STUDIES ON BILE ACID-BASED MOLECULAR RECEPTORS AND COENZYME ANALOGUES

by

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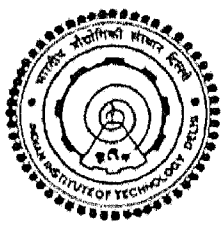
Department of Chemistry

Submitted
in fulfilment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

to the



INDIAN INSTITUTE OF TECHNOLOGY, DELHI

JULY, 2002

DEDICATED

TO

MY PARENTS

CERTIFICATE

This is to certify that the thesis entitled, "Studies on bile acid-based molecular receptors and coenzyme analogues", being submitted by Ms. Roopali Rai 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 her. Ms. Roopali Rai has worked under my supervision and guidance 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 the award of any degree or diploma.

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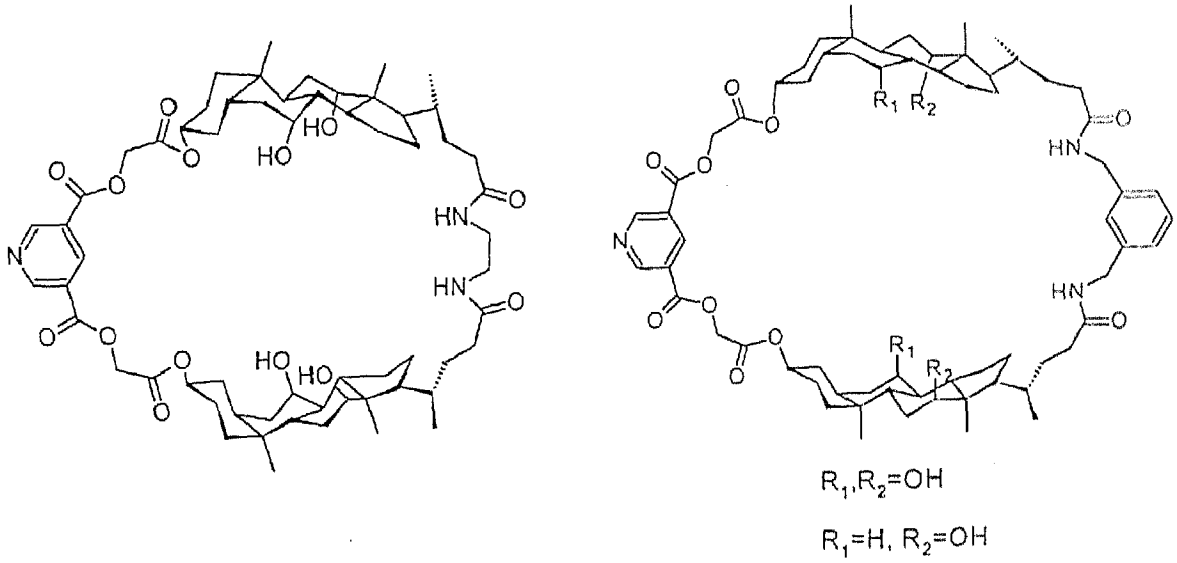
ABSTRACT

There has been considerable interest in recent years in designing molecular receptors based on bile acids. The interest in bile acid emerged due to its unique features such as chiral and rigid framework, and different reactivities of their hydroxyl groups. The present thesis deals with the design and reactivity of various steroid-based receptors and divided into four chapters. The first chapter presents a brief literature survey on supramolecular chemistry.

The second chapter describes the new synthetic methodology, which has been developed for the head-to-head cholaphanes having different types of spacers, based on cholic and deoxycholic acids. The limiting step in the synthesis is the selective bromoacetylation at 3α-position of bischolamides. The present study describes a modified step wherein treatment of bischolamides with two equivalents of $BrCH_2COBr$ in the presence of K_2CO_3 in $CHCl_3$ afforded the 3α-bromoacetylcholamides in nearly 70% yield in 10 min. The cyclization of the 3α-bromoacetylcholamides with Cs-salt of appropppriate dicarboxylic acids gave high yields of the desired cholaphanes. This modification has made the synthetic strategy much simpler and efficient and led to the synthesis of various head-to-head cholaphanes. The detailed structural features of these cholaphanes have been determined by using one- and two-dimensional NMR spectroscopy. The chapter describes the detailed assignments of ^{13}C NMR signals on the basis of DEPT $^{13}C\{^1H\}$ 135/90 and HSQC $\{^1H\text{-}^{13}C\}$ NMR studies.

An improved synthesis of pyridinocholaphane has also been developed. This involved the treatment of 3α-bromoacetyl succinimido deoxycholate with dicesium 3,5-

pyridine dicarboxylate in DMF followed by the addition of *m*-xylylenediamine in the same reaction mixture, which afforded the pyridinocholaphane in an overall yield of 90%.

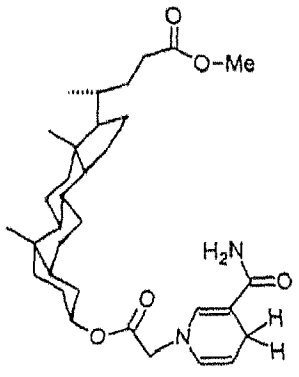


Pyridinocholaphanes

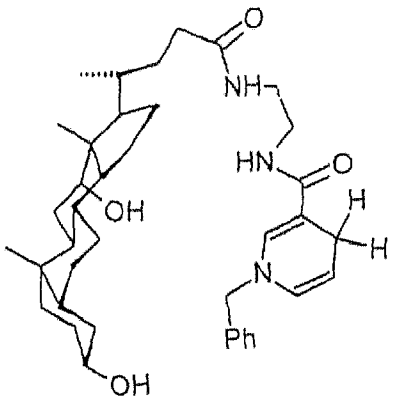
These efficient methodologies for the synthesis of head-to-head cholaphanes would find their application in the study of supramolecular chemistry of this class of compounds. The pyrinocholaphanes synthesized may serve as useful precursors for the synthesis of NADH analogues.

The design and synthesis of NAD^+/NADH analogues has been an area of considerable interest. NADH mimics based on crown ethers, cyclodextrins, cyclophanes, carbohydrates etc. are well reported, however, there is no report on steroid-based NADH mimics. The chapter three deals with the design of steroid-based NADH analogues Thus, acyclic steroidal NADH models have been synthesized, in

which the 1,4-dihydronicotinamide moiety is attached to the bile acid via its C-1 nitrogen (mimic I) and through amide bond (mimic II). The analogues are fully characterized by ^{1}H, ^{13}C NMR and FAB mass spectrometry. The corresponding deuterated analogues were also synthesized by carrying out the reduction of NAD^{+} analogues in D_2O. The reduction of NAD^{+} analogue to NADH analogue was effected by its treatment with $Na_2S_2O_4$ in phosphate buffer (pH 7). The reactivity of NADH mimics I and II was investigated towards reduction of carbonyl substrates. The NADH analogue I was found to be unreactive, however, the analogue II reduced the ethyl benzoylformate to ethyl mandelate in about 50% yield. The stereochemistry of the reduced product was studied by ^{1}H NMR spectroscopy by using NMR shift reagent. It was expected that the hydroxyl group at 12-position of the deoxycholic acid would also participate in the formation of the ternary complex, however the *ee* came out to be only ~4-5%. This small value of the *ee* indicates that there is very little involvement of the steroid molecule in the formation of the ternary complex.

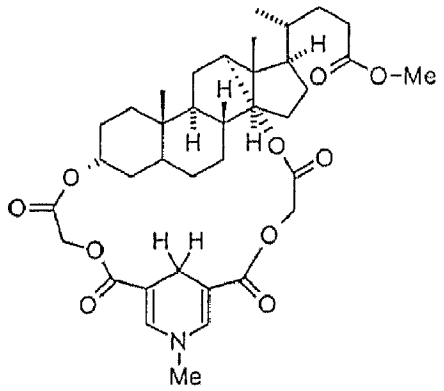


NADH mimic I



NADH mimic II

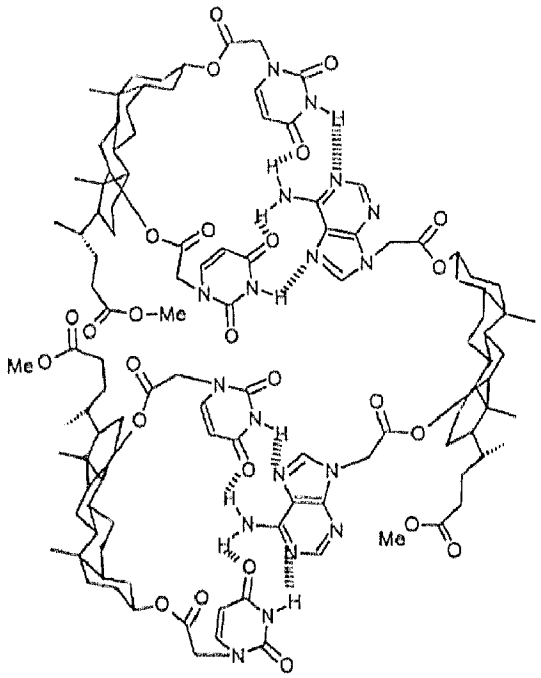
A cyclic NADH analogue **III** based on deoxycholic acid has also been synthesized and its reactivity towards the reduction of various carbonyl compounds has been investigated. To our surprise, this analogue completely failed to show any reactivity towards these compounds, presumably due to the steric effect of the steroid molecule which may prevent the formation of an effective ternary complex, which is essential for the reaction.



NADH mimic **III**

The chapter IV of the thesis describes an attempt to develop the receptors for recognition of adenine derivatives. The study describes the synthesis of deoxycholic acid derived receptor by incorporating uracil units at 3α- and 12α-positions that showed high affinity for steroidal adenine derivatives in $CDCl_3$. The recognition property was studied by utilizing ^1H NMR titration method. The analysis of the saturation data with a NMR software, WinEQNMR, gave the stoichiometry of the complex, 2:1. The formation of the 2:1 complex indicates that there is an ideal spacing between the two-uracil units of the receptor for simultaneous Watson-Crick and

Hoogsteen binding. Studies have also been carried out to show the comparative binding of steroidal adenine derivative with uracil and flavin derivatives.



2:1 complex of receptors based on deoxycholic acids

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