SYNTHESIS OF NOVEL AZA-NUCLEOSIDES AND A NEW STRATEGY TO (+)-BULGECININE, *TRANS*-4-HYDROXY-D-PROLINE

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by

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Submitted

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This thesis is dedicated to
my beloved parents
Mr. Ram Sagar Mishra
and
Mrs. Sarojni Mishra

CERTIFICATE

This is to certify that the thesis entitled **"SYNTHESIS OF NOVEL AZA-NUCLEOSIDES AND A NEW STRATEGY TO (+)-BULGECININE, *TRANS*-4-HYDROXY-D-PROLINE",** being submitted by **Mr. UMESH KUMAR MISHRA** to Indian Institute of Technology Delhi, for the award of the degree of **Doctor of Philosophy**, is a record of bonafide research work carried out by him. Mr. Umesh Kumar Mishra has worked under my supervision and guidance and has fulfilled all the requirements for the submission of a Ph.D. thesis, which to my knowledge has reached the requisite standard and is worthy of consideration for the award of Ph.D. degree.

The work embodied in this thesis has not been submitted, in part or full, to other University or Institute for the award of any degree or diploma.

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Umesh Kumar Mishra

ABSTRACT

The thesis titled **"SYNTHESIS OF NOVEL AZA-NUCLEOSIDES AND A NEW STRATEGY TO (+)-BULGECININE, *TRANS*-4-HYDROXY-D-PROLINE"** present the research work carried out on the synthesis of novel aza-nucleosides starting material tri-*O*-benzyl-D-glucal. On the other hand, (+)-Bulgecinine, polyhydroxypyrrolidine and *trans*-4-hydroxy-D-proline were synthesized from tri-*O*-acetyl-D-glucal as the convenient starting material.

Aza-nucleosides are nucleoside analogues wherein the oxygen atom of the furanose ring has been replaced with a nitrogen atom. Synthesis and development of aza-nucleosides have been the focus of attention, due to the vital role played by carbohydrates in a variety of biological process. A variety of novel nucleoside analogues have been discovered, which are potential inhibitors of glycosidases and purine nucleoside phosphorylase (PNP) enzyme. PNP catalyses the reversible phosphorylation of purine nucleosides to produce the corresponding purine base and ribose-1-phosphate.

Chapter I describes the synthesis of aza-nucleosides. This is the first example of the synthesis of double headed aza-nucleosides, wherein two nucleobases (either same or complimentary) were coupled with polyhydroxypyrrolidine. Single crystal X-ray structure of three compounds were obtained and their supramolecular assembly was investigated. The PNP inhibition of the purine based aza-nucleosides is in progress and the outcome of the results is awaited.

Chapter II describes the synthesis of four molecules namely (+)-bulgecinine, 3-hydroxy-2,5-dihydroxymethylpyrrolidine and two stereoisomers of 2-oxapyrrolizidin-3-one starting from 4,6-di-*O*-benzyl-D-glucal through divergent approach involving a

common intermediate. This is the first carbohydrate based approach to the synthesis of (+)-bulgecinine that proceeds with complete stereochemical integrity.

Chapter III describes a straight-forward, high yielding, highly stereoselective synthesis of *trans*-4-hydroxy-D-proline and *trans*-4-hydroxy-D-prolinol starting from 4,6-di-*O*-benzyl-D-glucal. This is perhaps the first carbohydrate based approach to the synthesis of *trans*-4-hydroxy-D-proline and prolinol that proceeds with complete stereochemical integrity.

सार

थिसिस शीर्षक ''**नॉवेल एजा-न्यूक्लियोसाइड और एक नई रणनीति (+)-बुलजेसिनीन, ट्रांस 4-हाइड्रॉक्सी-डी-प्रोलीन का संश्लेषण**" प्रस्तुत शोध कार्य नॉवेल एजा-न्यूक्लियोसाइड के संश्लेषण पर शुरू किया जो सामग्री त्रिकोणीय-ओ-बेंजिल-डी-ग्लुकल है। दूसरी ओर, सुविधाजनक शुरुआत सामग्री के रूप में ट्राई-ओ-एसिटाइल-डी-ग्लुकल से (+)-बुलजेसिनीन, पॉलीहाइड्रॉक्सीपिरोलिडीन और ट्रांस-4-हाइड्रॉक्सी-डी-प्रोलीन को संश्लेषित किया गया था।

एजा-न्यूक्लियोसाइड न्यूक्लियोसाइड एनालॉग्स हैं, जहां फूरेनोज रिंग के ऑक्सीजन परमाणु को नाइट्रोजन परमाणु के साथ बदल दिया गया है। विभिन्न जैविक प्रक्रिया में कार्बोहाइड्रेट द्वारा निभाई गई महत्वपूर्ण भूमिका के कारण, एजा-न्यूक्लियोसाइड का संश्लेषण और विकास ध्यान का केंद्र बिंदु रहा है। नॉवेल न्यूक्लियोसाइड एनालॉग्स की एक किस्म की खोज की गई है, जो ग्लाइकोसिडेस और प्यूरीन न्यूक्लियोसाइड फॉस्फोराइलेज (पीएनपी) एंजाइम के संभावित अवरोधक हैं। पीएनपी, इसी प्यूरीन बेस और राइबोस-1-फॉस्फेट के उत्पादन के लिए प्यूरीन न्यूक्लियोसाइड के प्रतिवर्ती फॉस्फोराइलेशन को उत्प्रेरित करता है।

अध्याय I एजा-न्यूक्लियोसाइड के संश्लेषण का वर्णन करता है। यह डबल हेडेड एजा-न्यूक्लियोसाइड्स के संश्लेषण का पहला उदाहरण है, जिसमें दो न्यूक्लियोबेस (या तो समान या मानार्थ) पॉलीहाइड्रॉक्सीपिरोलिडीन के साथ मिलकर किए गए थे। तीन यौगिकों के एकल क्रिस्टल एक्स-रे संरचना प्राप्त किए गए थे और उनकी सुपरमॉलेक्यूलर असेंबली की जांच की गई थी। प्यूरीन के बेस एजा-न्यूक्लियोसाइड का पीएनपी निषेध प्रगति पर है और परिणामों का इंतजार है।

अध्याय II में चार अणुओं के संश्लेषण का वर्णन है, (+)-बुलजेसिनीन, 3-हाइड्रॉक्सी-2,5-डायहाइड्रॉक्सीमिथाइलपिरोलिडीन और 2-ऑक्सापायरोलिज़िडिन-3-ओन के दो स्टीरियोइसोमर्स 4,6-डाई-ओ-बेन्ज़िल-डी-ग्लुकल से शुरू होते हैं। ग्लुकल गर्त विचलन दृष्टिकोण जिसमें एक सामान्य मध्यवर्ती शामिल है। यह (+)-बुलजेसिनीन के संश्लेषण के लिए पहला कार्बोहाइड्रेट आधारित दृष्टिकोण है जो पूर्ण स्टीरियोकेमिकल अखंडता के साथ आगे बढ़ता है।

अध्याय III में ट्रांस 4-हाइड्रॉक्सी-डी प्रोलीन और ट्रांस-4-हाइड्रॉक्सी-डी-प्रोलिनोल के 4,6- डाई-ओ-बेंजिल-डी-ग्लुकल से शुरू होने वाले एक सीधे-आगे, उच्च उपज, अत्यधिक स्टीरोसेलेक्टिव संश्लेषण का वर्णन है। यह ट्रांस-4-हाइड्रॉक्सी-डी-प्रोलीन और प्रोलिनोल के संश्लेषण के लिए शायद पहला कार्बोहाइड्रेट आधारित दृष्टिकोण है जो पूर्ण स्टिरियोकेमिकल अखंडता के साथ आगे बढ़ता है।

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

GENERAL EXPERIMENTAL CONSIDERATIONS

All solvents employed were purified by standard procedures. Anhydrous solvents were dried over sodium wire (THF, diethyl ether, benzene) or molecular sieves (CH_2Cl_2, $CHCl_3$, DMF).

Nitrogen or Argon gas used for creating inert atmosphere was freed from oxygen prior to entry into reaction vessel.

Commercially sourced TLC plates were used, and the spots were visualized by exposure to iodine, or by dipping in $KMnO_4$ and ninhydrin solution. Column chromatography was carried out on silica gel (230–400 mesh) using mixtures of hexane and ethyl acetate as eluent unless otherwise mentioned.

Optical rotations were recorded on an Autopol V (Rudolph Research Flanders, NJ) instrument. All the rotations were measured at 589 nm (sodium D' line).

All melting points reported in this thesis are uncorrected and were taken on an electric melting point apparatus (Ambassador, India).

Freeze-drying of samples was done on a Freezone 2.5 (Labconco, USA) lyophilizer.

Inhibition studies were carried out on Biotek Synergy 2 microplate reader.

IR spectra were taken within the range 4000–600 cm^{-1}either as KBr pellets or neat on a Nicolet (Madison, USA) FT-IR spectrophotometer (Model Protégé 460).

^{1}H-NMR spectra were recorded on a 300 MHz or 400 MHz Bruker Spectrospin DPX FT-NMR instruments. The solvents employed were $CDCl_3$, CD_3OD, D_2O or

DMSO-d_6 with Me_4Si as the internal standard. The multiplicities are denoted as s-singlet, brs-broad singlet, d-doublet, brm-broad multiplet, t-triplet, q-quartet, dt-doublet triplet and m-multiplet. ^{13}C-NMR spectra were recorded at 75 MHz or at 100 MHz instrument. The chemical shifts are reported in δ values (parts per million, ppm) relative to the internal standard Me_4Si.

High-resolution mass spectra were recorded with a Q-TOF Bruker instrument, using electrospray ionization (ESI) as the ionization method.

X-ray crystallography

Suitable crystal of compounds, have been carried out using BRUKER AXS SMART-APEX diffractometer equipped with CCD area detector (Kα=0.71073Å, monochromator: graphite). Frames were collected at T=298K by ω, φ and 2θ-rotation with full quadrant data collection strategy (four domains each with 600 frames) at 10s per frame with SMART. The measured intensities were reduced to F^2 and corrected for absorption with SAINT. Structure solution and refinement were carried out with the SHELXTL package by direct methods. Non-hydrogen atoms were refined anisotropically. All hydrogen atoms were included in idealized positions, and a riding model was used for the refinement.

COMMON ABBREVIATIONS

ent	**Enantiomer**
Cbz	Carbobenzyloxy
2,2-DMP	2,2-Dimethoxy propane
Boc	Tertiary-butoxycarbonyl
epi	Epimer
aq	Aqueous
Bn	Benzyl
Conc.	Concentrated
LDA	Lithium diisopropyl amide
c	Concentration
cat	Catalyst
COSY	Correlation Spectroscopy
HSQC	Heteronuclear Single Quantum Coherence
NOESY	Nuclear Overhauser Effect Spectroscopy
HMBC	Heteronuclear Multiple Bond Correlation
HETCOR	Heteronuclear Correlation
DCC	*N*, *N*'-dicyclohexylcarbodiimide
DCM	Dichloromethane
DEAD	Diethyl azodicarboxylate
DMAP	4-dimethylaminopyridine
DMF	*N*, *N*'-dimethylformamide
DMSO	Dimethylsulfoxide
ppm	Parts per million
dr	Diastereomeric ratio
DEPT	Distortionless Enhancement by Polarization Transfer
equiv	Equivalent
ESI	Electrospray ionization
CSA	Camphor sulphonic acid

g	Gram
GalNAcase	*N*-acetyl-α-D-galactosaminidase
h	Hour
HRMS	High Resolution Mass Spectrometry
Hz	Hertz
IC_{50}	Half maximal Inhibitory Concentration
^{i}Pr	Isopropyl
IR	Infrared
K_i	Inhibition Constant
LAH	Lithium Aluminum Hydride
Me	Methyl
mg	Milligrams
min	Minute
mL	Milliliters
mM	Millimolar
mmol	Millimoles
µM	Micromolar
M.p.	Melting Point
Ms	Mesyl
MS	Molecular sieves
MHz	Megahertz
m/z	mass-to-charge ratio
NBS	*N*-Bromosuccinimide
NIS	*N*-Iodosuccinimide
nM	Nanomolar
NMO	*N*-Methylmorpholine*N*-oxide
NMR	Nuclear magnetic resonance
Nu	Nucleophile
Pd/C	Palladium on activated carbon
Ph	Phenyl
***p*-TSA**	Para-toluenesulfonic acid
TsCl	Para-toluenesulfonyl chloride

Ts	Para-toluenesulfonyl
R_f	Retention factor
ref	Reference
rt	Room temperature
TBAF	Tertiary-butyldimethylsilyl fluoride
TBS	Tertiary-butyldimethylsilyl
TBSCl	Tertiary-butyldimethylsilyl chloride
TBSOTf	Tertiary-butyldimethylsilyltriflate
TBDPS	Tertiary-butyl diphenylsilyl
TMSCN	Trimethylsilyl cyanide
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMS	Tetramethylsilane
liq	Liquid
DIPEA	Diisopropyl ethyl amine
Py	Pyridine
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
ATP	Adenosine triphosphate
HIV	Human immunodeficiency virus
AIDS	Acquired immunodeficiency syndrome
US FDA	United states Food and Drug Administration
HBV	*Hepatitis B* virus
HCV	*Hepatitis C* virus
mRNA	Messenger Ribonucleic acid
AON	Antisense oligonucleotide
PNP	Purine nucleoside phosphorylase
Ipc	Diisopinocamphyl
de	Diastereomeric excess
***m*CPBA**	Meta chloroperbenzoic acid
TFA	Trifluoroacetic acid
Fmoc	Fluorenylmethyloxycarbonyl

DMTrCl	Dimethoxytrityl chloride
CbzCl	Carboxybenzyl chloride
DAST	Diethylaminosulfurtrifluoride
cAMP	Cyclic adenosine mono phosphate
cGMP	Cyclic guanosine mono phosphate
NAD^+	Nicotinamide adenine dinucleotide
$NADP^+$	Nicotinamide adenine dinucleotide phosphate
FAD	Flavin adenine dinucleotide
FMN	Flavin mono nucleotide
dGTP	Deoxyguanosine triphosphate