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Design and Synthesis of Bisubstrate Analogues for 3-Hydroxy-3-Methyl-Glutaryl Coenzyme A Reductase

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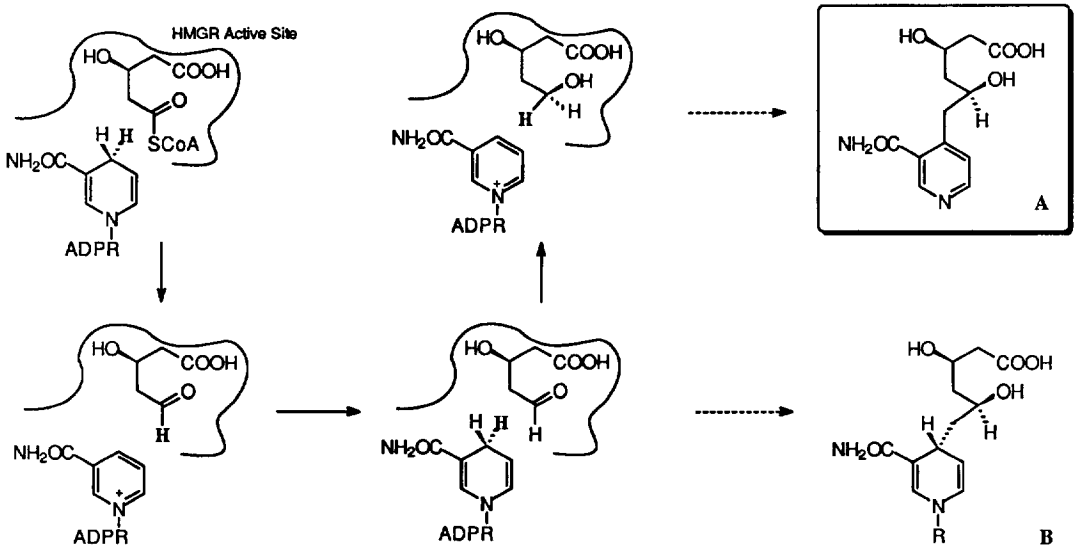
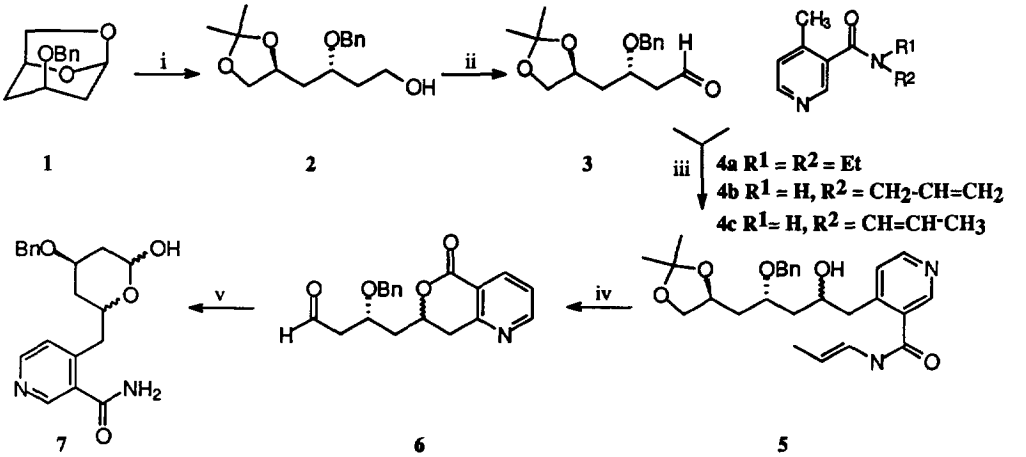
Abstract : The coupling of metalated pyridine derivatives with a chiral Weinreb amide derived from gluconolactone followed by stereoselective reduction of the resulting ketone are the key steps of the synthesis of bisubstrates analogues of the HMGCoA reduction by HMGR.

The efficacy of cholesterol biosynthesis inhibitors for the control of cholesterol blood level is now well recognised. Among these inhibitors, mevinic acids, the so-called statines, are potent competitive inhibitors of 3-hydroxy-3-methyl glutaryl coenzyme A reductase (HMGR) and have been thoroughly studied.[1] The protective effect of statines against coronary diseases by diminishing the LDL level,[2] and also by preventing the noxious oxidation of LDL responsible for atheroma formation[3] is now established. The chemistry of mevinic acids has been widely explored, favouring the emergence of efficient drugs.[4] Nevertheless there is still room for basic research aimed at the discovery of new lead compounds with enhanced tissue selectivity[5]. Moreover it is interesting to know in detail how these inhibitors interact with the enzyme.[6] In this context we embarked some years ago in a programme aimed at the discovery of new inhibitors of HMGR based on the concept of multisubstrate and/or transition state analogues.[7]



Scheme 1

HMGR needs the cofactor NADPH to reduce its substrate HMGCoA into mevalonic acid by two successive reductions of a carbonyl group. According to this principle, depicted in Scheme 1, compounds **A** and **B** bearing the 3-hydroxy-glutaryl moiety coupled with a pyridine or dihydropyridine ring are bisubstrate analogues and should be good candidates for inhibition. Some previously prepared related compounds having a two carbon bridge between the two moieties showed no activity *in vitro* against HMGR.[7,8] One possible explanation is that these compounds did not fulfil all the geometrical requirements (2 carbon link instead of 1). Although they are structurally related to synthetic mevinic acids, a "lactonic part" coupled with a lipophilic moiety, they did not interact with HMGR in the same way. In order to shed some light on this problem, we decided to prepare new analogues having a one-carbon bridge linking the lactonic moiety and a pyridine ring which could be further elaborated to a dihydropyridine.

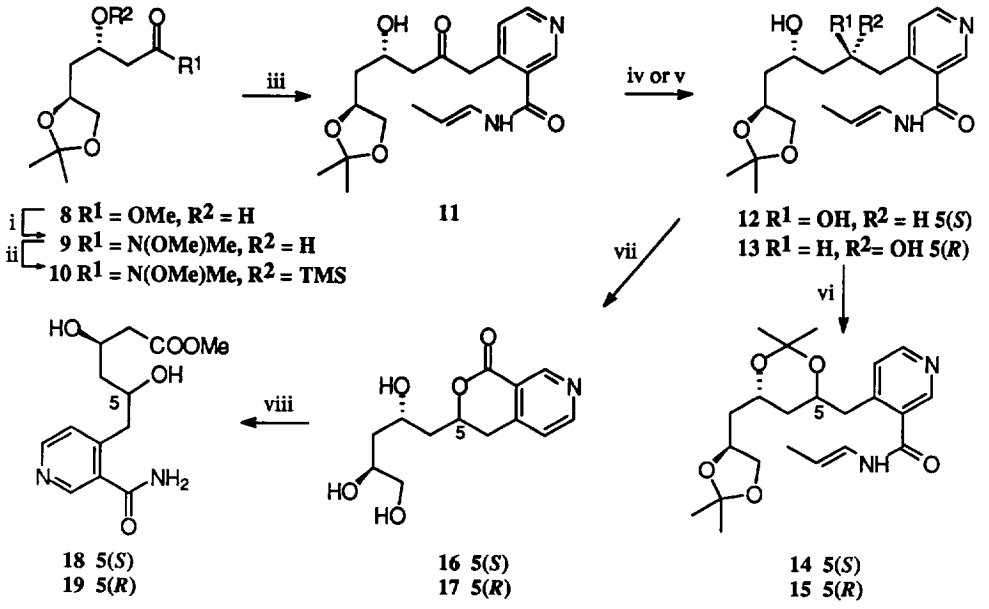


Scheme 2: Reagents; i: 1) Dowex, H^+, THF/H_2O, rflx, 2) $NaBH_4$, EtOH, 63%, 3) DMP, acetone, PTSA, 94%; ii: DMSO, $(COCl)_2$, NEt_3; iii: **4c**, *s*BuLi, THF -80°C, then **3**, to rt, 60%; iv: MeOH, HCl, rflx 18h, 2) $NaIO_4$, MeOH/H_2O, 80%; v: NH_3, MeOH

Aldehyde **3** was prepared from the known levoglucosan derivative **1**[6] by acid hydrolysis of the glycoside and reduction of the hemiacetal. The resulting 1,2 diol function was protected as an acetonide to provide the dideoxy alditol **2**. The resulting primary alcohol was oxidized to aldehyde **3**. The condensation of the anion derived from tertiary amide **4a**, prepared from 3-cyano-4-methyl-pyridine[7] using standard chemistry, was efficient (60%). However, the diethylamido group, necessary to ensure metalation at the benzylic position, was difficult to transform efficiently into the required primary amide. Thus we turned to another directing metalation group. The N-propenylamide **4c** was prepared by isomerisation of the corresponding N-allyl-amide **4b** using *t*BuOK in DMSO (81%).[9] Condensation of the corresponding anion with aldehyde **3** cleanly gave a 1/1 mixture of the two expected epimeric alcohols **5** in 60% yield. Acid hydrolysis of **5**, which cleaved the acetonide and promoted lactonisation, was followed by oxidative cleavage of the resulting diol to aldehyde **6**. Treatment of **6** with ammonia in methanol gave the primary amide **7** isolated as an unseparable mixture of stereoisomers.

We explored another strategy based on the highly stereo controlled reduction of β-hydroxy-keto derivatives. In this regard Weinreb amide **9** seemed to be an appropriate precursor of the required β-hydroxy-ketone system.[10] This compound was prepared from ester **8**, which can be obtained in quantities from D-glucono-1,5-lactone, by a straightforward route.[11] The best way to prepare amide **9** was the saponification of ester **8** followed by coupling of the resulting acid with N-methyl-N-methoxyamine hydrochloride. The

secondary alcohol was protected by trimethylsilylation to give amide **10**. The condensation of the lithium anion derived from **4c** with **10** proceeded uneventfully to give the hydroxyketone **11** in 82% yield after removal of the TMS group.



Scheme 3: Reagents; i: 1) MeOH, 1N NaOH, 50°C, 67%, 2) BOP, MeNHOMe, HCl, NEt_3, 77%; ii) TMSCl, CH_2Cl_2/pyridine, 94%; iii: 1) **4c**, *s*BuLi, THF -80°C, 20 min then **10**, 30 min, 2) Bu_4NF, THF 82%; iv: $Me_4N,HB(OAc)_3$, CH_3CN, AcOH -30°C, 87%; v: Et_2BOMe, air, THF then $NaBH_4$, -80°C; vi: DMP, acetone, PTSA, 94%; vii: 1) MeOH, 3N HCl, rflx; viii: 1) NH_3, MeOH, 2) $NaIO_4$, MeOH, H_2O, 48% four steps, 3) Ag_2O, MeOH rflx.

The last crucial step was to secure the stereochemistry at C-5. The stereochemistry of the reduction of HMGCoA and mevaldate by HMGR in the presence of NADPH is known, the hydride being transferred on the *Si* face of the carbonyl group (Scheme 1). Accordingly the stereochemistry of the reduction must provide the *anti* diol. Reduction of the ketone **11** according to Evans method gave the *anti* diol **12**.[12] As seen from ^{13}C nmr, about 5% of the *syn* isomer was detected in the crude reaction mixture. This was confirmed by the reduction of **11** using Narasaka's conditions,[13] which gave mainly the *syn* isomer (*syn/anti*, 8.5:1). The stereochemistry at the newly created chiral centre C-5 was confirmed by careful examination of the ^{13}C nmr spectra of the acetonides **14** and **15** prepared from diols **12** and **13**. The chemical shifts of the methyl groups of the six-membered ring isopropylidenes were 24.2 ppm in the *anti* isomer and 19.5 and 29.6 in the *syn* isomer.[14a] The chemical shifts of the quaternary carbons 101.1 and 99.28 ppm were also consistent with the assigned structures.[14b] The remainder of the synthesis consisted into removal of the propenyl protecting group of **12** and **13** in acidic medium to give lactones **16** and **17** respectively. Upon treatment with ammonia in methanol followed by sodium periodate cleavage of the 1,2-diol, the corresponding aldehydes were isolated as their hemiacetals which were immediately oxidised into methyl esters **18** and **19** in moderate yields with some starting material recovery.[15] These esters were submitted to saponification (NaOH, MeOH, H_2O) just before testing. None of these compounds showed any biological activity as inhibitors of rat liver HMGR even at 10^{-4} M concentration. Although compounds **18** and **19** bear all the required functionalities to mimic, the presence of the heterocyclic part of NADPH in the early stage of the reduction or the presence of NADP at the late stage, it did not sufficiently mimic the NADP(H) residues. This provides additional evidence that most interactions of

the NAD(H) residue with HMGR are located on the adenosine diphosphoribose part as recently seen from the X-ray crystal structure of HMGR of *P. mevalonii*.[16]

In conclusion, we have developed an efficient route to chiral elaborated structures derived from nicotinamide but, undoubtedly much work is still needed to obtain better analogues by incorporation of a mimic of the adenosine diphosphoribose.[17] We are currently applying this concept to obtain efficient bisubstrate analogues for reductase and deshydrogenase enzymes requiring cofactors NAD(P)H.

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