BILE ACID-BASED MOLECULAR RECOGNITION OF FLAVIN AND URACIL DERIVATIVES AND SYNTHESIS OF NADH ANALOGUES

by

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Department of Chemistry

Submitted

in fulfillment of the requirements of the degree of

Doctor of Philosophy

to the

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This thesis is dedicated to my grandmother

CERTIFICATE

This is to certify that the thesis entitled, **"Bile acid-based molecular recognition of flavin and uracil derivatives and synthesis of NADH analogues"**, being submitted by Mr. Prosenjit Chattopadhyay, to the Indian Institute of Technology, Delhi, for the award of the degree of 'Doctor of Philosophy in Chemistry', is a record of bonafide research work carried out by him. Mr. Prosenjit Chattopadhyay has worked under my guidance and supervision and has fulfilled all the requirements for the submission of this thesis, which to my knowledge has reached the requisite standard. The results embodied in this thesis have not been submitted in part or in full, to any other University or Institute for award of any degree or diploma.

P S Pandey 21.5.2008

(Pramod S. Pandey)

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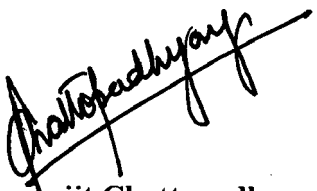
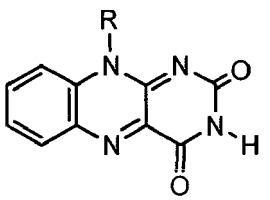


Prosenjit Chattopadhyay

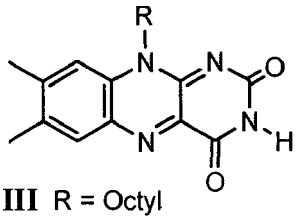
ABSTRACT

Bile acids, formed as end products of cholesterol metabolism in liver, have proved to be promising candidates in the field of molecular recognition because of its unique architecture. The present thesis deals with the synthesis and studies of bile acid-based acyclic and cyclic receptors containing 2,6-diaminopyridine for recognition of flavin and uracil derivatives. It also focuses on the design and synthesis of bile acid-based NADH analogues. The thesis has been divided into five chapters. The first chapter presents a brief literature survey on synthetic receptors that bind flavin analogues. It reviews the recent developments in flavin chemistry.

The second chapter describes the efficient synthesis of flavin analogues. We have synthesized 10-substituted isoalloxazines **I,II** by a two step method starting from *o*-phenylenediamine. The monoalkylated product can be selectively obtained by using amines in large excess. This modification has made the synthetic strategy much simpler and efficient and led to the synthesis of flavin analogues possessing a variety of substituents at *N*(10) position.



I R = Octyl
II R = Hexyl



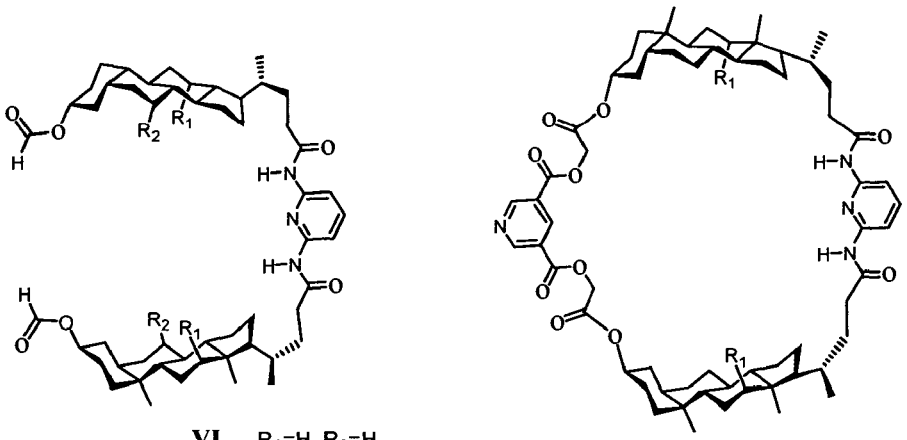
III R = Octyl
IV R = Hexyl
V R = Butyl

Flavin analogues

The second chapter also describes the synthesis of 7,8-dimethyl flavin analogues **III-V**, which are more biologically relevant flavins. The key step in this synthetic route was the alkylation of 4,5-dimethyl-2-nitroaniline with the help of sodium hydride in DMF. The

method is applicable to the synthesis of riboflavin analogues possessing a variety of substituents at *N*(10)-position, starting from readily available 3,4-dimethyl aniline. The analogues were fully characterized by ^{1}H and ^{13}C and HRMS (ES^{+}) spectral data. The flavin analogues have been used for binding studies with bile acid-based receptors, described in chapter 3.

Chapter 3 deals with the synthesis and study of various acyclic and cyclic bile acid-based 2,6-diaminopyridine systems for flavin recognition. Condensation of the acid chloride of formylated bile acids with 2,6-diaminopyridine afforded the acyclic bile acid-based receptors **VI-VIII** in good yields. We have also developed a modified route for the synthesis of the acyclic bile acid receptors by using ethyl chloroformate in the presence of triethylamine to give the corresponding anhydrides that were subsequently condensed with 2,6-diaminopyridine. This modified synthetic route is much cleaner, easy to handle and most importantly gives comparable yields. In case of deoxycholaphane, the limiting step in the synthesis is the selective bromoacetylation at 3α-position of bisdeoxycholamides. The present study describes a modified step wherein treatment of bisdeoxycholamides with two equivalents of bromoacetyl bromide in the presence of potassium carbonate in chloroform afforded the 3α,3α′-bisbromoacetyl deoxycholamides in nearly 70 % yield. The cyclisation of the 3α,3α′-bisbromoacetylcholamides with Cs-salt of pyridine-3,5-dicarboxylic acid gave high yield of the desired cholaphane. Dioctyldiamide of 2,6-diaminopyridine was synthesized for control experiments. The binding behaviour of the flavin analogues were determined with 2,6-diaminopyridine based receptors using ^{1}H NMR titration in $CDCl_3$.

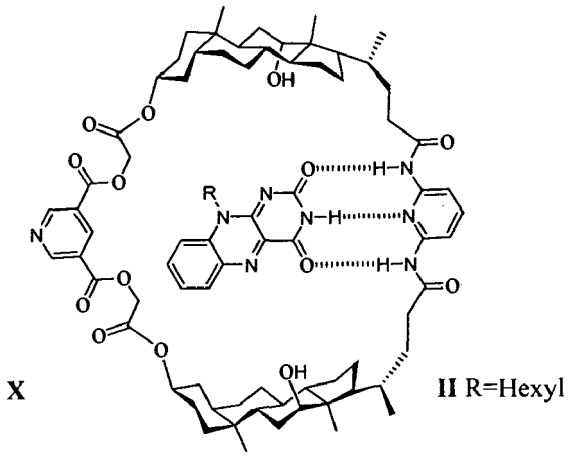


VI R_1=H, R_2=H
VII R_1=OCHO, R_2=H
VIII R_1=OCHO, R_2=OCHO

IX R_1=H
X R_1=OH

Bile acid-based acyclic and cyclic receptors

Addition of the flavin derivative to the solution of the receptor in $CDCl_3$ resulted in the downfield shift in the resonances of the amidic protons of the receptor. The change in the chemical shift of the amidic protons was followed as a function of increasing guest concentration until saturation of the chemical shift values was reached. Analysis of the saturation data with WinEQNMR software, a non-linear regression curve fitting program, revealed a 1:1 complexation.

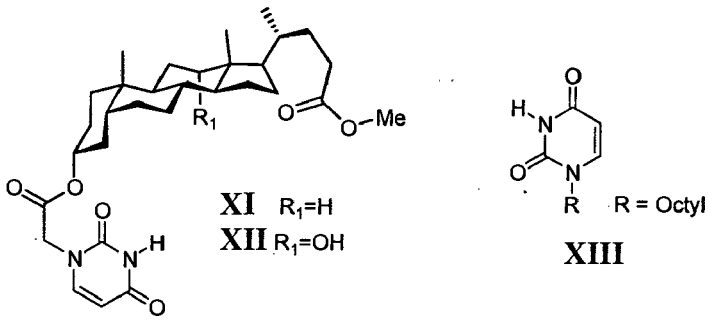


X **II** R=Hexyl

1:1 Complex formation

The complex stoichiometry was further determined by using Job's method of continuous variations. The binding constants of the complexes of the acyclic receptors with 10-hexylisoalloxazine were higher as compared to 7,8-dimethyl-*N*(10)-hexylisoalloxazine. A similar trend was observed for the complexation of 10-octylisoalloxazine and 7,8-dimethyl-*N*(10)-octylisoalloxazine. The unexpected decrease in the binding constants of 7,8-dimethyl flavin analogues with acyclic bile acid-based receptors, presumably, is due to the steric effects arising from the interaction of the methyl groups at the 7 and 8 positions and *N*(10) substituent of the flavin with the rigid steroidal framework. The binding affinity of 7,8-dimethyl-*N*(10)-butyl flavin is lower as compared to 7,8-dimethyl-*N*(10)-octyl flavin & 7,8-dimethyl-*N*(10)-hexyl flavin may be due to the dominant steric interactions of 7,8-dimethyl groups with the steroidal framework. In the case of the cholaphanes, there is no significant change in the binding constant with respect to 7,8-unsubstituted and substituted flavin analogues.

The design and synthesis of molecular receptors using weak non-covalent interactions is a flourishing field with many potential applications. Chapter 4 deals with the recognition properties of bile acid-based receptors with uracil derivatives.

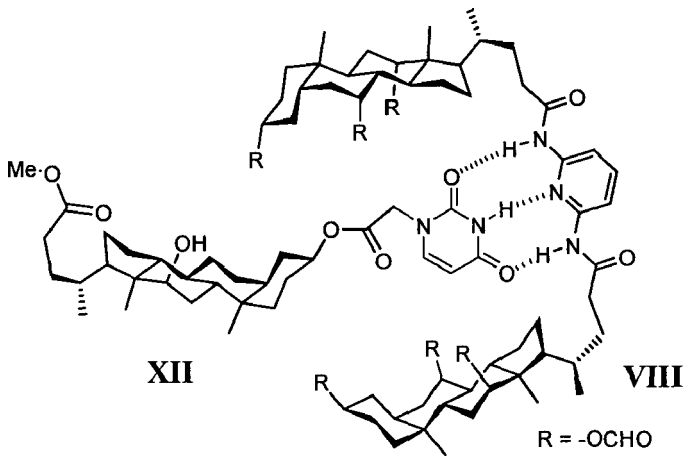


XI R_1=H
XII R_1=OH

XIII

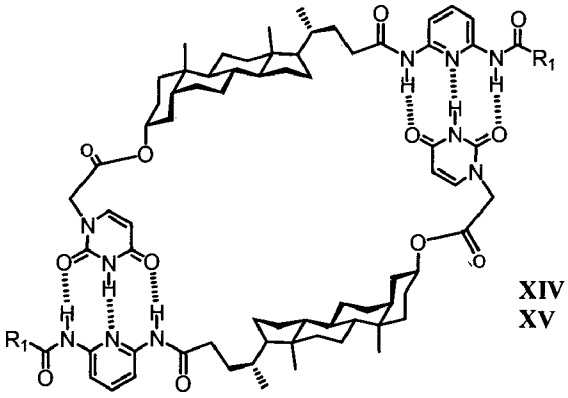
Uracil derivatives

The study describes the synthesis of uracil derivatives that showed high affinity for acyclic and cyclic bile acid-based receptors containing 2,6-diaminopyridine. The binding efficacy of the receptors containing 2,6-diaminopyridine for uracil derivatives were evaluated by ^{1}H NMR titrations in $CDCl_3$. Analysis of the saturation data with WinEQNMR software revealed a 1:1 complexation which was further confirmed by Job's plot.



1:1 Complex formation

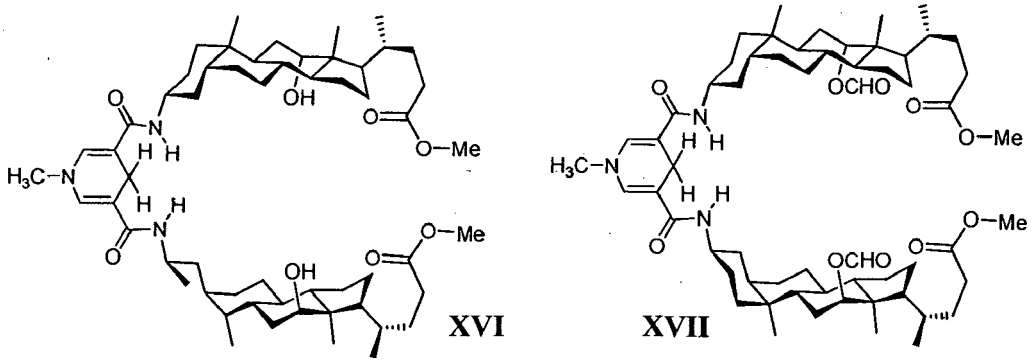
Nucleobases have been widely used as supramolecular motifs capable of forming strong hydrogen bonds to construct new biomaterials and complex nanostructures. In an effort to utilize the hydrogen bond interactions between uracil and 2,6-diaminopyridine to design new bile acid-based materials which may have unique supramolecular properties, we synthesized bile acid-based DAP analogues bearing uracil unit **XIV**,**XV**. These bile acid-conjugates exhibit unique ability to utilize hydrogen bonding to form molecular duplexes in non-polar solvents. The mode of assembly was established in solution by ^{1}H NMR dilution experiments, ESI-MS and size exclusion chromatography.



XIV $R_1=C_{11}H_{23}$
XV $R_1=CH_3$

Self-assembly of bile acid conjugate into dimeric species

In nature, many biological redox transformations are carried out stereoselectively in the presence of coenzymes NADH/NAD^+. Chapter 5 of this thesis deals with the design of bile acid-based NADH analogues. Acyclic NADH models have been synthesized, in which the 1,4-dihydronicotinamide moiety is attached to the bile acid. The reduction of the NAD^+ analogues to NADH analogues were accomplished by its treatment with sodium dithionite in phosphate buffer (pH 7). Unfortunately, both the NADH analogues were found to be ineffective in reducing ethyl benzoylformate. The lack of reactivity of the steroid based NADH analogues may be attributed to the steric hindrance created by the bulky bile acid units which does not allow the formation of the ternary complex required for the reaction.



XVI **XVII**

NADH analogues

Glossary of symbols and Abbreviations

%	percentage
δ	chemical shift
°C	degree centigrade
Ar	aryl
bs	broad singlet
d	doublet
ee	enantiomeric excess
DMF	*N,N'*-dimethylformamide
THF	tetrahydrofuran
DCC	dicyclohexylcarbodiimide
DEAD	diethyl azodicarboxylate
DCM	dichloromethane
DAP	2,6-diaminopyridine
DMAP	*N,N'*-4-dimethylaminopyridine
Fig.	figure
g	gram
mp	melting point
h	hour
min.	minute
IR	Infra red
J	coupling constant

m	multiplet
mg	milli gram
K_a	Association constant
MHz	mega hertz
Hz	hertz
mmol	millimole
M	molar
NMR	Nuclear Magnetic Resonance
rt	Room temperature
s	Singlet
TLC	Thin layer chromatography
TMS	Tetramethylsilane
t	triplet
m/z	mass/charge
M^+	molecular ion
FAB	fast atom bombardment
HRMS	High resolution mass spectrum
ESI	Electrospray ionization

NOTES

1. All the melting points reported in the thesis are uncorrected and were taken on an electrical melting point apparatus.
2. All the solvents were purified by routine methods and were distilled before use.
3. TLC was performed on silica gel plates using iodine for visualizing spots.
4. Purification of the compounds was done by flash chromatography using Spectrochem silica gel 230-400 mesh.
5. IR spectra were recorded on a Nicolet Protégé 460 FTIR Spectrometer, using potassium bromide pellets.
6. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Spectrospin DPX 300. Tetramethylsilane was used as internal reference and the chemical shifts are expressed as displacement (δ) in ppm downfield from tetramethylsilane.
7. Low resolution mass spectrum was recorded on a quadrupole mass spectrometer (VG Platform-II) using acetonitrile-water (1:1) as mobile phase. High resolution mass spectra (ES) were recorded on a VG-Fisons 'Autospec' spectrometer.
8. Elemental analyses were taken on Perkin Elmer 2400C Elemental Analyzer.
9. The solid compounds were dried under vacuum in the presence of P_2O_5.

CONTENTS