Carbohydrate-derived molecular diversity : synthesis and application in cell biology

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**NANYANG
TECHNOLOGICAL
UNIVERSITY**

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

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SCHOOL OF PHYSICAL AND MATHEMATICAL SCIENCES

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

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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-*cis*-Mannosides play important roles in cellular biology and therefore their synthesis is necessary for biological studies. However, efficient formation of 1,2-*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.

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 H_2O or $\text{H}_2\text{O}/\text{THF}$ 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.

INDEX OF ABBREVIATIONS

δ	chemical shift
Ac	acetyl
acac	acetylacetone
AIBN	azobisisobutyronitrile
Alloc	allyloxycarbonyl
aq.	aqueous
ATRP	atom transfer radical polymerization
BBB	blood brain barrier
BIEMA	2-(2-bromoisobutyryloxy)ethyl methacrylate
BIPHEPHOS	6,6'-{[3,3'-bis(1,1-dimethylethyl)-5,5'-dimethoxy[1,1'-bipheyl]-2,2'-diyl]bis(oxy)}bis{dibenzo[<i>d,f</i>][1,3,2]dioxaphosphepine}
Bn	benzyl
Boc	<i>tert</i> -butoxycarbonyl
br s	broad singlet
Bu	butyl
Bz	benzoyl
calcd.	calculated
CAN	ceric ammonium nitrate
cat.	catalytic
Cbz	benzyloxycarbonyl
CD	cyclodextrin
CDI	1,1'-Carbonyldiimidazole
CNS	central nervous system
CNTs	carbon nanotubes
COD	cyclooctadiene
CSA	camphorsulfonic acid
cm ⁻¹	inverse centimeter
Cy	cyclohexane; cyclohexyl
d	doublet
D-A	Diels-Alder
dba	dibenzylideneacetone

DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCE	dichloroethane
dd	doublets of doublet
ddd	doublets of doublets of doublet
DEAD	diethyl azodicarboxylate
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DHP	2,3-dihydropyran
DIAD	diisopropyl azodicarboxylate
DIBAL-H	diisobutylaluminium hydride
DIPEA	diisopropylethylamine
DMA	dimethylacetamide
DMAP	4-(<i>N,N</i> -dimethylamino)pyridine
DME	dimethoxyethane
DMEDA	<i>N,N'</i> -dimethylethylenediamine
DMF	dimethylformamide
DMP	Dess-Martin periodinane
DMSO	dimethyl sulfoxide
DPPA	diphenylphosphoryl azide
DPPDA	diphenyl phosphorazidate
dppp	1,3-bis(diphenylphosphino)propane
dq	doublets of quartet
dt	doublets of triplet
DTBMP	2,6-di- <i>tert</i> -butyl-4-methylpyridine
EDCI	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide
<i>ee</i>	enantiomeric excess
EI	electron ionization
eq.	equivalent
ESI	electronspray ionization
Et	ethyl
F-C	Friedel-Crafts
FITC	fluorescein isothiocyanate
FTIR	Fourier transform infrared spectroscopy
GalNAc	galactosamine
GlcNAc	glucosamine

h	hour
HA	hemagglutinin
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HPA	<i>Helix Pomatia</i> 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>i</i> Pr	isopropyl
<i>J</i>	coupling constants
KHMDS	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
<i>m</i> CPBA	<i>meta</i> -chloroperoxybenzoic acid
Me	methyl
MOM	methoxymethyl
min	minute
MS	mass spectrum
M.S.	molecular sieves
Ms	methanesulfonyl
MTBE	methyl <i>tert</i> -butyl ether
MVK	methyl vinyl ketone
MWNTs	multi-walled carbon nanotubes
NA	neuraminidase
NaHMDS	sodium hexamethyl disilazide
NAP	2-naphthylmethyl
NBA	<i>N</i> -bromoacetamide
NBS	<i>N</i> -bromosuccinimide
<i>n</i> Bu	<i>n</i> -butyl

NCS	<i>N</i> -chlorosuccinimide
NIS	<i>N</i> -iodosuccinimide
NMR	nuclear magnetic resonance
NMO	4-methylmorpholine <i>N</i> -oxide
NOESY	nuclear overhauser enhancement spectroscopy
Ns	4-nitrobenzenesulfonyl
OTf	trifluoromethanesulfonate
<i>p</i>	para
PCC	pyridinium chlorochromate
PDC	pyridinium dichromate
Pd/C	palladium on carbon
Ph	phenyl
Phth	phthalate
Piv	pivaloyl; trimethylacetyl
PLE	pig liver esterase
PMB	<i>p</i> -methoxybenzyl
PMP	<i>p</i> -methoxyphenyl
pN	piconewton (10^{-12} newtons)
ppm	parts per million
PPTS	pyridinium <i>p</i> -toluenesulfonate
q	quartet
RAFT	reversible addition-chain transfer fragmentation
rt	room temperature
s	singlet
SCVCP	self condensing vinyl copolymerization
SES	2-(trimethylsilyl)ethanesulfonyl
SPG	Schizophyllan
SWNTs	single-walled carbon nanotubes
t	triplet
TBAI	tributylammonium iodide
TBAF	tetrabutylammonium fluoride
TBDPS	<i>tert</i> -butyldiphenyl silyl
TBS	<i>tert</i> -butyldimethyl silyl
<i>t</i> Bu	<i>tert</i> -butyl

TEMPO	2,2,6,6-tetramethylpiperidine-1-oxyl
TES	triethylsilyl
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
Tf	trifluoromethanesulfonyl
THF	tetrahydrofuran
THP	tetrahydropyran
TLC	thin layer chromatography
TMS	trimethylsilyl
TPAP	tetrapropylammonium perruthenate
Tr	triphenylmethyl
Ts	<i>p</i> -toluenesulfonyl
v	volume

PART 1

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

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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.^[1] Thereafter, two serious pandemics in 1957 (the Asian flu) and 1968 (the Hong Kong flu) killed millions of people.^[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.

Figure 1. 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.

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.^[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 infection 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.

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.^[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**·H₃PO₄, 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.^[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.^[11] Tamiflu and oseltamivir could not readily pass the BBB, but they may penetrate into CNS when the BBB is impaired or immature.^[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.^[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 μ M 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.^[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 μ M 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 μ M oseltamivir was involved. Another NA

inhibitor, zanamivir, showed similar results as oseltamivir. Since sialic acid modulates neurite adhesion between hippocampal neurons,^[14] it is possible that oseltamivir regulates sialylation-mediated neurite adhesion and enhances interneurons network synchronicity.

Yoshino *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.^[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.^[16] Wei *et al.* found that a nonsynonymous single nucleotide polymorphism in human cytosolic sialidase is the target of Tamiflu.^[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 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^[18] 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.^[2b, 3, 19] For example, asymmetric Diels-Alder chemistry^[20] and Michael reaction^[21] have been exploited to generate the cyclohexene backbone. Commercially available compounds such as (–)-quinic acid,^[22] L-serine,^[23] xylose,^[24] mesoaziridine,^[25] substituted cyclohexanediene,^[26] lactone,^[27] pyridine,^[28] 2,6-dimethoxyphenol,^[29] D-mannitol,^[30] L-methionine,^[31] ethyl benzoate^[18a] and ribose^[32] 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).^[18b] Epoxide **7** was prepared through esterification and epoxidation from **6** according to the literature procedure.^[33] 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% H₃PO₄ 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) SOCl₂, EtOH, reflux; (b) 3-pentanone, TsOH; (c) MsCl, Et₃N, CH₂Cl₂, 0–5 °C, 80% for 3 steps; (d) TMSOTf, BH₃·Me₂S, and then KHCO₃, H₂O/EtOH, 65 °C, 1 h, 60–72%; (e) NaN₃, NH₄Cl, H₂O/EtOH, 70–75 °C, 86%; (f) Me₃P, MeCN, 2 h, 97%; (g) NaN₃, NH₄Cl, DMF, 70–80 °C; (h) Ac₂O, aq. NaCO₃, CH₂Cl₂, 44% for 2 steps; (i) i) Ra-Ni, H₂, EtOH, 16 h; ii) 85% H₃PO₄, 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 SOCl₂ in EtOH. After removal of the

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) SOCl_2 , EtOH, reflux, 3.5 h, 97%; (b) $\text{Me}_2\text{C}(\text{OMe})_2$, TsOH, EtOAc, 35 °C, 15–200 mbar, 95%; (c) MsCl, Et_3N , EtOAc, 30 min, 89%; (d) 3-pentanone, $\text{CF}_3\text{SO}_3\text{H}$, 98%; (e) Et_3SiH , TiCl_4 , CH_2Cl_2 , –34 °C, 2 h; (f) NaHCO_3 , $\text{H}_2\text{O}/\text{EtOH}$, 65 °C, 2.5 h, 80% for 2 steps; (g) allyl amine, $\text{MgBr}_2 \cdot \text{OEt}_2$, MTBE/MeCN, 55 °C, 16 h, and then $(\text{NH}_4)_2\text{SO}_4$, H_2O , 97%; (h) Pd/C, EtOH, $\text{H}_2\text{NCH}_2\text{CH}_2\text{OH}$, reflux, 3 h, and then $\text{H}_2\text{SO}_4/\text{H}_2\text{O}$, 77%; (i) i) PhCHO, *t*BuOMe; ii) MsCl, Et_3N , filtration; iii) allyl amine, 112 °C, 15 h; iv) HCl/ H_2O , 80%; (j) Ac_2O , AcOH, MsOH, *t*BuOMe, 15 h, 20 °C, 83%; (k) i) 10% Pd/C, EtOH, $\text{H}_2\text{NCH}_2\text{CH}_2\text{OH}$, reflux, 3 h; ii) H_3PO_4 , EtOH, 70% for 2 steps.

Reduction of **25** with $\text{Et}_3\text{SiH}/\text{TiCl}_4$ in CH_2Cl_2 at –34 °C afforded the major product **26**, which was converted to epoxide **18** upon heating with NaHCO_3 solution in EtOH/ H_2O .

Epoxide **18** was attacked by allyl amine in the presence of $\text{MgBr}_2 \cdot \text{OEt}_2$ in

*t*BuOMe/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 phosphonylation 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) *t*BuNH₂, 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).^[18e] Epoxide **18** was regioselectively ring-opened by *t*BuNH₂-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_3H . The secondary amine was efficiently acetylated by heating **33** with Ac_2O in the presence of NaOAc at $110\text{ }^\circ\text{C}$ 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/ $\text{Pd}(\text{OAc})_2/\text{PPh}_3$ and subsequent phosphonylation 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) $\text{Me}_2\text{C}(\text{OMe})_2$, TsOH, EtOAc, $35\text{ }^\circ\text{C}$, 95%; (b) BzCl , Et_3N , DMAP, CH_2Cl_2 , $0\text{ }^\circ\text{C}$ to rt, 5 h, 98%; (c)

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 Ph_3P and Et_3N . Acetylation of **46** with Ac_2O and Et_3N resulted in **47**, which was selectively ring-opened by 3-pentanol in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$. 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 , Et_3N , DMAP, EtOAc , 0°C , 1 h, 93%; (c) NaN_3 , $\text{Me}_2\text{CO}/\text{H}_2\text{O}$, 0°C , 4 h, 92%; (d) Ph_3P , THF , rt, 0.5 h, then Et_3N , H_2O , rt, 24 h, 84%; (e) Ac_2O , Et_3N , EtOAc , 0°C , 0.5 h, 98%; (f) $\text{BF}_3 \cdot \text{OEt}_2$, 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 Ph_3P 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 SO_2Cl_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_3N , CH_2Cl_2 , 0–5 °C, 1.5 h, 69% for 3 steps; (d) SO_2Cl_2 , pyridine, CH_2Cl_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) ZnCl_2 , 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, Et_3N , CH_2Cl_2 , rt; (g) 3-pentanol, $\text{BF}_3 \cdot \text{OEt}_2$, CH_2Cl_2 , 62% for 2 steps; (h) 20% H_2SO_4 , EtOH, 70 °C, 22 h, 68%.

The first approach, which is independent of shikimic acid and quinic acid, commenced with a ZnCl_2 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 bicyclic 62 was treated with NaHMDS in THF to give the alcohol 63. Mesylation of 63 with MsCl and Et_3N 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) *n*Bu₄NBr, KHMDS, DME, -20 °C, 10 min; (j) cat. Cu(OTf)₂, 3-pentanol, 0 °C, 12 h, 61%; (k) H₃PO₄, EtOH.

Corey's group^[20a] commenced their synthesis with an asymmetric D-A reaction between butadiene with trifluoroethyl acrylate, catalyzed by *S*-proline-derived catalyst **67** (Scheme 11).^[39] 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) *n*BuLi, 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 *t*BuOH. Subsequently an intramolecular addition of 3-hydroxyl group to the C-4 isocyanate group and an intermolecular addition of *t*BuOH 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 Et₃N produced β -cyanoenone **94**. Enone **94** was selectively reduced by LiAlH(O*t*Bu)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) TMSN₃, DMAP, rt, 2 h; (c) 1 N HCl, 4 °C, 10 min, 55% for 3 steps; (d) *t*BuOH, reflux, 13 h; (e) LiOH; (f) Ac₂O, Et₃N, DMAP, CH₂Cl₂, rt, 2.5 h; (g) Isobutyric anhydride, DMSO, then chiral HPLC, 53% for 4 steps; (h) i) TMSCN, Ni(COD)₂, COD; ii) NBS, Et₃N; (i) LiAlH(O*t*Bu)₃, 44% for 2 steps; (j) DEAD, PPh₃, 66%; (k) BF₃·OEt₂, 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 **85** is not stable under acidic conditions, they developed a D-A reaction catalyzed by Ba(OiPr)₂/F2-FujiCAPO (**106**) and CsF to give the adducts in 99% yield (d.r. 5:1) with 91% *ee* of **99** (Scheme 14). The mixture was hydrolyzed to acid, which was treated with DPPA and Et₃N to afford diacyl azide **89**. During this transformation, the desired product **89** derived from **99** could be purified by column chromatography. Carbamate **90** was prepared from **89** according to the same procedure as described above. Then carbamate amine was protected by acetyl group. Compound **100** was allylic substituted by dicyanomethyl acetate **101** catalyzed by [Pd₂(dba)₃]CHCl₃ and dppf to afford **102**. α -Epoxide was exclusively formed by the treatment of **102** with *in situ* generated trifluoroperacetic acid. Acetoxydicyanomethyl group was converted to ethoxycarbonyl and subsequently, epoxide opening occurred in a one pot reaction under K₂CO₃ in EtOH. Later, the configuration of 3-hydroxy group of **105** was inverted by Mitsunobu esterification and one-pot ethanolysis. Aziridine **76** was produced under Mitsunobu conditions using Me₂PPh and DIAD. The aziridine **76** was ring-opened by 3-pentanol in the presence of BF₃·Et₂O. Tamiflu was obtained after cleavage of the Boc group with TFA and treatment with H₃PO₄. The key steps in the fourth generation synthesis are an asymmetric D-A reaction to build the cyclohexene framework, a Curtius 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) $\text{Ba}(\text{OiPr})_2$, F2-FujiCAPO, CsF, THF, $-20\text{ }^\circ\text{C}$, 36-96 h, then aq. 1 M HCl, 91%; (b) aq. 2 M NaOH, MeOH, $60\text{ }^\circ\text{C}$, 10 h; (c) DPPA, Et_3N , THF, $0\text{ }^\circ\text{C}$, 21 h, 95% for 2 steps; (d) *t*BuOH, $80\text{ }^\circ\text{C}$ 13 h; (e) Ac_2O , Et_3N , DMAP (10 mol%), CH_2Cl_2 , rt, 2.5 h, 80% for 2 steps; (f) **101**, $[\text{Pd}_2(\text{dba})_3]\cdot\text{CHCl}_3$, dppf, toluene, $60\text{ }^\circ\text{C}$, 30 min, 85%; (g) TFAA, urea/ H_2O_2 , Na_2HPO_4 , CH_2Cl_2 , $4\text{ }^\circ\text{C}$, 2 h; h) K_2CO_3 , EtOH, rt, 5 h; (i) DEAD, PPh_3 , *p*-nitrobenzoic acid, THF, $-20\text{ }^\circ\text{C}$, 1.5 h; LiOH, EtOH, $-20\text{ }^\circ\text{C}$, 15 min, 65% for 3 steps; (j) DIAD, Me_2PPh , Et_3N , CH_2Cl_2 , $4\text{ }^\circ\text{C}$, 10 min, 76%; (k) $\text{BF}_3\cdot\text{OEt}_2$, 3-pentanol, $-20\text{ }^\circ\text{C}$, 15 min, 75%; (l) TFA; H_3PO_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 $\text{Y}(\text{OiPr})_3$ 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% $\text{Y}(\text{OiPr})_3$, **106**, TMSN_3 , $\text{CH}_3\text{CH}_2\text{CN}$, rt, 48 h, 96%, 91% *ee*; ii) recrystallization from *i*PrOH, 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 from *i*PrOH/hexane, 62% for 2 steps, >99% *ee*; (h) $\text{Ni}(\text{COD})_2$, COD, TMS-CN, THF, 60 °C, 65 h; (i) i) NBS, THF, 20 min; ii) Et_3N , 4 °C, 40 min; (j) $\text{LiAlH}(\text{OtBu})_3$, THF, 4 °C, 30 min, 60% for 3 steps; (k) DEAD, Ph_3P , THF, 4 °C, 1 h, 87%; (l) 3-pentanol, $\text{BF}_3 \cdot \text{OEt}_2$, 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

group to give **110**. Treatment of diamide **110** with $\text{SeO}_2^{[43]}$ 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 $\text{Ni}(\text{COD})_2$ gave **113**, which was converted to γ -keto nitrile **114** by treating with NBS and Et_3N . The ketone was selectively reduced to alcohol **115** with $\text{LiAlH}(\text{O}i\text{Bu})_3$. Applying Mitsunobu conditions to **115** generated aziridine **116**, which was then ring-opened by 3-pentanol in the presence of $\text{BF}_3 \cdot \text{OEt}_2$. 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_2O in pyridine. Hydrolysis of nitrile with HCl/EtOH and H_2O and concomitant cleavage of the Boc group provided oseltamivir **3**, which was finally converted to Tamiflu with H_3PO_4 salt formation.

Scheme 16. Shibasaki group's second generation synthesis of Tamiflu. Reagents and conditions: (a) i) I_2 , K_2CO_3 , KI, CHCl_3 , 60°C , 19 h, 83%; ii) DBU, CH_2Cl_2 , rt, 4 h, 85%; (b) Boc_2O , Et_3N , DMAP, CH_2Cl_2 , rt, 2 h, 99%; (c) AcSH , 2,6-lutidine, CHCl_3 , 60°C , 19 h, 83%; (d) Cs_2CO_3 , $n\text{BuOH}$, 4°C to rt, 16 h, 86%; (e) Dess-Martin periodinane, CH_2Cl_2 , 4°C to rt, 15 min, 90%; (f) $(\text{EtO})_2\text{P}(\text{O})\text{CN}$, LiCN, THF, -20°C , 4 h; (g) i) toluene, sealed tube, 140°C , 40 min; ii) aq. NH_4Cl , rt, 3 h, 78% for 3 steps; (h) DEAD, Ph_3P , $p\text{NO}_2\text{C}_6\text{H}_4\text{CO}_2\text{H}$, THF, 4°C , 15 min; ii) 1 M LiOH, $\text{H}_2\text{O}/\text{THF}$, -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 SeO₂ 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 I₂/K₂CO₃/KI in CHCl₃ followed by DBU provided oxazolidinone **120**, which was protected as a Boc derivative. Then, the azide was reduced to acetamide by AcSH. The resulting oxazolidinone **122** was hydrolyzed by Cs₂CO₃ in *n*BuOH 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 NH₄Cl 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 SeO₂ 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) $\text{Fe}_2(\text{CO})_9$, toluene, 55 °C, 86%; (b) Ph_3CPF_6 , CH_2Cl_2 , rt, 94%; (c) **129**, DIPEA, CH_2Cl_2 , 0 °C, 75%; (d) i) preparative HPLC, 47% for (–)-**130**; ii) HPF_6 , Et_2O , 0 °C, 94%; (e) i) BocNH_2 , DIPEA, CH_2Cl_2 , 0 °C, 86%; ii) H_2O_2 , NaOH , EtOH , 0 °C, 95%; (f) *m*CPBA, CH_2Cl_2 , –70 °C to rt, 95%; (g) NaN_3 , NH_4Cl , $\text{DME}/\text{EtOH}/\text{H}_2\text{O}$, 0 °C, 95%; (h) MsCl , Et_3N , CH_2Cl_2 , 0 °C; (i) Ph_3P , Et_3N , $\text{THF}/\text{H}_2\text{O}$, rt; (j) Ac_2O , pyridine, CH_2Cl_2 , 0 °C, 65%; (k) 3-pentanol, $\text{Cu}(\text{OTf})_2$, 0 °C, 48%. (l) H_3PO_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_3CPF_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 HPF_6 . Reaction of (–)-**128** with BocNH_2 and decomplexation with $\text{H}_2\text{O}_2/\text{NaOH}$ afforded **131**. The more electron-rich alkene of **131** was selectively epoxidized by *m*CPBA to furnish epoxide **132**, which was ring-opened by sodium azide. Treatment of **133** with MsCl followed by $\text{Ph}_3\text{P}/\text{Et}_3\text{N}$ resulted in aziridine **134**. Acetylation of **134** with Ac_2O 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, $\text{TsOH}\cdot\text{H}_2\text{O}$, acetone, 0 °C to rt, 0.5 h; (b) cat. SnBr_4 , NBA, H_2O , CH_3CN , 0 °C, 8 h, 75% for 2 steps; (c) LiHMDS , THF, –10 to 0 °C, 0.5 h; (d) 3-pentanol, $\text{BF}_3\cdot\text{OEt}_2$, –10 to 0 °C, 6 h, 73% for 2 steps; (e) conc. HCl , MeOH , 50 °C, 6 h, 94%; (f) $\text{AcOCMe}_2\text{COBr}$, THF, 0 °C to rt, 3.5 h; (g) LiBHET_3 , THF, 0 °C to rt, 2 h, 82% for 2 steps; (h) DPPA, DIAD, PPh_3 , THF, 40 °C, 24 h, 84%; (i) $[\text{Ni}(\text{CO})_2(\text{PPh}_3)_2]$, DIPEA, EtOH , THF, 80 °C, 24 h, 81%; (j) i) H_2 , Lindlar catalyst; ii) H_3PO_4 , EtOH , 50 °C, 6 h, 91%.

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 NaN_3 and *m*CPBA 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 SnBr_4 , resulted in bromoamide **138**,^[20a] which was converted to aziridine **139** under LiHMDS. Aziridine ring-opening with 3-pentanol was promoted by $\text{BF}_3 \cdot \text{OEt}_2$ 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 LiBHET_3 to give alcohol **143**. Mitsunobu reaction of alcohol **143** with DPPA furnished azide **144**. Treatment of **144** with stoichiometric amount of $\text{Ni}(\text{CO})_2(\text{PPh}_3)_2$ 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. Bu_4NOCN was used as a nitrogen source instead of DPPA to install amino group be introduced on C-5 (Scheme 19). Treatment of **143** with $\text{Bu}_4\text{NOCN}/\text{PPh}_3/ \text{DDQ}$ followed by *t*BuOH produced compound **145**. Since direct Pd-catalyzed carbonylation

of **145** failed, the bromide was converted to iodide to improve the reaction activity. Then, carbonylation of **146** proceeded successfully with $\text{Pd}(\text{OAc})_2$ as the catalyst in EtOH to give carboxylate **147**. Concurrent removal of the Boc group and salt formation with H_3PO_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 , $n\text{Bu}_4\text{NOCN}$, CH_3CN , rt, 18 h, then $t\text{BuOH}$, reflux, 24 h, 78%; (b) CuI , KI , DMEDA, $n\text{BuOH}$, 120°C , 24 h; (c) cat. $[\text{Pd}(\text{OAc})_2]$, CO , NaOAc , EtOH, rt, 24 h, 82% for 2 steps; (d) H_3PO_4 , EtOH, 50°C , 6 h, 81%.

The highlight of this work is that the carboxylate could be replaced by phosphonate by the phosphonylation 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_3N provided *N*-tosyloxy carbamate **150**, which was readily transformed to acylaziridine **151** in the presence of $\text{Cu}(\text{MeCN})_4\text{PF}_6$ and K_2CO_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, Et₃N, toluene, -78 to -30 °C, 5 h, 85% for 2 steps; (c) i) CDI, MeCN, 0 °C, 1 h; ii) NH₂OH·HCl, imidazole, 0 to 18 °C, 16 h, 56% at 88% conversion; (d) TsCl, Et₃N, Et₂O, 0 to 18 °C, 16 h, 79%; (e) Cu(MeCN)₄PF₆, K₂CO₃, MeCN, 3-pentanol, 0 to 18 °C, 16 h, 43%; (f) LiOH, 1,4-dioxane/water, 100 °C, 48 h, 85%; (h) AcCl, Et₃N, 0 to 18 °C, 1 h, 99%; (i) HCl, CH₃OH, 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 NaBH₄ 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 Boc₂O afforded **161**, which was oxidized with NaIO₄ and a catalytic amount of RuO₂·*n*H₂O to give **162**. Compound **162** was ammonolysed in *t*BuOH to **163**, which was in turn mesylated to **164** under MsCl and Et₃N. 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-Pentanyl was then introduced stereoselectively with the aid of BF₃·OEt₂. Deprotection and acetylation of **167** furnished **168**. Removal of the alloc group under Pd/C, Ph₃P 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 RuO₂·*n*H₂O 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, *t*BuOH/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, *t*BuOH, THF, 0 °C, 95%; (h) MsCl, Et₃N, CH₂Cl₂, rt, 1 h, 91%; (i) allyl alcohol, PhI(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.^[29] 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 KO^{*t*}Bu 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 Et₃N resulted in oxazolidinone **176** through Curtius rearrangement and subsequently intramolecular esterification. Cyclohexenol **177** was generated by treating **176** with Boc₂O and DMAP in toluene followed by catalytic amount of NaH. The hydroxyl group at C-4 was triflated with Tf₂O and then substituted by NaN₃ 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 H₃PO₄.

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-pentanyl methanesulfonate, KOtBu, 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) H₂ (100 bar), 5% Ru-Al₂O₃, EtOAc, 60 °C, 82%; (e) TMSCl, NaI, MeCN, cat. H₂O, 97%; (f) PLE pH 8 buffer, 98%; (g) DPPA, Et₃N, CH₂Cl₂, 40 °C, 81%; (h) Boc₂O, cat. DMAP, toluene, rt, then cat. NaH, toluene, reflux; (i) Tf₂O, pyridine, -10 °C, CH₂Cl₂, 85%; (j) NaN₃, acetone/H₂O, rt, 78%; (k) H₂, Ra-Co; (l) Ac₂O, Et₃N, then HBr-AcOH, EtOAc; (m) H₃PO₄, 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 isopropylidene, diol was subsequently reacted with *N*-hydroxy acetamide with the aid of NaIO₄ to furnish **183**.^[56] Cleavage of the N-O bond in the presence of [Mo(CO)₆]

afforded **184**, which was treated with $\text{MsCl}/\text{Et}_3\text{N}$ 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 NaN_3 . Treatment of **189** with DBU in CH_2Cl_2 afforded **190**, which could be converted to Tamiflu in 3 steps by Fang's method.

This formal synthesis utilized a commercially available and cheap starting 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) CH_3CONHOH , NaIO_4 , MeOH , rt, 70% for 2 steps; (d) $[\text{Mo}(\text{CO})_6]$, $\text{MeCN}/\text{H}_2\text{O}$ (15:1), 75%; (e) MsCl , Et_3N , DMAP, CH_2Cl_2 , rt, 54%; (f) CaCO_3 , $\text{EtOH}/\text{H}_2\text{O}$ (1:1), 72%; (g) $\text{Rh}/\text{Al}_2\text{O}_3$ (5 mol%), 60 *psi* H_2 , 85% aq. EtOH , 95%; (h) Ms_2O , Et_3N , CH_2Cl_2 , rt, 73%; (i) NaN_3 , acetone/ H_2O , rt, 86%; (j) DBU, CH_2Cl_2 , rt, 85%.

An alternative route to synthesize intermediate **77** was reported by Hudlicky *et al.* recently (Scheme 24).^[57] Treatment of **184** with $\text{CrO}_3/\text{Ac}_2\text{O}$ in CH_2Cl_2 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) CrO_3 , Ac_2O , CH_2Cl_2 , 4 °C, 5 min; (b) $\text{NH}_2\text{OH}\cdot\text{HCl}$, EtOH , pyridine, 75-82% for 2 steps; (c) 5% $\text{Rh}/\text{Al}_2\text{O}_3$, H_2 , aq. EtOH , Boc_2O , 93%; (d) NaOEt , EtOH , 94%; (e) 3-Iodopentane, Ag_2CO_3 .

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 starting 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) Boc_2O , Et_3N , THF, 90%; iii) Dimethoxypropane, acetone, $\text{BF}_3\cdot\text{OEt}_2$, 91%; iv) DIBAL-H, toluene, 70%; (b) i) *N*-(4-methoxybenzyl)-hydroxylamine, MgSO_4 , CH_2Cl_2 , 74%; ii) ZnBr_2 , allylmagnesium bromide, THF/ Et_2O , -70°C , 4 h; 87%; iii) $\text{Zn-Cu}(\text{OAc})_2$, $\text{AcOH}/\text{H}_2\text{O}$, 70°C ; iv) CbzCl , aq. NaHCO_3 , EtOAc ; c) OsO_4 , NMO, acetone/ H_2O , 89%; (d) $\text{Pd}(\text{OH})_2/\text{H}_2/\text{CH}_3\text{OH}$, 35°C ; (e) CbzCl , NaHCO_3 , $\text{H}_2\text{O}/\text{EtOAc}$, 86% for 2 steps; (f) TBDPSCl , imidazole, CH_2Cl_2 , rt, 96%; (g) i) $(\text{COCl})_2$, DMSO, CH_2Cl_2 , Et_3N , -78°C ; ii) $\text{Ph}_3\text{PCH}_3\text{Br}$, $n\text{BuLi}$, THF, -78°C to rt, 86% for 2 steps; (h) BiBr_3 , MeCN, rt, 89%; (i) i) $(\text{COCl})_2$, DMSO, CH_2Cl_2 , Et_3N , -78°C ; ii) vinylMgBr , ZnBr_2 , THF, -78 to -30°C , 56%; (j) MOMCl , DIPEA, CH_2Cl_2 , rt, 98%; (k) cat. **211**, CH_2Cl_2 , rt, 98%; (l) TBAF, THF, rt, 96%; (m) i) PCC, 4 Å M.S., CH_2Cl_2 , rt; ii) NaClO_2 , K_2HPO_4 , 2,3-dimethylbuta-1,3-diene, $t\text{BuOH}/\text{THF}/\text{H}_2\text{O}$, 10°C to rt, 88% for 2 steps; (n) EtOH , HOBt, EDCI, DIPEA, CH_2Cl_2 , rt, 85%; (o) 5% HCl/EtOH , 0°C to rt; (p) AcCl , Na_2CO_3 , EtOH , 0°C to rt, 83% for 2 steps; (q) $\text{Pd}(\text{OAc})_2$, Et_3SiH , Et_3N , CH_2Cl_2 , 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).^[31] 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^[61] 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, $-70\text{ }^{\circ}\text{C}$, 2.5 h, 91%; (b) *p*-anisidine, MgSO_4 , CH_2Cl_2 , $0\text{ }^{\circ}\text{C}$ to rt, overnight, quantitative; (c) DIPEA, **225**, CH_2Cl_2 , $-15\text{ }^{\circ}\text{C}$ to rt, overnight, 55%; (d) NCS, $\text{MeCN}/\text{EtOH}/\text{H}_2\text{O}$, rt, 40 min, quantitative; (e) α -pinene-decalins, NaHCO_3 , $155\text{ }^{\circ}\text{C}$, 6 h, 81%; (f) TFA, CH_2Cl_2 , $0\text{ }^{\circ}\text{C}$ to rt, 1.5 h; then $\text{PhthNCO}_2\text{Et}$, Et_3N , THF, $50\text{ }^{\circ}\text{C}$, 7.5 h, 86%; (g) CAN, $\text{MeCN}/\text{H}_2\text{O}$, $0\text{ }^{\circ}\text{C}$, 1 h, 80%; (h) LiHMDS, THF, $-78\text{ }^{\circ}\text{C}$, 15 min, AcCl , $-78\text{ }^{\circ}\text{C}$, 1 h, then EtSH , Et_3N , -78 to $-20\text{ }^{\circ}\text{C}$, 3.5 h, 94%; (i) $\text{Rh}(\text{acac})(\text{CO})_2$, BIPHEPHOS, CO/H_2 , THF, $65\text{ }^{\circ}\text{C}$, 7 h, 88%; (j) 10% Pd/C , Et_3SiH , CH_2Cl_2 , rt, 1.5 h; (k) $\text{Bn}_2\text{NH}\cdot\text{TFA}$, toluene, $50\text{ }^{\circ}\text{C}$, 10 h, 62% for 2 steps; (l) NaClO_2 , NaHPO_4 , 2-methyl-2-butene, *t*BuOH/THF/ H_2O , $0\text{ }^{\circ}\text{C}$, 1 h, rt, overnight, 86%; (m) NaBH_4 , *i*PrOH/ H_2O , rt, overnight, 93%; (n) K_2CO_3 , $\text{EtOH}/\text{H}_2\text{O}$, 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, $60\text{ }^{\circ}\text{C}$, 20 h, quantitative; (q) SOCl_2 , $80\text{ }^{\circ}\text{C}$, 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 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 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_3CCOCl , pyridine, 0 °C, 8 h, 89%; (b) i) PDC, Ac_2O , reflux, 1.5 h; ii) $\text{NH}_2\text{OH}\cdot\text{HCl}$, pyridine, 60 °C, 24 h, 82%; (c) LiAlH_4 , THF, reflux, 1.5 h, 88%; (d) i) Ac_2O , 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_2O , pyridine, CH_2Cl_2 , -15 °C, 2 h; ii) $\text{EtO}_2\text{CCH}_2\text{PO}(\text{OEt})_2$, 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_2O , pyridine, CH_2Cl_2 , -15 to -10 °C, 2 h; ii) KNO_2 , 18-crown-6, DMF, 40 °C, 24 h, 70%; (k) $\text{Cl}_3\text{CC}(\text{=NH})\text{OCH}_2\text{Et}$, $\text{CF}_3\text{SO}_3\text{H}$, CH_2Cl_2 , 25 °C, 24 h, 78%; (l) H_2 , Lindlar catalyst, EtOH, rt, 16 h, 85%; (m) H_3PO_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. 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, *t*BuOH/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).^[32] 1-Hydroxy and 2,3-hydroxy groups were protected by methyl group and 3-pentylidene respectively. After iodization under I₂/Ph₃P/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/H₂O 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 AlCl₃/Et₃SiH in CHCl₃. 5-Hydroxy group of **253** was mesylated by MsCl/Et₃N. 4-Hydroxy group of **254** was converted to triflate as a good leaving group, which was substituted by NaN₃ in acetone/H₂O. Aziridine **21** was generated from **256** through Staudinger reaction and cyclization. Finally, aziridine could be converted to Tamiflu by reported procedures in 3 steps.^[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) *n*Bu₃P, 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 starting material because it has the six-membered carbon skeleton (Scheme 30).^[27] 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 (η^3 -C₃H₅PdCl)₂ and Trost ligand. The resulting TMS ester was ethanolyzed in one pot to give **259**, which was further sulfonylated by

PhSSO₂Ph and KHMDS. The obtained diastereomers were oxidized by *m*CPBA which underwent thermal elimination in the presence of DBU to generate **261**. To obtain desired γ,δ -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 $\text{PhI}(\text{O}_2\text{CCMe}_3)_2$, SESNH_2 , $[\text{Rh}_2(\text{esp})_2]$ (bis-[rhodium($\alpha,\alpha,\alpha',\alpha'$ - tetramethyl-1,3-benzenedipropionic acid))] and MgO in chlorobenzene.^[65] Subsequently, aziridine was ring-opened by 3-pentanol in the presence of $\text{BF}_3 \cdot \text{OEt}_2$. After acetylation with Ac_2O 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**.

Scheme 30. Synthetic route to oseltamivir developed by Trost. Reagents and conditions: (a) 2.5 mol% $[(\eta^3\text{-C}_3\text{H}_5\text{PdCl})_2]$, 7.5 mol% (*R,R*)-**266**, trimethylsilyl phthalimide, THF, 40 °C, then $\text{TsOH} \cdot \text{H}_2\text{O}$, EtOH, reflux, 84%, 98% *ee*; (b) KHMDS, PhSSO₂Ph, THF, -78 °C to rt, 94%; (c) *m*CPBA, NaHCO₃, 0 °C, then DBU, 60 °C, toluene, 85%; (d) 2 mol% **267**, SESNH₂, $\text{PhI}(\text{O}_2\text{CCMe}_3)_2$, MgO, PhCl, 0 °C to rt, 86%;

(e) $\text{BF}_3 \cdot \text{Et}_2\text{O}$, 3-pentanol, 75 °C, 65%; (f) DMAP, pyridine, Ac_2O , Microwave, 150 °C, 1 h, 84%; (g) TBAF, THF, rt, 95%; (h) NH_2NH_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**, $\text{ClCH}_2\text{CO}_2\text{Et}$, CH_2Cl_2 , rt, 40 min; (b) i) **279**, Cs_2CO_3 , 0 °C, 3 h; ii) Cs_2CO_3 , EtOH, rt, 15 min; (c) $\text{CH}_3\text{C}_6\text{H}_4\text{SH}$, EtOH, -15 °C, 36 h, 70% for 3 steps; (d) TFA, CH_2Cl_2 , 2 h; (e) i) $(\text{COCl})_2$, cat. DMF, CH_2Cl_2 , 1 h; ii) NaN_3 , acetone/ H_2O , 0 °C, 20 min; (f) AcOH, Ac_2O , rt, 49 h; (g) Zn, TMSCl, EtOH, 70 °C, 2 h; (h) NH_3 , K_2CO_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 dipheylprolinol silyl ether **278**.^[66] The Michael adduct **270** was *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 NaN₃ successively resulted in acyl azide **275**. Crude **275** was treated with AcOH/Ac₂O 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 K₂CO₃ 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. 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*-methoxybenzyl (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.

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 NaN_3 . 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 PMBBBr 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. CrO_3 /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) PMBBBr, 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 CH_2Cl_2 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**.

Oxidant	Solvent	Isolated yield
PCC	CH_2Cl_2	52%
TPAP/NMO	CH_2Cl_2	-
IBX	DMSO	80%
DMP	CH_2Cl_2	86%

Heating aldehyde **291** with Cp_2TiMe_2 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

$\text{Ph}_3\text{PCH}_3\text{I}/\text{NaNH}_2$ afforded the alkene **283** in 10% yield while ylide reagent produced from $\text{Ph}_3\text{PCH}_3\text{Br}/n\text{BuLi}$ provided alkene **283** in 30% yield (Table 2). In hope of improving the yield, different bases such as *t*BuOK, NaH and LiHMDS were used, but proved to be inefficient. With *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_2O_3 instead of silica gel for column chromatography. With that, the isolated yield of alkene **283** was improved to 62%.

Table 2. Optimization of Wittig reaction of **291**.

Ylide reagent	Base	solvent	Temperature	Yield
$\text{Ph}_3\text{PCH}_3\text{I}$	NaNH_2	THF	0 °C to rt	10% ^[a]
$\text{Ph}_3\text{PCH}_3\text{Br}$	<i>n</i> BuLi	THF	−78 °C to rt	30% ^[a] , 62% ^[b]
$\text{Ph}_3\text{PCH}_3\text{Br}$	<i>t</i> BuOK	Et_2O	0 °C to rt	22% ^[a]
$\text{Ph}_3\text{PCH}_3\text{Br}$	NaH	THF	0 °C to rt	20% ^[a]
$\text{Ph}_3\text{PCH}_3\text{Br}$	LiHMDS	THF	−20 °C to rt	24% ^[a]

^[a] Isolated yield after column chromatography on silica gel; ^[b] isolated yield after column chromatography on neutral Al_2O_3 .

Table 3. Optimization of Claisen rearrangement of functionalized galactal **283**.

Reagent/solvent	Temp.(°C)	Time(h)	Yield
Bu ₃ N	180	12	-
xylene	180	12	-
Ph ₂ O	210	12	23%
silica gel/Ph ₂ O	200	12	-
dichlorobenzene	230	18	-
dichlorobenzene	240 (microwave)	0.7	10%
PdCl ₂ /toluene	110	12	-
Me ₃ Al/toluene	50	3	-
Me ₃ Al-PPh ₃ /DCM	25	2	-
Me ₃ Al-H ₂ O/DCM	-20	2	-
Me ₃ Al/2,4,6-tribromophenol/DCM	-78	3	-
BF ₃ /Et ₂ O	-60	2	-

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 Ph₂O. In addition, deprotected product was observed when Lewis acids such as AlMe₃ and BF₃·OEt₂ 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 unfavored.

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 °C in Ph₂O in a sealed tube.

Scheme 35. Synthesis of compound **298**. Reagents and conditions: (a) TBSCl, imidazole, DMF, 0 °C to rt, overnight, 81%; (b) PMBBBr, 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, *n*BuLi, 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, *n*BuLi, -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.

Reagents	Isolated yields
Oxone/EtOH	43%
I ₂ /KOH/EtOH	42%
NaClO ₂ /NaH ₂ PO ₄ , then EtI/K ₂ CO ₃	87%

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

Scheme 38. Conversion of **306** to alcohol **307**.

2.2.3 Introduction of two amino groups

With **307** in hand, we attempted to prepare compound **308** through sulfonamide,^[72] which is a common precursor for intermolecular aziridination. However, a mixture of ClSO₂NCO, 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).^[73]

Scheme 39. Formation of carbamate **300**.

Then conditions for intramolecular aziridination of **300** were optimized (Table 5). Conventional conditions, such as $\text{Rh}_2(\text{OAc})_4$, $\text{PhI}(\text{OAc})_2$, MgO , CH_2Cl_2 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 $\text{Rh}_2(\text{tfacam})_4$ and $\text{Cu}(\text{MeCN})_4\text{PF}_6$ could not promote the aziridination.

Table 5. Optimization of aziridination of carbamate **300**.

Catalyst	Auxiliary and solvent	Temp.	Product
-	PhIO , CH_2Cl_2	50 °C	-
$\text{Rh}_2(\text{OAc})_4$	$\text{PhI}(\text{OAc})_2$, MgO , CH_2Cl_2	rt	-
$\text{Rh}_2(\text{OAc})_4$	$\text{PhI}(\text{OAc})_2$, MgO , CH_2Cl_2	50 °C	309 (12%)
$\text{Rh}_2(\text{OAc})_4$	PhIO , MgO , CH_2Cl_2	50 °C	-
$\text{Rh}_2(\text{tfacam})_4$	$\text{PhI}(\text{OAc})_2$, MgO , CH_2Cl_2	rt	-
$\text{Rh}_2(\text{tfacam})_4$	PhIO , MgO , CH_2Cl_2	rt	-
$\text{Rh}_2(\text{tfacam})_4$	PhIO , MgO , CH_2Cl_2	50 °C	-
$\text{Cu}(\text{MeCN})_4\text{PF}_6$	PhIO , MgO , MeCN	rt	-
$\text{Cu}(\text{MeCN})_4\text{PF}_6$	PhIO , MgO , MeCN	50 °C	-
$\text{Cu}(\text{MeCN})_4\text{PF}_6$	$\text{PhI}(\text{OAc})_2$, MgO , MeCN	50 °C	-

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 $\text{TMSN}_3/\text{TBAF}$ was used as nucleophile. Alternative nucleophile such as NaN_3 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.

Catalyst	Reagents/solvent	Temp.	Yields
$\text{Rh}_2(\text{OAc})_4$	$\text{PhI}(\text{OAc})_2$, MgO , $\text{NaN}_3/\text{CH}_2\text{Cl}_2$	50 °C	trace 299 +10% 309
$\text{Rh}_2(\text{OAc})_4$	$\text{PhI}(\text{OAc})_2$, MgO , $\text{TMSN}_3/\text{CH}_2\text{Cl}_2$	50 °C	-

Inspired by Fleming's work, we considered enhancing the feasibility of accessing the nitrene species.^[49] As such, the substituted carbamate **311** was prepared in 77% yield (75% conversion) by treating alcohol **307** with $\text{CDI}/\text{NH}_2\text{OH}$ followed by $\text{TsCl}/\text{Et}_3\text{N}$ (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**.

Catalyst	Solvent	Temp. (°C)	Yield(¹ H NMR)
Rh ₂ (OAc) ₄	CH ₂ Cl ₂	25	63%
Cu(OTf) ₂	CH ₃ CN	25	78%
IPrCu	CH ₃ CN	25	83%
Cu(MeCN) ₄ PF ₆	CH ₃ CN	25	86%
(CuOTf) ₂ toluene	CH ₃ CN	25	91%

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.

Scheme 41. Azide ring-opening with *N*-containing nucleophiles.

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, whereas 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 TMSN_3 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

Base	Temperature	Time (h)	Product
Cs_2CO_3	rt	3	trace 317 +recovered 299
Cs_2CO_3	80 °C	2	318 + recovered 299
LiOH	rt	3	80% 317 + recovered 299
NaOH	rt	4	30% 317 + 318 + recovered 299

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 $\text{Cs}_2\text{CO}_3/\text{EtOH}$ 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

Base	Temperature	Product
Cs ₂ CO ₃	rt	15% 299 + recovered 319
Cs ₂ CO ₃	80 °C	321
LiOH/LiCl	rt	299 + recovered 319
NaOMe	rt	299

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_N2 reaction before the olefin formation. However, reaction of **325** with Tf₂O 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) Boc₂O, Et₃N, DMAP, CH₂Cl₂, rt, 2 h, 90%; (b) Cs₂CO₃, 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.

Base	Temperature	Solvent	Product
DBU	rt	CH ₃ CN	317 +recovered 299
DBU	80 °C	CH ₃ CN	327
NaH	rt	THF	317
NaOH	rt	EtOH	317 + 318

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).

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.^[25b] Oxidation of alcohol with Dess-Martin periodinane and subsequent selective reduction by $\text{LiAl}(\text{O}t\text{Bu})_3$ 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 $\text{BF}_3 \cdot \text{Et}_2\text{O}$ to furnish **336**. The Boc group was replaced by acetyl through treating **336** with TFA and subsequent $\text{Ac}_2\text{O}/\text{Et}_3\text{N}$ (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(O^{*t*}Bu)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%.

Figure 5. X-ray structure of compound **23**.

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_2CO_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_2CO_3 , EtOH, rt, 2 h, 72%, (c) AcCl, NaH, THF, 90%.

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

Scheme 49. Installation of 3-pentanyl group. Reagents and conditions: (a) i) DMP, CH_2Cl_2 , rt, 2 h; ii) $\text{LiAl}(\text{OtBu})_3$, THF, -20°C , 70%; (b) i) MsCl , Et_3N , CH_2Cl_2 ; ii) 3-pentanol, $\text{BF}_3 \cdot \text{Et}_2\text{O}$, 4 Å M.S., 52%.

The azido group in **23** was reduced to amine with PPh_3 in THF/ H_2O (90% yield). Finally, oseltamivir phosphate salt (Tamiflu) was obtained by treatment of **3** with H_3PO_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_2O , reflux, 2 h, 90%; (b) H_3PO_4 , EtOH, 80%; (c) LiOH, THF/ H_2O , 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).

Figure 8. Organization of actin network in PC12 cells with (left) or without oseltamivir carboxylate) treatment (0.5 mM for 20 h).

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.

Figure 9. Typical TIRFM images of a PC12 cell with (left) or without (right) OC treatment (0.5 mM for 20 h). Individual footprints of the subplasmalemmal vesicles can be resolved as green dots (highlighted by circles). The cell contours are outlined by the dashed lines.

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).^[74] 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 ($0.27 \pm 0.02 \mu\text{m}^2$ vs. $0.58 \pm 0.07 \mu\text{m}^2$) (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 $\pm$ 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

Part I Sugar-based synthesis of Tamiflu and its inhibitory effects on cell secretion

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 a 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.^[75] 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

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 stereoselective 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, *n*BuLi, THF, -78 °C, 1 h, rt, 1 h, 71%; (e) Ph₂O, 210 °C, 88%; (f) i) NaClO₂, NaH₂PO₄, 2-methyl-2-butene, *t*BuOH/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(O*t*Bu)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 premierTM 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.

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 MgCl₂, 2 CaCl₂, 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 Elektronik, 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 CaCl₂, 1 MgCl₂, 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):

tert-Butyldimethylsilyl chloride (11.6 g, 0.08 mol) was added portionwise to a solution of D-galactal (9.4 g, 0.06 mol) and imidazole (10.5 g, 0.15 mol) in anhydrous DMF (100 mL) at 0 °C. The mixture was allowed to warm to room temperature and stirred overnight. The mixture was diluted with ether (100 mL) and washed with water and brine. The organic layer was dried over Na₂SO₄, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (EtOAc/hexane = 1: 1) to give **288** (13.2 g, 79%) as a colorless oil. $[\alpha]_D^{23} = +9.8$ ($c = 0.5$ in CHCl₃); (lit.^[79] $[\alpha]_D^{20} = +17.7$ ($c = 1.26$ in CHCl₃)); ¹H NMR (400 MHz, CDCl₃): 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 (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, 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, 9H), 0.11 (s, 6H) ppm; ¹³C NMR (100 MHz, CDCl₃): δ = 144.5, 103.1, 75.7, 66.1, 64.2, 63.4, 25.8, 18.2, -5.5, -5.6 ppm; IR (neat): ν = 3385, 3053, 2926, 2855, 1458, 1261, 1107, 837 cm⁻¹; MS (m/z) 283 [M+Na]⁺; HRMS (ESI): calcd. for C₁₂H₂₄O₄SiNa [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 PMBBBr (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. $[\alpha]_D^{23} = +12.2$ ($c = 1.0$ 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; $[\alpha]_D^{22} = -97.2$ ($c = 1.0$ in CHCl_3); ^1H NMR (400 MHz, CDCl_3): $\delta = 7.30$ - 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; ^{13}C NMR (100 MHz, CDCl_3): $\delta = 159.4$, 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): $\nu = 3447$, 2934 , 1645 , 1612 , 1514 , 1248 , 1034 , 822 cm^{-1} ; MS (m/z) 410 $[\text{M}+\text{Na}]^+$; HRMS (ESI): calcd. for $\text{C}_{22}\text{H}_{26}\text{O}_6\text{Na}$ $[\text{M}+\text{Na}]^+$, 409.1627; found, 409.1626.

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

(291):

To a solution of **290** (11.2 g, 0.03 mol) dry CH_2Cl_2 (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 $\text{Na}_2\text{S}_2\text{O}_3/\text{NaHCO}_3$ (80 mL, $v/v=1:1$). The resulting solution was partitioned. The organic layer was washed with sat. NaHCO_3 and brine, dried over Na_2SO_4 , filtered and concentrated. The residue was purified by column

chromatography on silica gel (EtOAc/hexane = 1: 2) to give **291** (9.58 g, 86%) as a colorless oil. $[\alpha]_D^{22} = +28.8$ ($c = 0.55$ in CHCl_3); ^1H 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 $[\text{M}]^+$; HRMS (EI): calcd. for $\text{C}_{22}\text{H}_{24}\text{O}_6$ $[\text{M}]^+$, 384.1573; found, 384.1501.

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

To a suspension of methyltriphenylphosphonium bromide (14.5 g, 40.6 mmol) in anhydrous THF (60 mL) was added $n\text{BuLi}$ (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 $^{\circ}\text{C}$. Then a solution of aldehyde **291** (7.8 g, 20.3 mmol) in anhydrous THF (40 mL) was added dropwise. The suspension was stirred for 30 min at -78 $^{\circ}\text{C}$ and 1 h at room temperature. The mixture was then quenched with saturated aqueous NH_4Cl 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_2SO_4 . After removing the solvent, the residue was purified by flash chromatography on neutral Al_2O_3 (EtOAc/ hexane = 1: 10 to 1: 2) to

afford the alkene **283** (4.81 g, 62%) as a colorless oil. $[\alpha]_D^{23} = -42.4$ ($c = 0.63$ in CHCl_3); ^1H NMR (400 MHz, CDCl_3): $\delta = 7.30$ - 7.26 (m, 4H), 6.89 - 6.86 (m, 4H), 6.40 (dd, $J = 6.3, 1.5$ Hz, 1H), 6.07 (ddd, $J = 17.2, 10.5, 6.6$ Hz, 1H), 5.33 (dt, $J = 17.2, 1.3$ Hz, 1H), 5.23 (dt, $J = 11.7, 1.3$ Hz, 1H), 4.84 - 4.79 (m, 2H), 4.65 (d, $J = 11.7$ Hz, 1H), 4.56 (dd, $J = 14.9, 11.9$ Hz, 1H), 4.37 (d, $J = 6.4$ Hz, 1H), 4.21 - 4.20 (m, 1H), 3.81 - 3.80 (m containing 2s, 7H) ppm; ^{13}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) ppm; IR (neat): $\nu = 2999, 2904, 2835, 1645, 1612, 1514, 1248, 1034, 822$ cm^{-1} ; MS (m/z) 406 $[\text{M}+\text{Na}]^+$; HRMS (ESI): calcd. for $\text{C}_{23}\text{H}_{26}\text{O}_5\text{Na}$ $[\text{M}+\text{Na}]^+$, 405.1678; found, 405.1680.

(1S,5S,6R)-5,6-Di(4-methoxybenzyloxy)-3-cyclohexene-1-carbaldehyde (281):

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. ^1H NMR (400 MHz, CDCl_3): $\delta = 9.85$ (d, $J = 2.0$ Hz, 1H), 7.31 (d, $J = 8.5$ Hz, 2H), 7.21 (d, $J = 8.5$ Hz, 2H), 6.87 (d, $J = 9.3$ Hz, 4H), 5.87 - 5.78 (m, 2H), 4.71 - 4.59 (m, 3H), 4.39 (d, $J = 11.5$ Hz, 1H), 4.05 (d, $J = 4.0$ Hz, 1H), 3.81 (s, 3H), 3.80 (s, 3H), 3.76 (dd, $J = 11.0, 3.4$ Hz, 1H), 3.23 - 3.17 (m, 1H), 2.28 - 2.20 (m, 1H) ppm; ^{13}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 $[\text{M}+\text{Na}]^+$; HRMS (EI): calcd. for $\text{C}_{23}\text{H}_{26}\text{O}_5$ $[\text{M}]^+$, 382.1780; found, 382.1760.

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

To a solution of **281** (80.0 mg, 0.21 mmol) and 2-methyl-2-butene (0.3 mL) in a *t*BuOH/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),

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 $[\text{M}+\text{Na}]^+$; HRMS (ESI): calcd. for $\text{C}_{23}\text{H}_{36}\text{O}_5\text{SiNa}$ $[\text{M}+\text{Na}]^+$, 443.2230; found, 443.2214.

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

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. ^1H 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 $[\text{M}+\text{H}]^+$; HRMS (ESI): calcd. for $\text{C}_{12}\text{H}_{25}\text{O}_4\text{Si}$ $[\text{M}+\text{H}]^+$, 261.1522; found, 261.1519.

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

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, CDCl_3): 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; ^{13}C NMR (100 MHz, CDCl_3): 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 $[\text{M}+\text{Na}]^+$; HRMS (ESI): calcd. for $\text{C}_{28}\text{H}_{40}\text{O}_6\text{SiNa}$ $[\text{M}+\text{Na}]^+$, 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, CDCl_3): 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; ^{13}C NMR (100 MHz, CDCl_3): 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 $[\text{M}+\text{Na}]^+$; HRMS (ESI): calcd. for $\text{C}_{22}\text{H}_{26}\text{O}_6\text{Na}$ $[\text{M}+\text{Na}]^+$, 409.1627; found, 409.1630.

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

(296):

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. ^1H 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 $[\text{M}+\text{Na}]^+$; HRMS (ESI): calcd. for $\text{C}_{22}\text{H}_{24}\text{O}_6$ $[\text{M}+\text{Na}]^+$, 407.1471; found, 406.1478.

(2R,3S,4R)-3,4-Di(4-methoxybenzyloxy)-2-vinyl-2,3-dihydro-2H-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. ^1H NMR (400 MHz, CDCl_3): δ = 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): δ = 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): ν = 3007, 2901, 1645, 1614, 1514, 1248, 1036, 820, 750 cm^{-1} ; MS (m/z) 406 $[\text{M}+\text{Na}]^+$; HRMS (ESI): calcd. for $\text{C}_{23}\text{H}_{26}\text{O}_5\text{Na}$ $[\text{M}+\text{Na}]^+$, 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. ^1H NMR (400 MHz, CDCl_3): δ = 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): $\nu = 3005, 2911, 2835, 1724, 1611, 1512, 1248, 1034, 820\text{ cm}^{-1}$; HRMS (EI): calcd. for $\text{C}_{23}\text{H}_{26}\text{O}_5$ $[\text{M}]^+$, 382.1780; found, 382.1763.

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

To a solution of D-(+)-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_2Cl_2 and then washed with saturated aqueous NH_4Cl , H_2O and brine. The organic layer was dried over Na_2SO_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); $[\alpha]_{\text{D}}^{22} = -6.7$ ($c = 1.0$ in CHCl_3); ^1H 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): δ = 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): ν = 3292, 2906, 1639, 1518, 1254, 1096, 824, 756 cm^{-1} . MS (m/z) 265 $[\text{M}+\text{H}]^+$; HRMS (ESI): calcd. for $\text{C}_{14}\text{H}_{16}\text{O}_5\text{Na}$ $[\text{M}+\text{Na}]^+$, 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_2O , washed with saturated NH_4Cl solution and dried over Na_2SO_4 . Evaporation of the organic solvent under reduced pressure gave a crude product, which was purified by flash column chromatography on silica gel ($\text{EtOAc}/\text{hexane}$ = 1: 15) to afford **304** (20.4 g, 90%) as a colorless oil. $[\alpha]_{\text{D}}^{22} = -97.3$ (c = 1.0 in CHCl_3); ^1H NMR (400 MHz, CDCl_3): δ = 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): δ = 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): ν = 2932, 2858, 1640, 1520, 1249, 1103, 868 cm^{-1} ; MS (m/z) 379 $[\text{M}+\text{H}]^+$; HRMS (ESI): calcd. for $\text{C}_{14}\text{H}_{16}\text{O}_5$ $[\text{M}+\text{H}]^+$, 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 CH_2Cl_2 (100 mL), DIBAL-H (1 M in toluene, 68.6 mL, 68.6 mmol) was slowly added at $-15\text{ }^\circ\text{C}$ under nitrogen. The reaction mixture was stirred at $-15\text{ }^\circ\text{C}$ for 20 min and at rt for 2 h, then it was cooled to $0\text{ }^\circ\text{C}$ and MeOH (1 mL) was added dropwise. EtOAc (60 mL) and sodium potassium tartrate (20%- H_2O , 80 mL) were added at room temperature. The mixture was stirred for 1 h, extracted with EtOAc ($2 \times 60\text{ mL}$). The organic layers were washed with brine ($2 \times 100\text{ mL}$) and dried over Na_2SO_4 . 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. $[\alpha]_{\text{D}}^{22} = -20.6$ ($c = 1.0$ in CHCl_3); ^1H NMR (400 MHz, CDCl_3): $\delta = 7.28$ (d, $J = 8.6\text{ Hz}$, 2H), 6.88 (d, $J = 8.6\text{ Hz}$, 2H), 6.33 (d, $J = 6.0\text{ Hz}$, 1H), 4.77 (d, $J = 11.0\text{ Hz}$, 1H), 4.67 (dd, $J = 4.9, 2.9\text{ Hz}$, 1H), 4.63 (d, $J = 11.0\text{ Hz}$, 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, $J = 6.6\text{ Hz}$, 1H), 0.92 (s, 9H), 0.12 (s, 6H) ppm; ^{13}C NMR (100 MHz, CDCl_3): $\delta = 159.9, 143.3, 130.0, 129.6, 113.9, 103.4, 76.4, 73.5, 68.6, 55.3, 25.8, 18.0, -4.4, -4.7\text{ ppm}$; IR (neat): $\nu = 3445, 2932, 2859, 1651, 1516, 1250, 1096, 837\text{ cm}^{-1}$; MS (m/z) 404 $[\text{M}+\text{Na}]^+$; HRMS(ESI): calcd. for $\text{C}_{20}\text{H}_{32}\text{O}_5\text{SiNa}$ $[\text{M}+\text{Na}]^+$, 403.1917; found, 403.1902.

(2R,3R,4R)-4-(tert-Butyldimethylsilyloxy)-3-(4-methoxybenzyloxy)-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 CH_2Cl_2 (200 mL). The suspension was stirred for 2 h at room temperature under nitrogen. Saturated aqueous $\text{Na}_2\text{S}_2\text{O}_3$ (100 mL) and NaHCO_3 (100 mL) were added slowly to the reaction mixture. The resulting solution was separated. The organic layer was washed with saturated NaHCO_3 solution and brine, dried over Na_2SO_4 , 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 *n*BuLi (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 NH_4Cl 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 Na_2SO_4 . After removing the solvent, the residue was purified by flash chromatography on neutral Al_2O_3 (EtOAc/hexane = 1: 40) to afford the alkene **302** (10.1 g, 67%) as a colorless oil. $[\alpha]_D^{22} = -44.1$ ($c = 1.0$ in CHCl_3); ^1H NMR (400 MHz, CDCl_3): $\delta = 7.26$ (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 $[\text{M}+\text{Na}]^+$; HRMS (ESI): calcd. for $\text{C}_{21}\text{H}_{32}\text{O}_4\text{SiNa} [\text{M}+\text{Na}]^+$, 399.1968; found, 399.1976.

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

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. $[\alpha]_{\text{D}}^{22} = -37.8$ ($c = 1.2$ in CHCl_3); ^1H 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 $[\text{M}+\text{Na}]^+$; HRMS (ESI): calcd. for $\text{C}_{21}\text{H}_{32}\text{O}_4\text{SiNa} [\text{M}+\text{Na}]^+$, 399.1968; found, 399.1957.

Ethyl (1*S*,5*R*,6*R*)-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); ^1H 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 $[\text{M}+\text{Na}]^+$; HRMS (ESI): calcd. for $\text{C}_{23}\text{H}_{36}\text{O}_5\text{SiNa}$ $[\text{M}+\text{Na}]^+$, 443.2230; found, 443.2219.

Ethyl (1*S*,5*R*,6*R*)-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 $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O}$ (80 mL, v/v = 1:1). The mixture was stirred at room temperature. After 6 h, $\text{Na}_2\text{S}_2\text{O}_3$ (80 mL) was added to the mixture. The suspension was filtered. The

filtrate was extracted with CH_2Cl_2 . The organic layer was washed with saturated NaHCO_3 solution and brine successively, dried over Na_2SO_4 , filtered, and evaporated. The residue was purified by column chromatography on silica gel ($\text{EtOAc}/\text{hexane} = 1:8$) to give **307** (3.82 g, 92%) as a colorless oil. $[\alpha]_{\text{D}}^{22} = -42.5$ ($c = 1.03$ in CHCl_3); ^1H NMR (400 MHz, CDCl_3): $\delta = 5.71\text{--}5.57$ (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; ^{13}C NMR (100 MHz, CDCl_3): $\delta = 174.0, 128.4, 126.5, 74.8, 72.9, 60.6, 46.7, 28.3, 25.8, 18.1, 14.0, -4.2, -5.0$ ppm; IR (neat) $\nu = 3480, 2932, 2855, 1736, 1254, 1126, 837$ cm^{-1} ; MS (m/z) 323 $[\text{M}+\text{Na}]^+$; HRMS (ESI): calcd. for $\text{C}_{15}\text{H}_{28}\text{O}_4\text{SiNa}$ $[\text{M}+\text{Na}]^+$, 323.1655; found, 323.1642.

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

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 K_2CO_3 (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 ($\text{EtOAc}/\text{hexane} = 1:3$) to give **300** as a white solid (0.40 g, 87%). mp 71–73 °C; $[\alpha]_{\text{D}}^{22} = -50.1$ ($c = 1.0$ in CHCl_3); ^1H NMR (400 MHz,

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

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; $[\alpha]_{\text{D}}^{22} = -62.9$ ($c = 1.03$ in CHCl_3); ^1H 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 $[\text{M}+\text{Na}]^+$; HRMS (ESI): calcd. for $\text{C}_{16}\text{H}_{29}\text{NO}_6\text{SiNa}$ $[\text{M}+\text{Na}]^+$, 382.1662; found, 323.1660.

To a solution of **310** (2.6 g, 7.2 mmol) in Et_2O (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_2SO_4 . The solvent was removed under reduced pressure and the residue was purified by column chromatography on silica gel ($\text{EtOAc}/\text{hexane} = 1: 4$) to give compound **311** (3.29 g, 89%) as a white solid. mp 121-123 °C; $[\alpha]_{\text{D}}^{22} = -50.3$ ($c = 0.97$ in CHCl_3); ^1H 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): $\nu = 3366, 3019, 2930, 2857, 1734, 1381, 1215, 756$ cm^{-1} ; MS (m/z) 536 $[\text{M}+\text{Na}]^+$; HRMS (ESI): calcd. for $\text{C}_{23}\text{H}_{35}\text{NO}_8\text{SSiNa}$ $[\text{M}+\text{Na}]^+$, 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 4h 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. $[\alpha]_D^{23} = +14.1$ ($c = 1.0$ 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, 9H), 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, 1757, 1512, 1247, 1111, 837, 756 cm⁻¹; MS (m/z) 480 [M+H]⁺; HRMS (ESI) calcd. for C₂₄H₃₉N₂O₆Si [M+H]⁺, 479.2577; found 479.2570.

Ethyl (3a*R*,4*R*,6*S*,7*R*,7a*R*)-7-(*tert*-butyldimethylsilyloxy)-4-(4-methoxybenzylamino)-2-oxooctahydro benzoxazole-6-carboxylate (313):

$[\alpha]_D^{23} = +13.1$ ($c = 1.0$ in CHCl_3); ^1H NMR (400 MHz, CDCl_3): $\delta = 7.17$ (d, $J = 8.4$ Hz, 2H), 6.85 (d, $J = 8.4$ Hz, 2H), 5.76 (t, $J = 5.5$ Hz, 1H), 4.32-4.22 (m, 1H), 4.15-4.10 (m, 2H), 3.97 (dd, $J = 7.2, 4.8$ Hz, 1H), 3.79 (s, 3H), 3.74 (s, 1H), 3.22 (d, $J = 8.0$ Hz, 1H), 2.93-2.91 (m, 1H), 2.80-2.77 (m, 1H), 2.49 (q, $J = 7.0$ Hz, 1H), 2.22-2.20 (m, 1H), 1.23 (t, $J = 7.2$ Hz, 3H), 0.85 (s, 9H), 0.11 (s, 3H), 0.03 (s, 3H) ppm; ^{13}C NMR (100 MHz, CDCl_3): $\delta = 174.3, 164.0, 159.0, 130.4, 128.9, 114.0, 71.3, 71.1, 60.9, 55.3, 46.0, 44.1, 42.5, 37.2, 25.7, 24.1, 18.0, 13.9, -4.5, -5.1$ ppm; IR (neat) $\nu = 3306, 2930, 2855, 1732, 1514, 1248, 1115, 837, 756$ cm^{-1} ; MS (m/z) 480 $[\text{M}+\text{H}]^+$; HRMS (ESI) calcd. for $\text{C}_{24}\text{H}_{39}\text{N}_2\text{O}_6\text{Si}$ $[\text{M}+\text{H}]^+$, 479.2577; found 479.2577.

Ethyl (3a*R*,4*S*,6*S*,7*R*,7a*R*)-4-(allylamino)-7-(*tert*-butyldimethylsilyloxy)-2-oxooctahydro 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. $[\alpha]_D^{23} = +6.6$ ($c = 0.8$ in CHCl_3); ^1H 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) $\nu = 3306, 2930, 2857, 1732, 1250, 1111, 839, 756$ cm^{-1} ; MS (m/z) 400 $[\text{M}+\text{H}]^+$; HRMS (ESI) calcd. for $\text{C}_{19}\text{H}_{35}\text{N}_2\text{O}_5\text{Si}$ $[\text{M}+\text{H}]^+$, 399.2315; found 399.2312.

Ethyl (3a*R*,4*R*,6*S*,7*R*,7a*R*)-4-(allylamino)-7-(*tert*-butyldimethylsilyloxy)-2-oxoocta hydro benzoxazole-6-carboxylate (315**):**

$[\alpha]_D^{23} = 1.45$ ($c = 0.8$ in CHCl_3); ^1H 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 $[\text{M}+\text{H}]^+$; HRMS (ESI) calcd. for $\text{C}_{19}\text{H}_{35}\text{N}_2\text{O}_5\text{Si}$ $[\text{M}+\text{H}]^+$, 399.2315; found 399.2306.

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

The *N*-tosyloxy carbamate **311** (2.98 g, 5.8 mmol), K_2CO_3 (4.01 g, 29.0 mmol) and $(\text{CuOTf})_2 \cdot \text{toluene}$ (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_2Cl_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 $^\circ\text{C}$ under nitrogen. The mixture was warmed to room temperature and stirred for 4h. 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 $^\circ\text{C}$; $[\alpha]_{\text{D}}^{22} = +4.8$ ($c = 1.0$ in CHCl_3); ^1H 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 $[\text{M}+\text{Na}]^+$; HRMS (ESI): calcd. for $\text{C}_{16}\text{H}_{29}\text{N}_4\text{O}_5\text{Si}$ $[\text{M}+\text{H}]^+$, 385.1907; found, 385.1904.

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

Mp 140-142 °C; $[\alpha]_{\text{D}}^{22} = +64.8$ ($c = 1.5$ in CHCl_3); ^1H NMR (400 MHz, CDCl_3): δ = 5.71 (s, 1H), 4.60 (s, 1H), 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 $[\text{M}+\text{Na}]^+$; HRMS (ESI): calcd for $\text{C}_{16}\text{H}_{29}\text{N}_4\text{O}_5\text{Si}$ $[\text{M}+\text{H}]^+$, 385.1907; found, 385.1898.

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

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-dihydrobenzooxazole-6-carboxylate (327):

¹H NMR (300 MHz, CDCl₃): δ = 8.73 (br s, 1H), 7.96 (dd, *J* = 8.2, 1.4 Hz, 1H), 7.89 (d, *J* = 1.4 Hz, 1H), 7.12 (d, *J* = 8.2 Hz, 1H), 4.39 (q, *J* = 7.1 Hz, 2H), 1.41 (t, *J* = 7.1 Hz, 3H) ppm; ¹³C NMR (75 MHz, CDCl₃): δ = 165.7, 154.9, 143.5, 133.0, 126.6, 125.6, 111.4, 109.2, 61.3, 14.3 ppm.

Ethyl (3aR,4S,6S,7R,7aR)-4-azido-7-hydroxy-2-oxooctahydrobenzoxazole-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. ^1H NMR (400 MHz, CDCl_3): δ = 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): δ = 173.2, 159.4, 80.0, 70.8, 61.5, 57.4, 56.3, 41.5, 26.7, 14.1 ppm; IR (neat): ν = 3320, 2106, 1720, 1643, 1251, 748 cm^{-1} ; MS (m/z) 271 $[\text{M}+\text{H}]^+$; HRMS (ESI): calcd. for $\text{C}_{11}\text{H}_{16}\text{N}_4\text{O}_4\text{Na}$ $[\text{M}+\text{Na}]^+$, 293.0862; found, 293.0869.

Ethyl (3aR,4S,7aS)-4-azido-2-oxo-2,3,3a,4,5,7a-hexahydrobenzoxazole-6-carboxylate (326):

Et₃N (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 CH₂Cl₂ (15 mL) at 0 °C under N₂. The mixture was allowed to warm to room temperature and stirred for 2 h. The mixture was washed with aqueous NH₄Cl solution and brine. The organic phase was dried over Na₂SO₄, 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 CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ = 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; ¹³C NMR (100 MHz, CDCl₃): δ = 164.9, 152.6, 138.9, 132.5, 77.2, 61.7, 60.1, 55.0, 26.7, 14.1 ppm; IR (neat) ν = 3018, 2110, 1767, 1719, 1215, 762 cm⁻¹; MS (m/z) 253 [M+H]⁺; HRMS (ESI): calcd. for C₁₀H₁₂N₄O₄Na [M+Na]⁺, 275.0756; found, 275.0751.

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

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 CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ = 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,

$J = 7.1$ Hz, 2H), 4.09 (dt, $J = 7.2, 4.2$ Hz, 1H), 2.78 (dd, $J = 18.0, 4.2$ Hz, 1H), 2.50 (dd, $J = 18.0, 7.2$ Hz, 1H), 1.31 (t, $J = 7.1$ Hz, 3H) ppm; ^{13}C NMR (100 MHz, CDCl_3): $\delta = 164.8, 150.9, 149.4, 132.7, 129.7, 85.0, 69.8, 61.6, 57.5, 56.1, 27.8, 26.1, 14.1$ ppm; IR (neat): $\nu = 2982, 2106, 1817, 1721, 1253, 1080, 758\text{ cm}^{-1}$; MS (m/z) 376 $[\text{M}+\text{Na}]^+$; HRMS (ESI): calcd for $\text{C}_{15}\text{H}_{20}\text{N}_4\text{O}_6\text{Na}$ $[\text{M}+\text{Na}]^+$, 375.1281; found, 375.1217.

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

Cs_2CO_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. $[\alpha]_{\text{D}}^{22} = +61.7$ ($c = 0.7$ in CHCl_3); ^1H NMR (400 MHz, CDCl_3): $\delta = 6.87$ (d, $J = 1.4$ Hz, 1H), 5.14 (d, $J = 7.7$ Hz, 1H), 4.47 (s, 1H), 4.21 (q, $J = 7.1$ Hz, 2H), 3.79 (s, 2H), 2.91-2.81 (m, 2H), 2.35-2.29 (m, 1H), 1.46 (s, 9H), 1.30 (t, $J = 7.1$ Hz, 3H) ppm; ^{13}C NMR(100 MHz, CDCl_3): $\delta = 165.8, 155.9, 136.1, 130.5, 80.3, 65.1, 61.2, 56.8, 53.4, 29.6, 28.3, 14.1$ ppm; IR (neat) $\nu = 3366, 2978, 2104, 1713, 1699, 1490, 1250, 1163, 756\text{ cm}^{-1}$; MS (m/z) 350 $[\text{M}+\text{Na}]^+$; HRMS (ESI): calcd. for $\text{C}_{14}\text{H}_{22}\text{N}_4\text{O}_5\text{Na}$ $[\text{M}+\text{Na}]^+$, 349.1488; found, 349.1486.

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

DMP (0.38 g, 0.90 mmol) was added to a solution of compound **330** (0.20 g, 0.60 mmol) in dry CH_2Cl_2 (15 mL). The mixture was stirred 2 h at room temperature. Aqueous $\text{Na}_2\text{S}_2\text{O}_3$ and NaHCO_3 solution (30 mL, v/v = 1:1) was added portionwise. The resulting solution was separated. The organic phase was washed with NaHCO_3 solution (15 mL) and brine (15 mL), dried over Na_2SO_4 , concentrated. The crude product was dissolved in dry THF (20 mL) and added dropwise to a solution of $\text{LiAlH}(\text{O}t\text{Bu})_3$ (0.90 mL, 1 M in THF) at $-20\text{ }^\circ\text{C}$. The mixture was allowed to warm to room temperature and stirred 2 h. Saturated NH_4Cl 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 Na_2SO_4 . 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. $110\text{--}111\text{ }^\circ\text{C}$; $[\alpha]_{\text{D}}^{22} = +67.5$ ($c = 1.0$ in CHCl_3); ^1H NMR (400 MHz, CDCl_3): $\delta = 6.81$ (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; ^{13}C NMR (100 MHz, CDCl_3): $\delta = 165.7, 157.1, 138.5, 127.6, 81.1, 71.4, 61.1, 58.1, 57.9, 29.6, 28.2, 14.1$ ppm; IR (neat) $\nu = 3382, 2978, 2105, 1715, 1699, 1493, 1249, 1163, 756\text{ cm}^{-1}$; MS (m/z) 349

$[M+Na]^+$; HRMS (ESI): calcd. for $C_{14}H_{22}N_4O_5Na$ $[M+Na]^+$, 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):

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 $CHCl_3$); 1H NMR (400 MHz, $CDCl_3$): $\delta = 7.19$ (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, $CDCl_3$): $\delta = 165.5, 160.5, 133.3, 129.7, 82.5, 61.0, 52.7, 40.6, 33.3, 27.8, 25.9, 14.2$ ppm; IR (neat): $\nu = 3420, 3019, 2982, 2110, 1701, 1260, 1215, 1153, 756$ cm^{-1} ; MS (m/z) 331 $[M+Na]^+$; HRMS (ESI): calcd. for $C_{14}H_{20}N_4O_4Na$ $[M+Na]^+$, 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), $\text{BF}_3 \cdot \text{OEt}_2$ (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_2Cl_2 (3×20 mL) and the combined organic layer was dried over Na_2SO_4 . The solvent was removed under reduced pressure, and the residue was purified by column chromatography ($\text{EtOAc}/\text{hexane} = 1:6$) to give compound **336** (57 mg, 80%) as a white solid. ^1H 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 $[\text{M}-\text{Boc}+\text{H}]^+$; HRMS (ESI): calcd. for $\text{C}_{19}\text{H}_{32}\text{N}_4\text{O}_5\text{Na} [\text{M}+\text{Na}]^+$, 419.2270; found, 419.2267.

Ethyl (3aR,4S,7aS)-3-acetyl-4-azido-2-oxo-3a,4,5,7a-tetrahydrobenzooxazole-6-carboxylate (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 dissolved 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; $[\alpha]_D^{22} = -136.7$ ($c = 0.92$ 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 CHCl_3); ^1H NMR (400 MHz, CDCl_3): $\delta = 6.89$ (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, CDCl_3): $\delta = 171.1, 165.8, 136.1, 130.5, 64.8, 61.3, 56.3, 52.1, 29.6, 23.4, 14.1$ ppm; IR (neat): $\nu = 3340, 2106, 1643, 1252, 750$ cm^{-1} ; MS (m/z) 269 $[\text{M}+\text{H}]^+$; HRMS (ESI): calcd. for $\text{C}_{11}\text{H}_{16}\text{N}_4\text{O}_4\text{Na}$ $[\text{M}+\text{Na}]^+$, 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 CHCl_3); ^1H NMR (400 MHz, CDCl_3): $\delta = 6.80$ (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): $\nu = 3316, 2100, 1714, 1258\text{ cm}^{-1}$; MS (m/z) 269 $[\text{M}+\text{H}]^+$; HRMS (ESI): calcd. for $\text{C}_{11}\text{H}_{17}\text{N}_4\text{O}_4$ $[\text{M}+\text{H}]^+$, 269.1250; found, 269.1251.

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

To a solution of **190** (0.20 g, 0.74 mmol) in CH_2Cl_2 (15 mL), Et_3N (0.30 mL, 2.22 mmol) and MsCl (86.0 μL , 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_4Cl solution and brine successively. The organic phase was dried over Na_2SO_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 $\text{BF}_3 \cdot \text{OEt}_2$ (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_2Cl_2 (3×20 mL) and the combined organic layer was dried over Na_2SO_4 . The solvent was removed under reduced pressure, and the residue was purified by column chromatography ($\text{EtOAc}/\text{hexane} = 1:6$) to give compound **23** (0.13 g, 52%) as a white solid. mp 138-140 °C; $[\alpha]_{\text{D}}^{22} =$

–41.4 ($c = 1.02$ in CHCl_3); ^1H NMR (400 MHz, CDCl_3): $\delta = 6.77$ (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; ^{13}C NMR (100 MHz, CDCl_3): $\delta = 171.1, 165.8, 137.9, 128.1, 82.0, 73.4, 61.0, 57.9, 57.2, 30.5, 26.2, 25.6, 23.5, 14.1, 9.5, 9.2$ ppm; IR (neat): $\nu = 3390, 3019, 2102, 1709, 1215, 756$ cm^{-1} ; MS (m/z) 339 $[\text{M}+\text{H}]^+$; HRMS (ESI): calcd. for $\text{C}_{16}\text{H}_{26}\text{N}_4\text{O}_4\text{Na}$ $[\text{M}+\text{Na}]^+$, 361.1852; found, 361.1843.

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

PPh_3 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_2Cl_2 and washed with brine (20 mL). The organic layer was dried over Na_2SO_4 , 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. $[\alpha]_{\text{D}}^{22} = -58.1$ ($c = 1.0$ in CHCl_3); ^1H NMR (400 MHz, CDCl_3): $\delta = 6.76$ (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,

1H), 3.34-3.29 (m, 1H), 3.19 (dt, $J = 10.0, 5.4$ Hz, 1H), 2.73 (dd, $J = 17.7, 5.0$ Hz, 1H) 2.16-2.09 (m, 1H), 2.02 (s, 1H), 1.51-1.46 (m, 4H), 1.26 (t, $J = 7.1$ Hz, 3H), 0.90-0.85 (m, 6H) ppm; ^{13}C NMR (100 MHz, CDCl_3): $\delta = 171.0, 166.3, 137.6, 129.4, 81.6, 74.8, 60.8, 58.8, 49.1, 33.5, 26.2, 25.6, 23.6, 14.1, 9.5, 9.3$ ppm; IR (neat): $\nu = 3389, 2966, 1643, 1244, 735\text{ cm}^{-1}$; MS (m/z) 314 $[\text{M}+\text{H}]^+$; HRMS (ESI): calcd. for $\text{C}_{16}\text{H}_{29}\text{N}_2\text{O}_4$ $[\text{M}+\text{H}]^+$, 313.2127; found, 313.2126.

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); $[\alpha]_{\text{D}}^{22} = -35.3$ ($c = 1.0$ in H_2O) (lit.^[24] $[\alpha]_{\text{D}}^{20} = -35.8$ ($c = 1.0$ in H_2O)); ^1H NMR (400 MHz, D_2O): $\delta = 6.86$ (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; ^{13}C NMR (100 MHz, D_2O): $\delta = 175.2, 167.4, 137.8, 127.6, 84.3, 75.1, 62.4, 52.6, 49.1, 28.1, 25.4, 25.0, 22.3, 13.3, 8.5, 8.4$ ppm; ^{31}P NMR (121 MHz, D_2O) $\delta = 0.65$; MS (m/z) 314 $[\text{M}+\text{H}]^+$; HRMS (ESI): calcd. for $\text{C}_{16}\text{H}_{29}\text{N}_2\text{O}_4$ $[\text{M}+\text{H}]^+$, 313.2127; found, 313.2122.

(3R,4R,5S)-4-Acetamido-5-amino-3-(1-ethylpropoxy)-1-cyclohexene-1-carboxylic acid (5):

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); $[\alpha]_D^{22} = -135.3$ ($c = 0.5$ in H₂O) (lit.^[24] $[\alpha]_D^{20} = -143.2$ ($c = 0.4$ 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**

Empirical formula	C16 H26 N4 O4
Temperature	173(2) K
Wavelength	0.71073 Å
Space group	P2(1)2(1)2(1)
Unit cell dimensions	a = 4.8575(2) Å $\alpha = 90^\circ$ b = 17.0746(7) Å $\beta = 90^\circ$ c = 23.2669(9) Å $\gamma = 90^\circ$
Z	4
Density (calculated)	1.165 Mg/m ³
Absorption coefficient	0.085 mm ⁻¹
F(000)	728
Crystal size	0.34 x 0.08 x 0.06 mm ³
Reflections collected	24278
Independent reflections	2013 [R(int) = 0.0499]
Completeness to $\theta = 25.02^\circ$	99.6 %
Refinement method	Full-matrix least-squares on F ²
Goodness-of-fit on F ²	1.046
Final R indices [I > 2sigma(I)]	R1 = 0.0427, wR2 = 0.1017
R indices (all data)	R1 = 0.0652, wR2 = 0.1174
Largest diff. peak and hole	0.289 and -0.201 e.Å ⁻³

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PART 2

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

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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.^[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.^[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 ($-\text{COOH}$) 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.*^[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.^[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 *et al.*^[15] modified SWNTs with 2'-aminoethyl- β -D-galactopyranoside through amidation.

Figure 1. Gal-, Man-, and their Dendrons Functionalized SWNTs.

Sun *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

improve solubility, MWNTs should be functionalized with bulky functional groups like Gal₂ 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 β -D-galactopyranosides and α -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-methacryloyl-1,2:5,6-di-isopropylidene-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 C₁₈ 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 (C₁₈- α -MM). The mucin mimic polymers were assembled on the CNTs surface in aqueous media through hydrophobic interactions between the C₁₈ lipid and CNTs surface. As reported, the C₁₈ 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 C₁₈- α -MM functionalized SWNTs bundles are heavily entangled with one another to form a 3D-network. The resulting coated CNTs (C₁₈- 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. C₁₈- α -mucin mimic functionalized SWNTs.

Shinkai and coworkers functionalized SWNTs with supramolecular wrappings, which had Schizophyllan (SPG) carrying lactoside appendages (SPG-Lac).^[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 β -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 *et al.* threaded SWNTs with η -CD which was composed of 12 glucosidic units.^[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, α -, β -, and γ -CDs were also tested for the influence on the solubility of SWNTs by Ikeda *et al.*^[24] They mixed the SWNTs with different saccharides by a mechanochemical “high-speed vibration milling technique” (HSVM). Unmodified γ -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.^[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-*N*-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 π - π 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 wide spread 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, C₁₈- α -MM SWNTs were incubated with a solution of α -GalNAc-specific receptor such as lectin *Helix Pomatia* agglutinin (HPA) conjugated with fluorescein isothiocyanate. The C₁₈- α -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 C₁₈- α -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, C₁₈- α -MM coated CNTs were first bound to HPA which is hexavalent α -GalNAc binding lectin. This complex was then bound to cell surface glycoconjugates using HPA binding sites present on CNTs. To evaluate the first pathway, C₁₈- α -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 C_{18} - 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 C_{18} - 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), *Arachishypogaea* 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. In

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. (a) C₁₈-terminated, α -GalNAc-conjugated mucin mimic (C₁₈- α -MM) functionalized 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 (RCA₁₂₀, 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 RCA₁₂₀ incubated SPG-Lac/SWNTs revealed dense clustering of RCA₁₂₀ 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.^[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*.

Recently, the same group demonstrated the effectiveness of CNTs to display multivalent monosaccharide ligands (mannose or galactose) that bind effectively with

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

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 Gal₂-SWNTs were found to have adhesion to pathogenic *E. coli* O157:H7 to result in significant cell agglutination. Similarly, Man-SWNTs, Man₂-SWNTs and Man₄-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.^[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 *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.^[38] Cyclodextrins are a family of cyclic oligosaccharides with a hydrophilic

outer surface and lipophilic central cavity. These cyclodextrins can serve as multi-functional 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.^[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 *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

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dendritic configuration with *B. anthracis* and *B. subtilis*.^[41] As Dai *et al.* reported, “functionalization partitioning” of CNT (imparting 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

macromolecules into cells,^[45] and as nanofibrous scaffolds for tissue engineering.^[46] However, the nonideal interface between CNTs and the living cells largely restricts the nanotube applications in biology.^[47] To tackle this issue, hydrophobic nanotubes have been coated with positively charged polyelectrolytes, such as poly(ethylene)imine (PEI)^[48] and poly-L-lysine (PLL),^[49] in order to promote adhesion of negatively charged cell membrane. Charge groups can also be imparted to nanotube surface *via* chemical functionalization.^[50] Non-physiological strong electrostatic interactions between the highly charged surface and the cell membrane, nevertheless, may lead to cell death or cytotoxic effects.^[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.^[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.^[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.^[53] In particular, carbohydrates are important in mediating cell adhesion process through carbohydrate-carbohydrate or carbohydrate-receptor (lectin) interactions.^[53b, 54]

Figure 6. Structures of *O*-linked carbohydrate probes for PC12 cell culture and catecholamine detection.

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.

Scheme 2. Synthetic routes to GlcNAc-lipid **1** and GlcNAc-pyrene **2**. Reagents and conditions: (a) Ac_2O , pyridine, rt, overnight, 75%; (b) MeNH_2 , MeOH, THF, rt, 2 h, 70%; (c) CCl_3CN , DBU, CH_2Cl_2 , rt, 3 h, 75%; (d) **10**, TMSOTf, CH_2Cl_2 , 0 °C to rt, 3 h, 62%; (e) NaOMe, MeOH, rt, 3 h, Amberlyst-15 resin, 88%; (f) 1-pyrenebutanol, TMSOTf, CH_2Cl_2 , 0 °C to rt, 3 h, 85%; (g) NaOMe, MeOH, rt, 3 h, Amberlyst-15 resin, 42%.

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.^[57] Glycosylation reactions were performed in the presence of trimethylsilyl triflate,^[58] 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) Ac_2O , pyridine, 0 °C, 17 h, 90%; (b) MeNH_2 , MeOH, THF, rt, 2 h, 68%; (c) CCl_3CN , DBU, CH_2Cl_2 , rt, 3 h, 73%; (d) 1-pyrenebutanol, TMSOTf, CH_2Cl_2 , 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_2O , pyridine, 0°C , overnight, 92%; (b) MeNH_2 , MeOH , THF , rt, 2 h, 69%; (c) CCl_3CN , DBU, CH_2Cl_2 , rt, 3 h, 65%; (d) 1-pyrenebutanol, TMSOTf, CH_2Cl_2 , 0°C to rt, 3 h, 40%; (e) NaOMe , MeOH , rt, 3 h, Amberlyst-15 resin, 84%.

Scheme 5. Synthetic route to GalNAc-pyrene **5**. Reagents and conditions: (a) Ac_2O , pyridine, rt, overnight, 94%; (b) MeNH_2 , MeOH, THF, rt, 2 h, 83%; (c) CCl_3CN , DBU, CH_2Cl_2 , rt, 3 h, 72%; (d) 1-pyrenebutanol, TMSOTf, CH_2Cl_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.^[59] The SWNT-nets were functionalized by incubation with 5 mM glycoconjugates 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.*^[60] (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 μS to 0.1 μS 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

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

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

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 μm. (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 mm²) 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 *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^{2+} channels. Ca^{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 π - π

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^+) solution was introduced to PC12 cells cultured on glycosylated SWNT-net (Fig. 10b). When another common secretagogue, calcymycin (1 mM) which inserts into cell membrane and forms Ca^{2+} selective ion channel, was applied, similar current spikes were elicited. We hypothesized that each spike corresponds to Ca^{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 μm) positioned 1-2 μm away from the target cell to locally deliver high K^+ 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^+ solution and calcymycin were failed to produce any signals. It is arguable that the observed spike may be due to the acidic vesicular fluids ($\sim pH$ 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 $\sim 5\%$ 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 premierTM 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):

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):

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 $[\text{M}+\text{H}]^+$. Data were in agreement with those reported in the literature.^[69]

2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy- α -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 *in vacuo* and the residue was purified by flash column chromatography on silica gel ($\text{EtOAc}/\text{CH}_2\text{Cl}_2 = 1:1$ to $3:1$) to give compound **8** as a colorless oil (1.02 g, 70%). ^1H 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 $[\text{M}+\text{H}]^+$. Data were in agreement with those reported in the literature.^[70]

2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy- α -D-glucopyranosyl trichloroacetimidate**(9):**

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%). ^1H NMR (300 MHz, CDCl_3) δ = 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) δ = 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- β -D-glucopyranoside (11):

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 μ L, 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); ¹H 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 (dd, J = 4.7, 12.2 Hz, 1H), 4.12 (dd, 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; ¹³C 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); ^1H NMR (400 MHz, MeOD) δ = 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) δ = 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) ν = 3240, 2150, 1670, 1025, 852 cm^{-1} ; MS (m/z) 503 $[\text{M}+\text{H}]^+$; HRMS (ESI) calcd. for $\text{C}_{27}\text{H}_{51}\text{NO}_7\text{Na}$ $[\text{M}+\text{Na}]^+$, 524.3563; found, 524.3561.

4-(1-Pyrenyl)butyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy- β -D-glucopyranoside (12):

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 μL , 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); ^1H NMR (500 MHz, CDCl_3) δ = 8.26

(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) $\nu = 3289, 2940, 1748, 1231, 1044, 847, 754\text{ cm}^{-1}$; MS (m/z) 605 $[\text{M}+\text{H}]^+$; HRMS (ESI) calcd. for $\text{C}_{34}\text{H}_{37}\text{NO}_9$ $[\text{M}+\text{H}]^+$, 604.2546; found, 604.2545.

4-(1-Pyrenyl)butyl 2-acetamido-2-deoxy- β -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 $>160\text{ }^\circ\text{C}$ decomposed; $[\alpha]_{\text{D}}^{22} = -15.3$ ($c = 0.5$, MeOH); ^1H 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

(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; ^{13}C NMR (100 MHz, MeOD) $\delta = 173.7, 138.3, 132.9, 132.4, 131.2, 129.9, 128.6, 128.5, 128.3, 127.6, 127.0, 126.2$ (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) $\nu = 3265, 2374, 1653, 1034, 835\text{ cm}^{-1}$; MS (m/z) 500 $[\text{M}+\text{Na}]^+$; HRMS (ESI) calcd. for $\text{C}_{28}\text{H}_{31}\text{NO}_6$ $[\text{M}+\text{H}]^+$, 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.^[72] After addition of ice into the reaction mixture a powdery white solid precipitated. The precipitate was washed by water, NaHCO_3 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, CDCl_3) $\delta = 6.31$ (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; ^{13}C NMR (100 MHz, CDCl_3) $\delta = 170.6, 170.5,$

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**):

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**):

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%). ^1H NMR (400 MHz, CDCl_3) δ = 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) δ = 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**):

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; $[\alpha]_{\text{D}}^{22} = -8.2$ (c = 1, CHCl_3); ^1H NMR (500 MHz, CDCl_3) δ = 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) δ = 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) ν = 2941, 1753, 1369, 1225, 1038, 849, 756 cm^{-1} ; MS (m/z) 628 $[\text{M}+\text{Na}]^+$; HRMS (ESI) calcd. for $\text{C}_{34}\text{H}_{36}\text{O}_{10}\text{Na}$ $[\text{M}+\text{Na}]^+$, 627.2206; found, 627.2203.

4-(1-Pyrenyl)butyl β -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 $^{\circ}\text{C}$; $[\alpha]_{\text{D}}^{22} = -7.8$ ($c = 0.1$, MeOH); ^1H NMR (500 MHz, MeOD) δ = 8.25 (d, $J = 9.3$ Hz, 1H), 8.11 (dd, $J = 3.1$, 7.6 Hz, 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) δ = 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) $\nu = 3400, 1634, 1061, 840, 754 \text{ cm}^{-1}$; MS (m/z) 455 $[\text{M}+\text{NH}_4]^+$; HRMS (ESI) calcd. for $\text{C}_{26}\text{H}_{29}\text{O}_6$ $[\text{M}+\text{H}]^+$, 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 NaHCO_3 solution ($2 \times 50 \text{ mL}$) and 2 M H_2SO_4 ($2 \times 50 \text{ mL}$). Evaporation gives **19** (α and β mixture) as a white solid (1.99 g, 92%). ^1H NMR (300 MHz, CDCl_3) $\delta = 6.06$ (d, $J = 1.7 \text{ Hz}$, 1H), 5.84 (d, $J = 1.0 \text{ Hz}$, 1H), 5.46 (dd, $J = 1.0, 3.3 \text{ Hz}$, 1H), 5.24-5.34 (m, 4H), 5.12 (dd, $J = 3.3, 9.9 \text{ Hz}$, 1H), 4.23-4.32 (m, 2H), 4.12 (dd, $J = 2.4, 9.1 \text{ Hz}$, 2H), 4.04-4.08 (m, 1H), 3.79 (ddd, $J = 2.4, 5.2, 9.9 \text{ Hz}$, 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; ^{13}C NMR (100 MHz, CDCl_3) $\delta = 170.6, 170.1, 169.9, 169.7$ (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 $[\text{M}+\text{Na}]^+$. Data were in agreement with those reported in the literature.^[74]

2,3,4,6-Tetra-O-acetyl- α -D-mannopyranose (20):

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%). ^1H NMR (400 MHz, CDCl_3) δ = 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) δ = 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):

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%). ^1H NMR (400 MHz, CDCl_3) δ = 8.78 (s, 1H), 6.28 (s, 1H), 5.47 (s, 1H), 5.36-5.42 (m,

2H), 4.19-4.22 (dd, $J = 4.6, 11.9$ Hz, 1H), 4.14-4.20 (m, 2H), 2.19 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 2.00 (s, 3H) ppm; ^{13}C NMR (100 MHz, CDCl_3) $\delta = 170.5, 169.8, 169.7, 169.6, 159.7, 94.5, 90.5, 71.2, 68.8, 67.8, 65.4, 62.0, 20.7$ (2C), 20.6 ppm. Data were in agreement with those reported in the literature.^[75]

4-(1-Pyrenyl)butyl 2,3,4,6-tetra-O-acetyl- α -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; $[\alpha]_{\text{D}}^{22} = +35.2$ ($c = 1$, CHCl_3); ^1H 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 $[\text{M}+\text{Na}]^+$; HRMS (ESI) calcd. for $\text{C}_{34}\text{H}_{36}\text{O}_{10}\text{Na}$ $[\text{M}+\text{Na}]^+$, 627.2206; found, 627.2202.

4-(1-Pyrenyl)butyl α -D-mannopyranoside (4):

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; $[\alpha]_{\text{D}}^{22} = +31.2$ ($c = 0.5$, MeOH); ^1H NMR (500 MHz, CDCl_3) $\delta = 7.99$ -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; ^{13}C NMR (100 MHz, MeOD) $\delta = 136.2$, 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) $\nu = 3412$, 2087, 1638, 841, 752 cm^{-1} ; MS (m/z) 455 $[\text{M}+\text{NH}_4]^+$; HRMS (ESI) calcd. for $\text{C}_{26}\text{H}_{29}\text{O}_6$ $[\text{M}+\text{H}]^+$, 437.1964; found, 437.1969.

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

Compound **24** was prepared according to the same procedure as compound **7** (94% yield). ^1H NMR (400 MHz, CDCl_3) $\delta = 5.70$ (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),

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.^[74]

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

Compound **25** was prepared according to the same procedure as compound **8** and obtained by column chromatography on silica gel (EtOAc/CH₂Cl₂ = 1:1 to EtOAc/CH₂Cl₂/MeOH = 50:40:1) as a colorless oil (0.62 g 83%). ¹H NMR (400 MHz, CDCl₃) δ = 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; ¹³C NMR (100 MHz, CDCl₃) δ = 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 C₁₄H₂₁NO₉ [M+Na]⁺, 370.1114; found, 370.1111. Data were in agreement with those reported in the literature.^[70]

2-Acetamido-3,4,6-tri-O-acetyl-2-deoxy- α -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%). ^1H NMR (400 MHz, CDCl_3) δ = 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) δ = 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 $\text{C}_{16}\text{H}_{21}\text{N}_2\text{O}_9\text{Cl}_3$ $[\text{M}+\text{H}]^+$, 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- 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

light yellow powder (0.10 g, 30%). mp >148 °C decomposed; ^1H NMR (400 MHz, CDCl_3) δ = 8.24 (d, J = 9.2 Hz, 1H), 8.14 (dd, J = 7.6, 3.5 Hz, 2H), 8.10-8.07 (m, 2H), 8.02-7.95 (m, 3H), 7.83 (d, J = 7.8 Hz, 1H), 5.53 (d, J = 8.6 Hz, 1H), 5.33 (d, J = 3.0 Hz, 1H), 5.21 (dd, J = 11.2, 3.3 Hz, 1H), 4.55 (d, J = 8.4 Hz, 1H), 4.15-4.07 (m, 2H), 3.95-3.88 (m, 2H), 3.82 (t, J = 6.6 Hz, 1H), 3.48-3.42 (m, 1H), 3.36-3.30 (m, 2H), 2.13 (s, 3H), 2.01 (s, 3H), 1.97 (s, 3H), 1.93-1.80 (m, 2H), 1.75 (s, 3H), 1.72-1.69 (m, 2H) ppm; ^{13}C NMR (100 MHz, CDCl_3) δ = 170.4, 170.2, 136.6, 131.3, 130.7, 129.7, 128.5, 127.4, 127.1, 126.5, 125.8, 124.9, 124.8 (2C), 124.7, 124.6, 123.3, 100.8, 76.7, 70.4, 69.8, 69.5, 66.7, 61.4, 51.5, 33.0, 29.3, 28.0, 23.2, 20.6 (2C) ppm; IR (neat) ν = 2936, 2361, 1748, 1662, 1371, 1234, 1130, 1043 cm^{-1} ; MS (m/z) 604 $[\text{M}+\text{Na}]^+$; HRMS (ESI) calcd. for $\text{C}_{34}\text{H}_{37}\text{NO}_9$ $[\text{M}+\text{H}]^+$, 604.2547; found, 604.2542.

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/ CH_2Cl_2 (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) δ = 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) ν = 3352, 2924, 2864, 1630, 1541, 1078, 837 cm^{-1} ; MS (m/z) 478 $[\text{M}+\text{H}]^+$; HRMS (ESI) calcd. for $\text{C}_{28}\text{H}_{31}\text{NO}_6$ $[\text{M}+\text{H}]^+$, 478.2230; found, 478.2224.

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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 glycoproteins 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* mannoside 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_N2 -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_N2 -like manner. A 1,2-*cis* glycosidic bond is thus formed in a stereoselective fashion (Scheme 1).

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.^[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. 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).^[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, (β 1 $\rightarrow$ 6)-linked glycosides were obtained as the major product instead of the desired (β 1 $\rightarrow$ 4)-linked glycosides.

Scheme 3. Stork's silicon-tethered IAD. Reagents and conditions: (a) ROSiMe₂Cl, imidazole, THF, rt, 10 min, quantitative; (b) *m*CPBA, CH₂Cl₂, -25 °C to 0 °C; (c) Tf₂O, DTBMP, CH₂Cl₂, 4 Å M.S., -80 °C to rt, 61-73%; (d) ROH, Me₂SiCl₂, DMAP, imidazole, THF, -78 °C, 60-98%; (e) Tf₂O, DTBMP, CH₂Cl₂, 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.^[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, CH_2Cl_2 , 4 Å M.S.; (b) AgOTf, SnCl_2 , DTBMP, Et_2O for **10**; MeOTf, DTBMP, 4 Å M.S., CH_2Cl_2 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 mannoside **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,^[22, 23] similar to Hindsgaul's method.^[7] As mentioned in Hindsgaul's first research paper on IAD, the yields for glycosylations with bulky aglycons were unsatisfactory. 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, $-78\text{ }^{\circ}\text{C}$ to rt, 66-95%; (b) NIS, DTBMP, CH_2Cl_2 , $0\text{ }^{\circ}\text{C}$ 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 $50\text{ }^{\circ}\text{C}$ (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. Alkyl-mediated IAD developed by Fairbanks *et al.* Reagents and conditions: (a) Wilkinson's catalyst, *n*BuLi, 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).^[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.^[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, I₂, AgOTf, DTBMP, CH₂Cl₂, -78 °C to rt, 60-86%; (b) I₂, 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 α -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 β -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) KO^tBu, 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.^[28] 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_2O , HClO_4 ; (b) HBr , AcOH , 88% for two steps; (c) MeOH , 2,6-lutidine, CHCl_3 , 74%; (d) anhydrous K_2CO_3 , $\text{MeOH}/\text{CH}_2\text{Cl}_2$.

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 α -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.

Scheme 14. Synthesis of glycosyl donor **36** and **38**. Reagents and Conditions: (a) NaH, BnBr, DMF, 69%; (b) DAST, CH₂Cl₂, 0 °C, overnight, 86%, (c) anhydrous K₂CO₃, MeOH, 72%, (d) *p*-thiocresol, DCE, reflux, 48 h, 58%; (e) anhydrous K₂CO₃, MeOH/CH₂Cl₂, 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 CH₂Cl₂ afforded **37**, which was deprotected by K₂CO₃ 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) *n*BuLi, 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) *t*BuOK, 18-crown-6, benzene, reflux, 5 h, 56%; (b) NBS, BnOH, acetone, CH₂Cl₂, –40 °C; (c) *t*BuOK, 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 to our

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

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. 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 $^3J_{H-1,H-2}$ coupling constant

obtained in ^1H NMR spectrum to determine the anomeric configuration of mannopyranoside **51**. This is because $^3J_{\text{H-1,H-2}}$ coupling constant for both α - and β -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 β -mannoside by measuring the $J_{\text{C-1,H-1}}$ coupling constant. It was reported that the $J_{\text{C-1,H-1}}$ value for α -mannosides are usually > 170 Hz whereas that of β -mannosides are in the region of < 160 Hz.^[9] The $J_{\text{C-1,H-1}}$ coupling constant obtained from ^{13}C spectrum of **51** is 155.5 Hz.

Figure 2. NOESY Spectrum of β -mannoside **51**

To further verify the β 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**.

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, inverting the anomeric configuration to afford a 1,2-*cis* glycoside.

Figure 4. Proposed routes for the synthesis of β -mannoside **51**

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.

Scheme 21. Aglycon delivery of **49** in the presence of allyl alcohol.

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 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.

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_2Cl_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 acetylide 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. 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-mannopyranosyl 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 ($\times$ 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**)

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 ($\times 2$) 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 ($\times 2$), 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); $[\alpha]_D^{20} = -18.8$ ($c = 1.0$ in CHCl₃) (lit.^[30] $[\alpha]_D^{20} = -26.8$ ($c = 1$, CHCl₃)); ¹H NMR (400 MHz, CDCl₃): $\delta = 5.48$ (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), 3.27 (s, 3H), 2.11 (s, 3H), 2.06 (s, 3H), 2.04 (s, 3H), 1.73 (s, 3H) ppm; ¹³C NMR (100 MHz, CDCl₃): $\delta = 170.6, 170.3, 169.4, 124.5, 97.3, 76.5, 71.3, 70.6, 65.4, 62.3, 49.9, 24.3, 20.7$ (2C), 20.6 ppm; IR (neat): $\nu = 2900, 1746, 1373, 1231, 1047$ 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. ^1H NMR (400 MHz, CDCl_3): δ = 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_2O and then washed with ice water. The aqueous layer was extracted with Et_2O ($\times$ 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); $[\alpha]_{\text{D}}^{20}$ = +36.2 (c = 1.0 in CHCl_3) (lit.^[30] $[\alpha]_{\text{D}}^{20}$ = +34.4 (c = 6.0 in CHCl_3)); ^1H NMR (400 MHz, CDCl_3): δ = 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): δ = 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): ν = 2914, 2864, 1638,

1454, 1383, 1099, 1047, 735, 698 cm^{-1} ; MS (m/z) 529 $[\text{M}+\text{Na}]^+$; HRMS (ESI): calcd. for $\text{C}_{30}\text{H}_{34}\text{O}_7\text{Na}$ $[\text{M}+\text{Na}]^+$, 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_2Cl_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_2SO_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%). $[\alpha]_{\text{D}}^{20} = +12.5$ ($c = 1.0$ in CHCl_3) (lit.^[24] $[\alpha]_{\text{D}}^{23} = +13.3$ ($c = 1.0$ in CHCl_3)); ^1H NMR (400 MHz, CDCl_3): $\delta = 7.38\text{--}7.26$ (m, 13H), 7.19–7.17 (m, 2H), 5.64 (d, $J = 49.2$ Hz, 1H), 5.50 (s, 1H), 4.89 (d, $J = 10.8$ Hz, 1H), 4.74 (d, $J = 11.2$ Hz, 1H), 4.69 (d, $J = 12.2$ Hz, 1H), 4.58 (d, $J = 11.2$ Hz, 1H), 4.54 (d, $J = 11.2$ Hz, 1H), 4.52 (d, $J = 10.8$ Hz, 1H), 3.99 (s, 3H), 3.83 (dd, $J = 10.8, 1.9$ Hz, 1H), 3.73 (d, $J = 10.8$ Hz, 1H), 2.18 (s, 3H) ppm; ^{13}C NMR (100 MHz, CDCl_3): $\delta = 170.0, 138.1, 137.9, 137.5, 128.4, 128.3$ (2C), 128.0, 127.9, 127.8, 127.7 (2C), 105.5 (d, $J = 219.2$ Hz), 77.2, 75.2, 73.7 (2C), 73.4, 73.3, 72.0, 68.1, 67.0 (d, $J = 39.8$ Hz) ppm; IR (neat): $\nu = 3030, 2905, 2866, 1749, 1371, 1231, 1105, 737, 698$ cm^{-1} ; MS (m/z) 517 $[\text{M}+\text{Na}]^+$, 475 $[\text{M}-\text{F}+\text{H}]^+$; HRMS (ESI): calcd. for $\text{C}_{29}\text{H}_{31}\text{O}_6\text{FNa}$ $[\text{M}+\text{Na}]^+$, 517.2002; found, 517.1997.

2-O-acetyl-3,4,6-tri-O-benzyl- α -D-mannopyranosyl fluoride (36)

Anhydrous K_2CO_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 $CHCl_3$)); 1H 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 $C_{27}H_{29}O_5FNa$ $[M+Na]^+$, 475.1897; found, 475.1897.

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

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); $[\alpha]_D^{23} = +88.9$ ($c = 1.0$ in CHCl_3) (lit.^[25] $[\alpha]_D^{25} = +109.0$ ($c = 1.0$ in CHCl_3)); ^1H NMR (400 MHz, CDCl_3): $\delta = 7.35$ -7.26 (m, 15H), 7.19 (d, $J = 7.8$ Hz, 2H), 7.05 (d, $J = 8.0$ Hz, 2H), 5.60 (s, 1H), 5.46 (s, 1H), 4.88 (d, $J = 10.8$ Hz, 1H), 4.73 (d, $J = 11.2$ Hz, 1H), 4.66 (d, $J = 12.0$ Hz, 1H), 4.57 (d, $J = 11.2$ Hz, 1H), 4.51 (d, $J = 10.8$ Hz, 1H), 4.46 (d, $J = 12.0$ Hz, 1H), 4.34 (d, $J = 4.4$ Hz, 1H), 3.96-3.94 (m, 2H), 3.85 (dd, $J = 10.4, 4.6$ Hz, 1H), 3.72 (d, $J = 10.4$ Hz, 1H), 2.30 (s, 3H), 2.14 (s, 3H) ppm; ^{13}C NMR (100 MHz, CDCl_3): $\delta = 170.3, 138.3, 138.2, 137.9, 132.3, 129.8, 128.5, 128.3, 128.2, 127.9, 127.7, 127.6, 127.5, 86.5, 78.5, 75.2, 74.6, 73.3, 72.4, 71.9, 70.3, 68.9$ ppm; IR (neat): $\nu = 3030, 2916, 2868, 1746, 1703, 1495, 1454, 1371, 1230, 1101, 743, 698$ cm^{-1} ; MS (m/z) 621 $[\text{M}+\text{Na}]^+$; HRMS (ESI): calcd. for $\text{C}_{36}\text{H}_{38}\text{O}_6\text{SNa}$ $[\text{M}+\text{Na}]^+$, 621.2287; found, 621.2288.

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

Anhydrous K_2CO_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_2Cl_2 (5 mL). The mixture was stirred at room temperature for 3 h. The solvent was removed under reduced pressure. The

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%). $[\alpha]_D^{20} = +169.4$ ($c = 1.0$ in CHCl_3) (lit.^[25] $[\alpha]_D^{23} = +189.9$ ($c = 1.0$ in CHCl_3)); ^1H NMR (400 MHz, CDCl_3): $\delta = 7.38\text{--}7.26$ (m, 15H), 7.23–7.21 (m, 2H), 7.06 (d, $J = 8.0$ Hz, 2H), 5.55 (s, 1H), 4.85 (d, $J = 10.8$ Hz, 1H), 4.72 (s, 2H), 4.62 (d, $J = 12.0$ Hz, 1H), 4.53 (d, $J = 10.8$ Hz, 1H), 4.45 (d, $J = 12.0$ Hz, 1H), 4.34–4.31 (m, 1H), 4.26 (d, $J = 1.5$ Hz, 1H), 3.96–3.88 (m, 2H), 3.80 (dd, $J = 10.8, 4.7$ Hz, 1H), 3.70 (dd, $J = 10.8, 1.8$ Hz, 1H), 2.65 (d, $J = 2.6$ Hz, 1H), 2.30 (s, 3H) 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): $\nu = 3415, 3030, 2920, 2868, 1714, 1495, 1454, 1099, 750, 698$ cm^{-1} ; MS (m/z) 574 $[\text{M}+\text{NH}_4]^+$; HRMS (ESI): calcd. for $\text{C}_{34}\text{H}_{36}\text{O}_5\text{SNa}$ $[\text{M}+\text{Na}]^+$, 579.2181; found, 579.2183.

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

NaH (15.0 mg, 0.36 mmol, 60% in mineral oil) and propargyl bromide (55.0 μL , 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_2O and quenched with NH_4Cl solution. The resulting solution was separated. The organic phase was washed with water, brine, dried over Na_2SO_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 CHCl_3); ^1H NMR (400 MHz, CDCl_3): 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; ^{13}C NMR (100 MHz, CDCl_3): 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 $[\text{M}+\text{Na}]^+$, 471 $[\text{M}-\text{F}+\text{H}]^+$; HRMS (ESI): calcd. for $\text{C}_{30}\text{H}_{31}\text{O}_5\text{FNa}$ $[\text{M}+\text{Na}]^+$, 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%). $[\alpha]_{\text{D}}^{23}$ = +187.0 (c = 1.0 in CHCl_3); ^1H NMR (400 MHz, CDCl_3): 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; ^{13}C NMR (100 MHz,

CDCl₃): δ = 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): ν = 3028, 2916, 2866, 2120, 1495, 1454, 1207, 1088, 737, 698 cm⁻¹; MS (m/z) 617 [M+Na]⁺; HRMS (ESI): calcd. for C₃₇H₃₈O₅SNa [M+Na]⁺, 617.2338; found, 617.2356.

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

*n*BuLi (0.14 mL, 0.22 mmol, 1.6 M solution in hexanes) was slowly added to a solution of **39** (0.10 g, 0.20 mmol) in anhydrous THF (3 mL). The mixture was stirred 1 h at -78 °C. Then TMSCl (28.4 μ L, 0.23 mmol) was added. The mixture was stirred for 2 h at 0 °C before quenched with saturated NaHCO₃ solution. The resulting solution was extracted Et₂O. The organic phase was dried over Na₂SO₄, evaporated and purified by column chromatography (EtOAc/Hexane = 1:10) to give **41** as a colorless oil (0.18 g, 67%). [α]_D²³ = +68.4 (*c* = 1.0 in CHCl₃); ¹H NMR (500 MHz, CDCl₃): δ = 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; ¹³C NMR (125 MHz, CDCl₃): δ = 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):

$\nu = 3030, 2901, 2866, 2359, 2173, 1454, 1250, 1184, 1098, 884, 737, 698 \text{ cm}^{-1}$; MS (m/z) 586 $[\text{M}+\text{Na}]^+$; HRMS (ESI): calcd. for $\text{C}_{33}\text{H}_{39}\text{O}_5\text{FSiNa}$ $[\text{M}+\text{Na}]^+$, 585.2449; found, 585.2444.

4-Methylphenyl 2-*O*-allenyl-3,4,6-tri-*O*-benzyl-1-thio- α -D-mannopyranoside (**43**)

*t*BuOK (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%). $[\alpha]_{\text{D}}^{23} = +126.4$ ($c = 1.0$ in CHCl_3); ^1H NMR (400 MHz, CDCl_3): $\delta = 7.41\text{--}7.21$ (m, 15H), 7.06 (d, $J = 8.2$ Hz, 2H), 6.82 (t, $J = 6.0$ Hz, 2H), 5.55 (s, 1H), 5.42–5.37 (m, 2H), 4.92 (d, $J = 10.8$ Hz, 1H), 4.73–4.67 (m, 2H), 4.65 (d, $J = 12.0$ Hz, 1H), 4.54 (d, $J = 10.8$ Hz, 1H), 4.47 (d, $J = 12.0$ Hz, 1H), 4.29–4.27 (m, 2H), 4.02 (t, $J = 9.4$ Hz, 1H), 3.92 (dd, $J = 9.4, 3.2$ Hz, 1H), 3.82 (dd, $J = 10.8, 4.8$ Hz, 1H), 3.73 (dd, $J = 10.8, 1.8$ Hz, 1H), 2.31 (s, 3H) ppm; ^{13}C NMR (100 MHz, CDCl_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 $[\text{M}+\text{H}]^+$; HRMS (ESI): calcd. for $\text{C}_{37}\text{H}_{38}\text{O}_5\text{SNa}$ $[\text{M}+\text{Na}]^+$, 617.2338; found, 617.2336.

(3,3-Diethoxy-1-propynyl)trimethylsilane (48)

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. The

reaction mixture was then cooled to room temperature and stirred overnight. It was then diluted with CH_2Cl_2 and washed with aqueous solution of ammonium chloride and 10% HCl in the ratio of 1:1 ($\times 3$). 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**: ^1H NMR (400 MHz, CDCl_3): δ = 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): δ = 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): ν = 3028, 2880, 2174, 1714, 1455, 1102, 737, 698 cm^{-1} ; MS (m/z) 733 $[\text{M}+\text{Na}]^+$; HRMS (ESI): calcd. for $\text{C}_{42}\text{H}_{50}\text{O}_6\text{SSiNa}$ $[\text{M}+\text{Na}]^+$, 733.2995; found, 733.2997. **(R)-49**: ^1H NMR (400 MHz, CDCl_3): δ = 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): δ = 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): ν = 3030,

2880, 2175, 1712, 1454, 1103, 737, 698 cm^{-1} ; MS (m/z) 733 $[\text{M}+\text{Na}]^+$; HRMS (ESI): calcd. for $\text{C}_{42}\text{H}_{50}\text{O}_6\text{SSiNa}$ $[\text{M}+\text{Na}]^+$, 733.2995; found, 733.2980.

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

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_2Cl_2 (3 mL) at 0 $^\circ\text{C}$ under nitrogen protection. The mixture was allowed to warm to room temperature and stirred overnight. It was then diluted with CH_2Cl_2 and washed with aqueous solution of ammonium chloride and 10% HCl in the ratio of 1:1 ($\times 3$). 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%). $[\alpha]_{\text{D}}^{22} = -22.5$ ($c = 0.65$ in CHCl_3); ^1H NMR (400 MHz, CDCl_3): $\delta = 7.39\text{--}7.19$ (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 $[\text{M}+\text{Na}]^+$; HRMS (ESI) calcd. for $\text{C}_{29}\text{H}_{34}\text{O}_6\text{Na}$ $[\text{M}+\text{Na}]^+$, 501.2253; found, 501.2249.

6-*O*-*tert*-Butyldimethylsilyl-1,2-*O*-(*exo*-methoxyethylidene)- β -D-mannopyranose (54)

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 ($\times$ 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-*O*-*tert*-Butyldimethylsilyl-3,4-di-*O*-propargyl-1,2-*O*-(*exo*-methoxyethylidene)- β -D-mannopyranose (55)

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 Et₂O and 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 **55** as a colorless oil (0.25 g, 55%). $[\alpha]_D^{23} = +34.2$ ($c = 1.0$ in CHCl₃); ¹H NMR (400 MHz, CDCl₃): $\delta = 5.39$ (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): $\nu = 3287, 2930, 2857, 2359, 2118, 1746, 1371, 1252, 1099, 837$ cm⁻¹; MS (m/z) 427 [M+H]⁺; HRMS (ESI) calcd. for C₂₁H₃₄O₇SiNa [M+Na]⁺, 449.1972; found, 449.1972.

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

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%). $[\alpha]_D^{23} = +26.4$ ($c = 1.0$ in CHCl₃); ¹H NMR (400 MHz, CDCl₃): $\delta = 5.43$ (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), 3.33 (m, 1H), 3.27 (s, 3H), 2.47 (dt, $J = 7.1, 2.2$ Hz, 2H), 2.26 (s, 1H), 1.67 (s, 3H) ppm; IR (neat): $\nu = 3279, 2932, 2118, 1732, 1373, 1242, 1057$ cm^{-1} ; MS (m/z) 313 $[\text{M}+\text{H}]^+$; HRMS (ESI) calcd. for $\text{C}_{15}\text{H}_{20}\text{O}_7\text{Na}$ $[\text{M}+\text{Na}]^+$, 335.1107; found, 335.1105.

4-Methylphenyl 2-*O*-propargyl-3,4,6-tri-*O*-benzyl-1-thio- α -D-mannopyranoside-silver complex (**59**)

A solution of **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 **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 Et_2O ($\times 2$). The combined organic layers was dried, filtered and evaporated under reduce pressure to give back the starting material **40** (0.16 g, 65%).

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Part 4

Recyclable Sulfonated Amorphous Carbon Catalyzed Friedel-Crafts Alkylation of Indoles with α,β - Unsaturated Carbonyl Compounds in Water

J. Ma, S. Ng, Y. Yong, X.-Z. Luo, X. Wang, X.-W. Liu, *Chem. Asian J.*
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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] $\text{ZrOCl}_2 \cdot 8\text{H}_2\text{O}$ ^[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 Recyclable sulfonated amorphous carbon catalyzed Friedel-Crafts alkylation of indoles with α,β -unsaturated carbonyl compounds in water

with ionic liquid in water.^[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 α,β -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.^[11] Consisting of flexible polycyclic carbon sheets which bear phenolic hydroxyl (-OH), carboxylic acid (-COOH) and sulfonic acid (-SO₃H) 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.^[12] Initially used as a green catalyst in the esterification of fatty acids in biodiesel production,^[11c] it was soon reported to demonstrate high catalytic efficiency in other reactions such as hydrolysis^[12a] and hydration.^[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.^[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 SO₃H groups,^[12b] the density of SO₃H 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 2θ 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**.

Figure 2. XRD profile for the sulfonated carbon based solid acid.

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^[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 SO₃H 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 H₂O/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]

Entry	Catalyst	Catalyst	Solvent	Time [h]	Yield [%] ^[b]
1	1a	1.0	H ₂ O	6	96
2	1a	2.0	H ₂ O	3	96
3	1a	5.0	H ₂ O	1	96
4	1a	10.0	H ₂ O	0.5	94
5 ^[c]	1b	29 mg	H ₂ O	48	43
6 ^[c]	1c	29 mg	H ₂ O	8	95
7	black carbon	29 mg	H ₂ O	24	18
8 ^[d]	1a	5.0	H ₂ O/THF	1	93
9	1a	5.0	THF	1	84
10	1a	5.0	CH ₂ Cl ₂	1	79
11	1a	5.0	MeCN	1	77
12	1a	5.0	MeOH	1	66
13	1a	5.0	Toluene	1	19
14	H ₂ SO ₄	5.0	H ₂ O	1	40
15	H ₂ SO ₄	100.0	H ₂ O	1	63

^[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 H₂O or H₂O/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 H₂O/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

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

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]

Entry	Michael Donor	Michael Acceptor	Product	Time [h]	Yield [%] ^[b]
1 ^[c, d]				1	96
2 ^[c, d]				1	89
3 ^[c]				0.5	90

4 ^[c]				0.5	85
	5	7	16		
5 ^[c]				0.5	88
	6	7	17		
6 ^[d]				2	91
	2	8	18		
7				4	81
	3	8	19		
8				2	86
	4	8	20		
9				3	82
	5	8	21		
10				3	80
	6	8	22		
11 ^[c]				48	78
	2	9	23		
12 ^[d]				48	76
	2	10			

			24		
13 ^[c]				48	72
	2	11	25		
14				4	12+60 ^[f]
	2	12	26 27		
15 ^[c]				6	67
	4	12	28		

^[a] Unless specified, Michael donor (0.20 mmol) was reacted with Michael acceptor (0.22 mmol) in H₂O/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] H₂O (1 mL) was used. ^[e] H₂O/THF (0.5 mL/0.5 mL) was used. ^[f] Conversion yield calculated based on indole.

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 β -nitrostyrene gave satisfactory yield of Michael adducts (Table 2, entries 11 and 12). Other substrates such as α,β -unsaturated ester, aldehydes and nitriles are found to be less appropriate for Michael reaction with indole under the same catalytic system.

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 of 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 triindolylcyclohexane 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 monoindolyl 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. 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 H₂O/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 ^1H NMR spectra and 77.0 ppm for ^{13}C NMR spectra in CDCl_3). Chemical shift (δ) 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 premierTM 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.

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.^[3b,3d,3h] The remaining solid acid catalyst was dried under reduced pressure to remove all the volatile components, and then reused in the next run.

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

(Eluent: Hexane/EtOAc = 4:1, 96% yield); ¹H NMR (400 MHz, CDCl₃) δ = 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; ¹³C NMR (100 MHz, CDCl₃) δ = 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 C₁₂H₁₄NO [M+H]⁺, 188.1075; found, 188.1082.

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

(Eluent: Hexane/EtOAc = 2:1, 89% yield); ^1H NMR (300 MHz, CDCl_3) δ = 8.18 (br s, 1H), 7.70 (d, J = 1.5 Hz, 1H), 7.28-7.18 (m, 2H), 6.97 (d, J = 2.1 Hz, 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) δ = 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 $[\text{M}-\text{Br}+\text{H}]^+$; HRMS (ESI) calcd. for $\text{C}_{12}\text{H}_{13}\text{NOBr}$ $[\text{M}+\text{H}]^+$, 266.0181; found, 266.0174.

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

(Eluent: Hexane/EtOAc = 4:1, 90% yield); ^1H NMR (300 MHz, CDCl_3) δ = 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) δ = 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 $[\text{M}+\text{H}]^+$; HRMS (ESI) calcd. for $\text{C}_{13}\text{H}_{16}\text{NO}$ $[\text{M}+\text{H}]^+$, 202.1232; found, 202.1194.

4-(7-Methyl-1H-indol-3-yl)butan-2-one (16)

(Eluent: Hexane/EtOAc = 4:1, 85% yield); ^1H NMR (300 MHz, CDCl_3) δ = 7.94 (br s, 1H), 7.46 (d, J = 7.5 Hz, 1H), 7.09-6.99 (m, 3H), 3.06 (t, J = 7.5 Hz, 2H), 2.85 (t, J = 7.5 Hz, 2H), 2.48 (s, 3H), 2.15 (s, 3H) ppm; ^{13}C NMR (75 MHz, CDCl_3) δ = 208.9, 135.8, 126.6, 122.5, 121.2, 120.4, 119.5, 116.3, 115.5, 44.1, 30.0, 19.5, 16.5 ppm; MS (m/z) 202 $[\text{M}+\text{H}]^+$; HRMS (ESI) calcd. for $\text{C}_{13}\text{H}_{16}\text{NO}$ $[\text{M}+\text{H}]^+$, 202.1232; found, 202.1234.

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

(Eluent: Hexane/EtOAc = 8:1, 88% yield); ^1H NMR (300 MHz, CDCl_3) δ = 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) δ = 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 $[\text{M}+\text{H}]^+$; HRMS (ESI) calcd. for $\text{C}_{13}\text{H}_{16}\text{NO}$ $[\text{M}+\text{H}]^+$, 202.1232; found, 202.1230.

5-(1*H*-Indol-3-yl)hexan-3-one (18)

(Eluent: Hexane/EtOAc = 6:1, 91% yield); ^1H NMR (300 MHz, CDCl_3) δ = 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) δ = 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 $[\text{M}+\text{H}]^+$; HRMS (ESI) calcd. for $\text{C}_{14}\text{H}_{18}\text{NO}$ $[\text{M}+\text{H}]^+$, 216.1388; found, 216.1378.

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

(Eluent: Hexane/EtOAc = 6:1, 81% yield); ^1H NMR (300 MHz, CDCl_3) δ = 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) δ =

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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); 1H NMR (300 MHz, $CDCl_3$) δ = 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, $CDCl_3$) δ = 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)

(Eluent: Hexane/EtOAc = 8:1, 82% yield); ^1H NMR (300 MHz, CDCl_3) δ = 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) δ = 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 $[\text{M}+\text{H}]^+$; HRMS (ESI) calcd. for $\text{C}_{15}\text{H}_{20}\text{NO}$ $[\text{M}+\text{H}]^+$, 230.1545; found, 230.1540.

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

(Eluent: Hexane/EtOAc = 12:1, 80% yield); ^1H NMR (300 MHz, CDCl_3) δ = 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) δ = 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 $[\text{M}+\text{H}]^+$; HRMS (ESI) calcd. for $\text{C}_{15}\text{H}_{20}\text{NO}$ $[\text{M}+\text{H}]^+$, 230.1545; found, 230.1541.

3-(1*H*-Indol-3-yl)-1,3-diphenylpropan-1-one (23)

(Eluent: Hexane/EtOAc = 6:1, 78% yield); ^1H NMR (300 MHz, CDCl_3) δ = 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) δ = 198.6, 144.2, 137.1, 136.6, 133.0, 128.5, 128.4, 128.1, 127.8, 126.6, 126.3, 122.1, 121.4, 119.5, 119.3, 119.2, 111.1, 45.2, 38.2 ppm; MS (m/z) 326 $[\text{M}+\text{H}]^+$; HRMS (ESI) calcd. for $\text{C}_{23}\text{H}_{20}\text{NO}$ $[\text{M}+\text{H}]^+$, 326.1545; found, 326.1539.

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

(Eluent: Hexane/EtOAc = 6:1, 76% yield); ^1H NMR (400 MHz, CDCl_3) δ = 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) δ = 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;

MS (m/z) 267 $[M+H]^+$; HRMS (ESI) calcd. for $C_{16}H_{15}N_2O_2$ $[M+H]^+$, 267.1134; found, 267.1135.

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

(Eluent: Hexane/EtOAc = 4:1, 72% yield); 1H NMR (400 MHz, $CDCl_3$) δ = 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; ^{13}C NMR (100 MHz, $CDCl_3$) δ = 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 $C_{13}H_{14}NO$ $[M+H]^+$, 200.1075; found, 200.1074.

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

(Eluent: Hexane/EtOAc = 6:1, 12% yield); ^1H NMR (300 MHz, CDCl_3) δ = 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) δ = 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 $[\text{M}+\text{H}]^+$; HRMS (ESI) calcd. for $\text{C}_{14}\text{H}_{16}\text{NO}$ $[\text{M}+\text{H}]^+$, 214.1232; found, 214.1224.

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

(Eluent: Hexane/EtOAc = 4:1, 60% yield); ^1H NMR (400 MHz, CDCl_3) δ = 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) δ = 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, $CDCl_3$) δ = 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, 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, $CDCl_3$) δ = 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.

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