, J = 6.5 Hz, 1H), 3.63-3.54 (m, 1H), 2.97 (dd, J = 17.5, 5.4 Hz, 1H), 2.56-2.49 (m, 1H), 2.01 (s, 1H), 1.58-1.47 (m, 4H), 1.29 (t, J = 7.1 Hz, 3H), 0.87 (dt, J = 17.5, 7.4 Hz, 6H) ppm; \textsuperscript{13}C NMR (100 MHz, D_2O): δ = 175.2, 167.4, 137.8, 127.6, 84.3, 75.1, 62.4, 52.6, 49.1, 28.1, 25.4, 25.0, 22.3, 13.3, 8.5, 8.4 ppm; \textsuperscript{31}P NMR (121 MHz, D_2O) δ = 0.65; MS (m/z) 314 [M+H]\textsuperscript{+}; HRMS (ESI): calcd. for C\textsubscript{16}H\textsubscript{29}N\textsubscript{2}O\textsubscript{4} [M+H]\textsuperscript{+}, 313.2127; found, 313.2126.

\((3R,4R,5S)-4\text{-Acetamido\text{-5-amino\text{-3\text{-\textdagger{}(1-ethylpropoxy)\text{-1\text{-cyclohexene\text{-1\text{-carboxylic acid}}}}}}})\)
A solution of lithium hydroxide (3.6 mg, 0.15 mmol) in 0.1 mL water was added to a solution of oseltamivir 3 (31.2 mg, 0.1 mmol) in THF (1 mL). The mixture was stirred for 3 h at room temperature and acidified to pH = 6 with Amberlite IR-120. The mixture was filtered and washed with methanol. The filtrate was evaporated to give crude product, which was further purified by HPLC (0.1% TFA in MeCN/H₂O) to give 5-TFA (21.8 mg, 57%) as a white solid. mp. 194-196 °C (lit.[24] mp. 185-187 °C); [α]₂²² = −135.3 (c = 0.5 in H₂O) (lit.[24] [α]₀²₀ = −143.2 (c = 0.4 in H₂O)); ¹H NMR (300 MHz, D₂O): δ = 6.79 (s, 1H), 4.25 (d, J = 9 Hz, 1H), 3.98 (dd, J = 11.7, 9.0 Hz, 1H), 3.56-3.42 (m, 2H), 2.87 (dd, J = 17.5, 5.4 Hz, 1H), 2.48-2.38 (m, 1H), 2.00 (s, 1H), 1.52-1.35 (m, 4H), 0.87 (dt, J = 17.5, 7.4 Hz, 6H) ppm; ¹³C NMR (100 MHz, D₂O): δ = 175.3, 169.0, 138.4, 127.5, 84.4, 75.1, 52.6, 49.2, 28.2, 25.4, 25.0, 22.3, 8.4 ppm; IR (neat): ν = 3529, 2966, 1753, 1646, 1241, 1050, 736 cm⁻¹; MS (m/z) 285 [M+H]⁺; HRMS (ESI): calcd. for C₁₄H₂₅N₂O₄ [M+H]⁺, 285.1814; found, 285.1825.
### Table 11. Data collection and refinement statistics for compound 23

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5. REFERENCES


[14] H. Cremer, G. Chazal, A. Carleton, C. Goridis, J. D. Vincent, P. M. Lledo, 


PART 2

Interfacing Glycosylated Carbon-Nanotube-Network Devices with Living Cells to Detect Dynamic Secretion of Biomolecules


1. INTRODUCTION

Carbon nanotubes (CNTs) are a unique class of materials which will revolutionize future technologies. Due to their unique electrical, thermal, structural, and physiochemical properties, in recent years CNTs have attracted very substantial attention in a wide variety of scientific disciplines including electronics, catalysis and biomedicine. Of particular interest are their potential applications in medicine/biology, especially as biomarkers for cancer treatment, biosensors and drug delivery carriers.\(^1\) CNTs are functionalizable with a wide variety of organic, inorganic and biological moieties. This functionalization can serve not only to improve the solubility of CNTs, but also to provide an opportunity for the fabrication of novel devices. Highly purified and well functionalized CNTs appear to be less toxic.\(^2\) Recent progress in chemical modification and bio-functionalization have made it possible to generate a new class of bioactive nanotubes that are bound to nucleic acids,\(^3\) DNA,\(^4\) proteins,\(^5\) mono and oligosaccharides and others.\(^6\) Among the various biomolecules, carbohydrate functionalized CNTs could provide a great means for specific cell surface multivalent binding with the benefit of low cytotoxicity and high solubility in the cell culture system.

Glycosylation\(^7\) is one of the most ubiquitous forms of post-translational modification, with more than 50% of the human proteome estimated to be glycosylated.\(^8\) Carbohydrates, in the forms of glycoproteins, glycolipids, and glycans, are the key constituents of cell membrane and extracellular matrix, playing pivotal roles in cell-cell communication, cell-protein interactions and molecular recognition of antibodies.
and hormones.\cite{9} Due to the presence of multiple hydroxyl functional groups on each monomeric unit, carbohydrates are capable of forming many different combinatorial structures from a relatively small number of sugar units. Each sugar moiety could potentially carry a specific biological message, thus widening the probability of reactivity that is possible from a limited number of monomers. The chemical diversity and complexity of carbohydrates have bestowed glycans with a vast array of biological functions. Recently, sugar code and glycomics are becoming commonly used terminologies.

Over the past two decades, considerable evidence has been presented to demonstrate that carbohydrates have tremendous potential for encoding biological information in a wide variety of physiological and pathological processes.\cite{10} However, the extent to which the sugar code has been deciphered is still very limited despite many great efforts. This embarrassment is mainly caused by the lack of pure, structurally defined complex oligosaccharides and glycoconjugates and the lack of methods for molecular glycobiology study. Isolation of homogeneous pure polysaccharides from highly heterogeneous natural sources remains a daunting task. Synthesis of complex carbohydrates also presents a great challenge to organic chemists. On the other hand, multivalent interactions, which are characterized by simultaneous binding of multiple ligands to multiple receptors, are prevalent in biological systems, but the methods to realize multivalent interactions are very limited until functionalization of nanoscale scaffolds comes into practice.

Research on carbohydrate modified CNTs has made tremendous progress, which has left a great impression on glycobiology and nanotechnology. Technical advances in
the synthesis and structure analysis of glycosylated CNTs, together with expanding knowledge of their interaction with pathogenic cell surfaces, have enabled researchers to develop new biomedical instruments and to penetrate into this challenging field. In this review, we describe current efforts on the fabrication of carbohydrate decorated CNTs and their biomedical and biological applications.

1.1 Carbohydrate functionalized CNTs

The poor solubility of CNTs is the major limitation to their application in biology. Many studies have shown that biocompatible modifications have made it possible to solubilize and disperse CNTs in water and increase their bio-functionalities. High hydrophilicity of carbohydrates enables them to functionalize nanotubes either through covalent bonding or through noncovalent wrapping.

1.1.1 Covalent functionalization

The most common method for covalent functionalization of CNTs involves reactions with carboxylic acid (–COOH) residues on CNTs. These carboxylic acid groups are usually introduced by oxidation using strong acids such as sulfuric acid, and they occur predominantly at the more reactive ends or sidewalls of CNTs.[11]

Coupling is the most frequently used method to functionalize CNTs. For example, protected or unprotected sugars can be coupled with carboxylic acids on CNTs. The Resasco group has functionalized single-walled carbon nanotubes (SWNTs) with glucosamine to achieve the solubilization of SWNTs in water.[6b] Carboxylic groups of CNTs were first converted to acyl chlorides, which were then grafted with glucosamine by the formation of the amide bonds between glucosamine and SWNTs.
These glucosamine grafted SWNTs conserved their integrity in solution. Fu et al.\cite{12} have demonstrated that the amide and/or ester groups formed during the functionalization can be hydrolyzed in acidic or basic media. By using this defunctionalization method, the SWNTs could be effectively recovered from the homogeneous solutions.

Benjamin and coworkers have synthesized $N$-acetyl-$d$-glucosamine (GlcNAc) derivative modified SWNTs.\cite{13} This composite preparation could be achieved in two ways (Scheme 1). In the convergent method, 1-azido-2,3,5-triiodobenzoyl–GlcNAc was directly coupled to SWNTs, which allowed higher levels of glycan incorporation. In the sequential route, carbohydrate coupled SWNTs were furnished by the direct Staudinger coupling of preactivated carboxylates on oxidized SWNTs with iminophosphoranes derived from GlcNAc-azide. The resulting SWNTs were further subjected to Steglich esterification to introduce TIBz (2,3,5-triiodo benzoyl) tags on SWNTs.

**Scheme 1.** Sequential and Convergent Syntheses of I-Tagged SWNTs.
Alternatively, carbohydrates could be grafted onto CNTs through other linkers. Functionalization of SWNTs with β-galactosides at the terminal positions increases their aqueous solubility due to the adsorption of lectin molecules on the sidewalls. For example, the Kimizuka group\[^{14}\] and Sun \textit{et al.}\[^{15}\] modified SWNTs with 2'-aminoethyl-β-d-galactopyranoside through amidation.

\[\text{Gal-SWNT} \rightarrow \text{Gal-MWNT}\]

\[\text{Man-SWNT} \rightarrow \text{Man-MWNT}\]

\[\text{Gal}_4\text{-SWNT} \rightarrow \text{Gal}_4\text{-MWNT}\]

\[\text{Man}_4\text{-SWNT} \rightarrow \text{Man}_4\text{-MWNT}\]

\textbf{Figure 1.} Gal-, Man-, and their Dendrons Functionalized SWNTs.

Sun \textit{et al.} found that the size of the functional group relative to that of CNTs is a critical factor in determining the solubilization extent of the CNTs via chemical functionalization.\[^{12}\] It has been observed that the solubility of CNTs significantly decreased when Gal-SWNTs have been changed to Gal-MWNTs because the multi-walled carbon nanotubes (MWNTs) were considerably larger than SWNTs. In order to
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improve solubility, MWNTs should be functionalized with bulky functional groups like Gal$_2$ to drag the nanotube into the solution. The higher order sugar dendrons are usually more effective in the solubilization of SWNTs. This phenomenon was further demonstrated by a series of dendritic $\beta$-D-galactopyranosides and $\alpha$-D-mannopyranosides having a terminal amino group (Figure 1).$^{[12]}$

Direct grafting of a large number of polymer chains or biomolecules onto CNTs without linkers may alter their native structure and properties. Indirect grafting of polymer chains or small functional molecules on CNTs with long linkers will resolve this problem. Hong and coworkers$^{[16]}$ reported the functionalization of MWNTs with long polymer chains, which contain highly reactive maleic anhydride groups, by employing reversible addition-chain transfer fragmentation (RAFT) copolymerization. Subsequently, many small functional biomolecules such as amino sugars could be grafted onto the surface of MWNTs. This method significantly increases the solubility of MWNTs in organic solvents and water, and more importantly, the intrinsic structure of MWNTs remains unaltered.

The water soluble and biocompatible CNTs in conjugation with glycopolymers are of enormous interest to scientists in the field of tissue engineering and biomaterials. Atom transfer radical polymerization (ATRP) proved to be the most efficient and versatile approach to functionalize CNTs with glycopolymers. Gao and coworkers$^{[17]}$ have demonstrated the grafting of biocompatible and hyperbranched glycopolymers onto the surfaces of MWNTs by ATRP of 3-methacyryloyl-1,2:5,6-di-isopropylidene-$\alpha$-D-glucofuranose (MAIG) and self condensing vinyl copolymerization (SCVCP) of MAIG and inimer, 2-(2-bromoisobutyryloxy)ethyl methacrylate (BIEMA) (Figure 2a).
After deprotection in formic acid, the resulting high density multihydroxy glycopolymer (polyMAG) functionalized MWNTs (Figure 2b) have fascinating potential in the fields of bio-nanotechnology and tissue engineering. Another successful implementation of ATRP technique has been demonstrated by Narian et al.\[18\] They functionalized SWNTs with bioinspired sugar poly(lactobionamidoethyl methacrylate) (polyLAMA) and phosphocholine polymeric structures via surface-initiated ATRP. The functionalized CNTs with cyclic carbohydrate moieties (Figure 2c) have shown good aqueous dispersion.

**Figure 2.** Polymer-functionalized SWNTs.

1.1.2 Noncovalent functionalization

Covalent functionalization improves the solubility of CNTs, but it often compromises the desirable properties of CNTs such as the electronic properties of SWNTs. In contrast, noncovalent modification of CNTs could maintain their intrinsic properties and integrate the characters of decorations. Polymers, surfactants, peptides, nucleic acids, proteins, and carbohydrates have been used for wrapping CNTs through noncovalent functionalization.\[19\]
Bertozzi and coworkers complexed SWNTs with mucin glycosylated glycopolymers that are designed to mimic natural cell surface mucins (Figure 3).[^6a] A C\textsubscript{18} lipid with polymers comprising of a poly(methyl vinyl ketone) backbone decorated with α-N-Acetylgalactosamine (α-GalNAc) residues was incorporated at one end of a mucin mimic polymer (C\textsubscript{18}-α-MM). The mucin mimic polymers were assembled on the CNTs surface in aqueous media through hydrophobic interactions between the C\textsubscript{18} lipid and CNTs surface. As reported, the C\textsubscript{18} on the mucin mimic polymer is essential for the solubilization of the CNTs because the lipid rapidly precipitates from solutions of polymers identical to the mucin mimics. The C\textsubscript{18}-α-MM functionalized SWNTs bundles are heavily entangled with one another to form a 3D-network. The resulting coated CNTs (C\textsubscript{18}-α-MM-CNTs) are soluble in water[^20]. Bertozzi et al. further studied the complexation of CNTs with biofunctional glycodendrimers based on 2,3-bis(hydroxymethyl propionic acid). These composite dendrimers, possessing peripheral carbohydrates and a pyrene tail were attached to the SWNTs surface through π-π interactions. The resulting SWNTs-glycodendrimer composite geometry resembles multi-antenna N-linked glycans[^21].

![Figure 3. C\textsubscript{18}-α-mucin mimic functionalized SWNTs.](image-url)
Shinkai and coworkers functionalized SWNTs with supramolecular wrappings, which had Schizophyllan (SPG) carrying lactoside appendages (SPG-Lac).\textsuperscript{[22]} SPG can be dissociated into single strands in DMSO and can also be re-constituted in water. Taking advantage of this unique property of SPG, SPG-Lac/SWNTs were synthesized by mixing SPG-Lac in DMSO with SWNTs dispersed in water. Besides excellent water solubility of this composite, SPG has the one structural advantage of possessing the pendent $\beta$-1,6-glycosides with 1,2-diols which are suitable for chemical modifications.

Cyclodextrins (CDs) are known to selectively form inclusion complexes and promote solubility. Dodziuk \textit{et al.} threaded SWNTs with $\eta$-CD which was composed of 12 glucosidic units.\textsuperscript{[23]} The complexes could be dissolved in water and used to estimate the number of types of SWNTs present in bulk in the resulting solution. D-Glucose, maltoheptaose, $\alpha$-, $\beta$-, and $\gamma$-CDs were also tested for the influence on the solubility of SWNTs by Ikeda \textit{et al.}\textsuperscript{[24]} They mixed the SWNTs with different saccharides by a mechanochemical “high-speed vibration milling technique” (HSV). Unmodified $\gamma$-CD was deemed to be the most efficient in promoting SWNTs solubility in water. Inspired by the fact that CDs of appropriate dimensions and correct stoichiometries will dissolve fullerenes such as C60 and C70 in water; the Stoddart group wrapped starch, the macrocyclic analogues of CDs, helically around iodine with SWNTs to improve the solubility of SWNTs in water.\textsuperscript{[6c]} The process is reversible at high temperatures to separate SWNTs. SWNTs can be precipitated after mixing the starched CNTs with glucosidases. It is possible to use these readily prepared starch complexes to purify SWNTs at ambient conditions. They also fabricated pyrene-
modified β-cyclodextrin decorated SWNTs/field-effect transistor devices which behave as chemical sensors in aqueous solution.[25]

Shinohara et al.[26] synthesized photoluminescence active multivalent carbohydrate based CNTs by employing poly(p-β-acryloylamidophenyl-α-glucopyranoside) (PAP-α-Glc) with an estimated Mn 71000. A random helical structure of the hybrid was formed as polymer wrapped SWNTs by hydrophobic interaction.

1.2 Bioapplications of carbohydrate functionalized CNTs

Different kinds of nano structures such as CNTs, graphene, nanoparticles and quantum dots find their suitable places in the nanotechnology world and each of them differs from each other in their physicochemical properties and in their applications. CNTs possess many advantages over other nanomaterials in biomedical applications. Graphene is a single sheet of graphite and it has unique mechanical, electronic and thermal properties. However, implementation of graphene has not been established due to difficulties in producing high quality graphene single layers and the inability to scale up in industrial level, which in turn limits the application of these materials.[27] Carbon nanoparticles, usually exist in the form of carbon black with primary particle size ranging from 12 to 100 nm. Due to their unique concentric graphene layer symmetry, most of the carbon nanoparticles have hydrophobic, low-polarity surfaces that present challenges for dispersion and functionalization. The small size and large surface area of nanoparticles can lead to particle-particle aggregation, making physical handling of nanoparticles difficult in liquid and dry forms. In biomedical imaging,
SWCNTs can be used as a contrasting agent in NIR fluorescence and photoacoustic imaging of cells and animals to achieve the multimodality optical imaging. Quantum dots or nanoparticles normally only have a single imaging functionality. Unlike the fluorescent quantum dots, CNTs contain less heavy metals and thus have a safer chemical composition. The ability of CNTs to attach to aromatic chemotherapy drugs such as doxorubicin through supramolecular $\pi-\pi$ stacking thereby achieves ultra-high loading capacity. This makes CNTs superior to other nanomaterials based drug carriers.[28] The high optical absorbance capacity of CNTs over other nanomaterials gives rise to their widespread usage in photothermal therapy, chemotherapy and gene therapy. Besides, needle-like CNTs have the tendency to penetrate cellular membranes, possess high potential to carry multiple moieties at higher density and superior flow dynamics when compared with spherical nanoparticles.[29]

Compared to the conventional method, CNTs sensors are more sensitive and reliable. The large surface area of CNTs allows the binding of a high density of biomolecules. Thus signal strength is amplified when the charge changes in the surrounding bio-environment. The high sensor performance leads to lower detection limits. Moreover, functionalized CNTs could probe various specific recognition through different decorations.

Meanwhile, carbohydrates play a vital role in biological functions such as cell-cell recognition, immunological response, metastasis and fertilization. The improvement of biocompatibility and the introduction of bio-functionalities by integration of carbohydrate with CNTs paved a new way to bio-nanotechnology. An increasing
number of examples have demonstrated that they could be applied in biological and biomedical systems.[1a, 30]

1.2.1 Recognition with proteins

Mucins are known to participate in molecular recognition on cell surface. To determine whether these functions are achievable with respect to the nanotube surface, $C_{18}$-$\alpha$-MM SWNTs were incubated with a solution of $\alpha$-GalNAc-specific receptor such as lectin *Helix Pomatia* agglutinin (HPA) conjugated with fluorescein isothiocyanate. The $C_{18}$-$\alpha$-MM SWNTs were analyzed for bound lectin by fluorescence spectroscopy and found to exhibit significant fluorescence, which could be attributed to bound fluorescein. HPA-FITC (Fluorescein isothiocyanate conjugate HPA) labeling of the $C_{18}$-$\alpha$-MM SWNTs was inhibited when 0.2 M free GalNAc was present in the solution thus confirming that fluorescent labeling was dependent on a receptor–ligand interaction. This methodology allows mucin mimics to be adsorbed on CNTs surface to display carbohydrates capable of molecular recognitions.[6a]

CNTs modified with mucin mimics are highly beneficial as they show the following advantages (1) mucin modified CNTs are water soluble, (2) they resist non-specific protein binding, and (3) they bind specifically to biomolecules through receptor-ligand interactions. In order to explore the intricate details of interface between functionalized CNTs and cell surface, two paths were proposed (Figure 4).[20] In the first path, $C_{18}$-$\alpha$-MM coated CNTs were first bound to HPA which is hexavalent $\alpha$-GalNAc binding lectin. This complex was then bound to cell surface glycoconjugates using HPA binding sites present on CNTs. To evaluate the first pathway, $C_{18}$-$\alpha$-MM-
CNTs were complexed with HPA-FITC and subsequently the CNTs were incubated with the Chinese hamster ovary (CHO) cells. Flow cytometry analysis showed the formation of α-GalNAc-HPA complexes on both the CNTs and cell surfaces. No fluorescent labeling was observed in a control experiment where CNTs coated with C18-β-linked Gal-NAc residues did not bind HPA. In the second path, HPA was first bound to cell surface glycoconjugates and then these HPA binding sites were bound to α-GalNAc residues on C18-α-MM coated CNTs. The cytotoxicity of glycopolymer-coated CNTs was evaluated using CHO cells and Jurkat cells. The results showed that the modified CNTs had very good biocompatibility and nontoxicity.[20]

In subsequent work, Bertozzi et al. studied the interaction between CNTs coated with glycan based mucin mimic glycopolymers and cell surface glycoconjugates.[21] The main aim of their study was to evaluate the cytotoxicity and explore the targeting capability of glycodendrimer functionalized SWNTs in a biological system. The functionalized material was found to possess interesting sensing and targeting applications. To investigate the specific binding of SWNTs bound glycodendrimers to receptors, SWNT composites were incubated with fluorescein isothiocyanate (FITC) conjugate lectins such as Canavalia ensiformis agglutinin (Con A), Arachis hypogaea agglutinin (PNA) and Phosphocarpus tetragonolobus agglutinin (PTA) which recognize α-mannose, lactose and β-galactose respectively. It was found that Con A treated G-Man-SWNTs (α-D-mannopyranoside glycodendrimer) showed significant fluorescence whereas only background fluorescence was observed for PTA or PNA treated G-Man-SWNTs. Similarly, G-Gal-SWNTs (β-D-galactopyranoside glycodendrimer) bound to FITC-conjugated PTA but not to PNA or Con A.
subsequent experiments, the authors tried to evaluate the cytotoxicity of glycodendrimer coated SWNTs on HEK293 cells. The proliferation rate of the cells cultured with glycodendrimer coated SWNTs was found to be the same as that of the cells grown in the absence of SWNTs, whereas unmodified SWNTs significantly hindered the growth of HEK 293 cells. It demonstrates the effectiveness of thin-coated glycodendrimers to passivate SWNTs against cytotoxicity. Bertozzi group developed a new approach to fabricate CNTs with biomimics.[6a],[20] These biomimic CNTs did not show any cytotoxicity and led to the integration of CNTs into aqueous biological systems successfully. This technique paved a way for biosensor application including carbohydrate-proteins interaction study, the introduction of myriad functional epitopes, and delivery agents that target specific cell-surface receptors.

![Figure 4](attachment:image.png)

**Figure 4.** (a) C\textsubscript{18}-terminated, α-GalNAc-conjugated mucin mimic (C\textsubscript{18}-α-MM) functionlized SWNTs. (b) Schematic presentation of interfacing CNTs on cell surfaces via carbohydrate-receptor binding.

It was found that SPG-Lac/SWNTs composite showed specific binding to a Au-surface immobilized with *ricinus communis* agglutinin (RCA\textsubscript{120}, β-Lac-specific).[22a]
The specific interactions between SPG-Lac/SWNTs composite and lectins were visualized by AFM spectroscopy and confirmed by confocal laser scanning microscopic (CLSM) observations using FITC labeled lectins. AFM images of RCA\textsubscript{120} incubated SPG-Lac/SWNTs revealed dense clustering of RCA\textsubscript{120} on the surface of SPG-Lac/SWNTs composite to cover the inherent stripped patterns of the SPG. The unique character of SPG allows it to be chemically modified and can be used as one-dimensional host. The exterior surfaces of the resultant nanocomposites can be utilized as interaction sites for the construction of supermolecular architectures. The wrapping effect of SPG suppressed the strong interpolymer interactions among appendages to maintain their intrinsic functionality.

Individual dispersion of CNTs in solvent facilitates their usage in biological applications. Shinohara synthesized photoluminescence active multivalent carbohydrate based CNTs, which provided an alternative technique to detect carbohydrate recognition in proteins by virtue of exploiting the characteristic photoluminescence signals emitted by multivalent CNTs conjugates.\textsuperscript{[26]} It is an attractive method for biosensing applications because of their essential fluorescence and high sensitivity to minor external stimulus.

The large surface area of CNTs can provide a high density of proteins at one time, which can amplify enormous signal once charge changes in the surrounding biological environment and lower the detection limit. The good conductivity also enhances the recognition sensitivity when a CNT-based device was applied.
1.2.2 Recognition with bacteria

There has been an enormous interest in exploiting functionalized CNTs for their remarkable capability of interaction with bacterial cells. In a recent study, it has been shown that a large number of *Escherichia Coli (E. Coli)* cells have been inactivated following direct contact with highly purified SWNTs in the aqueous phase.\[^{[31]}\] As bacteria are enveloped with carbohydrates, it is conceivable that glycosylated CNTs may recognize and biocompatibly interact with specific bacterial species.

Gu *et al.* reported that SWNTs tethered with galactose derivatives exhibited strong cell adhesion with *E. Coli* resulting in significant cell agglutination.\[^{[15]}\] These galactose functionalized nanotubes (Gal-SWNTs), each displaying multiple copies of the sugar, were found to have adhesion to *E. Coli* O157:H7 to result in significant cell agglutination (Figure 5). Moreover, the galactose pairs displayed on MWNTs prove to be more efficient in the binding with bacteria cells.\[^{[32]}\]

![Figure 5. Galactose derivative functionalized SWNTs interact with receptors on pathogenic *E. Coli.*](image)

Recently, the same group demonstrated the effectiveness of CNTs to display multivalent monosaccharide ligands (mannose or galactose) that bind effectively with
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*Bacillus anthracis* (*B. anthracis*) spores in the presence of divalent cation like Ca$^{2+}$.[33]

As a result of aggregation, the colony forming unit value has been reduced to 97.7%, which could be attributed to divalent cation-mediated multivalent carbohydrate-carbohydrate interactions. The role of Ca$^{2+}$ in binding and agglutination of *B. anthracis* spores was confirmed by the addition of ethylene diamine tetraacetic acid (EDTA) to the Man-SWNTs. It was observed that Ca$^{2+}$ were removed by chelating with the chelating agent EDTA, as evidenced by the de-aggregation of the *B. anthracis* spores, which suggests the requirement of Ca$^{2+}$ in the aggregation of spores. Surprisingly, polymeric nanomaterials like polystyrene beads functionalized with the same monosaccharide ligands showed no binding affinity with the spores, which indicates the uniqueness of SWNTs as a semi-flexible platform for multivalent displaying of the monosaccharides.

When compared to monovalent ligands, multivalent ligands are more effective in binding with receptors with greater avidity and specificity, thereby acting as powerful inhibitors.[34] Whiteside *et al.* hypothesized that interaction between biological entities which have multiple ligands and receptors commonly involve polyvalency.[35] These multiple ligands have a great number of characteristics than that of mono ligands. Polyvalent interactions are much stronger than monovalent interactions. Sun and coworkers demonstrated this phenomenon by increasing the population of sugar moieties in the functionalized CNTs. By varying the functionality on CNTs from mono to tetrasaccharides, it was observed that there was considerable improvement in solubility and related properties. When galactose molecules were displayed in pairs in the sugar dendron functionalized CNTs, their binding with the pathogenic *E. Coli* for
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Cell agglutination was improved marginally. Thus, enhanced carbohydrate valency and higher order sugar dendrons were more effective in the solubilization of SWNTs. Gal-SWNTs and Gal$_2$-SWNTs were found to have adhesion to pathogenic *E. coli* O157:H7 to result in significant cell agglutination. Similarly, Man-SWNTs, Man$_2$-SWNTs and Man$_4$-SWNTs were capable of binding and aggregating *B. subtilis* spores in the presence of calcium cation.[12]

Sun *et al.* demonstrated that CNTs are elegant scaffolds for displaying multiple copies of sugar molecules.[15] [12, 32-33] These sugar-functionalized CNTs have proved to be efficient in the binding with bacteria cells and have chemical and biochemical potential. It is possible to apply them to detect pathogens and inhibit bacterial infections after optimization of the binding and investigation of related mechanistic details.

1.2.3 Interfacing with mammalian cells

Dai and coworkers demonstrated the ability of CNTs to penetrate mammalian cells to deliver various cargos into cells including small peptides, proteins and nucleic acids.[36] Intracellular delivery of biomolecules using nanotube carriers are nonspecific for various adherent and nonadherent mammalian cell lines including HeLa, 3T3 fibroblast, HL60 and Jurkats cells. Biocompatibility is a major concern when foreign substances are introduced inside living systems. Several groups have reported that relatively pure, well-solubilized and well functionalized CNTs appear to exert no apparent adverse effects on cell viability after internalizing mammalian cells.[36-37] For example, glucosamine functionalized SWNTs can increase 3T3 fibroblasts viability at...
concentration lower than 0.5% and no significant influence on 3T3 cell metabolic activity was observed.\cite{37} It is anticipated that glycosylation of CNTs would facilitate cellular internalization of CNTs and non-cytotoxic delivery of therapeutics.

### 1.2.4 Application in nanomedicine

One of the important areas of nanotechnology is “nanomedicine”. According to the National Institute of Health (NIH) Nanomedicine Roadmap Initiative refers to highly specific medical intervention at the molecular level for diagnosis, prevention and treatment of diseases. Nanomedicine has tremendous potential for revolutionizing therapeutics and diagnostics under the premise of astute nanodevises. The unique properties of CNTs such as ultra lightweight, ordered structure with high aspect ratio and high surface area make CNTs a potential tool for nanomedicine. The \textit{in vivo} toxicological and pharmacological studies carried out so far indicate that functionalized CNTs can be developed for nanomedicine. This could be explained on the grounds that functionalization renders the surface of CNTs water soluble, compatible with biological fluids and leads to their rapid excretion through the renal route thus minimizing unwanted tissue accumulation. CNTs functionalized with sugars could emerge as new alternative and efficient carrier systems for transporting translocating therapeutic molecules due to their low toxicity, biocompatibility and, moreover, they are not immunogenic. For example, Jung and coworkers reported that noncovalent association of CNTs with cyclodextrins could lead to the fabrication of biosensors, which detect biomaterials like proteins, nucleic acids, enzymes and antibodies.\cite{38} Cyclodextrins are a family of cyclic oligosaccharides with a hydrophilic
outer surface and lipophilic central cavity. These cyclodextrins can serve as multifunctional drug carriers through the formation of inclusion complexes or they can form cyclodextrin/drug conjugates. Thus the combined use of cyclodextrin, CNTs and a drug is capable of alleviating the undesirable properties of drug molecules, improving efficacy and reducing side effects. In the first step CNTs were wrapped with cyclodextrins with the aid of an enzyme having cyclodextrin forming activity such as cyclodextrin glucanotransferase, α-glucosidase, amylopullulanase, isopullulanase or neopullulanase. Subsequently, a receptor corresponding to specific biomaterials was attached to cyclodextrin wrapped CNTs. This method facilitates the selective recognition of biomolecules with CD-wrapped CNTs. Thus they can be used as a powerful tool for targeted delivery applications for proteins, genes and other therapeutics.[39]

The development of functionalized CNTs for bio-applications has drawn great attention in the past few years. In this review, the fabrication of various carbohydrate functionalized CNTs and their bio-applications have been summarized. These modifications provide various methods to construct biocompatible CNTs. Either covalent or noncovalent, functionalized CNTs fully lay out their unique intrinsic properties and increase their solubility in solvents and biosystems. As demonstrated, carbohydrate functionalized CNTs are successfully utilized to detect bacteria, bind to specific lectins, probe cellular activities as biosensors, and deliver glycomimetic drug molecules into cells.

Various approaches have been explored for the conjugation of proteins with nanotubes and stringent investigations are being carried out to understand the mechanism of
interaction between CNTs and biological molecules such as proteins, peptides and DNA. It is essential to understand the nanotube interactions with the bio-environment and cellular systems at the cellular level to fabricate next generation biosensors and to explore the potential therapeutic and cytotoxic effects. Due to the impermeability of cell membranes to foreign substrates, there has always been a need to fabricate new transporters which can penetrate cell membranes to facilitate drug and gene delivery. While previous research suggested that the nanotube uptake was accomplished via insertion and diffusion through the lipid bilayer of cell membrane, there is still uncertainty in the uptake mechanism. In future, carbohydrate functionalized water soluble nanotubes may find applications in the specific gene or drug delivery due to their biocompatibility, degradability, cell penetrating ability, and more importantly, the ability to localize in the nucleus of the cell without the need of nuclear localizing signals. For example, recently it was shown that glycopolymer functionalized CNTs could be successfully used as in vitro gene delivery transporting agents and they have the potential to replace the other commercially available gene delivery vehicles such as lipofectamine 2000 due to their high transfection efficiency.\textsuperscript{[40]} Even though there are considerable numbers of good results coming out, we still need to address transfection efficiencies. When it comes to the counter attack of bioterrorism, carbohydrate functionalized CNTs may provide a solution in the near future. As reported above in this review, sugar functionalized CNTs could interact with \textit{B. anthracis} spores in the presence of divalent cations, thus diminishing the most lethal action of anthrax infection, thereby countering anthrax-based bioterrorism. In this regard; recently, more systematic work has been carried out to investigate the intricate details of interaction between mannose or galactose functionalized CNTs and their
dendric configuration with *B. anthracis* and *B. subtilis*.\[^{41}\] As Dai et al. reported, “functionalization partitioning” of CNT (impacting multiple chemical species on the surface of same nanotube) could be adapted in functionalizing CNTs, thus the combination of water soluble PEG moieties, fluorescent tags and carbohydrates on the same CNT could lead to the construction of fascinating new technologies.\[^{28}\] These experiments clearly demonstrate that carbohydrate functionalized CNTs could be applied in a plethora of applications, such as fabrication of biosensors to use against bioterrorism.

Glycobiology is one of the last frontiers of science to be conquered and it is going to be at the cutting edge of large numbers of discoveries and therapies over the next decade.\[^{42}\] It is true that glycochemistry and glycobiology are strongly connected with organic, pharmaceutical, medicinal chemistry and their importance is profound and wide ranging. Although the synthesis and analysis of carbohydrates has been developed rapidly for the past few years, there is still a great need to develop new technologies to display them and evaluate their biological properties. CNTs with well-defined carbohydrate composition provide an excellent multivalency-displaying platform to study carbohydrate-mediated interactions, which are central to many biological events and diseases. Carbohydrate functionalized CNTs are developing into a powerful glyconanotechnology that will revolutionize glycobiological studies.

CNTs have spurred much interest in their biological applications due to their notable structural, electrical and physiochemical properties.\[^{43}\] Since their discovery, CNTs have been employed as nanoelectronic biosensors for rapid detection of various biomolecules with remarkable sensitivity,\[^{44}\] as nanovectors to transport...
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macromolecules into cells,\textsuperscript{[45]} and as nanofibrous scaffolds for tissue engineering.\textsuperscript{[46]} However, the nonideal interface between CNTs and the living cells largely restricts the nanotube applications in biology.\textsuperscript{[47]} To tackle this issue, hydrophobic nanotubes have been coated with positively charged polyelectrolytes, such as poly(ethyleneimine) (PEI)\textsuperscript{[48]} and poly-l-lysine (PLL),\textsuperscript{[49]} in order to promote adhesion of negatively charged cell membrane. Charge groups can also be imparted to nanotube surface via chemical functionalization.\textsuperscript{[50]} Non-physiological strong electrostatic interactions between the highly charged surface and the cell membrane, nevertheless, may lead to cell death or cytotoxic effects.\textsuperscript{[51]} Moreover, the introduced surface charges and chemical modifications often significantly alter the electrical properties of nanotubes, which is undesirable when nanotubes are used as electrical sensing elements. Alternatively, CNTs has been coated with adhesion proteins found in extracellular matrix.\textsuperscript{[52]} Nevertheless, the thick gel-like layer formed by the adhesion proteins precludes the intimate contact between the nanotubes and the cells. And the charge moieties on these macromolecules also may give adverse influences on the nanotube electrical properties.

Therefore, new functionalized strategies, ensuring biocompatible interactions between CNTs and living systems and preservation of functionalities of both, are needed to be developed. Carbohydrates, a major component of cell membrane, have recently been utilized to decorate CNTs in order to establish natural contact with living cells.\textsuperscript{[20-21]} Carbohydrates, in the forms of glycans, glycolipids, glycoproteins and other glycoconjugates, consist in the surfaces of both eukaryotic and bacterial cells and critically involve in a wide variety of biological processes such as cell growth, cell-cell
interaction and communication, immune recognition and responses, and signal transduction.\textsuperscript{[53]} In particular, carbohydrates are important in mediating cell adhesion process through carbohydrate-carbohydrate or carbohydrate-receptor (lectin) interactions.\textsuperscript{[53b, 54]}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{structures.png}
\caption{Structures of O-linked carbohydrate probes for PC12 cell culture and catecholamine detection.}
\end{figure}

In this work, we designed a few glycoside compounds and fabricated thin-film networks of SWNTs with these glycosides. Monosaccharides are abundantly found in
living systems, especially, N-acetyl-D-glucosamine (GlcNAc), N-acetyl-D-galactosamine (GalNAc), D-glucose, or D-mannose. Synthesized glycosides containing these sugar moieties (Figure 6) were anchored onto nanotubes via either a pyrene or a lipid tail (GalNAc was not included due to the extremely poor solubility in solvents). It was demonstrated that GlcNAc modified SWNT-net could biocompatibly interface with living neuroendocrine PC12 cells, and supported their adhesion and proliferation. In addition, we showed that the glycosylated SWNT-net devices were able to detect triggered secretion of catecholamines from individual PC12 cells with high temporal resolution.\[55\]
2. RESULTS AND DISCUSSION

The synthetic routes of (16-allyloxy)hexadecyl-2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranoside (GlcNAc-lipid, 1) and 4-(1-Pyrenyl)butyl 2-acetamido-2-deoxy-β-D-glucopyranoside (GlcNAc-pyrene, 2) are depicted in scheme 2.

In brief, \(N\)-acetyl-D-glucosamine was acetylated by acetic anhydride in pyridine to produce pentacetyl carbohydrate.\(^{[56]}\) The anomeric acetyl group was then cleaved by
methyl amine and subsequently transformed to trichloroacetimidate. Glycosylation reactions were performed in the presence of trimethylsilyl triflate, followed by deacetylation to generate GlcNAc-lipid 1 or GlcNAc-pyrene 2. 4-(1-Pyrenyl)butyl β-D-Glucopyranoside (Glc-pyrene, 3), 4-(1-Pyrenyl)butyl α-D-Mannopyranoside (Man-pyrene, 4) and 4-(1-Pyrenyl)butyl 2-acetamido-2-deoxy-β-D-galactopyranoside (GalNAc-pyrene, 5) were similarly synthesized (Scheme 3, 4 and 5).

**Scheme 3.** Synthetic route to Glc-pyrene 3. Reagents and conditions: (a) Ac₂O, pyridine, 0 °C, 17 h, 90%; (b) MeNH₂, MeOH, THF, rt, 2 h, 68%; (c) CCl₃CN, DBU, CH₂Cl₂, rt, 3 h, 73%; (d) 1-pyrenebutanol, TMSOTf, CH₂Cl₂, 0 °C to rt, 3 h, 42%; (e) NaOMe, MeOH, rt, 3 h, Amberlyst-15 resin, 96%.
Scheme 4. Synthetic route to Man-pyrene 4. Reagents and conditions: (a) Ac\(_2\)O, pyridine, 0 °C, overnight, 92%; (b) MeNH\(_2\), MeOH, THF, rt, 2 h, 69%; (c) CCl\(_3\)CN, DBU, CH\(_2\)Cl\(_2\), rt, 3 h, 65%; (d) 1-pyrenebutanol, TMSOTf, CH\(_2\)Cl\(_2\), 0 °C to rt, 3 h, 40%; (e) NaOMe, MeOH, rt, 3 h, Amberlyst-15 resin, 84%.

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Scheme 5. Synthetic route to GalNAc-pyrene 5. Reagents and conditions: (a) Ac$_2$O, pyridine, rt, overnight, 94%; (b) MeNH$_2$, MeOH, THF, rt, 2 h, 83%; (c) CCl$_3$CN, DBU, CH$_2$Cl$_2$, rt, 3 h, 72%; (d) 1-pyrenebutanol, TMSOTf, CH$_2$Cl$_2$, 0 °C to rt, 3 h, 30%; (e) NaOMe, MeOH, rt, 3 h, Amberlyst-15 resin, 40%.

After these glycosides were prepared, they were used for functionalizing the SWNT-net. Thin-film devices comprising of small SWNT bundles (10-30 nm) were prepared through phase separation facilitated self-assembly and drop-casted onto glass coverslip. The SWNT-nets were functionalized by incubation with 5 mM glyco-conjugates for 24 hours, followed by deionized water rinsing to remove unbound compounds. The hydrophobic lipid tail anchors onto nanotubes via hydrophobic interaction, while the aromatic pyrene tail firmly attaches to the nanotube sidewall via π-π stacking interactions, which has been described by Mioskowski et al. and Fukumura et al. (Fig. 7).

Figure 7. Schematic illustration of GlcNAc-pyrene functionalization onto SWNTs.
The effectiveness of functionalization was visualized using fluorescein isothiocyanate (FITC) conjugated carbohydrate binding lectins such as FITC conjugated Helix Pomatia Agglutinin (HPA) and FITC conjugated Concanavalin A (conA). HPA selectively binds to GlcNAc, whereas conA selectively binds to glucose and mannose. The functionalized SWNT-net was then incubated with 5 mM FITC-conjugated lectin for 24 hours, followed by rinsing to remove unattached lectin and fluorescence imaging. As shown by the fluorescence labeling, GlcNAc-pyrene uniformly covered the SWNT-net (Fig. 8a, left) whereas GlcNAc-lipid formed inhomogeneous patches on the SWNT-net (data not shown), indicating that pyrene is a better linker as compared to the lipid. In contrast, the bare SWNT-net only showed background fluorescence (Fig 8a, right) because FITC-HPA was unable to bind to the nanotube without GlcNAc bait.

The conductance of SWNT-net can be sensitively modulated by the gating voltage applied to the solution that baths the SWNTs. In a typical example shown in Fig. 8b, nanotube conductance dropped from 6.3 $\mu$S to 0.1 $\mu$S as the gating voltage varied from $-1$ to 1 V (red circles). This demonstrates that our SWNT-net device is highly sensitive to the electrochemical perturbation in the solution, therefore eligible for electrical biosensing. Glycosylation does not compromise the nanotube properties (Fig. 8b, blue triangle). Interestingly, the field-effect of nanotube is enhanced instead upon glycosylation as evidenced by increased nanotube conductance and on-off ratio in response to voltage gating. SWNT-net is superior to single carbon nanotube base devices because they can be more readily fabricated and have much lower 1/f noise that commonly plagues single nanotube devices.\[61\] SWNT-net is especially

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advantageous to probe cellular activities, because it could serve as cell growth substrate and interface with cells with large contact area.

![Fluorescence staining of GlcNAc functionalized SWNT-net (left) and bare SWNT-net (right) by HPA. The scale bar corresponds to 500 μm. (b) Electrical characterization of SWNT-net before (red circle) and after (blue triangle) glycosylation of GlcNAc. Biasing at 400 mV, the source-drain current (I_{ds}) was measured while varying the gating voltage (V_g) applied to the bath solution via an Ag/AgCl electrode.](image)

**Figure 8.** (a) Fluorescence staining of GlcNAc functionalized SWNT-net (left) and bare SWNT-net (right) by HPA. The scale bar corresponds to 500 μm. (b) Electrical characterization of SWNT-net before (red circle) and after (blue triangle) glycosylation of GlcNAc. Biasing at 400 mV, the source-drain current (I_{ds}) was measured while varying the gating voltage (V_g) applied to the bath solution via an Ag/AgCl electrode.

When PC12 cells were cultured on bare SWNT-net, they abnormally aggregated into sparsely dispersed clumps on the substrate (Fig. 9a, left) and their growth was apparently impaired. For comparison, we examined the growth of PC12 cells on SWNT-nets functionalized with GlcNAc, glucose or mannose, which are sugar terminal residues of many membrane glyco-conjugates relevant to various biological processes. In particular, GlcNAc is a major moiety of many proteoglycans which are important cell adhesion molecules found on both cell membrane and extracellular matrix.\(^{[62]}\) PC12 cells possess abundant proteoglycans, especially heparin sulfate that consists of a core protein with one or more covalently attached glycosaminoglycan polymerized from GlcNAc and glucuronic acid.\(^{[63]}\) As anticipated, GlcNAc-pyrene

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decorated SWNT-nets, in contrast to bare SWNT-nets, supported normal confluent growth of PC12 cells (Fig. 9a, right), suggesting the facilitating role of GlcNAc on adhesion of PC12 cells. It has been shown that noncovalent interactions between similar glycans can provide strong adhesion force (200 – 300 pN) comparable to that of protein-protein interaction.\(^{[64]}\) Therefore, it is plausible that a nanotube attached with many GlcNAc residues can strongly interact with glycosaminoglycan on PC12 cell membrane. In addition, specific lectins on cell membrane can bind with GlcNAc on nanotube and thus promote cell adhesion as well.\(^{[65]}\) In comparison to GlcNAc-pyrene, Glc-pyrene and Man-pyrene appear to significantly inhibit cell adhesion and, therefore, cell growth.

**Figure 9.** (a) Phase-contrast images of PC12 cells cultured on bare SWNT-net (left) and GlcNAc-SWNTs (right). Scale bar = 100 \(\mu\)m. (b) Proliferation curves of PC12 cells grown bare SWNT-net (red circles), or SWNT-net modified with GlcNAc (green squares), or glucose (yellow triangles), or mannose (blue diamonds). Each data points are the average from 40 different areas (3 mm\(^2\)) in three different substrates.
Proliferation curves of cells cultured on bare SWNT-net or SWNT-net functionalized with different sugar moieties are shown in Fig. 9b. These experiments were conducted in parallel and PC12 cells of the same initial density were introduced onto the respective substrates. As seen, PC12 cells grown on GlcNAc functionalized SWNT-nets can proliferate well, evident by 160.0% increase in cell density after 3 days culturing. In contrast, proliferation of PC12 cells was largely inhibited on bare SWNT-nets. In this case, the cell density only increased by 60.9% after 3 days. On both glucose and mannose coated nanotubes, PC12 cells can not adhere well and their density decreased by 57.5% and 56.1% in 3 days, respectively.

As demonstrated above, appropriate glycosylation enables biocompatible interfacing between SWNT-net and living cells without compromising SWNT functionalities. Therefore, glycosylated SWNT-net provides a new platform to probe dynamic biological processes, for example, to continuously monitor biomolecular release from living cells. CNTs-devices have already been used as nanoelectronic biosensors to detect various biomolecules.\[66\] But these realizations are essentially limited to static \textit{in vitro} measurements.

PC12 cells is a widely used cell model to study exocytosis, a fundamental and dynamic biological process in many cell types underlying, for instances, hormone secretion from endocrine cells and neurotransmitter secretion from neurons. As illustrated in Fig. 10a, membrane depolarization (i.e., increase in intracellular potential) resulting from certain physiological stimulation opens voltage gate Ca\textsuperscript{2+} channels. Ca\textsuperscript{2+} influx triggers actions of several sets of protein machineries, which drive immobilization and final fusion of large dense core secretory vesicles. Upon vesicle
fusion with the plasma membrane, catecholamine molecules such as dopamine, norepinephrine and epinephrine inside the vesicle are discharged into the extracellular space.

**Figure 10.** (a) Schematic illustration of triggered exocytosis of catecholamines from PC12 cells and subsequent detection by SWNT-net device. (b) CNTs responses to exocytosis of PC12 cells triggered by high K\(^+\) stimulation. The SWNT-net was biased at V\(_{ds}\)=0.4 V. (c) Stimulation of single PC12 cell through micropipette perfusion of high K\(^+\) solution. (d) Transient perfusion of 1 mM dopamine or norepinephrine on glycosylated SWNTs-net results in current spikes. In contrast, perfusion of acetylcholine, acidic solution (pH 5.0), and high K\(^+\) did not cause any appreciable responses.

After being released into the narrow interface gap between the cell and the SWNT-net, catecholamines quickly diffuse onto the nanotubes and interact with them through \(\pi-\pi\)
stacking between the aromatic ring of the catecholamine molecule and the sidewalls of nanotube. As the current flows at the surface of nanotubes, the conductance of nanotubes is highly sensitive to the electrochemical perturbations at the surface induced by the interacting molecules. Thus, it is possible that triggered catecholamine release from PC12 cells can be revealed by changes in nanotube current.

Indeed, a train of current spikes were detected when high potassium (K\textsuperscript{+}) solution was introduced to PC12 cells cultured on glycosylated SWNT-net (Fig. 10b). When another common secretagog, calcymycin (1 mM) which inserts into cell membrane and forms Ca\textsuperscript{2+} selective ion channel, was applied, similar current spikes were elicited. We hypothesized that each spike corresponds to Ca\textsuperscript{2+} dependent exocytosis of catecholamines from single PC12 cell. To test this hypothesis, we used an application pipette (with a tip size of 1-2 \textmu m) positioned 1-2 \textmu m away from the target cell to locally deliver high K\textsuperscript{+} solution or calcymycin, which exclusively stimulate this one cell. Single nanotube current spike, with similar amplitude and time scale as observed in Fig. 10b, was resulted from such local stimulation (Fig. 10c), suggesting that each recorded spike in Fig. 10b is due to single cell activity. Furthermore, when a puff of bath solution contained 1 mM dopamine or norepinephrine was delivered onto a cell-free and 400 mV-biased glycosylated SWNT-net via an application micropipette, a current spike was also induced (Fig. 10d), indicating that local discharge of catecholamines can cause transient increase in nanotube current as observed in the experiment depicted in Fig. 10b. High K\textsuperscript{+} solution and calcymycin were failed to produce any signals. It is arguable that the observed spike may be due to the acidic vesicular fluids (~pH 5.0-6.0). This possibility was excluded by the observation that
nanotubes are not responsive to the micropipette application of bath solution of low pH (5.0). Upon Ca$^{2+}$ triggering, PC12 cells also release acetylcholine from small synaptic-like vesicles. But as shown in Fig. 3d, acetylcholine was not able to modulate nanotube current. Moreover, it is not likely that the recorded transient signals are originated from constitutively secreted molecules (e.g., metabolites) from the cells because, in comparison to triggered rapid exocytosis, the constitutive secretion is a slow and constant process. The spiky nanotube responses were only observed after application of the secretagogues which lead to Ca$^{2+}$ dependent exocytosis.

Exocytosis of biomolecules are usually analyzed by biochemical assays which only provide averaged ensemble measurements from a population of cells with low temporal resolution and sensitivity. Although the state-of-the-art electrophysiological methods, such as membrane capacitance measurement and carbon micro-fiber amperometry, provide high sensitivity and temporal resolution, they require specialized equipments and high skills. These methods are of very low-throughput due to tedious procedures. In addition, membrane capacitance measurement is invasive and carbon fiber microelectrode (typically 5 μm in size) can only detect the release of electroactive molecules from ~5% of the total area of cell surface. Comparing to these conventional methods, our nanotube approach provides a novel alternative to detect exocytosis from individual cells.
3. CONCLUSION

In summary, we demonstrated that non-covalent functionalization with bioactive sugar moieties confers biocompatibility to CNTs without compromising their intrinsic sensing capabilities. Glycosylated SWNT-net can directly interface with living cells by supporting their adhesion and growth, and detect dynamic biomolecular release from cells. Comparing to conventional methods to detect exocytosis, our approach provides real-time and noninvasive measurements from living cells with high sensitivity, high temporal resolution, high throughput and ease of use.[67]
4. EXPERIMENTAL SECTION

**General:** Unless otherwise noted, all reactions were carried out in oven-dried glassware under an atmosphere of N₂. All reagents and solvents were obtained from commercial suppliers and used without further purification. Evaporation of organic solutions was achieved by rotary evaporation with a water bath temperature below 40 °C. Product purification by flash column chromatography was accomplished using silica gel 60 (0.010–0.063 mm). Chromatograms were visualized by fluorescence quenching with UV light at 254 nm or by staining using basic solution of KMnO₄. Technical grade solvents were used for chromatography and were distilled prior to use. Optical rotations were measured in CHCl₃ or MeOH on a Schmidt + Haensch polarimeter with a 1 cm cell (c given in g/100 mL). IR spectra were recorded using FTIR Restige-21 (Shimadzu). NMR spectra were recorded at room temperature on 300 MHz Bruker ACF 300, 400 MHz Bruker DPX 400, 500 MHz Bruker AMX 500, and 400 MHz JEOL ECA 400 NMR spectrometers. The residual solvent signals were taken as the reference (7.26 ppm for ¹H NMR spectra and 77.0 ppm for ¹³C NMR spectra in CDCl₃, 3.31 ppm for ¹H NMR spectra and 49.0 ppm for ¹³C NMR spectra in CD₃OD). Chemical shift (δ) is reported in ppm, coupling constants (J) are given in Hz.

The following abbreviations classify the multiplicity: s = singlet, d = doublet, t = triplet, m = multiplet or unresolved, br = broad signal. LCMS (ESI) spectra were recorded on Finnigan LCQ Deca XP MAX. HRMS (ESI) spectra were recorded on a Waters Q-Tof premier™ mass spectrometer. SWNT-net preparation, cell culture and electrical recording were conducted by Chen Peng’s group from SCBE.
Culture PC12 cells on fabricated SWNT-net devices: SWNT-net on coverslip (8-10 mm²) was prepared as literature described. Two electrodes (source and drain) were then prepared across the SWNT-net using conductive silver paint (RS Component Pte Ltd). Finally, silicon rubber (Dow Corning 3140 Mil-A-46146 RTV coating) was used to insulate the electrodes and define the chamber (2-3 cm²) for cell culturing and recording. PC12 cells were seeded onto glycosylated SWNTs-net devices, which were previously sterilized by 70% ethanol and deionized water rinsing, 2-3 days before experiments. Subsequently, they were cultured in advanced RPMI-1640 medium supplemented with 10% fetal bovine Serum, 5% horse serum, and 1% penicillin streptomycin), and maintained at 37 °C in a humid atmosphere with 5% CO₂/95% air.

Electrical recording: SWNT-net current was monitored at a voltage bias of 400 mV, using a semiconductor device analyzer (Agilent Technologies, B1500A). And SWNT-net was bathed in a solution containing (in mM, titrated to pH 7.2): 10 HEPES, 140 NaCl, 1 MgCl₂, 5.5 KCl, and 2 CaCl₂. High potassium solution to depolarize PC12 cells and stimulate their secretion contains (in mM, titrated to pH 7.2): 10 HEPES, 40 NaCl, 1 MgCl₂, 105 KCl, and 6 CaCl₂.

16-Allyloxy-1-hexadecanol (10):

\[
\text{HO} \quad \begin{array}{c} \text{O} \\ 16 \end{array} \quad \text{C} \\
\]

NaH (60% in mineral oil, 44.0 mg, 1.1 mmol) was added to a suspension of hexadecanediol (258.4 mg, 1.0 mmol) in 2.5 mL of anhydrous DMF. Then allyl bromide (95.2 μL, 1.1 mmol) was added dropwise. The mixture was stirred overnight.
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at room temperature. The resulting mixture was diluted with 30 mL of Et₂O and washed with 20 mL of 10% K₂CO₃ solution. The organic phase was dried over Na₂SO₄, filtered and evaporated. The residue was purified by column chromatography on silica gel (EtOAc/Hexane = 1:6 to 1:3) to give 10 as a white solid (92.0 mg, 31%).

mp 58-59 °C; ¹H NMR (400 MHz, CDCl₃) δ = 5.86-5.96 (m, 1H), 5.26 (dt, J = 1.5, 17.2 Hz, 1H), 5.16 (dd, J = 1.5, 10.4 Hz, 1H), 3.94-3.96 (m, 2H), 3.63 (t, J = 6.7 Hz, 2H), 3.41 (t, J = 6.7 Hz, 2H), 1.52-1.61 (m, 4H), 1.25 (br s, 24H) ppm; ¹³C NMR (100 MHz, CDCl₃) δ = 135.1, 116.7, 71.8, 70.5, 63.0, 32.8, 29.7, 29.6 (2C), 29.5, 29.4, 26.2, 25.7 ppm; IR (neat) ν = 3377, 2950, 2851, 1724, 1470, 1096, 756 cm⁻¹; MS (m/z) 299 [M+H]⁺; HRMS (ESI) calcd. for C₁₉H₃₉O₂ [M+H]⁺, 299.2950; found, 299.2947.

2-Acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-α-D-glucopyranose (7):

![Structure of 2-Acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-α-D-glucopyranose (7)](image)

A solution of D-glucosamine hydrochloride 6·HCl (2.0 g, 9.3 mmol) in a mixture of 15 mL of anhydrous pyridine and 10 mL of acetic anhydride was stirred overnight at room temperature.[68] The mixture was diluted with 60 mL of chloroform and washed successively with 50 mL of cold water, 50 mL of saturated sodium bicarbonate solution and then with portions of a 10% solution of cupric sulfate until disappearance of the deep blue pyridine-copper complex, and finally with water. After drying over Na₂SO₄, the chloroform was removed in vacuo and the crude product 7 was used directly to the next step without purification (75%). ¹H NMR (500 MHz, CDCl₃) δ =
6.15 (d, J = 3.6 Hz, 1H), 5.64 (d, J = 9.1 Hz, 1H), 5.17-5.25 (m, 2H), 4.47 (ddd, J = 3.6, 9.1, 10.4 Hz, 1H), 4.23 (dd, J = 4.1, 12.5 Hz, 1H), 4.05 (dd, J = 2.3, 12.5 Hz, 1H), 3.97-4.00 (m, 1H), 2.18 (s, 3H), 2.07 (s, 3H), 2.04 (s, 3H), 2.03 (s, 3H), 1.92 (s, 3H) ppm; $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta =$ 171.7, 170.7, 169.9, 169.1, 168.6, 90.6, 70.6, 69.7, 67.4, 61.5, 51.0, 23.0, 20.9, 20.7 ppm; MS (m/z) 390 [M+H]$^+$. Data were in agreement with those reported in the literature.$^{[69]}$

2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-$\alpha$-D-glucopyranose (8):

![Structure of 2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-$\alpha$-D-glucopyranose (8)]

A solution of methylamine in methanol (2 M, 4 mL) was added to a solution of 7 (1.64 g, 4.2 mmol) in THF (20 mL) at room temperature and the mixture was stirred for 2 h. The mixture was concentrated $\text{in vacuo}$ and the residue was purified by flash column chromatography on silica gel (EtOAc/CH$_2$Cl$_2$ = 1:1 to 3:1) to give compound 8 as a colorless oil (1.02 g, 70%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta =$ 6.10 (d, J = 9.4 Hz, 1H), 5.27 (t, J = 10.1 Hz, 1H), 5.21 (t, J = 3.7 Hz, 1H), 5.07-5.12 (m, 2H), 4.17-4.27 (m, 3H), 4.06-4.11 (m, 1H), 2.01 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.94 (s, 3H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta =$ 171.4, 171.0, 170.6, 169.5, 91.4, 71.0, 68.3, 67.3, 62.1, 52.3, 23.0, 20.7 (2C), 20.6 ppm; MS (m/z) 248 [M+H]$^+$. Data were in agreement with those reported in the literature.$^{[70]}$
2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-\(\alpha\)-d-glucopyranosyl trichloroacetimidate (9):

\[
\begin{align*}
\text{Cl}_3C\text{NH} \\
\text{O} \\
\text{AcO} \\
\text{O} \\
\text{O} \\
\text{NHAc} \\
\text{OAc}
\end{align*}
\]

To a stirred solution of 8 (0.98 g, 2.8 mmol) in dry dichloromethane (15 mL) were added trichloroacetonitrile (1.12 mL, 11.2 mmol) and DBU (0.08 mL, 0.56 mmol). The reaction mixture was stirred for 3 h at room temperature. The crude product was then concentrated and purified by flash column chromatography on silica gel (EtOAc/hexane = 1:1) to give imidate 9 as a light yellow foamy solid (1.04 g, 75%).

\(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta = 8.79\) (br. s, 1H), 6.36 (d, \(J = 3.6\) Hz, 1H), 5.66 (d, \(J = 9.0\) Hz, 1H), 5.21-5.35 (m, 2H), 4.55 (ddd, \(J = 9.8, 9.8, 3.6\) Hz, 1H), 4.25 (dd, \(J = 4.8, 12.9\) Hz, 1H), 4.08-4.15 (m, 2H), 2.04-2.09 (s, 9H), 1.93 (s, 3H) ppm; \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta = 171.6, 170.6, 170.0, 169.1, 160.3, 94.8, 90.8, 70.7, 70.2, 67.3, 61.4, 51.6, 23.0, 20.7, 20.6\) (2C) ppm. Data were in agreement with those reported in the literature.\(^{[71]}\)

(16-Allyloxy)hexadecyl-2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-\(\beta\)-D-glucopyranoside (11):

\[
\begin{align*}
\text{AcO} \\
\text{O} \\
\text{AcO'} \\
\text{O} \\
\text{NHAc} \\
\text{OAc}
\end{align*}
\]
To a stirred solution of 10 (50.0 mg, 0.17 mmol) and 9 (142.0 mg, 0.29 mmol) in dichloromethane (3 mL) was slowly added TMSOTf (31.0 µL, 0.17 mmol) at 0 °C. The reaction mixture was stirred for 3 h at room temperature. After removal of solvent, the residue was purified by flash column chromatography on silica gel (EtOAc/hexane = 1:5 to 3:5) to give compound 11 as a white solid (66.0 mg, 62%). mp 107-108 °C; $[\alpha]_D^{22} = -12.1$ (c = 0.2, CHCl₃); $^1$H NMR (400 MHz, CDCl₃) δ = 5.85 (m, 1H), 5.46 (d, $J = 8.5$ Hz, 1H), 5.24-5.34 (m, 2 H), 5.15-5.18 (m, 1H), 5.06 (t, $J = 9.8$ Hz, 1H), 4.68 (d, $J = 8.3$ Hz, 1H), 4.26 (ddd, $J = 4.7$, 12.2 Hz, 1H), 4.12 (ddd, $J = 2.4$, 12.2 Hz, 1H), 3.96 (d, $J = 5.6$ Hz, 2H), 3.76-3.88 (m, 2H), 3.69 (ddd, $J = 2.4$, 4.6, 9.8 Hz, 1H), 3.45-3.49 (m, 1H), 3.40 (t, $J = 6.7$ Hz, 2H), 2.08 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 1.94 (s, 3H), 1.54-1.61 (m, 5H), 1.24 (br s, 24H) ppm; $^{13}$C NMR (100 MHz, CDCl₃) δ = 170.9, 170.7, 170.1, 169.4, 135.1, 116.7, 100.6, 72.3, 71.8, 71.7, 70.5, 70.0, 68.7, 62.2, 54.9, 29.7, 29.6 (2C), 29.5, 29.4, 29.3, 26.2, 25.8, 23.3, 20.7 (2C), 20.6 ppm; IR (neat) ν = 3292, 2918, 2850, 1746, 1659, 1231, 1049 cm⁻¹; MS (m/z) 629 [M+H]⁺; HRMS (ESI) calcd. for C₃₃H₅₈NO₁₀ [M+H]⁺, 628.4061; found, 628.4059.

(16-allyloxy)hexadecyl-2-acetamido-2-deoxy-β-D-glucopyranoside (1):

To a stirred solution of 11 (33.0 mg, 0.053 mmol) in methanol (1 mL) was added sodium methoxide (0.9 mg, 0.016 mmol) at room temperature. The reaction mixture...
was stirred for 3 h. The mixture was neutralized with Amberlyst-15 resin and filtered. The filtrate was concentrated to afford compound 1 as a pale solid (23.0 mg, 88%). mp 155-156 °C; \([\alpha]_D^{22} = -18.0\) (c = 0.25, MeOH); \(^1\)H NMR (400 MHz, MeOD) \(\delta = 5.86-5.95\) (m, 1H), 5.26 (dd, \(J = 1.6, 17.2\) Hz, 1 H), 5.14-5.16 (m, 1H), 4.38 (d, \(J = 8.4\) Hz, 1 H), 3.95-3.97 (m, 2H), 3.85-3.89 (m, 2H), 3.60-3.70 (m, 2H), 3.42-3.46 (m, 4H), 3.25-3.28 (m, 2H), 1.97 (s, 3H), 1.52-1.58 (m, 4H), 1.29 (br s, 24H) ppm; \(^{13}\)C NMR (100 MHz, MeOD) \(\delta = 173.6, 136.3, 117.0, 102.7, 78.0, 76.1, 72.8, 72.2, 71.5, 70.6, 62.8, 57.5, 30.8, 30.7, 30.6, 27.3, 27.2, 23.0\) ppm; IR (neat) \(\nu = 3240, 2150, 1670, 1025, 852\) cm\(^{-1}\); MS (m/z) 503 [M+H]\(^+\); HRMS (ESI) calcd. for C\(_{27}\)H\(_{51}\)NO\(_7\)Na [M+Na]\(^+\), 524.3563; found, 524.3561.

\(4-(1\text{-Pyrenyl})\text{butyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-}\beta\text{-D-glucopyranoside (12):}\)

\[\begin{align*}
\text{AcO} & \quad \text{OAc} \\
\text{OAc} & \quad \text{NHAc} \\
\text{OAc} & \quad \text{OAc} \\
\text{AcO} & \quad \text{OAc}
\end{align*}\]

To a stirred solution of 1-pyrenebutanol (54.9 mg, 0.2 mmol) and 9 (167.2 mg, 0.34 mmol) in dichloromethane (3 mL) was slowly added TMSOTf (36.2 \(\mu\)L, 0.2 mmol) at 0 °C. The reaction mixture was stirred for 3 h at room temperature. After removal of solvent, the residue was purified by flash column chromatography on silica gel (EtOAc/hexane = 1:5 to 3:5) to give 12 as a yellow solid (102.9 mg, 85%). mp >140 °C decomposed; \([\alpha]_D^{22} = -18.8\) (c = 1, CHCl\(_3\)); \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta = 8.26\).
Part 2 Chapter 4 Experimental section

(d, $J = 9.3$ Hz, 1H), 8.14-8.17 (m, 2H), 8.09-8.11 (m, 2H), 7.97-8.03 (m, 3H), 7.85 (d, $J = 7.8$ Hz, 1H), 5.44 (d, $J = 8.0$ Hz, 1H), 5.23 (t, $J = 9.7$ Hz, 1H), 5.04 (t, $J = 9.7$ Hz, 1H), 4.59 (d, $J = 8.5$ Hz, 1H), 4.23 (dd, $J = 4.7$, 12.2 Hz, 1H), 4.09 (dd, $J = 2.4$, 12.2 Hz, 1H), 3.91 (dt, $J = 6.2$, 9.7 Hz, 1H), 3.80 (dt, $J = 8.5$, 10.5 Hz, 1H), 3.61 (ddd, $J = 2.4$, 4.7, 9.7 Hz, 1H), 3.48 (dt, $J = 6.7$, 9.7 Hz, 1H), 3.32-3.37 (m, 2H), 2.01-2.04 (s, 9H), 1.86-1.92 (m, 2H), 1.78 (s, 3H), 1.74-1.76 (m, 2H) ppm; $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta = 170.8$, 170.7, 170.1, 169.4, 136.6, 131.4, 130.8, 129.8, 128.5, 127.5, 127.2, 126.6, 125.8, 125.0 (2C), 124.9, 124.8, 124.7, 123.3, 100.6, 72.3, 71.7, 69.5, 68.6, 62.1, 54.8, 33.1, 29.4, 28.1, 23.2, 20.7, 20.6 (2C) ppm; IR (neat) $\nu = 3289$, 2940, 1748, 1231, 1044, 847, 754 cm$^{-1}$; MS (m/z) 605 [M+H]$^+$/HRMS (ESI) calcd. for C$_{34}$H$_{37}$NO$_9$ [M+H]$^+$, 604.2546; found, 604.2545.

4-(1-Pyrenyl)butyl 2-acetamido-2-deoxy-\(\beta\)-D-glucopyranoside (2):

To a stirred solution of 12 (45.0 mg, 0.07 mmol) in methanol (3 mL) was added sodium methoxide (1.1 mg, 0.02 mmol) at room temperature. The reaction mixture was stirred for 3 h and then neutralized with Amberlyst-15 resin followed by filtration. The filtrate was concentrated to afford compound 2 as a yellow solid (14.0 mg, 42%). mp >160 °C decomposed; $[\alpha]_D^{22} = -15.3$ (c = 0.5, MeOH); $^1$H NMR (400 MHz, MeOD) $\delta = 8.33$ (d, $J = 9.2$ Hz, 1H), 8.12-8.19 (m, 4H), 8.03 (d, $J = 1.6$ Hz, 2H), 7.99
Part 2 Chapter 4 Experimental section

(t, J = 7.7 Hz, 1H), 7.90 (d, J = 7.7 Hz, 1H), 4.40 (d, J = 8.4 Hz, 1H), 3.97-4.01 (m, 1H), 3.85-3.88 (m, 1H), 3.61-3.70 (m, 2H), 3.54-3.57 (m, 1H), 3.35-3.44 (m, 3H), 3.25-3.31 (m, 2H), 1.88-2.00 (m, 2H), 1.71-1.78 (m, 5H) ppm; 13C NMR (100 MHz, MeOD) δ = 173.7, 138.3, 132.9, 132.4, 131.2, 129.9, 128.6, 128.5, 128.3, 127.6, 127.0, 126.2 (2C), 125.9, 125.7, 124.6, 102.8, 78.0, 76.1, 72.2, 70.5, 62.8, 57.4, 34.1, 33.1, 31.8, 30.8, 30.7, 30.6, 30.5, 29.9, 23.8, 22.9, 14.4 ppm; IR (neat) ν = 3265, 2374, 1653, 1034, 835 cm⁻¹; MS (m/z) 500 [M+Na]⁺; HRMS (ESI) calcd. for C₂₈H₃₁NO₆ [M+H]⁺, 478.2230; found, 478.2219.

1,2,3,4,6-Penta-O-acetyl-α-d-glucopyranose (14):

Acetic anhydride (7.9 mL, 84 mmol) was added dropwise to a stirred solution of d-glucose 13 (2.0 g, 11 mmol) in anhydrous pyridine (10 mL) at 0 °C, and the stirring continued at the same temperature for 17 h. After addition of ice into the reaction mixture a powdery white solid precipitated. The precipitate was washed by water, NaHCO₃ solution, 2 N HCl solution and water successively. After drying in vacuo, 14 (α and β mixture) was obtained as a white solid (3.96 g, 90%). ¹H NMR (400 MHz, CDCl₃) δ = 6.31 (d, J = 3.6 Hz, 1H), 5.70 (d, J = 8.0 Hz, 1H), 5.45 (t, J = 9.8 Hz, 1H), 5.24 (t, J = 9.4 Hz, 1H), 5.06-5.15 (m, 4H), 4.27 (dd, J = 4.0, 8.4 Hz, 2H), 4.06-4.11 (m, 3H), 3.83 (dt, J = 2.0, 10.0 Hz, 1H), 2.16 (s, 3H), 2.10 (s, 3H), 2.07 (s, 3H), 2.02 (s, 3H), 2.01 (s, 12H), 2.00 (s, 6H) ppm; 13C NMR (100 MHz, CDCl₃) δ = 170.6, 170.5,
170.2, 170.0, 169.6, 169.3, 169.2, 168.9, 168.7, 91.6, 89.0, 72.7 (2C), 70.2, 69.8, 69.1, 67.8, 67.7, 61.4, 20.8 (2C), 20.6 (2C), 20.5, 20.4 ppm; MS (m/z) 413 [M+Na]+. Data were in agreement with those reported in the literature.[73]

**2,3,4,6-Tetra-O-acetyl-α-D-glucopyranose (15):**

![Structure of 2,3,4,6-Tetra-O-acetyl-α-D-glucopyranose (15)](attachment)

Compound 15 was prepared according to the same procedure as compound 8 and purified by flash column chromatography on silica gel (EtOAc/Hexane = 1:3 to 1:1) to be obtained as a colorless oil (0.64 g, 68%). ¹H NMR (400 MHz, CDCl₃) δ = 5.53 (t, J = 9.9, Hz, 1H), 5.45-5.46 (m, 1H), 5.08 (t, J = 9.9 Hz, 1 H), 4.89 (dd, J = 3.6, 9.9 Hz, 1H), 4.21-4.28 (m, 2H), 4.11-4.14 (m, 1H), 3.44 (br s, 1H), 2.09 (s, 3H), 2.08 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H) ppm. ¹³C NMR (100 MHz, CDCl₃) δ = 170.8, 170.2, 170.1, 169.6, 90.1, 71.0, 69.8, 68.5, 67.2, 61.9, 20.7 (3C), 20.6 ppm. Data were in agreement with those reported in the literature.[70]

**2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl trichloroacetimidate (16):**

![Structure of 2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl trichloroacetimidate (16)](attachment)
Compound 16 was prepared according to the same procedure as compound 9. The crude product was purified by flash column chromatography on silica gel (EtOAc/hexane = 1:3) to give imidate 16 as a colorless oil (0.64 g, 73%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ = 8.69 (br s, 1H), 6.55 (d, $J = 3.6$ Hz, 1H), 5.55 (t, $J = 10.0$ Hz, 1H), 5.17 (t, $J = 10.0$ Hz, 1H), 5.12 (dd, $J = 3.9$, 10.0 Hz, 1H), 4.26 (dd, $J = 3.9$, 12.3 Hz, 1H), 4.19-4.22 (m, 1H), 4.12 (dd, $J = 1.7$, 12.3 Hz, 1H), 2.07 (s, 3H), 2.04 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ = 170.5, 170.0, 169.8, 169.5, 160.8, 92.9, 90.6, 70.0, 69.8, 69.7, 67.8, 61.3, 20.6, 20.5, 20.4 ppm. Data were in agreement with those reported in the literature.\(^{71}\)

4-(1-Pyrenyl)butyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (17):

![Chemical Structure](image)

Compound 17 was prepared according to the same procedure as compound 12. The crude product was purified by flash column chromatography on silica gel (EtOAc/hexane = 1:5 to 1:1) to give compound 17 as a light yellow solid (51.6 mg, 42%). mp 70-72 °C; $[\alpha]_D^{22} = -8.2$ (c = 1, CHCl$_3$); $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ = 8.25 (d, $J = 9.3$ Hz, 1H), 8.14-8.17 (m, 2H), 8.09-8.11 (m, 2H), 7.97-8.03 (m, 3H), 7.84 (d, $J = 7.8$ Hz, 1H), 5.18 (t, $J = 9.6$ Hz, 1H), 5.09 (t, $J = 9.6$ Hz, 1H), 5.00 (dd, $J = 8.1$, 9.6 Hz, 1H), 4.44 (d, $J = 8.1$ Hz, 1H), 4.24 (dd, $J = 4.7$, 12.3 Hz, 1H), 4.09-4.14 (m, 1H), 3.93 (dt, $J = 6.2$, 9.8 Hz, 1H), 3.61 (ddd, $J = 2.4$, 4.7, 9.8 Hz, 1H), 3.50 (dt, $J
= 6.6, 9.8 Hz, 1H), 3.29-3.39 (m, 2H), 2.05 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H), 1.85-1.95 (m, 5H), 1.73-1.79 (m, 2H) ppm; $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ = 170.6, 170.2, 169.3, 169.2, 136.5, 131.3, 130.8, 129.7, 128.5, 127.4, 127.2, 127.1, 126.5, 125.8, 125.0, 124.9, 124.8, 124.7 (2C), 123.3, 100.7, 72.8, 71.6, 71.3, 69.8, 68.4, 61.9, 33.0, 29.3, 28.0, 20.5 (3C) ppm; IR (neat) $\nu$ = 2941, 1753, 1369, 1225, 1038, 849, 756 cm$^{-1}$; MS (m/z) 628 [M+Na]$^+$; HRMS (ESI) calcd. for C$_{34}$H$_{36}$O$_{10}$Na [M+Na]$^+$, 627.2206; found, 627.2203.

4-(1-Pyrenyl)butyl $\beta$-D-glucopyranoside (3):

![Chemical structure of 4-(1-Pyrenyl)butyl $\beta$-D-glucopyranoside (3)]

Compound 3 was prepared according to the same procedure as compound 9 and obtained as a brown solid (26.2 mg, 96%). mp 102-104 °C; $[\alpha]_D^{22}$ = −7.8 (c = 0.1, MeOH); $^1$H NMR (500 MHz, MeOD) $\delta$ = 8.25 (d, $J$ = 9.3 Hz, 1H), 8.11 (dd, $J$ = 3.1, 7.6Hz, 2H), 8.04 (d, $J$ = 8.2 Hz, 2H), 7.91-7.96 (m, 3H), 7.81 (d, $J$ = 7.8 Hz, 1H), 3.95 (dt, $J$ = 6.6, 9.6 Hz, 1H), 3.85 (dd, $J$ = 2.1, 11.8 Hz, 1H), 3.67 (dd, $J$ = 5.6, 11.8 Hz, 1H), 4.25 (d, $J$ = 7.8 Hz, 1H), 3.57 (dt, $J$ = 6.6, 9.6 Hz, 1H), 3.23-3.37 (m, 5H), 3.18-3.121 (m, 1H), 1.85-1.92 (m, 2H), 1.73-1.78 (m, 2H) ppm; $^{13}$C NMR (100 MHz, MeOD) $\delta$ = 138.2, 132.8, 132.3, 131.1, 129.8, 128.5, 128.4, 128.1, 127.5, 126.9, 126.1 (2C), 125.8 (2C), 125.7, 124.5, 104.3, 78.1, 77.9, 75.1, 71.7, 70.5, 62.8, 34.0, 30.7,
29.5 ppm; IR (neat) ν = 3400, 1634, 1061, 840, 754 cm⁻¹; MS (m/z) 455 [M+NH₄]⁺; HRMS (ESI) calcd. for C₂₆H₂₉O₆ [M+H]⁺, 437.1964; found, 437.1958.

1,2,3,4,6-Penta-O-acetyl-D-mannopyranose (19):

Acetic anhydride (4.0 mL, 41.6 mmol) was added dropwise to a stirred solution of D-Mannose 18 (1.0 g, 5.55 mmol) in anhydrous pyridine (5 mL) at 0 °C. The mixture was allowed to warm to room temperature and stirred overnight. Then the mixture was poured to ice-water and extracted with EtOAc. The organic phase was washed with saturated NaHCO₃ solution (2 × 50 mL) and 2 M H₂SO₄ (2 × 50 mL). Evaporation gives 19 (α and β mixture) as a white solid (1.99 g, 92%). ¹H NMR (300 MHz, CDCl₃) δ = 6.06 (d, J = 1.7 Hz, 1H), 5.84 (d, J = 1.0 Hz, 1H), 5.46 (dd, J = 1.0 3.3 Hz, 1H), 5.24-5.34 (m, 4H), 5.12 (dd, J = 3.3, 9.9 Hz, 1H), 4.23-4.32 (m, 2H), 4.12 (dd, J = 2.4, 9.1 Hz, 2H), 4.04-4.08 (m, 1H), 3.79 (dd, J = 2.4, 5.2, 9.9 Hz, 1H), 2.19 (s, 3H), 2.16 (s, 3H), 2.15 (s, 3H), 2.08 (s, 3H), 2.07 (s, 6H), 2.03 (s, 6H), 1.98 (s, 6H) ppm; ¹³C NMR (100 MHz, CDCl₃) δ = 170.6, 170.1, 169.9, 169.7 (2C), 169.5 (2C), 168.3, 168.0, 90.5, 90.3, 73.2, 70.6, 70.5, 68.7, 68.3, 68.1, 65.5, 65.3, 62.0 (2C), 20.8, 20.7 (3C), 20.6 (2C), 20.5 ppm; MS (m/z) 413 [M+Na]⁺. Data were in agreement with those reported in the literature.[⁷⁴]
2,3,4,6-Tetra-O-acetyl-α-D-mannopyranose (20):

\[
\text{AcO} \quad \text{OH} \\
\text{AcO} \quad \text{OAc} \\
\text{OAc}
\]

Compound 20 was prepared according to the same procedure as compound 8. The crude product was purified by flash column chromatography on silica gel (EtOAc/Hexane = 1:3 to 1:1) to give compound 20 as a foamy solid (0.83 g, 69%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ = 5.40 (dd, $J$ = 3.3, 9.9 Hz, 1H), 5.29 (d, $J$ = 9.9 Hz, 1H), 5.22-5.26 (m, 2H), 4.21-4.25 (m, 2H), 4.11-4.15 (m, 1H), 3.85 (d, $J$ = 4.2 Hz, 1H), 2.15 (s, 3H), 2.09 (s, 3H), 2.04 (s, 3H), 1.99 (s, 3H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ = 170.9, 170.2, 170.1, 169.8, 92.1, 70.0, 68.8, 68.4, 66.1, 62.5, 20.9, 20.7 (2C) ppm. Data were in agreement with those reported in the literature.$^{[70]}

2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyl trichloroacetimidate (21):

\[
\text{AcO} \quad \text{O} \quad \text{NH} \\
\text{AcO} \quad \text{OAc} \\
\text{OAc}
\]

Compound 21 was prepared according to the same procedure as compound 9. The crude product was then concentrated and purified by flash column chromatography on silica gel (EtOAc/hexane = 1:3) to give imidate 21 as colorless oil (0.73 g, 65%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ = 8.78 (s, 1H), 6.28 (s, 1H), 5.47 (s, 1H), 5.36-5.42 (m,
4-(1-Pyrenyl)butyl 2,3,4,6-tetra-O-acetyl-\(\alpha\)-D-mannopyranoside (22):

Compound 22 was prepared according to the same procedure as compound 12 and purified by flash column chromatography on silica gel (EtOAc/hexane = 1:5 to 1:1) to give a light yellow solid (0.25 g, 40%). mp 66-68 °C; \([\alpha]_D^{22} +35.2\) (c = 1, CHCl\(_3\)); \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta = 8.27\) (d, \(J = 9.2\) Hz, 1H), 8.11-8.18 (m, 4H), 7.97-8.05 (m, 3H), 7.88 (d, \(J = 7.8\) Hz, 1H), 5.36 (dd, \(J = 3.4\) Hz, 10 Hz, 1H), 5.24-5.29 (m, 2H), 4.80 (s, 1H), 4.24 (dd, \(J = 5.2\) , 12.2 Hz, 1H), 4.06 (dd, \(J = 2.2\) , 12.2 Hz, 1H), 3.94-3.98 (m, 1H), 3.73 (dt, \(J = 6.6, 9.6\) Hz, 1H), 3.50 (dt, \(J = 6.6, 9.6\) Hz, 1H), 3.39 (t, \(J = 7.6\)Hz, 2H), 2.15 (s, 3H), 2.05 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.91-1.97 (m, 2H), 1.77-1.82 (m, 2H) ppm; \(^13\)C NMR (125 MHz, CDCl\(_3\)) \(\delta = 170.6, 170.1, 169.9, 169.7, 136.3, 131.4, 130.9, 129.8, 128.6, 127.5, 127.3 (2C), 126.6, 125.8, 125.1, 125.0, 124.9, 124.8, 124.7, 123.3, 97.6, 69.7, 69.1, 68.4 (2C), 66.2, 62.5, 33.1, 29.2, 28.2, 20.9, 20.7 ppm; IR (neat) \(\nu = 2940, 1749, 1630, 1223, 1047, 847, 752\) cm\(^{-1}\); MS (m/z) 628 [M+Na]\(^+\); HRMS (ESI) calcd. for C\(_{34}\)H\(_{36}\)O\(_{10}\)Na [M+Na]\(^+\), 627.2206; found, 627.2202.

Part 2 Interfacing glycosylated carbon-nanotube-network devices with living cells to detect dynamic secretion of biomolecules
4-(1-Pyrenyl)butyl α-D-mannopyranoside (4):

![Chemical structure of 4](image)

Compound 4 was prepared according to the same procedure as compound 2 and obtained as a light yellow solid (38.1 mg, 84%). mp 98-99 °C; [α]_D^22 = +31.2 (c = 0.5, MeOH); ^1H NMR (500 MHz, CDCl_3) δ = 7.99-8.02 (m, 2H), 7.89-7.914 (M, 2H), 7.82-7.85 (m, 3H), 7.60 (d, J = 7.8 Hz, 1H), 5.11 (br s, 3H), 4.74 (s, 1H), 4.57 (br s, 1H), 3.85-3.95 (m, 4H), 3.72 (d, J = 11.1, 1H), 3.49-3.51 (m, 2H), 3.18-3.22 (m, 1H), 3.78 (t, J = 7.5 Hz, 2H), 1.64-1.67 (m, 2H), 1.49-1.51 (m, 2H) ppm; ^13C NMR (100 MHz, MeOD) δ = 136.2, 131.3, 130.7, 129.6, 128.4, 127.3, 127.1, 127.0, 126.4, 125.6, 124.9, 124.8, 124.7, 124.6 (2C), 123.1 100.0, 72.2, 71.6, 71.0, 67.6, 66.1, 60.9, 32.9, 29.2, 27.9 ppm; IR (neat) ν = 3412, 2087, 1638, 841, 752 cm⁻¹; MS (m/z) 455 [M+NH₄]^+; HRMS (ESI) calcd. for C_{26}H_{29}O_{6} [M+H]^+, 437.1964; found, 437.1969.

2-Acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-β-D-galactopyranose (24):

![Chemical structure of 24](image)

Compounjd 24 was prepared according to the same procedure as compound 7 (94% yield). ^1H NMR (400 MHz, CDCl_3) δ = 5.70 (d, J = 8.8 Hz, 1H), 5.39 (d, J = 9.5 Hz, 1H), 5.37 (d, J = 3.0 Hz, 1H), 5.08 (dd, J = 11.3, 3.3 Hz, 1H), 4.44 (q, J = 9.9 Hz, 1H), 3.74-3.77 (m, 4H), 3.47-3.51 (m, 3H), 2.02-2.05 (m, 6H), 2.00 (s, 3H), 1.86 (s, 3H), 1.21-1.23 (m, 4H) ppm; IR (neat) ν = 3412, 2087, 1638, 841, 752 cm⁻¹; MS (m/z) 645 [M+NH₄]^+; HRMS (ESI) calcd. for C_{30}H_{36}O_{10} [M+H]^+, 645.2514; found, 645.2519.
4.19-4.09 (m, 2H), 4.02 (t, $J = 6.5$ Hz, 1H), 2.17 (s, 3H), 2.13 (s, 3H), 2.05 (s, 3H), 2.02 (s, 3H), 1.94 (s, 3H) ppm. Data were in agreement with those reported in the literature.\cite{74}

2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-$\beta$-D-galactopyranose (25):

\[
\begin{align*}
\text{AcO} & \quad \text{O} & \quad \text{OH} \\
\text{AcO} & \quad \text{O} & \quad \text{NHAc}
\end{align*}
\]

Compound 25 was prepared according to the same procedure as compound 8 and obtained by column chromatography on silica gel (EtOAc/CH$_2$Cl$_2$ = 1:1 to EtOAc/CH$_2$Cl$_2$/MeOH = 50:40:1) as a colorless oil (0.62 g 83%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ = 6.12 (d, $J = 9.5$ Hz, 1H), 5.35 (d, $J = 2.6$ Hz, 1H), 5.27 (s, 1H), 5.24 (d, $J = 3.2$ Hz, 1H), 5.21 (d, $J = 3.2$ Hz, 1H), 4.88 (s, 1H), 4.51-4.45 (m, 1H), 4.41 (t, $J = 6.7$ Hz, 1H), 4.11-4.02 (m, 3H), 2.13 (s, 3H), 2.02 (s, 3H), 1.97 (s, 3H), 1.96 (s, 3H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ = 171.1, 170.8, 170.6, 170.4, 92.1, 68.0, 67.5, 66.4, 62.1, 48.1, 23.2, 20.8, 20.7 ppm; MS (m/z) 348 [M+H]$^+$; HRMS (ESI) calcd. for C$_{14}$H$_{21}$NO$_9$ [M+Na]$^+$, 370.1114; found, 370.1111. Data were in agreement with those reported in the literature.\cite{70}

2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy-$\alpha$-D-galactopyranosyl trichloroacetimidate (26):
Compound 26 was prepared according to the same procedure as compound 9 and obtained by column chromatography on silica gel (EtOAc/hexane = 1:5 to 1:1) as a light yellow porous solid (0.58 g, 72%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ = 8.77 (s, 1H), 6.39 (d, $J$ = 3.6 Hz, 1H), 5.62 (d, $J$ = 9.2 Hz, 1H), 5.47 (d, $J$ = 2.8 Hz, 1H), 5.27 (dd, $J$ = 11.5, 3.2 Hz, 1H), 4.81-4.75 (m, 1H), 4.34 (t, $J$ = 6.6 Hz, 1H), 4.16 (dd, $J$ = 11.3, 6.7 Hz, 1H), 4.05 (dd, $J$ = 11.3, 6.6 Hz, 1H), 2.17 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.94 (s, 3H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ = 171.0, 170.3, 170.2, 170.1, 160.3, 95.4, 90.9, 69.1, 67.9, 66.6, 61.3, 47.4, 23.1, 20.7, 20.6 (2C) ppm; HRMS (ESI) calcd. for C$_{16}$H$_{21}$N$_2$O$_9$Cl$_3$ [M+H]$^+$, 491.0391; found, 491.0402. Data were in agreement with those reported in the literature.[71]

4-(1-Pyrenyl)butyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-galactopyranoside (27):

Compound 27 was prepared according to the same procedure as compound 12 and obtained by column chromatography on silica gel (EtOAc/hexane = 1.5:1 to 2:1) as a
4-(1-Pyrenyl)butyl 2-acetamido-2-deoxy-β-D-galactopyranoside (5):

To a stirred solution of 27 (90.0 mg, 0.15 mmol) in methanol/CH₂Cl₂ (5 mL, v/v = 1.5: 1) was added sodium methoxide (1.1 mg, 0.02 mmol) at room temperature. The reaction mixture was stirred for 3 h and then neutralized with Amberlyst-15 resin followed by filtration. The filtrate was concentrated to afford compound 5 as a gray yellow powder (28.7 mg, 40%). mp >170 °C decomposed; ¹H NMR (400 MHz, MeOD) δ = 8.35 (d, J = 9.3 Hz, 1H), 8.19-8.12 (m, 4H), 8.06-7.97 (m, 3H), 7.91 (d, J
= 7.8 Hz, 1H), 4.37 (d, J = 8.4 Hz, 1H), 4.02-3.97 (m, 1H), 3.91 (dd, J = 10.5, 8.7 Hz, 1H), 3.81 (d, J = 3.0 Hz, 1H), 3.78-3.70 (m, 2H), 3.58-3.53 (m, 2H), 3.48-3.45 (m, 2H), 3.40-3.35 (m, 2H), 2.01-1.84 (m, 3H), 1.79-1.72 (m, 4H) ppm; $^{13}$C NMR (100 MHz, MeOD) $\delta$ = 172.5, 136.8, 131.5, 131.0, 128.5, 127.2 (2C), 127.0, 126.3, 125.6, 124.9, 124.8, 124.5, 124.4, 123.1, 97.4, 71.1, 69.1, 68.3, 67.5, 61.5, 50.3, 32.8, 29.2, 28.4, 21.3 ppm; IR (neat) $\nu$ = 3352, 2924, 2864, 1630, 1541, 1078, 837 cm$^{-1}$; MS (m/z) 478 [M+H]$^+$; HRMS (ESI) calcd. for C$_{28}$H$_{31}$NO$_6$ [M+H]$^+$, 478.2230; found, 478.2224.
5. REFERENCES


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*Part 2 Interfacing glycosylated carbon-nanotube-network devices with living cells to detect dynamic secretion of biomolecules*
PART 3

Propargyl-mediated Intramolecular Aglycon Delivery (IAD)
1. INTRODUCTION

1,2-cis-Mannosides (β-mannosides) play vital roles in cellular biology. Besides being important components in natural plant polysaccharides, they are also essential moieties in the many glycans and glyproteins of higher organisms.[1] Diverse oligosaccharides play the role of “glycocode” in cell signaling, recognition and communication. 1,2-cis-β-Mannosides exist in many biological glycoconjugates and oligosaccharides. For example, a β-mannose residue linkage to C-4 of GlcNAc is the core structure of asparagine-linked oligosaccharides, which are common in all N-linked glycoproteins. One role of mannosidases in cellular events is the reprogramming of N-glycan synthesis during metastatic process.[2] Some antibiotics and antigens, such as Everninomicin, also contains a β-mannose moiety.[3]

Efficient synthesis of oligosaccharides and glycoconjugates is important to facilitate studies on their impact on biological functions. Thus, glycosylation which leads to the synthesis of oligosaccharides is very important in carbohydrate chemistry. To establish absolute control of the anomeric stereochemistry during glycosylation, much investigation has been carried out on the usage of stereochemistry at the C-2 position on sugar skeletons. While 1,2-trans glycosidic bonds can be formed with high stereoselectivity by taking advantage of neighboring group participation at the C-2 position, the efficient formation of 1,2-cis glycosidic linkage, especially 1,2-cis mannose formation, is difficult and challenging in current carbohydrate chemistry. There are two major difficulties associated with the synthesis of β-mannosides. Firstly, axial configuration at the anomeric carbon is favored in the presence of an electronegative group due to the anomeric effect. Neighboring group participation of
mannose also results in the formation of α-mannoside. Secondly, β-face attack was disfavored due to the steric hindrance of the axially orientated C-2 functional groups.

In the past, 1,2-cis-mannosides were mainly synthesized through intermolecular reactions: (1) S$_2$N$_2$-like reaction with α-glycosyl halides by using insoluble silver salts,$^4$ (2) inversion reactions at C-2 of β-D-glucosides,$^5$ (3) activation of anomeric position with good leaving groups followed by nucleophilic substitution of glycosyl acceptors.$^6$ Lately, a more general method termed as intramolecular aglycon delivery (IAD), originally developed by Hindsgaul$^7$-9] and Stork$^{10}$ then further promoted by Ogawa et al.$^{11-15}$ has drawn much attention for its elegance and potential to become an important tool for the formation of 1,2-cis glycosidic bonds.$^{16-18}$ The glycosyl acceptor, which is also known as the aglycon in IAD, is first tethered to the 2-position of the glycosyl donor. Next, activation of the compound gives rise to the intramolecular delivery of the aglycon to the anomeric position of the glycosyl donor via a 5-membered ring transition state in an S$_2$N$_2$-like manner. A 1,2-cis glycosidic bond is thus formed in a stereoselective fashion (Scheme 1).

![Scheme 1](image.png)

**Scheme 1.** 1,2-cis-Mannoside formation through intramolecular aglycon delivery.
The first strategy reported by Hindsgaul and Barresi made use of a mixed acetal tether.\cite{7} Treatment of enol ether 1 with different alcohols in the presence of acid (CSA or TsOH) afforded mixed acetal, which was then treated with NIS to yield 1,2-cis-glycosides as the only products (Scheme 2). However this strategy was very sensitive to steric effects. Increasing the bulk of glycosyl acceptors or donors were found to significantly decrease the yields of mixed acetal. The addition of DTBMP, a hindered base, was found to increase glycosylation yields.

![Scheme 2](image)

**Scheme 2.** First IAD reaction reported by Hindsgaul’s group. Reagents and conditions: (a) ROH, TsOH, CH₂Cl₂, −40 °C, 10 min, 55-74%; (b) NIS, DTBMP, CH₂Cl₂, 0 °C to rt, 51-77%.

Stork is the pioneer who introduced silicon-tethered IAD. Mannosyl sulfoxides were used as glycosyl donors in their investigation (Scheme 3).\cite{10,19} Initially, the tethering procedure was conducted as follows: Dimethylchlorosilyl ethers derived from aglycon alcohols were linked to the hydroxyl group at C-2 of a mannosyl thioglycoside to give mixed silyl acetals, which were subsequently oxidised to the mannosyl sulfoxides. Treatment of the tethering intermediates with Tf₂O and DTBMP provided β-mannosides in exclusive stereoselectivity. Later, they optimized the tethering procedures after many investigations. Mannosyl sulfoxides were prepared before
tethering and the tethering step was accomplished by mixing equimolar amounts of glycosyl donor, aglycon alcohol and dimethyldichlorosilane. However, there remains a limitation in this method. When $O$-$4$ glucose or glucosamine acceptors were used, $(\beta 1\rightarrow 6)$-linked glycosides were obtained as the major product instead of the desired $(\beta 1\rightarrow 4)$-linked glycosides.

**Scheme 3.** Stork’s silicon-tethered IAD. Reagents and conditions: (a) ROSiMe$_2$Cl, imidazole, THF, rt, 10 min, quantitative; (b) $m$CPBA, CH$_2$Cl$_2$, $-25$ °C to $0$ °C; (c) Tf$_2$O, DTBMP, CH$_2$Cl$_2$, 4 Å M.S., $-80$ °C to rt, 61-73%; (d) ROH, Me$_2$SiCl$_2$, DMAP, imidazole, THF, $-78$ °C, 60-98%; (e) Tf$_2$O, DTBMP, CH$_2$Cl$_2$, 4 Å M.S., $-100$ °C to rt, 12-92%.

Oxidation of the PMB group would generate an oxycarbonium ion which could be captured by alcohols to give mixed acetals. With this concept in mind, Ogawa and Ito
introduced PMB at the C-2 position of a glycosyl donor as an IAD methodology.\cite{13, 20, 21} Mannosyl fluorides and methyl thioglycosides were employed as glycosyl donors in their investigation (Scheme 4). β-Mannosides were formed exclusively in all cases. They have successively synthesized a glycosphingolipid and many N-linked glycan compounds with this elegant strategy.

Scheme 4. PMB-mediated IAD developed by Ogawa and Ito. Reagents and conditions: (a) DDQ, ROH, CH₂Cl₂, 4 Å M.S.; (b) AgOTf, SnCl₂, DTBMP, Et₂O for 10; MeOTf, DTBMP, 4 Å M.S., CH₂Cl₂ or DCE for 11.

Later, the 2-naphthylmethyl (NAP) group was introduced to assist IAD due to the similar properties it shares with the PMB group. As shown in Scheme 5, glycosyl acceptor 16 was tethered to 2-O-NAP-protected mannose 15 and subsequently delivered to give β-mannoside 18 in 90% yield. In fact, NAP-assisted IAD showed a higher efficacy than that of PMB.
Scheme 5. NAP-assisted IAD for β-Mannopyranoside. Reagents and conditions: (a) DDQ, 4 Å M.S., DCE, rt, quantity; (b) i) MeOTf, DTBMP, DCE, rt; ii) Ac₂O, pyridine, 90%.

Fairbanks and coworkers have reported several interesting IAD methodologies which are based on the electrophilic tethering to enol ethers to generate mixed acetals,

similar to Hindsgaul’s method. Fairbanks and coworkers then developed an iodonium-mediated IAD system to overcome the difficulty. N-iodosuccinimide (NIS) was used for both tethering and activation steps and thiophenyl glycosides were used as glycosyl donors in their investigation (Scheme 6). All mannosides were produced in β-form and isolated in good yields. The yields were also found to be good when the reaction was conducted in a one-pot manner.
Scheme 6. Iodonium-mediated IAD developed by Fairbanks et al. Reagents and conditions: (a) ROH, NIS, THF, $-78 \, ^\circ\text{C}$ to rt, 66-95%; (b) NIS, DTBMP, CH$_2$Cl$_2$, 0 $^\circ\text{C}$ to rt, 63-100%.

Subsequently, Fairbanks et al. reported an allyl-mediated IAD method.$^{[24]}$ The 2-O-allyl group was first isomerized to 2-O-propenyl ether. When thioglycosides were used as glycosyl donors, IAD reaction proceeded smoothly with only simple aglycon alcohols. However, tethering was found to proceed sluggishly once the alcohol increases in bulk. Competition of intermolecular reaction is one of the major limitations associated with one-pot process. To overcome this problem, they have used glycosyl fluorides as alternative donors. The optimized IAD condition was then found to be a combination of SnCl$_2$, AgOTf and DTBMP in DCE at 50 $^\circ\text{C}$ (Scheme 7). It was also shown that tethering efficiency in the employment of hindered alcohols could be increased through the extending of reaction time.
Scheme 7. Allyl-mediated IAD developed by Fairbanks et al. Reagents and conditions: (a) Wilkinson’s catalyst, nBuLi, THF, 70 °C, 96%; (b) ROH, NIS, DCE, −40 °C to rt, 4 Å M.S., 37-99%; (b) AgOTf, DTBMP, SnCl2, DCE, 50 °C; then TFA, H2O or NIS, H2O, 49-75%.

To improve the moderate efficiency during the tethering of hindered alcohols, 2-O-vinyl thioglycosides were used as glycosyl donors by Fairbanks (Scheme 8).\textsuperscript{[25]} The tethering step was conducted in the presence of I2, AgOTf and DTBMP in CH2Cl2. The vinyl-mediated approach offers greater efficiency in the tethering of hindered aglycon to glycosyl donor as compared to the allyl-mediated system which in turn supersedes the original Hindsgaul system.\textsuperscript{[7]} However, this advantage is greatly compromised when the vinyl-mediated approach gives more difficult intramolecular glycosylation in comparison to both the allyl system and the Hindsgaul method, with the latter providing the easiest access to this glycosylation.
Scheme 8. Vinyl-mediated IAD developed by Fairbanks et al. Reagents and conditions: (a) ROH, I\(_2\), AgOTf, DTBMP, CH\(_2\)Cl\(_2\), −78 °C to rt, 60-86%; (b) I\(_2\), AgOTf, DTBMP, MeCN, −20 °C to rt, 52-79%.

This phenomenon was explained by the possible inductive effect on the stability of the carbocation produced after intramolecular glycosylation (Scheme 9).

Scheme 9. Intermediates in Hindsgaul’s, allyl- and vinyl-mediated IAD approaches
To increase the stability of the carbocation intermediate, 2-\(O\)-propargyl ether was used by Fairbanks et al. in a similar IAD strategy.\(^{[26, 27]}\) The advantage of this approach over the earlier methodologies was that the allylic carbocation produced after the intramolecular step could give another allylic cation by conjugation with the alkene group at the \(\alpha\)-position. Formation of these resonance structures could stabilize the carbocation intermediate (Scheme 10).

**Scheme 10.** Intermediates in Fairbanks’s allenyl-mediated IAD approach

The feasibility was investigated by the IAD reaction of compound 27. Propargyl ether 27 was first isomerized to allene 28, which was treated with \(I_2/AgOTf/DTBMP\) and glycosyl acceptor to give the mixed acetal in excellent yield. Subsequent IAD was conducted in the presence of \(Me_2S/Tf_2O/DTBMP\) to give the desired \(\beta\)-mannoside in excellent yield as well (Scheme 11). The result showed that allenyl-mediated IAD was able to improve both tethering and glycosylation efficiency. Fairbanks et al. successfully synthesized the core \(N\)-glycan pentasaccharide by using this strategy.
Scheme 11. Intermediates in Fairbanks’s allenyl-mediated IAD approach. Reagents and conditions: (a) KOtBu, Et₂O, 66%; (b) ROH, I₂, AgOTf, DTBMP, CH₂Cl₂, −78 °C to rt, 70-88%; (c) Me₂S, Tf₂O, DTBMP, CH₂Cl₂, 0 °C to rt, 32-81%.

Thus far, several acetal-mediated and silyl-mediated IAD methods have been reported. Many efforts have been made to improve both tethering and glycosylation efficiency. Protecting groups, promoters and solvents were important factors in IAD reactions. Despite the limitation on a few bulky glycosyl acceptors and the compromise of efficiency by application in convergent synthesis of oligosaccharides, IAD is nevertheless a good method for the preparation of various 1,2-cis-mannosides.
2. RESULTS AND DISCUSSION

Although propargyl-mediated IAD was reported by Fairbanks’s group, it is worthy to note that the propargyl group was converted to an allene group before tethering in their methodology. Thus, effectively speaking, it is only a platform for the formation of an allene group before any IAD is being executed. Therefore, we envisaged the greater capacity in the direct use of propargyl group in IAD.

Scheme 12. Intermediates in proposed propargyl-mediated IAD approach

In our proposed propargyl-mediated approach, there are three possible resonance structures for the intermediate formed after the intramolecular delivery step (Scheme 12). While we do not know if this stabilization effect of carbocation intermediate is as great as in the case of Fairbanks’s allenyl-mediated approach, we believe that efficient glycosylation is still achievable to produce satisfactory yield of β-mannosides. Furthermore, it was reported by Loh’s group that homopropargylic alcohols could be separated with excellent yield from allenic compounds via a simple separation procedure due to the formation of an insoluble silver acetylide complex. If we can take advantage of this special characteristic of propargyl functional group and apply it in the synthesis of oligosaccharides, it will be a breakthrough in the purification of oligosaccharides. After all, oligosaccharides, especially the larger and more highly
charged ones, are notorious for being hard to purify. Due to this limitation, difficulties are met in many areas, such as the study of their structure, mechanism and structure-activity relationship in medicinal chemistry. Consequently, enormous amount of money, time and effort are spent in purification of oligosaccharides. Hence, the vast but hidden potential in propargyl-mediated IAD in carbohydrate chemistry therefore deserves our efforts.

Scheme 13. Synthesis of compound 33. Reagents and conditions: (a) Ac$_2$O, HClO$_4$; (b) HBr, AcOH, 88% for two steps; (c) MeOH, 2,6-lutidine, CHCl$_3$, 74%; (d) anhydrous K$_2$CO$_3$, MeOH/CH$_2$Cl$_2$.

To kick start our investigation on IAD, we need to synthesize several glycosyl donors. Bromide 31 was first synthesized from d-mannose using a one-pot approach via the intermediate acetyl mannose 30. d-Mannose was treated with acetic anhydride and a catalytic amount of perchloric acid to give 30 before hydrogen bromide in acetic acid was added to yield 31. In the second step, both stereoisomers first gave a unique oxonium ion intermediate by releasing the activated leaving group at C-1. Intramolecular nucleophilic attack of neighboring C-2 acetyl group gave a second oxonium ion intermediate which was followed by the second nucleophilic attack of bromide from the $\alpha$-face to form a single stereoisomer 31. Treatment of 31 with 2,6-
lutidine and methanol in chloroform yielded the desired ortho ester 32. Subsequent removal of acetyl groups from 32 using anhydrous potassium carbonate in methanol and dichloromethane gave the deprotected ortho ester 33 (Scheme 13). Compound 33 is an important precursor for several glycosyl donors and aglycons.

\[ \text{Scheme 14. Synthesis of glycosyl donor 36 and 38. Reagents and Conditions: (a) NaH, BnBr, DMF, 69%; (b) DAST, CH}_2\text{Cl}_2, 0^\circ\text{C, overnight, 86%, (c) anhydrous K}_2\text{CO}_3, \text{MeOH, 72%, (d) p-thiocresol, DCE, reflux, 48 h, 58%; (e) anhydrous K}_2\text{CO}_3, \text{MeOH/CH}_2\text{Cl}_2, 64\%.} \]

Both thiomannoside and fluoro-mannoside were synthesized to be used as glycosyl donors. Etherification of 33 by treatment with benzyl bromide and sodium hydride in DMF gave O-benzyl ortho ester 34. Refluxing 34 with p-thiocresol in DCE for 48 h yielded the acetate 35. It was followed by deacetylation of 35 using anhydrous potassium carbonate in methanol and dichloromethane to afford the required alcohol 36 (Scheme 14). Treatment of 34 with DAST in CH\(_2\)Cl\(_2\) afforded 37, which was deprotected by K\(_2\)CO\(_3\) in MeOH to yield mannosyl donor 38.
In the IAD concept, the forward tethering approach involves the linker being initially attached to the glycosyl donor at the 2-position before the aglycon is coupled to the donor. In the reverse approach, the tethering linker is first attached to the aglycon at the position to be glycosylated before it is tethered to the donor which has a free hydroxy group at the 2-position (Figure 1).

Figure 1. Ito’s $p$-Methoxybenzyl-mediated IAD approach illustrating the difference between forward and reverse tethering.

The forward tethering method was attempted, in which the propargyl group was first linked to the glycosyl donor. Treatment of alcohol $36$ and $38$ with sodium hydride and propargyl bromide in DMF gave propargyl ethers $39$ and $40$ respectively (Scheme 15).
Scheme 15. Synthesis of compound 39 and 40. Reagents and conditions: propargyl bromide, NaH, DMF, 0 °C, 30 min, 88% for 39; 86% for 40.

We envisioned the tethering of glycosyl acceptors to the donor by radical reactions. The terminal alkyne was first protected by a TMS group to give 41. Compound 41 could be converted to bromide in the presence of NBS, Bz₂O₂ in CCl₄ to give bromide 42, which was then replaced by glycosyl acceptors. Unfortunately, this reaction yielded a complex mixture of products and as a result, the desired compound 42 could not be isolated (Scheme 16).

Scheme 16. Attempt to tethering mannosyl acceptor. Reagents and conditions: (a) nBuLi, TMSCl, THF, −78 to 0 °C, 3 h, 67%; (b) NBS, Bz₂O₂, CCl₄.

We even tried to prepare the mixed acetal with benzyl alcohol in stepwise manner. The propargyl group was firstly isomerized to allene 43. Unfortunately, treatment of allene 43 with NBS and BnOH in CH₂Cl₂ was unable to afford 44, which could be eliminated to give propargyl acetal 45 (Scheme 17).
Scheme 17. Synthetic route to compound 45. Reagents and conditions: (a) tBuOK, 18-crown-6, benzene, reflux, 5 h, 56%; (b) NBS, BnOH, acetone, CH₂Cl₂, −40 °C; (c) tBuOK, pentane, rt.

Alternative reverse tethering method was tried since the forward tethering was not smooth. 3,3-Diethoxy-1-propyne 46 was treated with 2,6-lutidine and TESOTf in CH₂Cl₂ at 0 °C for 1 h before 38 was added dropwise at the same temperature (Scheme 18).[29] However, we did not get our desired mixed acetal 47. We then synthesized (3,3-diethoxy-1-propynyl)trimethylsilane 48 to prevent any possible side reactions which might hinder the tethering.

Scheme 18. Attempt to synthesis of compound 47. Reagents and Conditions: (a) 2,6-lutidine, Et₃SiOTf, CH₂Cl₂.

Thioglycoside 38 and 48 were then used as substrates for the synthesis of corresponding acetal 49. We repeated the previous procedure using 48 and
delight, the desired compound 49 was formed as a mixture of diastereomers. Next, we tried to replace the ethoxy group with an alternative aglycon. We began the crucial tethering by first treating glycosyl donor 49 with 2,6-lutidine and triethylsilyl triflate in dichloromethane at 0 °C for 1 h. This is an attempt to remove the ethoxy group to give an electrophilic lutidinium salt intermediate, before benzyl alcohol was added dropwise to the reaction mixture at the same temperature. Unfortunately, we did not get the desired mixed acetal 50. We switched to a different method which made use of (S)-CSA as a catalyst. To our disappointment, tethered mixed acetal 50 was not formed. NMR data of products showed that sugar moiety was cleaved instead of the anticipated ethoxy group, affording (3-benzyloxy-3-ethoxy-1-propynyl)trimethyl silane (Scheme 19).

Scheme 19. Synthetic plan of compound 50. Reagents and Conditions: (a) 2,6-lutidine, TESOTf, CH₂Cl₂, 53%; (b) 2,6-lutidine, TESOTf, BnOH, CH₂Cl₂; or CSA, BnOH, CH₂Cl₂.

A reasonable explanation for the unsatisfactory results in both attempts is the great difficulty in inducing the selective removal of the ethoxy group in acetal 49, to form the required electrophilic lutidinium salt intermediate before any nucleophilic attack.
Part 3 Chapter 2 Results and discussion

by benzyl alcohol could possibly take place. This is in line with the fact that ethoxy, being a poor leaving group, requires harsh conditions for removal. The challenge is further complexed by the fact that the sugar moiety is such a bulky group and therefore likely to be preferentially cleaved. Hence, the desired electrophilic sugar-containing intermediate was not formed in both occasions and consequently spelt the absence of any desired mixed acetal 50.

![Scheme 20](attachment:image.png)

**Scheme 20.** The formation of compound 51. Reagents and Conditions: (a) NIS, DTBMP, 43%.

Fortunately, the outcome was positive when we used 49 as a tethered mixed acetal. An attempt to induce IAD was made by treating 49 with DTBMP and NIS in dichloromethane. To our delight, the ethoxy group was successfully delivered to the β anomeric position, forming a free hydroxy group at C-2 (Scheme 20). No α-isomer was observed in the reaction mixture. It marks the accomplishment of our first IAD β-mannoside 51 using the propargyl-mediated approach. While this positive result proves the feasibility of using (3,3-dialkoxy-1-propynyl)trimethylsilane as a tether in IAD strategy, amendments must be made since our aim is to efficiently deliver other aglycons of even higher complexity.

A crucial analytical consideration when constructing β-mannosides is the determination of the stereochemistry of the glycosidic linkage. Unlike in the cases of *gluco* and *galacto* pyranosides, we could not use the \( ^3J_{\text{H-1,H-2}} \) coupling constant
obtained in $^1$H NMR spectrum to determine the anomeric configuration of mannopyranoside 51. This is because $^3J_{H-1,H-2}$ coupling constant for both $\alpha$- and $\beta$-mannosides are approximately 1-2 Hz, due to the gauche relationship between H-1 and H-2 protons. Instead, we confirmed 51 to be a $\beta$-mannoside by measuring the $J_{C-1,H-1}$ coupling constant. It was reported that the $J_{C-1,H-1}$ value for $\alpha$-mannosides are usually $> 170$ Hz whereas that of $\beta$-mannosides are in the region of $< 160$ Hz.$^{[9]}$ The $J_{C-1,H-1}$ coupling constant obtained from $^{13}$C spectrum of 51 is 155.5 Hz.

![NOESY Spectrum of $\beta$-mannoside 51](image)

**Figure 2.** NOESY Spectrum of $\beta$-mannoside 51

To further verify the $\beta$ anomeric configuration of 51, we made use of Nuclear Overhauser Effect Spectroscopy (NOESY). From the spectrum, we can see strong signals between H-1 and H-2, H-1 and H-3, H-1 and H-5 very clearly. However, if the
configuration is α, it is not possible to observe the signals between H-1 and H-3 as well as H-1 and H-5 (Figure 2).

We also separated the acetal 49 mixture for the IAD reaction to test whether the efficiency of IAD is affected by the stereochemistry of the acetal (Figure 3). The configurations of (S)-49 and (R)-49 were determined by NOESY NMR spectra. Strong signal was observed between the acetal proton and H-1 of (S)-49, which was not observed in the spectrum of (R)-49. The IAD reaction of (S)-49 and (R)-49 were then conducted at the same conditions, providing compound 51 in 39% and 41% respectively. This result demonstrated that the stereochemistry of the mixed acetal did not seriously affect the efficiency of IAD.

![Figure 3. Chemical structures of (R)-49 and (S)-49.](image)

To date, the exact mechanism of IAD is still obscure and might vary according to several factors, such as the conditions and reagents used, the type of leaving group at C-1 of the glycosyl donor, the size of the aglycon and etc. However, it is believed to generally occur via a 5-membered ring transition state in a S_N2-like manner. This is not in terms of rate law since the reaction is clearly unimolecular. It is S_N2-like with reference to the mechanistic relationship. Overall, it is a backside attack of the inner nucleophile at C-1, inversing the anomeric configuration to afford a 1,2-cis glycoside.
We hereby propose two possible routes for the formation of β-mannoside 51 (Figure 4). When tethered mixed acetal 49 is treated with oxidant N-iodosuccinimide, the STol group at C-1 is activated to be a good leaving group. However, the actual expulsion of the leaving group can either take place in an S_N1 manner as shown in route 1 or in an S_N2-like manner with the simultaneous nucleophilic backside attack of the ethoxy group as reflected in route 2. Route 1 follows a S_N1 then S_N2-like sequence with the formation of oxonium ion intermediate A before the intramolecular nucleophilic attack of the ethoxy group at C-1 occurs to give oxonium ion intermediate B. On the other hand, the intramolecular delivery step in route 2 is a concerted process which affords oxonium ion intermediate B directly.
To investigate this mechanism, we repeated the IAD reaction of 49 with the addition of 1 eq. of allyl alcohol (Scheme 21). If there is no formation of allyl mannoside and the yield of 51 is not affected significantly, the synthesis could be proven to be indeed intramolecular and also via a concerted process as shown in route 2, where there is no formation of oxonium intermediate A for allyl alcohol to do a nucleophilic attack. On the contrary, formation of allyl mannoside suggests that the presence of alcohol in the reaction mixture can successfully compete with the intended intramolecular process. If that is the case, then the synthesis of 51 most likely took place via route 1 where the formation of oxonium ion intermediate A gives allyl alcohol an opportunity to attack anomeric C-1 from the α-face to afford α-mannoside. LCMS spectrum of the reaction mixture showed the presence of both intramolecular product 51 and intermolecular glycosylation product 52, which indicated that 51 was formed via proposed route 1.

Scheme 21. Aglycon delivery of 49 in the presence of allyl alcohol.
Scheme 22. Reverse tethering and propargyl-mediated IAD methodology.

The methodology of substituting the ethoxy group in a mixed acetal, e.g. compound 49, with different aglycons to form the desired tethered mixed acetal, seems to be problematic. However, the success in synthesizing compound 51 using (3,3-diethoxy-1-propynyl)trimethylsilane inspired us to come up with another strategy (Scheme 22) that could possibly help us avoid the shortcomings in Scheme 19. In this revised approach, we opt for reverse tethering, by synthesizing (3,3-dialkoxy-1-propynyl)trimethylsilane that contains the desired aglycon at both alkoxy positions. Next, it is to be tethered to a glycosyl donor in a fashion similar to step a in Scheme 19. This will bypass the problem of regioselective removal of ethoxy group in Scheme 19 since both alkoxy groups are identical. To investigate this potential method, (3-trimethylsilyl)-2-propynal 53 was synthesized as a precursor for different types of (3,3-dialkoxy-1-propynyl)trimethylsilane. We would like to use a variety of conditions and reagents to first succeed and then optimize this scheme.
Part 3 Chapter 2 Results and discussion

Scheme 23. Synthesis of compound 56. Reagents and Conditions: (a) TBSCl, imidazole, CH₂Cl₂, 82%; (b)NaH, propargyl bromide, DMF, 55%; (c) TBAF, THF, 85%.

While tethering strategies were being refined, we continue to synthesize and collect a library of different potential glycosyl donors and aglycons. Since the propargyl group of 49 was lost after delivering, compound 56 was synthesized to retain the propargyl group for easy purification. Precursor ortho ester 33 underwent regioselective silylation at the C-6 position using TBSCl and imidazole in dichloromethane to yield desired diol 54. Treatment with propargyl bromide and sodium hydride in dimethylformamide then afforded the fully protected ortho ester 55. Reacting 55 with TBAF in THF ensured selective deprotection giving us the desired primary alcohol 56 (Scheme 23). Since tethering reactions are sensitive to steric effect, primary alcohols should be more receptive to the coupling reactions in our IAD approach, as compared to their secondary alcohol structural isomers.
Scheme 24. Proposed synthesis of compound 58. Reagents and Conditions: (a) PPTS, CH$_2$Cl$_2$.

After we obtained 56, it was combined with (3-trimethylsilyl)-2-propynal 53 and treated with PPTS in dichloromethane in an attempt to form acetal 57. Although it was unsuccessful, we will continue to vary the conditions and reagents in hope of getting the desired product. This is because we conceptualize the reaction of 57 with a glycosyl donor, e.g. compound 38, to yield the desired mixed acetal 58 (Scheme 24). After which, we will be able to carry out intramolecular glycosylation to test the general practicability of the reversed tethering strategy proposed in Scheme 20.

We wanted to test the feasibility of forming a sugar-silver complex that can be separated and recovered easily. Hence, we followed the reported procedure by reacting 40 with silver nitrate and calcium carbonate in aqueous acetone. To our delight, a white silver acetylde precipitate 59 was indeed afforded. The precipitate was isolated.
by filtration before it was hydrolyzed with 1M hydrochloric acid. After a simple extraction, we successfully recovered 65% of propargyl ether 40 (Scheme 25).

![Scheme 25](image)

**Scheme 25.** Reversible silver salt formation from terminal alkyne. Reagents and Conditions: (a) AgNO₃, CaCO₃, acetone/H₂O; (b) 1M HCl, 75%.

A possible explanation for the partial loss of 40 is the hydrolysis of the STol group at C-1 in hydrochloric acid. In our future investigation, we will be aware of possible groups in oligosaccharides which are prone to hydrolysis under such acidic condition. While we do yearn for a higher yield, at least this attempt shows the potential in the easy purification of oligosaccharides via silver complex formation to propargyl groups. It is an attainable concept which deserves further exploration. We would also like to vary the position as well as the number of propargyl groups in sugar to investigate the effect of these factors on the efficiency of sugar-silver complex formation.
3. CONCLUSION AND FUTURE PLAN

We have shown the validity of (3,3-dialkoxy-1-propynyl)trimethylsilane as a tethering tool in IAD strategy and synthesized several potential glycosyl donors and aglycons. We have also explored the possibility of using propargyl group to obtain easy purification of oligosaccharides via the formation of silver acetylide complex.

In the future, we would like to find a general method to tether different glycosyl acceptors to the donor. Since ethoxy group is a poor leaving group in the forward tethering method, better leaving groups such as acetyl group will be introduced instead of ethoxy group. We also plan to protect 3-hydroxy group on the glycosyl donor with TMS group. Then, the 3-hydroxy group could potentially trap the transient alkyne carbocation intramolecularly after the delivery of the aglycon while the propargyl group will be kept for easy purification via the formation of silver complex.
4. EXPERIMENTAL SECTION

2,3,4,6-Tetra-O-acetyl-α-D-manno-\text{yranosyl} bromide (31)

0.1 mL of 70% perchloric acid was added to a suspension of D-mannose (0.10 g, 0.55 mmol) in acetic anhydride (3.9 mL, 41 mmol) at 0 °C. The remaining D-mannose (0.90 g, 4.95 mmol) were then added in small portions to the reaction mixture. The suspension was allowed to warm to room temperature and stirred until a clear yellow solution obtained. 33% hydrogen bromide in acetic acid (4.8 mL, 27.5 mmol) was added to it and the reaction mixture was stirred for 2 h. It was then diluted with CH₂Cl₂ and washed with ice water. The layers were separated and the organic layer was extracted with aqueous NaHCO₃ solution (× 2), dried over anhydrous Na₂SO₄, filtered and evaporated under reduce pressure to give crude 31 as a light yellow solid (2.00 g, 88%). ¹H NMR (400 MHz, CDCl₃): δ = 6.28 (s, 1H), 5.70 (dd, J = 10.1, 3.3 Hz, 1H), 5.44 (s, 1H), 5.33 (dd, J = 10.1 Hz, 1H), 4.31 (dd, J = 12.5, 4.8 Hz, 1H), 4.21 (dd, J = 10.1, 4.8 Hz, 1H), 4.13 (d, J = 12.5 Hz, 1H), 2.16 (s, 3H), 2.09 (s, 3H), 2.06 (s, 3H), 2.00 (s, 3H) ppm. Data were in agreement with those reported in the literature.³⁰

3,4,6-Tri-O-acetyl-1,2-O-(exo-methoxyethylidene)-β-D-mannopyranose (32)

Part 3 Propargyl-mediated intramolecular aglycon delivery (IAD)
Under an atmosphere of nitrogen, MeOH (4.1 mL, 10.4 mmol) was added to a stirred solution of 31 (1.00 g, 2.6 mmol) and 2,6-lutidine (0.45 mL, 3.9 mmol) in CHCl₃ (8 mL) for 2 h. Reaction mixture was dried in vacuum and then co-evaporated with toluene (× 2) to give brown syrup. It was then diluted with CH₂Cl₂ and washed with ice water. The layers were separated and the organic layer was extracted with aqueous solution of ammonium chloride and 10% HCl in the ration of 1:1 (× 2), dried over anhydrous sodium sulfate, filtered and evaporated under reduce pressure. Purification of the residue by column chromatography (EtOAc/Hexane = 1:3 to 1:1) on silica gel was carried out to afford 32 as a white solid (0.7 g, 74%). mp 106-108 °C (lit.[30] mp 110-111 °C); [α]D²⁰ = −18.8 (c = 1.0 in CHCl₃) (lit.[30] [α]D²⁰ = −26.8 (c = 1, CHCl₃));

^1^H NMR (400 MHz, CDCl₃): δ = 5.48 (d, J = 2.5 Hz, 1H), 5.30 ( t, J = 9.9 Hz, 1H), 5.13 (dd, J = 9.9, 4.0 Hz, 1H), 4.60 (dd, J = 4.0, 2.5 Hz, 1H), 4.33 (dd, J = 12.1, 4.9 Hz, 1H), 4.13 (dd, J = 12.1,2.6 Hz, 1H), 3.69-3.65 (m, 1H), 4.13 (dd, J = 12.1, 2.6 Hz, 1H), 3.27 (s, 3H), 2.11 (s, 3H), 2.06(s, 3H), 2.04 (s, 3H), 1.73 (s, 3H) ppm; ^1^C NMR (100 MHz, CDCl₃): δ = 170.6, 170.3, 169.4, 124.5, 97.3, 76.5, 71.3, 70.6, 65.4, 62.3, 49.9, 24.3, 20.7 (2C), 20.6 ppm; IR (neat): ν = 2900, 1746, 1373, 1231, 1047 cm⁻¹; MS (m/z) 385 [M+Na]⁺; HRMS (ESI): calcd. for C₁₅H₂₂O₁₀Na [M+Na]⁺, 385.1111; found, 385.1100.

1,2-O-(exo-methoxyethylidene)-β-D-mannopyranose (33)

Anhydrous K₂CO₃ (67.7 mg, 0.49 mmol) was added to a solution of 32 (1.76 g, 4.9 mmol) dissolved in MeOH (10 ml) and CH₂Cl₂ (5 mL). The reaction mixture was
stirred for 2 h before it was evaporated and dried under high vacuum to afford 33 as a foamy solid in quantitative yield. It was used for the next step without further purification. $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ = 5.52 (d, $J$ = 2.7 Hz, 1H), 4.53 (t, $J$ = 3.2 Hz, 1H), 3.90-3.70 (m, 4H), 3.37-3.33 (m, 1H), 3.31 (s, 3H), 2.70 (br s, 1H), 2.48 (br s, 1H), 2.14 (br s, 1H) ppm.

3,4,6-Tri-O-benzyl-1,2-O-(exo-methoxyethylidene)-β-D-mannopyranose (34)

Sodium hydride (0.32 g, 7.9 mmol) was added to a solution of 33 (0.49 g, 2.07 mmol) dissolved in dry DMF (10 mL) at 0 °C under an atmosphere of nitrogen. The resulting mixture was stirred for 1 h before benzyl bromide (0.82 mL, 7.9 mmol) was added dropwise at 0 °C. It was stirred for 2 h before it was diluted with Et$_2$O and then washed with ice water. The aqueous layer was extracted with Et$_2$O ($\times$ 2). The combined organic layers was dried, filtered and evaporated under vacuum to give crude product which was purified via column chromatography (EtOAc/Hexane = 1:8 to 1:4) to afford 34 as a white solid (0.72 g, 69%). mp 76-78 °C (lit.$^{[30]}$ mp 73-76 °C); [α]$_D^{20}$ = +36.2 (c = 1.0 in CHCl$_3$) (lit.$^{[30]}$ [α]$_D^{20}$ = +34.4 (c = 6.0 in CHCl$_3$)); $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ = 7.42-7.27 (m, 15H), 5.36 (d, $J$ = 2.4 Hz, 1H), 4.54-4.92 (m, 6H), 4.40 (t, $J$ = 3.2 Hz, 1H), 3.93 (t, $J$ = 9.3 Hz, 1H), 3.78-3.70 (m, 3H), 3.43 (dq, $J$ = 9.3, 2.4 Hz, 1H), 3.30 (s, 3H), 1.75 (s, 3H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ = 138.2, 137.8, 128.5, 128.4, 128.3, 128.0, 127.9, 127.7, 127.5, 124.0, 97.5, 79.0, 77.1, 75.2, 74.2, 74.1, 73.3, 72.3, 69.0, 49.7, 24.4 ppm; IR (neat): v = 2914, 2864, 1638,
1454, 1383, 1099, 1047, 735, 698 cm\(^{-1}\); MS (m/z) 529 [M+Na]\(^+\); HRMS (ESI): calcd. for C\(_{30}\)H\(_{34}\)O\(_7\)Na [M+Na]\(^+\), 529.2202; found, 529.2201.

**2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl fluoride (35)**

(Diethylamino)sulfur trifluoride (0.47 mL, 3.56 mmol) was added dropwise to a solution of 34 (1.2 g, 2.37 mmol) in dry CH\(_2\)Cl\(_2\) (20 mL) at 0 °C under N\(_2\). The mixture was stirred overnight at this temperature. The mixture was warmed to room temperature before diluting with ether. The resulting solution was washed with saturated NaHCO\(_3\) solution and brine. The organic phase was dried over Na\(_2\)SO\(_4\), filtered and concentrated. The residue was purified by column chromatography (EtOAc/hexane = 1:15 to 1:6) to give 35 as a colorless oil (0.96 g, 82%). \([\alpha]_D^{20} = +12.5 \ (c = 1.0 \text{ in CHCl}_3) \) (lit.\([24]\) \([\alpha]_D^{23} = +13.3 \ (c = 1.0 \text{ in CHCl}_3)\)); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta = 7.38-7.26 \text{ (m, 13H), 7.19-7.17 \text{ (m, 2H), 5.64 \ (d, } J = 49.2 \text{ Hz, 1H), 5.50 \ (s, 1H), 4.89 \ (d, } J = 10.8 \text{ Hz, 1H), 4.74 \ (d, } J = 11.2 \text{ Hz, 1H), 4.69 \ (d, } J = 12.2 \text{ Hz, 1H), 4.58 \ (d, } J = 11.2 \text{ Hz, 1H), 4.54 \ (d, } J = 11.2 \text{ Hz, 1H), 4.52 \ (d, } J = 10.8 \text{ Hz, 1H), 3.99 \ (s, 3H), 3.83 \ (dd, } J = 10. 8, 1.9 \text{ Hz, 1H), 3.73 \ (d, } J = 10.8 \text{ Hz, 1H), 2.18 \ (s, 3H) \text{ ppm; } ^{13}\text{C NMR (100 MHz, CDCl}_3\text{): } \delta = 170.0, 138.1, 137.9, 137.5, 128.4, 128.3 \ (2C), 128.0, 127.9, 127.8, 127.7 \ (2C), 105.5 \ (d, } J = 219.2 \text{ Hz), 77.2, 75.2, 73.7 \ (2C), 73.4, 73.3, 72.0, 68.1, 67.0 \ (d, } J = 39.8 \text{ Hz) ppm; IR (neat): } v = 3030, 2905, 2866, 1749, 1371, 1231, 1105, 737, 698 \text{ cm}^{-1}; \text{ MS (m/z) 517 [M+Na]}^{+}, 475 [M-F+H]^{+}; \text{ HRMS (ESI): calcd. for C}_{29}\text{H}_{33}\text{O}_{7}\text{FNa [M+Na]}^{+}, 517.2002; \text{ found, 517.1997.}
2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl fluoride (36)

Anhydrous K$_2$CO$_3$ (19.3 mg, 0.14 mmol) was added to a solution of 35 (0.70 g, 1.42 mmol) dissolved in MeOH (10 mL). The mixture was stirred at room temperature for 3 h. Solvent was removed under reduced pressure and the residue was purified by column chromatography (EtOAc/Hexane = 1:10 to 1:4) to give 36 as a colorless oil (0.46 g, 72%). $[\alpha]_D^{20} = +36.6$ ($c = 1.0$ in CHCl$_3$) (lit.$^{[24]}$ $[\alpha]_D^{22} = +15.3$ ($c = 1.25$ in CHCl$_3$)); $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ = 7.35-7.28 (m, 13H), 7.20-7.17 (m, 2H), 5.68 (d, $J$ = 49.4 Hz, 1H), 4.84 (d, $J$ = 10.8 Hz, 1H), 4.72 (dd, $J$ = 15.4, 11.6 Hz, 2H), 4.66 (d, $J$ = 12.2 Hz, 1H), 4.55 (d, $J$ = 10.8 Hz, 1H), 4.53 (d, $J$ = 12.2 Hz, 1H), 4.12 (s, 1H), 3.98-3.87 (m, 3H), 3.78 (dd, $J$ = 11.0, 3.5 Hz, 1H), 3.71 (d, $J$ = 11.0 Hz, 1H), 2.61 (s, 1H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ = 138.0, 137.9, 137.5, 128.6, 128.4 (2C), 128.1, 127.9 (3C), 127.8, 127.7, 107.2 (d, $J$ = 215.9 Hz), 79.1, 75.2, 73.5, 73.4, 73.3, 73.2, 72.4, 68.2, 67.2 (d, $J$ = 39.6 Hz) ppm; IR (neat): $\nu$ = 3437, 3030, 2909, 2868, 1497, 1454, 1365, 1175, 1099, 750, 698 cm$^{-1}$; MS (m/z) 475 (100%) [M+Na]$^+$, 433 [M-F+H]$^+$; HRMS (ESI): calcd. for C$_{27}$H$_{30}$O$_5$FNa [M+Na]$^+$, 475.1897; found, 475.1897.

4-Methylphenyl 2-O-acetyl-3,4,6-tri-O-benzyl-1-thio-α-D-mannopyranoside (37)
Part 3 Chapter 4 Experimental section

\( p \)-Thiocresol (0.2 g, 2.12 mmol) was added to a solution of 34 (0.715 g, 1.41 mmol) dissolved in DCE (5 mL). The reaction mixture was refluxed for 48 h under an atmosphere of nitrogen. It was cooled to room temperature before solvent was removed under reduced pressure. The residue was then purified via column chromatography (EtOAc/Hexane = 1:15 to 1:8) to afford 37 as a white solid (0.49 g, 58%). mp 81-83 °C (lit.[25] mp 77-78 °C); \([\alpha]_D^{23} = +88.9 \ (c = 1.0 \ \text{in CHCl}_3)\) (lit.[25] \([\alpha]_D^{25} = +109.0 \ (c = 1.0 \ \text{in CHCl}_3)\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta = 7.35-7.26 \ (m, 15\text{H}), 7.19 \ (d, J = 7.8 \text{ Hz}, 2\text{H}), 7.05 \ (d, J = 8.0 \text{ Hz}, 2\text{H}), 5.60 \ (s, 1\text{H}), 5.46 \ (s, 1\text{H}), 4.88 \ (d, J = 10.8 \text{ Hz}, 1\text{H}), 4.73 \ (d, J = 11.2 \text{ Hz}, 1\text{H}), 4.66 \ (d, J = 12.0 \text{ Hz}, 1\text{H}), 4.57 \ (d, J = 11.2 \text{ Hz}, 1\text{H}), 4.51 \ (d, J = 10.8 \text{ Hz}, 1\text{H}), 4.46 \ (d, J = 12.0 \text{ Hz}, 1\text{H}), 4.34 \ (d, J = 4.4 \text{ Hz}, 1\text{H}), 3.96-3.94 \ (m, 2\text{H}), 3.85 \ (dd, J = 10.4, 4.6 \text{ Hz}, 1\text{H}), 3.72 \ (d, J = 10.4 \text{ Hz}, 1\text{H}), 2.30 \ (s, 3\text{H}), 2.14 \ (s, 3\text{H}) \text{ ppm}; \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta = 170.3, 138.3, 138.2, 137.9, 132.3, 129.8, 128.5, 128.3, 128.2, 127.9, 127.7, 127.6, 127.5, 86.5, 78.5, 75.2, 74.6, 73.3, 72.4, 71.9, 70.3, 68.9 \text{ ppm};\) IR (neat): \(\nu = 3030, 2916, 2868, 1746, 1703, 1495, 1454, 1371, 1230, 1101, 743, 698 \text{ cm}^{-1};\) MS (m/z) 621 \([\text{M+Na}^+]\); HRMS (ESI): calcd. for \(C_{36}H_{38}O_6SNa\) \([\text{M+Na}^+]\), 621.2287; found, 621.2288.

\(4\)-Methylphenyl 3,4,6-tri-\(O\)-benzyl-1-thio-\(\alpha\)-D-mannopyranoside (38)

Anhydrous K\(_2\)CO\(_3\) (10.3 mg, 0.075 mmol) was added to a solution of 37 (0.47 g, 0.75 mmol) dissolved in MeOH (5 mL) and CH\(_2\)Cl\(_2\) (5 mL). The mixture was stirred at room temperature for 3 h. The solvent was removed under reduced pressure. The
Part 3 Chapter 4 Experimental section

Part 3 Propargyl-mediated intramolecular aglycon delivery (IAD)

residue was then purified via column chromatography (EtOAc/Hexane = 1:10 to 1:4) to give 38 as a colorless oil (0.12 g, 64%). \([\alpha]_D^{20} = +169.4 \ (c = 1.0 \ \text{in CHCl}_3) \) (lit.\[^{[25]}\]
\([\alpha]_D^{23} = +189.9 \ (c = 1.0 \ \text{in CHCl}_3))\); \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta = 7.38-7.26 \ (m, 15\text{H}), 7.23-7.21 \ (m, 2\text{H}), 7.06 \ (d, J = 8.0 \ \text{Hz}, 2\text{H}), 5.55 \ (s, 1\text{H}), 4.85 \ (d, J = 10.8 \ \text{Hz}, 1\text{H}), 4.72 \ (s, 2\text{H}), 4.62 \ (d, J = 12.0 \ \text{Hz}, 1\text{H}), 4.53 \ (d, J = 10.8 \ \text{Hz}, 1\text{H}), 4.45 \ (d, J = 12.0 \ \text{Hz}, 1\text{H}), 4.34-4.31 \ (m, 1\text{H}), 4.26 \ (d, J = 1.5 \ \text{Hz}, 1\text{H}), 3.96-3.88 \ (m, 2\text{H}), 3.80 \ (dd, J = 10.8, 4.7 \ \text{Hz}, 1\text{H}), 3.70 \ (dd, J = 10.8, 1.8 \ \text{Hz}, 1\text{H}), 2.65 \ (d, J = 2.6 \ \text{Hz}, 1\text{H}), 2.30 \ (s, 3\text{H}) \) ppm; \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta = 138.2, 137.7, 137.6, 132.2, 129.9, 129.8, 128.6, 128.4, 128.3, 128.1, 128.0, 127.9, 127.8, 127.7, 127.5, 87.6, 80.3, 75.2, 74.5, 73.3, 72.2, 72.1, 69.8, 68.9, 21.1 \) ppm; IR (neat): \(\nu = 3415, 3030, 2920, 2868, 1714, 1495, 1454, 1099, 750, 698 \ \text{cm}^{-1}\); MS (m/z) 574 [M+NH\(_4\)]\(^+\); HRMS (ESI): calcd. for C\(_{34}\)H\(_{36}\)O\(_5\)Na [M+Na]\(^+\), 579.2181; found, 579.2183.

2-O-propargyl-3,4,6-tri-O-benzyl-\(\alpha\)-D-mannopyranosyl fluoride (39)

NaH (15.0 mg, 0.36 mmol, 60% in mineral oil) and propargyl bromide (55.0 \(\mu\)L, 0.50 mmol) were added to a solution of 36 (0.15 g, 0.33 mmol) in dry DMF (5 mL) at 0 °C under N\(_2\). The mixture was stirred 30 mins at this temperature. The reaction mixture was then diluted with Et\(_2\)O and quenched with NH\(_4\)Cl solution. The resulting solution was separated. The organic phase was washed with water, brine, dried over Na\(_2\)SO\(_4\) and concentrated. The crude compound was purified by column chromatography (EtOAc/Hexane = 1:10) to give 39 as a colorless oil (0.15 g, 83%). \([\alpha]_D^{21} = +66.6 \ (c = \)
1.0 in CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ = 7.40-7.28 (m, 13H), 7.18-7.16 (m, 2H), 5.72 (dd, J = 50.3, 1.6 Hz, 1H), 4.87 (d, J = 10.7 Hz, 1H), 4.76 (dd, J = 23.3, 11.5 Hz, 2H), 4.67 (d, J = 12.2 Hz, 1H), 4.53 (d, J = 12.2 Hz, 1H), 4.51 (d, J = 10.7 Hz, 1H), 4.47-4.38 (m, 2H), 4.17 (s, 1H), 4.02-3.91 (m, 3H), 3.77 (dd, J = 11.0, 4.2 Hz, 1H), 3.70 (d, J = 11.0 Hz, 1H), 2.49 (t, J = 2.2 Hz, 1H) ppm; ¹³C NMR (100 MHz, CDCl₃): δ = 138.1, 138.0, 137.8, 128.5, 128.3, 127.9 (2C), 127.7, 127.6, 106.3 (d, J = 220.5 Hz), 79.3, 79.0, 75.3 (d, J = 18.4 Hz), 74.0, 73.8, 73.4, 72.8, 72.7, 72.4, 68.3, 58.7 ppm; IR (neat): ν = 3286, 2911, 2866, 2118, 1497, 1454, 1364, 1184, 1098, 750, 698 cm⁻¹; MS (m/z) 513 [M+Na]⁺, 471 [M-F+H]⁺; HRMS (ESI): calcd. for C₃₀H₃₁O₅FNa [M+Na]⁺, 513.2053; found, 513.2028.

4-Methylphenyl 2-O-propargyl-3,4,6-tri-O-benzyl-1-thio-α-D-mannopyranoside (40)

Prepared according to the same procedure as compound 39 and purified by column chromatography (EtOAc/Hexane = 1:6) to give 40 as a colorless oil (0.18 g, 86%). [α]D²³ = +187.0 (c = 1.0 in CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ = 7.43-7.26 (m, 15H), 7.22-7.20 (m, 2H), 7.07 (d, J = 8.0 Hz, 2H), 5.61 (s, 1H), 4.91 (d, J = 10.8 Hz, 1H), 4.82 (d, J = 11.5 Hz, 1H), 4.71 (d, J = 11.5 Hz, 1H), 4.65 (d, J = 12.0 Hz, 1H), 4.52 (d, J = 10.8 Hz, 1H), 4.48 (d, J = 12.0 Hz, 1H), 4.45-4.29 (m, 4H), 3.99 (t, J = 9.4 Hz, 1H), 3.92 (dd, J = 9.4, 2.9 Hz, 1H), 3.82 (dd, J = 10.8, 4.9 Hz, 1H), 3.74 (d, J = 10.8 Hz, 1H), 2.45 (t, J = 2.2 Hz, 1H), 2.32 (s, 3H) ppm; ¹³C NMR (100 MHz, CDCl₃):
CDCl$_3$): $\delta = 138.4, 138.3, 137.9, 137.6, 132.0, 130.4, 129.7, 128.4, 128.3, 128.2, 128.1,
127.9, 127.8, 127.7, 127.6, 127.4, 85.8, 79.9, 79.5, 75.3, 75.2, 74.9, 73.3, 72.5, 72.2,
69.0, 57.3, 21.1 ppm; IR (neat): $\nu = 3028, 2916, 2866, 2120, 1495, 1454, 1207, 1088,
737, 698$ cm$^{-1}$; MS (m/z) 617 [M+Na]$^+$; HRMS (ESI): calcd. for C$_{37}$H$_{38}$O$_5$SNa
[M+Na]$^+$, 617.2338; found, 617.2356.

2-O-(1-Trimethylpropargyl)-3,4,6-tri-O-benzyl-\(\alpha\)-D-mannopyranosyl fluoride (41)

\[ \text{BnO} \quad \text{O-F} \quad \text{BnO} \quad \text{O} \quad \text{O} \quad \text{TMS} \]

\text{nBuLi (0.14 mL, 0.22 mmol, 1.6 M solution in hexanes) was slowly added to a}
\text{solution of 39 (0.10 g, 0.20 mmol) in anhydrous THF (3 mL). The mixture was stirred}
\text{1 h at } -78 \degree C. Then TMSCl (28.4 } \mu\text{L, 0.23 mmol) was added. The mixture was stirred}
\text{for 2 h at } 0 \degree C \text{ before quenched with saturated NaHCO}_3 \text{ solution. The resulting}
\text{solution was extracted Et}_2\text{O. The organic phase was dried over Na}_2\text{SO}_4, \text{ evaporated}
\text{and purified by column chromatography (EtOAc/Hexane = 1:10) to give 41 as a}
colorless oil (0.18 g, 67\%). $[\alpha]_{D}^{23} = +68.4 \ (c = 1.0 \ \text{in CHCl}_3)$; $^1\text{H NMR (500 MHz,}
\text{CDCl}_3)$: $\delta = 7.41-7.28 \ (m, 13H)$, 7.17-7.15 (m, 2H), 5.75 (dd, $J = 50.2$, 1.6 Hz, 1H),
4.87 (d, $J = 10.8$ Hz, 1H), 4.81 (d, $J = 11.6$ Hz, 1H), 4.71 (d, $J = 11.6$ Hz, 1H), 4.67 (d,
$J = 12.2$ Hz, 1H), 4.53 (d, $J = 12.2$ Hz, 1H), 4.51 (d, $J = 10.8$ Hz, 1H), 4.46-4.39 (m,
2H), 4.21 (s, 1H), 4.01-3.90 (m, 3H), 3.78 (dd, $J = 11.0$, 4.4 Hz, 1H), 3.71 (dd, $J =
11.0$, 1.5 Hz, 1H), 0.20 (s, 9H) ppm; $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta = 138.1, 138.0,
137.8, 128.5, 128.3, 127.9 \ (3C)$, 127.8, 127.7, 127.6, 106.8 (d, $J = 220.3$ Hz), 101.1,
92.6, 78.8, 75.2, 73.9, 73.6 (d, $J = 42.8$ Hz), 72.4 (2C), 72.1, 68.4, 59.4 ppm; IR (neat):
\( \nu = 3030, 2901, 2866, 2173, 1454, 1250, 1184, 1098, 884, 737, 698 \text{ cm}^{-1} \); MS (m/z) 586 [M+Na]+; HRMS (ESI): calcd. for \( \text{C}_{33}\text{H}_{39}\text{O}_{5}\text{SiNa} \) [M+Na]+, 585.2449; found, 585.2444.

4-Methylphenyl 2-\( \text{O} \)-allenyl-3,4,6-tri-\( \text{O} \)-benzyl-1-thio-\( \alpha \)-\( \text{d} \)-mannopyranoside (43)

\[ \text{rtBuOK (9.1 mg, 0.08 mmol) and 18-crown-6 (5 mg) were added to a solution of 40 (0.16 g, 0.27mmol) in dry benzene. The mixture was heated at reflux for 5 h under N}_2. \]  
After cooling to room temperature, the solvent was removed under reduced pressure. The residue was flashed by column chromatography (EtOAc/Hexane = 1:15) to give 43 as a colorless oil (0.09 g, 56%). \( \alpha \)\( \text{D} \)\( _{25} \) = +126.4 (c = 1.0 in CHCl\text{3}); \( ^1\text{H} \) NMR (400 MHz, CDCl\text{3}): \( \delta = 7.41-7.21 \) (m, 15H), 7.06 (d, \( J = 8.2 \text{ Hz} \), 2H), 6.82 (t, \( J = 6.0 \text{ Hz} \), 2H), 5.55 (s, 1H), 5.42-5.37 (m, 2H), 4.92 (d, \( J = 10.8 \text{ Hz} \), 1H), 4.73-4.67 (m, 2H), 4.65 (d, \( J = 12.0 \text{ Hz} \), 1H), 4.54 (d, \( J = 10.8 \text{ Hz} \), 1H), 4.47 (d, \( J = 12.0 \text{ Hz} \), 1H), 4.29-4.27 (m, 2H), 4.02 (t, \( J = 9.4 \text{ Hz} \), 1H), 3.92 (dd, \( J = 9.4, 3.2 \text{ Hz} \), 1H), 3.82 (dd, \( J = 10.8, 4.8 \text{ Hz} \), 1H), 3.73 (dd, \( J = 10.8, 1.8 \text{ Hz} \), 1H), 2.31 (s, 3H) ppm; \( ^{13}\text{C} \) NMR (100 MHz, CDCl\text{3}): \( \delta = 200.4, 138.5, 138.4, 138.0, 137.9, 132.6, 130.3, 129.9, 128.6, 128.5, 128.4, 128.2, 128.1, 128.0, 127.9, 127.8, 127.6, 121.2, 92.2, 85.4, 79.2, 75.4, 75.3, 74.9, 73.4, 72.7, 72.3, 69.1, 21.2 ppm; IR (neat): \( \nu = 3030, 2914, 2866, 1952, 1495, 1454, 1192, 1101, 737, 696 \text{ cm}^{-1} \); MS (m/z) 595 [M+H]+; HRMS (ESI): calcd. for \( \text{C}_{37}\text{H}_{38}\text{O}_{5}\text{SnNa} \) [M+Na]+, 617.2338; found, 617.2336.
Methyl lithium (3.3 mL, 5.25 mmol, 1.6 M solution in Et₂O) was added dropwise to a solution of lithium bromide (0.46 g, 5.25 mmol) and 3,3-diethoxy-1-propyne (0.5 mL, 3.5 mmol) in THF (5 mL) at 0 °C under nitrogen protection. The mixture was stirred for 15 minutes before TMSCl (0.67 mL, 5.25 mmol) was added, followed by dry DMSO. The mixture was allowed to warm to room temperature and stirred for 3 h. Reaction mixture was diluted with Et₂O and then washed with ice water. The aqueous layer was extracted with Et₂O (× 2). The combined organic layers were dried, filtered and evaporated under vacuum to give crude product 48 as a colorless liquid (0.60 g, 71%). ¹H NMR (400 MHz, CDCl₃): δ = 5.23 (s, 1H), 3.74 (dq, J = 9.5, 7.1 Hz, 2H), 3.57 (dq, J = 9.5, 7.1 Hz, 2H), 1.23 (t, J = 7.1 Hz, 6H), 0.18 (s, 9H) ppm; ¹³C NMR (100 MHz, CDCl₃): δ = 100.0, 91.2, 90.3, 60.8, 15.1, –0.3 ppm.

4-Methylphenyl 3,4,6-tri-O-benzyl-2-(3-trimethylsilyl-1-ethoxy)propargyl-1-thio-α-D-mannopyranoside (49)

2,6-lutidine (0.15 ml, 1.26 mmol) and Et₃SiOTf (0.185 mL, 0.82 mmol) were added to a solution of acetal 48 (0.1 g, 0.42 mmol) dissolved in dry CH₂Cl₂ (4 mL) at 0 °C under an atmosphere of nitrogen. The reaction mixture was stirred for 1 h before a dissolved solution 38 (0.25 g, 0.46 mmol) in CH₂Cl₂ was added dropwise at 0 °C.
reaction mixture was then cooled to room temperature and stirred overnight. It was then diluted with CH$_2$Cl$_2$ and washed with aqueous solution of ammonium chloride and 10% HCl in the ratio of 1:1 (×3). The combined organic layers was dried over anhydrous sodium sulfate, filtered and evaporated under reduce pressure to give brown oil. The residue was then purified by column chromatography (EtOAc/Hexane = 1:30 to 1:8) to afford 49 as a colorless oil (0.158 g, 53%). ($S$)-49: $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ = 7.42-7.26 (m, 15H), 7.17 (d, $J = 5.4$ Hz, 2H), 7.02 (d, $J = 8.0$ Hz, 2H), 5.80 (s, 1H), 5.39 (s, 1H), 4.91 (d, $J = 10.7$ Hz, 1H), 4.83 (d, $J = 11.9$ Hz, 1H), 4.66-4.61 (m, 3H), 4.50 (dd, $J = 11.4$, 17.3 Hz, 2H), 4.31-4.28 (m, 1H), 3.99-3.91 (m, 2H), 3.90-3.81 (m, 2H), 3.73 (d, $J = 10.8$ Hz, 1H), 3.51 (dt, $J = 16.6$, 7.1 Hz, 1H), 2.28 (s, 3H), 1.08 (t, $J = 7.0$ Hz, 3H), 0.06 (s, 9H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ = 138.4 (2C), 138.2, 137.1, 131.3, 131.0, 129.6, 128.3 (2C), 128.2, 128.0, 127.9, 127.6, 127.5 (3C), 99.7, 91.7, 91.3, 87.2, 79.7, 75.1, 74.8, 73.3, 72.5, 72.3, 71.6, 69.2, 62.8, 21.0, 14.7, −0.5 ppm; IR (neat): $\nu$ = 3028, 2880, 2174, 1714, 1455, 1102, 737, 698 cm$^{-1}$; MS (m/z) 733 [M+Na]$^+$; HRMS (ESI): calcd. for C$_{42}$H$_{50}$O$_6$SSiNa [M+Na]$^+$, 733.2995; found, 733.2997. ($R$)-49: $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ = 7.42-7.26 (m, 15H), 7.21 (d, $J = 5.3$ Hz, 2H), 7.05 (d, $J = 8.0$ Hz, 2H), 5.70 (s, 1H), 5.52 (s, 1H), 4.89 (d, $J = 10.8$ Hz, 1H), 4.83 (d, $J = 11.5$ Hz, 1H), 4.75-4.74 (m, 1H), 4.61 (d, $J = 12.6$ Hz, 2H), 4.53 (d, $J = 10.8$ Hz, 1H), 4.48 (d, $J = 12.0$ Hz, 1H), 4.29 (dd, $J = 9.6$, 3.8 Hz, 1H), 3.8 (t, $J = 9.6$ Hz, 1H), 3.91-3.82 (m, 3H), 3.73 (d, $J = 9.6$ Hz, 1H), 3.57 (dt, $J = 9.6$, 7.0 Hz, 1H), 2.30 (s, 3H), 1.13 (t, $J = 7.0$ Hz, 3H), 0.17 (s, 9H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ = 138.5 (2C), 137.8, 137.4, 132.1, 130.7, 129.7, 128.4, 128.3, 128.2, 128.1, 128.0, 127.7, 127.6, 127.5, 127.3, 99.3, 92.4, 92.1, 87.5, 79.0, 75.2, 74.8, 73.1, 72.8, 72.6, 71.5, 69.2, 62.8, 21.1, 14.8, −0.3 ppm; IR (neat): $\nu$ = 3030,
2880, 2175, 1712, 1454, 1103, 737, 698 cm$^{-1}$; MS (m/z) 733 [M+Na]$^+$; HRMS (ESI): calcd. for C$_{42}$H$_{50}$O$_6$SSiNa [M+Na]$^+$, 733.2995; found, 733.2980.

3,4,6-Tri-O-benzyl-1-ethyl-β-D-mannopyranoside (51)

\[
\begin{align*}
\text{BnO} & \quad \text{OEt} \\
\text{BnO} & \quad \text{OEt} \\
\text{BnO} & \quad \text{OH} \\
\end{align*}
\]

NIS (63 mg, 0.28 mmol) was added to a solution of 49 (40 mg, 0.056 mmol) and DTBMP (0.068 mL, 0.28 mmol) in dry CH$_2$Cl$_2$ (3 mL) at 0 °C under nitrogen protection. The mixture was allowed to warm to room temperature and stirred overnight. It was then diluted with CH$_2$Cl$_2$ and washed with aqueous solution of ammonium chloride and 10% HCl in the ratio of 1:1 (× 3). The combined organic layers were dried over anhydrous sodium sulfate, filtered and evaporated under reduce pressure to give brown oil. The residue was then purified by column chromatography to afford 51 as a colorless oil (12 mg, 43%). \([\alpha]_D^{22} = -22.5\) (c = 0.65 in CHCl$_3$); $^1$H NMR (400 MHz, CDCl$_3$): $\delta = 7.39$-$7.19\) (m, 15H), 4.89 (d, $J = 11.9$ Hz, 1H), 4.78 (d, $J = 11.9$ Hz, 1H), 4.67 (d, $J = 11.9$ Hz, 1H), 4.63-4.52 (m, 3H), 4.43 (s, 1H), 4.09 (d, $J = 2.5$ Hz, 1H), 4.03-3.96 (m, 1H), 3.85 (t, $J = 9.4$ Hz, 1H), 3.78 (d, $J = 10.8$ Hz, 1H), 3.70 (dd, $J = 10.8, 5.3$ Hz, 1H), 3.63-3.55 (m, 2H), 3.44-3.41 (m, 1H), 2.34 (s, 1H), 1.25 (t, $J = 7.0$ Hz, 3H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta = 138.2$ (2C), 137.8, 128.5, 128.4, 128.3, 128.1, 127.9, 127.8 (2C), 127.7, 127.5, 99.5, 81.6, 75.2 (2C), 74.3, 73.5, 71.4, 69.3, 68.4, 65.1, 15.1 ppm; IR (neat): $\nu = 3470, 3028, 2866, 1713, 1452, 1108, 737, 698$ cm$^{-1}$; MS (m/z) 501 [M+Na]$^+$; HRMS (ESI) calcd. for C$_{29}$H$_{34}$O$_6$Na [M+Na]$^+$, 501.2253; found, 501.2249.

Part 3 Propargyl-mediated intramolecular aglycon delivery (IAD)
6-\textit{O-}tert-Butyldimethylsilyl-1,2-\textit{O-}(exo-methoxyethylidene)-\textbeta-D-mannopyranose (54)

![Chemical structure of 54](image)

TBSCl (0.82 g, 5.44 mmol) was added dropwise to a dissolved solution of 33 (1.26 g, 5.3 mmol), imidazole (0.74 g, 0.11 mmol) and DMAP (32.4 mg, 0.027 mmol) in dry DMF (10 mL) at 0 °C under an atmosphere of nitrogen. Reaction mixture was stirred overnight before it was diluted with Et₂O and then washed with ice water. The aqueous layer was extracted with Et₂O (× 2). The combined organic layers was dried, filtered and evaporated under vacuum to give crude product which was purified via column chromatography to afford 54 as colorless oil (1.52 g, 82%). ¹H NMR (400 MHz, CDCl₃): δ = 5.42 (d, J = 2.4 Hz, 1H), 4.48 (t, J = 3.1 Hz, 1H), 3.92 (dd, J = 10.6, 4.4 Hz, 1H), 3.84-3.73 (m, 3H), 3.51 (s, 1H), 3.27 (s, 3H), 3.25 (t, J = 4.4 Hz, 1H), 3.03 (d, J = 6.5 Hz, 1H), 1.65 (s, 3H), 0.87 (s, 9H), 0.07 (s, 6H) ppm; MS (m/z) 351 [M+H]⁺; HRMS (ESI) calcd. for C₁₅H₃₀O₇SiNa [M+Na]⁺, 373.1658; found, 373.1655.

6-\textit{O-}tert-Butyldimethylsilyl-3,4-di-\textit{O-}propargyl-1,2-\textit{O-}(exo-methoxyethylidene)-\textbeta-D-mannopyranose (55)

![Chemical structure of 55](image)
Sodium hydride (0.11 g, 2.71 mmol) was added to a solution of 54 (0.38 g, 1.08 mmol) dissolved in dry DMF (7 mL) at 0 °C under an atmosphere of nitrogen. The resulting mixture was stirred for 1 h before propargyl bromide (0.3 mL, 2.71 mmol) was added dropwise at 0 °C. It was stirred for 2 h before it was diluted with Et<sub>2</sub>O and washed with ice water. The aqueous layer was extracted with Et<sub>2</sub>O (× 2). The combined organic layers was dried, filtered and evaporated under vacuum to give crude product which was purified via column chromatography to afford 55 as a colorless oil (0.25 g, 55%). [α]<sub>D</sub><sup>23</sup> = +34.2 (c = 1.0 in CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ = 5.39 (d, J = 2.4 Hz, 1H), 4.58-4.43 (m, 4H), 4.33 (dd, J = 15.1, 2.4 Hz, 1H), 3.90-3.77 (m, 4H), 3.30 (s, 3H), 3.24 (d, J = 9.0 Hz, 1H), 2.45 (dt, J = 8.4, 2.4 Hz, 2H), 1.70 (s, 3H), 0.89 (s, 9H), 0.07 (s, 6H) ppm; IR (neat): ν = 3287, 2930, 2857, 2359, 2118, 1746, 1371, 1252, 1099, 837 cm<sup>-1</sup>; MS (m/z) 427 [M+H]<sup>+</sup>; HRMS (ESI) calcd. for C<sub>21</sub>H<sub>34</sub>O<sub>7</sub>SiNa [M+Na]<sup>+</sup>, 449.1972; found, 449.1972.

3,4-Di-O-propargyl-1,2-O-(exo-methoxyethylidene)-β-D-mannopyranose (56)

![3,4-Di-O-propargyl-1,2-O-(exo-methoxyethylidene)-β-D-mannopyranose (56)](image)

1M TBAF solution in THF (0.52 mL, 0.52 mmol) was added dropwise to a dissolved solution of 55 (0.11 g, 0.25 mmol) in THF (5 mL) and the reaction mixture was stirred at room temperature for 3 h. It was then evaporated under vacuum to give crude product which was purified by column chromatography to give 56 as colorless oil (66.1 mg, 85%). [α]<sub>D</sub><sup>23</sup> = +26.4 (c = 1.0 in CHCl<sub>3</sub>); <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>): δ = 5.43 (d, J = 2.3 Hz, 1H), 4.59 (t, J = 3.0 Hz, 1H), 4.43-4.33 (m, 4H), 3.70-3.90 (m, 4H), 2.45 (dt, J = 8.4, 2.4 Hz, 2H), 1.70 (s, 3H), 0.89 (s, 9H), 0.07 (s, 6H) ppm; IR (neat): ν = 3287, 2930, 2857, 2359, 2118, 1746, 1371, 1252, 1099, 837 cm<sup>-1</sup>; MS (m/z) 427 [M+H]<sup>+</sup>; HRMS (ESI) calcd. for C<sub>21</sub>H<sub>34</sub>O<sub>7</sub>SiNa [M+Na]<sup>+</sup>, 449.1972; found, 449.1972.
4-Methylphenyl 2-O-propargyl-3,4,6-tri-O-benzyl-1-thio-\(\alpha\)-D-mannopyranoside-silver complex (59)

A solution of \(\mathbf{40}\) (0.25 g, 0.424 mmol) in 2.5 mL of acetone was added dropwise to a solution of AgNO\(_3\) (85 mg, 0.50 mmol) and CaCO\(_3\) (50 mg, 0.50 mmol) in acetone/water (1.5 mL/1.5 mL) under nitrogen protection in the dark. It was then stirred at room temperature for 5 h before TLC check showed the complete consumption of \(\mathbf{40}\). The solvent was decanted and the residue white precipitate was treated with 1M of aq. HCl (3 mL) and stirred vigorously for 5 minutes before the reaction mixture was extracted with \(\text{Et}_2\text{O}\) (\(\times\) 2). The combined organic layers was dried, filtered and evaporated under reduce pressure to give back the starting material \(\mathbf{40}\) (0.16 g, 65%).
5. REFERENCES


Part 4

Recyclable Sulfonated Amorphous Carbon Catalyzed Friedel-Crafts Alkylation of Indoles with $\alpha,\beta$-Unsaturated Carbonyl Compounds in Water

1. INTRODUCTION

Indole derivatives are currently being explored as privileged structures for drug design and discovery due to their high binding affinity to many receptors.\[1\] Among them, 3-substituted indoles, which are important precursors in the synthesis of many biologically active compounds and natural products, have drawn the most attention.\[2\] They are known to be easily synthesized via Michael-type Friedel-Crafts (F-C) reactions of indoles with α,β-unsaturated carbonyl compounds, in the presence of either Brønsted or Lewis acids.\[3\] However, due to the homogeneous nature of these conventional acids, large stoichiometric amounts and tedious isolation procedures are required. In view of the increasing emphasis on the use of environmentally friendly processes, solid acid catalysts have been proposed as an attractive alternative to conventional liquid acid catalysts due to their simple work up, ease of recovery and reusability.\[4\] To date, several silica-supported solid acid catalysts have been developed and employed in the F-C reaction of indoles with electron-deficient olefin.\[3h, 5\] Other heterogeneous catalytic systems, which include nano TiO$_2$,\[6\] ZrOCl$_2·8$H$_2$O\[7\] and heteropolyacids\[3d, 8\], have also been reported. Unfortunately, their heterogeneous nature does not guarantee the consistency of catalytic performance after their recovery.\[3d, 5b, 8\] In fact, many of the silica-supported catalysts are mechanically unstable in water and consequently not reusable in reality.\[9\] Moreover, the use of transition metals and non-benign solvents still remains as an issue in many of these solid acid catalytic systems. A few years ago, Kobayashi and his co-workers developed a neutral catalytic system by combining silica-supported sodium sulfonate...
Part 4 Chapter 1 Introduction

with ionic liquid in water.\textsuperscript{[10]} While the neutral system allowed the Michael reaction of acid-labile substrates, the reaction time required was generally longer than that of other solid acid catalysts. Despite the recent advances, there is still a demand for other environmentally friendly and efficient alternatives for the F-C reactions of indoles with \(\alpha,\beta\)-unsaturated carbonyl compounds.

A novel carbon-based solid acid derived from carbohydrates has attracted considerable attention for its potential as an inexpensive, environmentally benign and stable catalyst with high catalytic performance since 2005.\textsuperscript{[11]} Consisting of flexible polycyclic carbon sheets which bear phenolic hydroxyl (-OH), carboxylic acid (-COOH) and sulfonic acid (-SO\(_3\)H) groups, the catalyst can be readily prepared by incomplete carbonization of natural carbohydrates, such as glucose, cellulose and starch, followed by sulfonation of the resulting amorphous carbon.\textsuperscript{[12]} Initially used as a green catalyst in the esterification of fatty acids in biodiesel production,\textsuperscript{[11c]} it was soon reported to demonstrate high catalytic efficiency in other reactions such as hydrolysis\textsuperscript{[12a]} and hydration.\textsuperscript{[12b]} Inspired by these reports, we envisaged the use of this carbon-based solid acid in catalyzing F-C alkylation to synthesize 3-substituted indole in an environmentally benign fashion.\textsuperscript{[3f, 12a, 13]} To the best of our knowledge, this is the first demonstration of carbon-based solid acid in catalyzing carbon-carbon bond formation.
2. RESULTS AND DISCUSSION

The sulfonated carbon-based solid acid 1a was prepared according to literature method (Figure 1).[11c] The elements content of 1a was determined by elemental analysis and the result obtained for sulfur content was 1.13 wt%. Since all sulfur atoms in the carbon material were in the form of SO$_3$H groups,[12b] the density of SO$_3$H groups in 1a was thus estimated to be 0.35 mmol/g. The solid acid powder was further characterized by X-ray diffraction (XRD). Two broad peaks are observed at 20 angles of 10°–33° and 35°–50° (Figure 2). The XRD pattern is in good agreement with literature,[12a, 12b] showing that the prepared solid acid is in the form of amorphous carbon.

![Figure 1. Schematic presentation for preparation of the sulfonated carbon-based solid acid 1a.](image)

![Figure 2. XRD profile for the sulfonated carbon based solid acid.](image)
The catalytic performance of 1a was first examined using the F-C reaction of indole with methyl vinyl ketone (MVK) as a model reaction. To our delight, the reaction took place smoothly in water\cite{3d, 14} at ambient temperature to give isolated product in excellent yield (Table 1, entry 1). Investigation shows that the reaction time required for complete conversion is inversely proportional to the amount of 1a loaded (Table 1, entries 1–4). However, along with an accelerated reaction rate, the yield remained consistent about 94% to 96% as the amount of 1a employed from 2 to 10 mol% (Table 1, entry 4). Thus, 5 mol% of 1a was determined to be the optimal amount of catalyst loading.

Next, phosphonated (1b) and perchlorated carbon solid acid (1c) were synthesized by treating the black amorphous carbon with concentrated phosphoric acid and perchloric acid respectively. They were investigated for their catalytic efficiencies in the model reaction. Less satisfactory results (Table 1, entries 5 and 6) were obtained than that of sulfonated 1a. This suggested that the presence of SO$_3$H groups was crucial to the high catalytic performance of the solid acid. To validate this hypothesis, a non-sulfonated black carbon, the precursor of 1a, was used in a control experiment. As anticipated, very low yield of product was obtained even after prolonged reaction time (Table 1, entry 7).

We also examined the solvent effect by screening different solvent systems (Table 1, entries 8–13). While the reaction exhibited the best yield in water, the use of solvent mixture H$_2$O/THF in the composition of 4:1 was found to afford comparably good yield as well (Table 1, entry 8). Other solvents provided less satisfactory results, ranging from low to moderate yields (Table 1, entries 9–13).
Table 1. Catalyst screening and reaction condition optimization.\[^{[a]}\]

![Chemical Structure](image.png)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Catalyst</th>
<th>Catalyst Solvent</th>
<th>Time [h]</th>
<th>Yield [%][^{[b]}]</th>
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<td>96</td>
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<tr>
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<td>1a</td>
<td>2.0 H₂O</td>
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<tr>
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<td>5.0 H₂O</td>
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<tr>
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<td>29 mg H₂O</td>
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<td>8[^{[d]}]</td>
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\[^{[a]}\] Indole (0.2 mmol) was reacted with MVK (0.4 mmol) in solvent (1 mL) at room temperature in the presence of the catalyst. \[^{[b]}\] Isolated yields after purification. \[^{[c]}\] The densities of the phosphoric and perchloric acid groups were not determined. \[^{[d]}\] H₂O/THF in the ratio of 4:1 was used.
Treatment of indole and MVK with 5 mol% of sulfuric acid as a liquid catalyst only brought forth small amount of Michael adducts (Table 1, entry 14). Even we raised the amount of sulfuric acid up to one equivalent, limited growth in the amount of product was observed, giving rise to only a moderate yield of product (Table 1, entry 15). These sluggish results show that 1a is a superior acid catalyst to its conventional homogenous counterpart.

Encouraged by the positive results from the preliminary studies, we investigated the efficiency of 1a in alkylation of indole and its derivatives with various α,β-unsaturated carbonyl compounds under optimized conditions. Most reactions proceeded smoothly in either H$_2$O or H$_2$O/THF solvent mixture to furnish the corresponding Michael adducts in moderate to excellent yields (Table 2). Moreover, the advantage of our catalytic system is that protection of the indole’s NH functional group is unnecessary.

In view of its environmental and economical advantages, water is the preferred solvent system in the course of our investigation. While some reactions were able to proceed smoothly in water to afford excellent yield, others were more sluggish as shown by their prolonged reaction time and the unsatisfactory yields obtained. Poor solubility of substrates and products in water, especially those of higher complexity, is probably the reason behind these results. We would then opt for an alternative solvent system of H$_2$O/THF in the composition of 4:1, which gave comparable good results in our earlier screening of solvents. As anticipated, a remarkable improvement in both the reaction time and isolated yield were observed when we switched to this solvent system.

The reaction of indole and its derivatives with MVK proceeded smoothly at ambient temperature in the presence of 5 mol% of solid acid catalyst 1a to give 85%–96% of...
isolated yield without side reactions of dimerization or polymerization (Table 2, entries 1–5). It is noteworthy that both electronic and architectural modification of the indole aromatic ring could be accomplished without compromising the good yield. The excellent yield (91%) could also be achieved in the Michael reaction of indole with 4-hexen-3-one (Table 2, entry 6). After all, the Michael acceptor employed is much more sterically hindered in comparison to MVK due to the elongation of the aliphatic chain. Reactions of various indoles derivatives with 4-hexene-3-one also gave satisfactory results (Table 2, entries 7–10).

**Table 2.** Michael-type Friedel-Crafts reactions of indole and derivatives with α,β-unsaturated carbonyl compounds[a]

\[
\begin{align*}
R^1 & \text{NH} + R^2 & \text{R}^3 \text{O} & 5 \text{ mol}\% 1a \\
{\text{H}_2\text{O} or \text{H}_2\text{O}/\text{THF} (4:1), r.t.} & \rightarrow & R^1 & \text{NH} \text{O} \text{R}^3 \\
\end{align*}
\]

<table>
<thead>
<tr>
<th>Entry</th>
<th>Michael Donor</th>
<th>Michael Acceptor</th>
<th>Product</th>
<th>Time [h]</th>
<th>Yield [%][b]</th>
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<td>13</td>
<td>1</td>
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<td>2[c, d]</td>
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<tr>
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</table>

Part 4 Recyclable sulfonated amorphous carbon catalyzed Friedel-Crafts alkylation of indoles with α,β–unsaturated carbonyl compounds in water
Part 4 Recyclable sulfonated amorphous carbon catalyzed Friedel-Crafts alkylation of indoles with α,β-unsaturated carbonyl compounds in water
Part 4 Recyclable sulfonated amorphous carbon catalyzed Friedel-Crafts alkylation of indoles with \(\alpha,\beta\)-unsaturated carbonyl compounds in water

To further validate the effectiveness of 1a in promoting the F-C reaction of indole with unreactive electron-deficient olefin, several Michael acceptors, which are notorious for their poor reactivity to nucleophilic attack were employed (Table 2, entries 11–15). Among them, chalcone and \(\beta\)-nitrostyrene gave satisfactory yield of Michael adducts (Table 2, entries 11 and 12). Other substrates such as \(\alpha,\beta\)-unsaturated ester, aldehydes and nitriles are found to be less appropriate for Michael reaction with indole under the same catalytic system.

[a] Unless specified, Michael donor (0.20 mmol) was reacted with Michael acceptor (0.22 mmol) in \(\text{H}_2\text{O}/\text{THF}\) (0.8 mL/0.2 mL) at room temperature in the presence of 1a (5 mol%). [b] Isolated yields after purification. [c] MVK (0.40 mmol) was used. [d] \(\text{H}_2\text{O}\) (1 mL) was used. [e] \(\text{H}_2\text{O}/\text{THF}\) (0.5 mL/0.5 mL) was used. [f] Conversion yield calculated based on indole.
Cyclic enones, such as 2-cyclopentenone and 2-cyclohexenone, are generally less reactive than acyclic enones. Therefore they tend to proceed sluggishly, affording low yield even under prolonged reaction time.\(^{[3h]}\) Catalyst 1a was demonstrated to be effective in promoting the F-C reaction of indole with 2-cyclopentenone. The reaction proceeded smoothly to give the desired monoindolyl cyclopentanone in a moderate yield of 72% (Table 2, entry 13). Complication was observed when 2-cyclohexenone was employed as the Michael acceptor, giving triindolycyclohexane as the major product (Table 2, entry 14). It is prone to overreact with indole under acidic conditions, making it difficult to obtain mono-substituted product with satisfactory yield. The regioselectivity was affected due to the competition between 1,4-addition and 1,2-addition of indole to 2-cyclohexenone.\(^{[15]}\) The difference of the formation of major product when using 2-cyclohexenone and 2-cyclopentenone as acceptors is accounted by torsional strain effect of their corresponding monoindolyl products.\(^{[16]}\) Nevertheless, the replacement of indole with 2-methylindole gave an increased regioselectivity of the Michael addition to 2-cyclohexenone. The corresponding 1,4-conjugated monoindoyl adduct was obtained as the major product in moderate yield of 67% (Table 2, entry 15).

To validate the reusability of 1a, 5 mol% catalyst was initially employed in the F-C reaction of indole (0.2 mmol) with MVK (0.4 mmol) in water (1 mL) for 1 h at room temperature. After recovery via a simple filtration and removal of volatile solvents under vacuum, the catalyst was reused in catalyzing the same model reaction without further activation. The reaction proceeded smoothly even after 5 runs, without any extension of reaction time or marked loss in yield (Figure 3). These positive results...
indicated that the sulfonated carbon-based solid acid 1a could be recycled up to 5 times with no noticeable loss in its catalytic efficiency.

![Figure 3](image-url)  
**Figure 3.** Catalytic performance of recovered 1a on F-C reaction of indole with MVK.
3. CONCLUSION

In conclusion, we have explored the efficiency of the carbon-based solid acid in catalyzing F-C reaction of indole and its derivatives with a variety of α,β-unsaturated carbonyl compounds in water or H\(_2\)O/THF solvents at ambient temperature. It is worthy to highlight the water-tolerant property of the carbon-based solid acid since it is capable of exhibiting consistent high catalytic activity without being poisoned by water\(^{[14]}\). Furthermore, the solid acid, derived from D-glucose which is accessible from nature in abundance, is considerably inexpensive. Most importantly, easy recovery of catalyst upon completion of reaction is achievable via simple filtration and the recovered catalyst is reusable up to 5 times without any significant loss in catalytic performance. All in all, this carbon-based solid acid catalytic system serves as an environmentally benign tool for the efficient synthesis of 3-substituted indole derivatives.
4. EXPERIMENTAL SECTION

**General:** All reagents and solvents were obtained from commercial suppliers (Sigma-Aldrich and Alfa Aesar) and used without further purification unless otherwise stated. Methyl vinyl ketone, 2-cyclopentenone and 2-cyclohexenone were distilled prior to use. Evaporation of organic solutions was achieved by rotary evaporation with a water bath temperature below 40 °C. Product purification by flash column chromatography was accomplished using silica gel 60 (0.010–0.063 mm). Chromatograms were visualized by fluorescence quenching with UV light at 254 nm or by staining using base solution of potassium permanganate. Technical grade solvents were used for chromatography and were distilled prior to use. NMR spectra were recorded at room temperature on 300 MHz Bruker ACF 300, 400 MHz Bruker DPX 400 spectrometers. The residual solvent signals were taken as the reference (7.26 ppm for $^1$H NMR spectra and 77.0 ppm for $^{13}$C NMR spectra in CDCl$_3$. Chemical shift ($\delta$) is reported in ppm, coupling constants ($J$) are given in Hz. The following abbreviations classify the multiplicity: br s = broad singlet, s = singlet, d = doublet, t = triplet, m = multiplet or unresolved. LCMS (ESI) spectra were recorded on Finnigan LCQ Deca XP MAX. HRMS (ESI) spectra were recorded on a Waters Q-Tof premier™ mass spectrometer. Elemental analysis was measured on Perkin Elmer Series II CHNS/O Analyzer 2400. Powder X-ray diffraction (XRD) was collected from Shimadzu 6000 diffractometer. Carbonization of D-glucose was carried out on Carbolite 1200 °C three zone tube furnace.
Part 4 Chapter 4 Experimental section

Preparation of carbon-based solid acid catalyst (1a):

D-Glucose powder (2 g) was heated at 400 °C for 15 h under N₂ flow to produce a black carbon solid. The solid was ground to fine powder and heated in 20 mL of conc. H₂SO₄ (>96%) at 150 °C under N₂. After heating for 15 h and then cooling to room temperature, the mixture was diluted with 100 mL of distilled water. The black precipitate was collected by filtration and washed repeatedly with hot distilled water (>80 °C) until pH 7 was observed in filtrate. The resulting black solid was then washed with methanol (30 mL) followed by diethyl ether (30 mL). It was further dried at 60 °C in vacuo prior to use. Elemental analysis revealed that sulfur content is 1.13 wt% which is equivalent to 0.35 mmol SO₃H per gram of catalyst.

A typical procedure for Michael-type Friedel-Crafts reaction of indoles with α,β-unsaturated carbonyl compounds:

To a stirred mixture of indole (23.4 mg, 0.20 mmol) and 1a (29.0 mg, 0.01 mmol) in H₂O (1 mL) or H₂O/THF (0.8 mL/0.2 mL), Michael acceptor (0.22 mmol) was added in one portion. Reaction mixture was stirred at room temperature and the progress of reaction was monitored by TLC checking. After completion of reaction, mixture was diluted with ethyl acetate (2 mL), filtered and washed successively with water (5 mL), ethyl acetate (3 × 5 mL) and diethyl ether (5 mL). Filtrate was collected and the organic layer was separated. The aqueous layer was extracted with ethyl acetate (3 × 5 mL). Combined organic layers were dried with Na₂SO₄, filtered, and concentrated under reduced pressure to afford the crude product, which was purified by column chromatography on silica gel to give the corresponding product. All compounds were
characterized on the basis of their spectroscopic data and by comparison with those reported in the literature. The remaining solid acid catalyst was dried under reduced pressure to remove all the volatile components, and then reused in the next run.

**4-(1H-Indol-3-yl)butan-2-one (13)**

![Image of 4-(1H-Indol-3-yl)butan-2-one (13)](image)

(Eluent: Hexane/EtOAc = 4:1, 96% yield); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ = 7.99 (br s, 1H), 7.60 (d, $J$ = 7.8 Hz, 1H), 7.36 (d, $J$ = 8.0 Hz, 1H), 7.20 (t, $J$ = 7.5 Hz, 1H), 7.13 (t, $J$ = 7.4 Hz, 1H), 6.99 (s, 1H), 3.06 (t, $J$ = 7.4 Hz, 2H), 2.86 (t, $J$ = 7.4 Hz, 2H), 2.15 (s, 3H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ = 208.8, 136.3, 127.1, 122.0, 121.4, 119.3, 118.6, 115.2, 111.1, 44.1, 30.0, 19.3 ppm; MS (m/z) 188 [M+H]$^+$; HRMS (ESI) calcd. for C$_{12}$H$_{14}$NO [M+H]+$^+$, 188.1075; found, 188.1082.

**4-(5-Bromo-1H-indol-3-yl)butan-2-one (14)**

![Image of 4-(5-Bromo-1H-indol-3-yl)butan-2-one (14)](image)
Part 4 Chapter 4 Experimental section

(Eluent: Hexane/EtOAc = 2:1, 89% yield); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ = 8.18 (br s, 1H), 7.70 (d, $J$ = 1.5 Hz, 1H), 7.28-7.18 (m, 2H), 6.97 (d, $J$ = 2.1Hz, 1H), 2.99 (t, $J$ = 7.2 Hz, 2H), 2.82 (t, $J$ = 7.2 Hz, 2H), 2.15 (s, 3H) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ = 208.6, 134.8, 128.9, 124.7, 122.8, 121.2, 114.8, 112.6, 112.5, 43.8, 30.0, 19.0 ppm; MS (m/z) 188 [M-Br+H]$^+$; HRMS (ESI) calcd. for C$_{12}$H$_{13}$NOBr [M+H]$^+$, 266.0181; found, 266.0174.

4-(2-Methyl-1H-Indol-3-yl)butan-2-one (15)

(Eluent: Hexane/EtOAc = 4:1, 90% yield); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ = 7.80 (br s, 1H), 7.50-7.47 (m, 1H), 7.27-7.24 (m, 1H), 7.16-7.06 (m, 2H), 2.99 (t, $J$ = 7.4 Hz, 2H), 2.78 (t, $J$ = 7.4 Hz, 2H), 2.38 (s, 3H), 2.11 (s, 3H) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ = 209.0, 135.3, 131.1, 128.2, 120.9, 119.1, 117.7, 110.4, 110.2, 44.1, 30.2, 18.4, 11.5 ppm; MS (m/z) 202 [M+H]$^+$; HRMS (ESI) calcd. for C$_{13}$H$_{16}$NO [M+H]$^+$, 202.1232; found, 202.1194.

4-(7-Methyl-1H-indol-3-yl)butan-2-one (16)
Part 4 Recyclable sulfonated amorphous carbon catalyzed Friedel-Crafts alkylation of indoles with $\alpha,\beta$-unsaturated carbonyl compounds in water

4-(1-Methyl-1H-indol-3-yl)butan-2-one (17)

(Eluent: Hexane/EtOAc = 8:1, 88% yield); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ = 7.59 (d, $J$ = 8.1 Hz, 1H), 7.32-7.22 (m, 2H), 7.15-7.10 (m, 1H), 6.85 (s, 1H), 3.74 (s, 3H), 3.06 (t, $J$ = 7.4 Hz, 2H), 2.85 (t, $J$ = 7.4 Hz, 2H), 2.15 (s, 3H) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ = 208.7, 137.0, 127.5, 126.3, 121.5, 118.7, 118.6, 113.6, 109.2, 44.3, 32.5, 30.0, 19.2 ppm; MS (m/z) 202 [M+H]$^+$; HRMS (ESI) calcd. for C$_{13}$H$_{16}$NO [M+H]$^+$, 202.1232; found, 202.1230.
5-((1H-Indol-3-yl)hexan-3-one (18)

![Structure of 5-((1H-Indol-3-yl)hexan-3-one](image)

(Eluent: Hexane/EtOAc = 6:1, 91% yield); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ = 8.11 (br s, 1H), 7.67 (d, $J = 7.5$ Hz, 1H), 7.35 (d, $J = 7.8$ Hz, 1H), 7.21 (t, $J = 7.1$ Hz, 1H), 7.14 (t, $J = 7.5$ Hz, 1H), 6.95 (d, $J = 1.8$ Hz, 1H), 3.74-3.62 (m, 1H), 2.94 (dd, $J = 15.9$, 6.0 Hz, 1H), 2.71 (dd, $J = 15.9$, 8.3 Hz, 1H), 2.39 (q, $J = 7.2$ Hz, 2H), 1.40 (d, $J = 6.9$ Hz, 3H), 1.02 (t, $J = 7.2$ Hz, 3H) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ = 211.4, 136.5, 126.2, 121.9, 121.0, 120.1, 119.1 (overlapped), 111.2, 50.2, 36.4, 27.0, 21.2, 7.6 ppm; MS (m/z) 216 [M+H$^+$]; HRMS (ESI) calcd. for C$_{14}$H$_{18}$NO [M+H$^+$], 216.1388; found, 216.1378.

5-((5-Bromo-1H-indol-3-yl)hexan-3-one (19)

![Structure of 5-((5-Bromo-1H-indol-3-yl)hexan-3-one](image)

(Eluent: Hexane/EtOAc = 6:1, 81% yield); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ = 8.05 (br s, 1H), 7.76 (s, 1H), 7.28-7.20 (m, 2H), 6.97 (d, $J = 2.1$ Hz, 1H), 3.64-3.53 (m, 1H), 2.87 (dd, $J = 15.9$, 6.0 Hz, 1H), 2.68 (dd, $J = 15.9$, 8.1 Hz, 1H), 2.37 (q, $J = 7.2$ Hz, 2H), 1.35 (d, $J = 6.9$ Hz, 3H), 1.00 (t, $J = 7.2$ Hz, 3H) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta =$ Part 4 Recyclable sulfonated amorphous carbon catalyzed Friedel-Crafts alkylation of indoles with $\alpha,\beta$-unsaturated carbonyl compounds in water
210.9, 135.1, 128.0, 124.8, 121.7, 121.4, 120.9, 112.7, 112.5, 50.0, 36.4, 26.9, 21.2, 7.7 ppm; MS (m/z) 294 [M+H]+; HRMS (ESI) calcd. for C_{14}H_{17}NOBr[M+H]^+, 294.0494; found, 294.0495.

5-(2-Methyl-1H-indol-3-yl)hexan-3-one (20)

(Eluent: Hexane/EtOAc = 8:1, 86% yield); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ = 7.82 (br s, 1H), 7.66-7.63 (m, 1H), 7.26-7.24 (m, 1H), 7.14-7.05 (m, 2H), 3.66-3.54 (m, 1H), 3.03 (dd, $J$ = 15.8, 7.7 Hz, 1H), 2.82 (dd, $J$ = 15.8, 6.8 Hz, 1H), 2.38 (s, 3H), 2.35-2.12 (m, 2H), 1.44 (d, $J$ = 6.9 Hz, 3H), 0.93 (t, $J$ = 7.4 Hz, 3H) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ = 211.5, 135.5, 130.4, 126.9, 120.5, 118.9, 118.8, 115.0, 110.5, 49.1, 36.6, 27.2, 21.1, 11.9, 7.6 ppm; MS (m/z) 230 [M+H]^+; HRMS (ESI) calcd. for C$_{15}$H$_{20}$NO [M+H]^+, 230.1545; found, 230.1540.

5-(7-Methyl-1H-indol-3-yl)hexan-3-one (21)

Part 4 Recyclable sulfonated amorphous carbon catalyzed Friedel-Crafts alkylation of indoles with $\alpha,\beta$-unsaturated carbonyl compounds in water
(Eluent: Hexane/EtOAc = 8:1, 82% yield); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ = 7.97 (br s, 1H), 7.52 (d, $J$ = 7.8 Hz, 1H), 7.09-6.97 (m, 3H), 3.72-3.60 (m, 1H), 2.93 (dd, $J$ = 15.9, 6.0 Hz, 1H), 2.70 (dd, $J$ = 15.9, 8.4 Hz, 1H), 2.48 (s, 3H), 2.38 (q, $J$ = 7.2 Hz, 2H), 1.39 (d, $J$ = 6.9 Hz, 3H), 1.02 (t, $J$ = 7.2 Hz, 3H) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ = 211.3, 136.1, 125.7, 122.5, 121.6, 120.4, 119.9, 119.4, 116.9, 50.2, 36.4, 27.2, 21.2, 16.6, 7.7 ppm; MS (m/z) 230 [M+H]$^+$; HRMS (ESI) calcd. for C$_{15}$H$_{20}$NO [M+H]$^+$, 230.1545; found, 230.1540.

5-(1-Methyl-1H-indol-3-yl)hexan-3-one (22)

(Eluent: Hexane/EtOAc = 12:1, 80% yield); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ = 7.65 (d, $J$ = 7.8 Hz, 1H), 7.32-7.21 (m, 2H), 7.12 (dd, $J$ = 7.8, 6.9 Hz, 1H), 6.84 (s, 1H), 3.74 (s, 3H), 3.72-3.60 (m, 1H), 2.92 (dd, $J$ = 15.9, 6.0 Hz, 1H), 2.69 (dd, $J$ = 15.9, 8.4 Hz, 1H), 2.38 (q, $J$ = 7.2 Hz, 2H), 1.39 (d, $J$ = 6.9 Hz, 3H), 1.02 (t, $J$ = 7.2 Hz, 3H) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ = 211.1, 137.1, 126.6, 125.0, 121.5, 119.6, 119.2, 118.6, 109.3, 50.4, 36.4, 32.5, 27.0, 21.4, 7.6 ppm; MS (m/z) 230 [M+H]$^+$; HRMS (ESI) calcd. for C$_{15}$H$_{20}$NO [M+H]$^+$, 230.1545; found, 230.1541.
3-(1H-Indol-3-yl)-1,3-diphenylpropan-1-one (23)

(Eluent: Hexane/EtOAc = 6:1, 78% yield); $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ = 7.99 (br s, 1H), 7.95-7.93 (m, 2H), 7.55 (t, $J$ = 7.2 Hz, 1H), 7.46-7.41 (m, 3H), 7.35 (t, $J$ = 7.4 Hz, 2H), 7.30-7.24 (m, 3H), 7.19-7.13 (m, 2H), 7.05-6.98 (m, 2H), 5.09 (t, $J$ = 7.2 Hz, 1H), 3.84 (dd, $J$ = 16.5, 6.9 Hz, 1H), 3.74 (dd, $J$ = 16.5, 7.7 Hz, 1H) ppm; $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ = 198.6, 144.2, 137.1, 136.6, 133.0, 128.5, 128.4, 128.1, 127.8, 126.8, 126.3, 122.1, 121.4, 119.5, 119.3, 119.2, 111.1, 45.2, 38.2 ppm; MS (m/z) 326 [M+H]$^+$; HRMS (ESI) calcd. for C$_{23}$H$_{20}$NO [M+H]$^+$, 326.1545; found, 326.1539.

3-(1-Phenyl-2-nitroethyl)-1H-indole (24)

(Eluent: Hexane/EtOAc = 6:1, 76% yield); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ = 8.07 (br s, 1H), 7.47 (d, $J$ = 8.0 Hz, 1H), 7.36-7.27 (m, 6H), 7.22 (t, $J$ = 7.5 Hz, 1H), 7.10 (t, $J$ = 7.5 Hz, 1H), 7.00 (s, 1H), 5.21 (t, $J$ = 8.0 Hz, 1H), 5.07 (dd, $J$ = 12.5, 7.7 Hz, 1H), 4.95 (dd, $J$ = 12.5, 8.4 Hz, 1H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ = 139.1, 136.4, 128.9, 127.7, 127.5, 126.0, 122.6, 121.6, 119.9, 118.9, 114.3, 111.4, 79.5, 41.5 ppm;

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Part 4 Recyclable sulfonated amorphous carbon catalyzed Friedel-Crafts alkylation of indoles with α,β-unsaturated carbonyl compounds in water

3-(1H-Indol-3-yl)cyclopentanone (25)

\[
\text{N}H\quad O
\]

(Eluent: Hexane/EtOAc = 4:1, 72% yield); \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \(\delta = 8.12\) (br s, 1H), 7.65 (d, \(J = 7.9\) Hz, 1H), 7.39 (d, \(J = 8.1\) Hz, 1H), 7.24 (dt, \(J = 8.1, 1.1\) Hz 1H), 7.15 (dt, \(J = 7.9, 0.8\) Hz, 1H), 6.99 (d, \(J = 2.2\) Hz, 1H), 3.77-3.69 (m, 1H), 2.78 (dd, \(J = 18.2, 7.8\) Hz, 1H), 2.58-2.50 (m, 1H), 2.49-2.45 (m, 1H), 2.44-2.41 (m, 1H), 2.38-2.29 (m, 1H), 2.20-2.12 (m, 1H) ppm; \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}) \(\delta = 219.5, 136.7, 126.6, 122.3, 119.9, 119.4, 119.0, 118.5, 111.3, 45.3, 38.1, 33.7, 29.8\) ppm; MS (m/z) 199 [M]\(^{+}\); HRMS (ESI) calcd. for C\textsubscript{13}H\textsubscript{14}NO [M+H]\(^{+}\), 200.1075; found, 200.1074.

3-(1H-Indol-3-yl)cyclohexanone (26)

\[
\text{N}H\quad O
\]

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Part 4 Recyclable sulfonated amorphous carbon catalyzed Friedel-Crafts alkylation of indoles with \( \alpha,\beta \)-unsaturated carbonyl compounds in water

(Eluent: Hexane/EtOAc = 6:1, 12% yield); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta = \) 8.03 (br s, 1H), 7.63 (d, \( J = 7.8 \) Hz, 1H), 7.38 (d, \( J = 8.1 \) Hz, 1H), 7.21 (dt, \( J = 7.6, 1.1 \) Hz, 1H), 7.13 (dt, \( J = 7.5, 1.1 \) Hz, 1H), 6.99 (d, \( J = 1.8 \) Hz, 1H), 3.49-3.42 (m, 1H), 2.85-2.77 (m, 1H), 2.64 (ddd, \( J = 14.0, 10.6, 1.1 \) Hz, 1H), 2.48-2.40 (m, 2H), 2.30-2.25 (m, 1H), 2.10-1.81 (m, 3H) ppm; \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \( \delta = \) 211.8, 136.4, 126.1, 122.2, 120.3, 119.7, 119.4, 119.0, 111.3, 48.1, 41.6, 35.9, 31.7, 24.9 ppm; MS (m/z) 214 [M+H]\(^+\); HRMS (ESI) calcd. for C\(_{14}\)H\(_{16}\)NO [M+H]\(^+\), 214.1232; found, 214.1224.

1,1,3-Tris(1H-Indol-3-yl)cyclohexene (27)

(Eluent: Hexane/EtOAc = 4:1, 60% yield); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta = \) 8.08 (br s, 1H), 7.86 (br s, 1H), 7.78 (br s, 1H), 7.67 (d, \( J = 8.0 \) Hz, 1H), 7.54 (d, \( J = 8.0 \) Hz, 1H), 7.47-7.45 (m, 2H), 7.34 (dd, \( J = 11.3, 8.1 \) Hz, 2H), 7.26-7.25 (m, 1H), 7.17-7.13 (m, 1H), 7.12-7.08 (m, 1H), 7.07-6.99 (m, 2H), 6.97 (d, \( J = 2.2 \) Hz, 1H), 6.93-6.88 (m, 2H), 6.87 (d, \( J = 2.4 \) Hz, 1H), 3.33-3.27 (m, 2H), 3.04-3.01 (m, 1H), 2.42 (t, \( J = 13.4 \) Hz, 1H), 2.30 (dt, \( J = 13.4, 4.2 \) Hz, 1H), 2.22-2.19 (m, 1H), 2.00-1.95 (m, 1H), 1.89-1.85 (m, 1H), 1.71-1.65 (m, 1H) ppm; \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \( \delta = \) 137.1, 137.0, 136.3, 126.7, 126.6, 126.4, 125.8, 123.2, 122.7, 121.9, 121.8, 121.4, 121.2, 121.1, 120.7, 120.5, 119.5, 119.4, 118.9, 118.7, 118.5, 111.2, 111.1, 111.0, 43.9, 40.3, 36.6, 34.0,
31.0, 23.3 ppm; MS (m/z) 430 [M+H]+; HRMS (ESI) calcd. for C_{30}H_{28}N_{3} [M+H]^+, 430.2283; found, 430.2282.

3-(2-Methyl-1H-Indol-3-yl)cyclohexanone (28)

(Eluent: Hexane/EtOAc = 7:1, 67% yield); \( ^1H \) NMR (300 MHz, CDCl₃) \( \delta = 7.82 \) (br s, 1H), 7.66 (d, \( J = 7.5 \) Hz, 1H), 7.29 (dd, \( J = 7.1, 1.4 \) Hz, 1H), 7.15-7.05 (m, 2H), 3.22 (tt, \( J = 12.9, 3.8 \) Hz, 1H), 3.00 (t, \( J = 13.8 \) Hz, 1H), 2.55-2.47 (m, 3H), 2.41-2.28 (m, 4H), 2.26-2.18 (m, 1H), 2.03-1.99 (m, 1H), 1.83-1.75 (m, 1H) ppm; \( ^{13}C \) NMR (75 MHz, CDCl₃) \( \delta = 211.7, 135.4, 130.1, 126.9,120.9, 119.1, 118.8, 113.8, 110.6, 48.1, 41.5, 37.3, 31.5, 26.1, 12.1 \) ppm; MS (m/z) 228 [M+H]+; HRMS (ESI) calcd. for C_{15}H_{18}NO [M+H]^+, 228.1388; found, 228.1384.
5. REFERENCES


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Part 4 Chapter 5 References


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