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Template-Directed Condensations between Acyl Units

by

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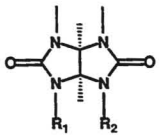
To my wife Huiming and my daughter Cana

ABSTRACT

Glycoluril **1** acts as a bifunctional template, allowing efficient and repetitive Claisen-type condensations between attached acyl units. Thus acetoacetyl derivative **3** was prepared from **1** via **2**. After conversion to the crotonate derivative **4** (2 steps, β-carbonyl reduction and dehydration), a second cycle of acylation and condensation gave crotonyl-transferred product **5**. Repetition of the sequence gave the 8-carbon derivative **6**. Reduction of **4** with L-Selectride afforded the butyryl derivative **7**, allowing further acetylation and condensation reactions leading towards long fatty acid chains. The product ratio from condensation of the acetyl butyryl derivative of **1** is 4:1 in favour of deprotonation of the acetyl group and transfer of the butyryl group. Reaction of **7** with lithium benzyloxide gave recovered **1** and benzyl butyrate. Treatment of crotonyl acetyl



1. $R_1 = R_2 = H$
2. $R_1 = R_2 = Ac$
3. $R_1 = CH_3COCH_2CO$, $R_2 = H$
4. $R_1 = CH_3CH{=}CHCO$, $R_2 = H$
5. $R_1 = CH_3CH{=}CHCOCH_2CO$, $R_2 = H$
6. $R_1 = CH_3(CH{=}CH)_2COCH_2CO$, $R_2 = H$
7. $R_1 = CH_3(CH_2)_2CO$, $R_2 = H$
8. $R_1 = CH_3CH{=}CHCO$, $R_2 = Ac$
9. $R_1 = CH_3CH_2(Ac)CHCO$, $R_2 = H$

derivative **8** with L-Selectride produced only the acetyl transferred condensation product **9**. A similar regioselectivity was observed with L-Selectride reduction of other α,β-unsaturated compounds (*e.g.*, the acetyl acryloyl derivative). Results of isotopic-labelling experiments support an intramolecular acyl transfer mechanism for these condensations. This system mimics some of the features of polyketide and fatty acid synthases, allowing intramolecular acyl transfer and repetitive additions of acetate units to make linear carbon chains while the growing chain remains attached to the template. This is a potentially useful method for the synthesis of some natural products and some useful organic intermediates, such as putative oligoketide biosynthetic precursors.

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LIST OF ABBREVIATIONS

Ac	Acetyl
ACPSH (or HSACP)	Acyl Carrier Protein (with Thiol Group SH)
Anal.	Analysis
Bn	Benzyl
Bu	Butyl
Calcd	Calculated
CIMS	Chemical Ionization Mass Spectrometry
cm	Centimetre
COSY	Correlated Spectroscopy
d	Doublet
dd	Doublet of Doublets
dt	Doublet of Triplets
EIMS	Electron Impact Ionization Mass Spectrometry
Enz	Enzyme
equiv.	Equivalent
Et	Ethyl
FAS	Fatty Acid Synthase
Fig.	Figure
FTIR	Fourier Transformed Infrared Spectroscopy
h	Hour(s)

HMDSA	Hexamethyldisilazane
HREIMS	High Resolution Electron Impact Ionization Mass Spectrometry
HSCoA	Coenzyme A (with Thiol Group SH)
IR	Infrared spectroscopy
LAH	Lithium Aluminium Hydride
LDA	Lithium Diisopropylamide
M^+	Molecular Ion
Me	Methyl
m/e	Mass/Charge Ratio
mL	Millilitre
mmole	Millimole
m.p.	Melting Point
MS	Mass Spectrometry
nm	Nanometre
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser Enhancement
PKS	Polyketide Synthase
ppm	Parts Per Million
pTsOH	p-Toluenesulphonic Acid
q	Quartet
s	Singlet

S.M.	Starting Material
t	Triplet
TBDMS	*tert*-Butyldimethylsilyl
TFA	Trifluoroacetic Acid
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TMS	Tetramethylsilane
TMS-	Trimethylsilyl
UV	Ultraviolet

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CHAPTER 1. INTRODUCTION

1.1 Enzymes as Catalysts[1,2]

Enzymes catalyze biochemical transformations to provide materials and energy for living systems. Most enzymes are special proteins built from amino acids through amide bond connections. A few RNAs have also been found to have catalytic activities. The major features of enzyme-catalyzed reactions include a high degree of specificity as well as high efficiency; these attributes have attracted the interest of organic chemists. Most enzyme-catalyzed reactions take place on a special area of the protein catalyst surface, which is referred to as the *active site*. Chemical transformations carried out at the active site follow the general principles of organic and physical chemistry. However, specific amino acid side chains with a well defined geometrical alignment can provide multiple catalytic functionalities which operate simultaneously. This simultaneous action can provide a new and efficient reaction pathway in which the rate-determining step has a lower free energy of activation than that of the uncatalyzed reaction. Usually, all transition state energies in an enzyme-catalyzed reaction are lower than those of the corresponding uncatalyzed process; however, there are at least two more transition states corresponding to the binding of substrates and release of products in the catalytic reaction.

The initial binding step is one of the most important processes and the large binding energy ($\Delta H_{binding}$ is large and negative) provides the first driving force for enzyme catalysis. Enzyme-substrate complex formation in a binding process involves a large loss of entropy ($\Delta S_{binding}$ is also large and negative), which can be offset by the binding energy according to the free energy equation:

$$\Delta G_{binding} = \Delta H_{binding} - T\Delta S_{binding}$$

The net value of the free energy change $\Delta G_{binding}$ must still be negative to give efficient binding. This binding process leads all catalytic groups to act co-operatively and substrates to be optimally oriented (spatially and stereo-electronically) on the same molecule. Therefore the subsequent step of an enzyme-catalyzed transformation actually proceeds in an "intramolecular" fashion and the free energy of activation ($\Delta G^{\ddagger}$) is substantially lowered due to little further loss of entropy in the transition state ($\Delta S^{\ddagger} \sim 0$).

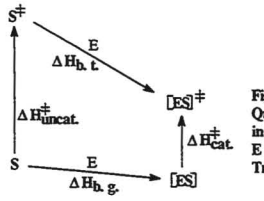
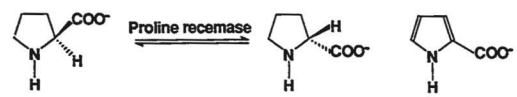


Fig. 1.1.1
Qualitative Illustration of Enthalpy Changes in Enzymatic Binding and Catalysis.
E is for Enzyme; S for Substrate; b.t. for Binding Transition State; b.g. for Binding Ground State.

Although the initial binding step of substrate to an enzyme is very efficient, the enzyme has evolved to bind the transition state of the rate-limiting step in the catalyzed reaction even more favourably than the ground state of the substrate.[3] This can result in $\Delta H^{\ddagger}_{cat.} < \Delta H^{\ddagger}_{uncat.}$ due to $\Delta H_{b.g.} > \Delta H_{b.t.}$, as shown in Fig. 1.1.1 ($\Delta H_{b.g.}$ is the enthalpy change of binding the ground state of the substrate to the enzyme and $\Delta H_{b.t.}$ is that of binding the uncatalyzed transition state; S is substrate).

The evidence to support the stronger binding between an enzyme and the transition state comes from two early experiments.[4,5] A stable compound that has a structure similar to the transition state of an enzyme catalyzed reaction can be a competitive inhibitor of that enzyme (Transition State Analog Inhibitor).[6,7] For example, proline racemase from *Clostridium sticklandii* can bind pyrrole-2-carboxylic acid 160-fold stronger than the substrate L-proline as shown in Fig. 1.1.2. Pyrrole-2-carboxylic acid can hence act as a competitive inhibitor of the proline racemase. The α-carbon atom of the substrate assumes a near-planar



L-Proline **D-Proline** **Pyrrole-2-carboxylic acid**

Fig. 1.1.2 Proline Racemase Inhibited by Pyrrole-2-carboxylic Acid.

configuration at some point in the reaction. Tight binding would therefore be expected for analogues with sp^2 hybridization at this position.[4] In the second experiment, triosephosphate isomerase was inhibited by a transition state analog 2-phosphoglycollate, as discussed by Wolfenden [5]

The free energy of activation of an enzyme-catalyzed reaction is lowered due to this discriminating binding as well as the entropy advantage and a large rate acceleration is therefore observed.

1.2 Biosynthesis of Fatty Acids[1,8,9,10]

Fatty acids are carboxylic acids which usually contain long linear hydrocarbon chains. An even number of carbon atoms ranging from 2 (acetic acid) to 20 (arachidic acid) is very common for fatty acids found in biological systems. Short chain fatty acids (10 carbon atoms or fewer) are intermediates in many pathways of metabolism and are found, for example, in milk. Longer fatty acids with 12 to 18 carbon atoms exist predominantly as lipid and fat constituents and can be easily obtained in large quantity from fat hydrolysis. Arachidic acid (C_{20}) is an intermediate during the biosynthesis of prostaglandins.

Phospholipids and glycolipids, which contain fatty acids as constituents,

are the important structural components of cell membranes. However, there is a much larger quantity of the fatty acid, which functions as a reservoir of stored energy in the form of triacylglycerols esters. The oxidation of hydrocarbon chains of fatty acids to CO_2 and H_2O provides one of the major pathways to supply energy for biological activities.

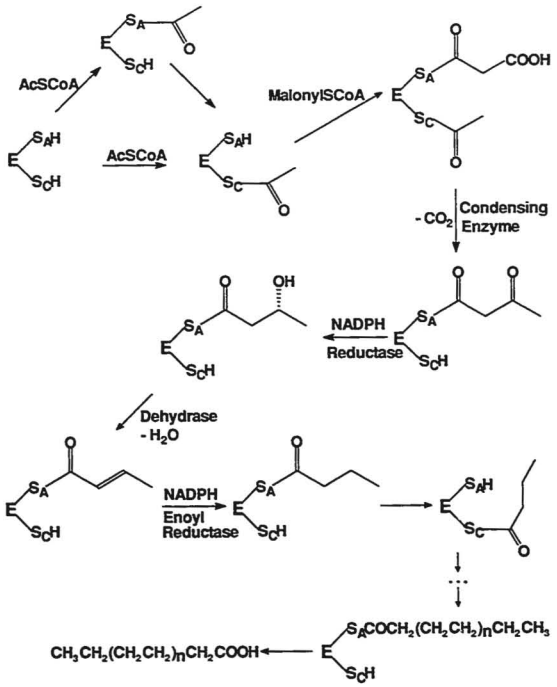
Due to the importance of fatty acids for the survival and well-being of organisms and their ubiquitous nature, they are classified as primary metabolites. The biosynthesis of fatty acids has been widely studied for more than a half century and the intricate biochemical mechanisms have been well understood. Fatty acid biosynthesis occurs in the cytoplasm with an acetyl group from acetyl-SCoA as a starter unit; malonyl-SCoA provides the subsequent building blocks.

Fatty acid synthase (FAS) contains seven enzymes of different functions and an acyl carrier protein (ACPSH or HSACP). One of the enzymes is the 3-ketoacyi-SACP synthase (referred to simply as condensing enzyme). FAS directs the repeated Claisen-type condensations between acyl and malonyl groups and uses 2 thiol groups as binding sites for each cycle of condensation. One of the active site thiol groups is contributed from one of the ACPSHs and the other from those of the condensing enzymes.

In all organisms, fatty acids are constructed by following an essentially

universal head-to-tail sequence (Scheme 1.2.1): the acetate unit at the methyl terminal is the starter unit and subsequent ones are sequentially added to the carbonyl terminal. However, two basic types of FAS with structural differences have been identified. At one extreme, Type I FASs are single enzymes which possess all the catalytic functions required to assemble the fatty acid. These functions are present in several different domains of the multifunctional proteins.[11] Type I FAS is usually found in eukaryotic organisms. At the other extreme, FASs in prokaryotic micro organisms and plants are classified as the Type II FASs, which consist of a group of seven dissociable enzymes and an ACPSH, all of which can be isolated separately.[12] In this case, each separate enzyme is monofunctional and catalyses a single step such as condensation, β-keto reduction, dehydration or enoyl reduction, *etc.*

Until recently, it was generally accepted that the Type II FASs contain two dissociable condensing enzymes (I and II) which catalyze condensations between a malonyl-SACP and an acyl thiol ester. Fatty acids with chain length less than 16 carbon atoms are assembled on condensing enzyme I, while condensing enzyme II plays the role of lengthening the fatty acids from C_{16} to C_{18} and beyond. However, a third condensing enzyme, condensing enzyme III has been reported, first from spinach[13] and then from *E. coli.*[14] The condensing enzyme III is often found in higher plants and is probably general for Type II FASs.[15] The function of condensing enzyme III is to mediate the first decarboxylative



Scheme 1.2.1. Steps in Fatty Acid Biosynthesis
(S_A = Thiol on ACPSH; S_C = Active Site Thiol on Condensing Enzyme)

condensation in fatty acid biosynthesis between malonyl-SACP and acetyl-SCoA to generate acetoacetyl-SACP. In the first binding step, one acetyl group from acetyl-SCoA was generally believed to be transferred to an ACPSH and then to the active site thiol of a condensing enzyme.[8,9] Recent studies, however, have shown that condensing enzyme III has preference for directly binding acetyl-SCoA; that is, acetyl-SCoA is a better substrate than acetyl-SACP and is directly used by condensing enzyme III without transacylation on to an HSACP or another thiol residue.[13,14,15] The condensing enzyme III is sometimes called 'short chain 3-ketoacyl-SACP synthase' because when the condensing enzymes I and II are selectively inhibited, the condensing enzyme III can generate C_6-acyl-SACP product.

In the next binding step, a malonyl group from malonyl-SCoA is transferred to the ACP. The chain elongation is then initiated by enzyme-catalyzed decarboxylation of the malonyl unit to generate a transient enolate. The following acetyl transfer takes place immediately from the active site thiol of the condensing enzyme to the ACP-bound enolate *via* Claisen-type condensation. This acetyl or acyl transfer is truely intramolecular for Type I FAS, as the FAS is a single multifunctional protein including the segments of HSACP and condensing enzyme. On Type II FAS, which is formed as a complex of individual enzymes, the acyl transfer is also "intramolecular-like". It is proposed that the formation of the new C-C bond between acetyl and malonyl groups could occur concertedly

with loss of carbon dioxide in order to explain why malonyl-SCoA is the source of the C_2 units that are added to the starting acetyl unit. No experimental evidence has been established to distinguish these two mechanisms (concerted and transient enolate). However, mechanistic studies using deuterium-labelled malonyl-SCoA demonstrated that the decarboxylative condensation in the fatty acid biosynthesis proceeds with inversion of configuration at the methylene position of the malonyl unit.[16,17] It is evident that an important aspect of enzyme catalysis involves orientation of acetyl (or acyl) and malonyl moieties on the enzyme surface. This optimal orientation greatly contributes to the facilitated formation of a "unimolecular" transition state.

Acetoacetyl-SACP is then reduced stereospecifically in the presence of NADPH to produce exclusively the enzyme-bound (3R)-hydroxy intermediate. Next, dehydration produces a 2-(E)-enoyl-SACP species. A further reduction mediated by NADPH produces a saturated acyl-SACP intermediate, completing the first cycle of transformations. The second cycle starts with the transfer of the butyryl group (as a new starting unit) from butyryl-SACP to the condensing enzyme and addition of a malonyl group from malonyl-SCoA to the HSACP. Further transformations involve repetition of the steps that occur when the acetyl group is the starter unit, namely decarboxylative condensation, carbonyl reduction, dehydration and olefinic reduction. The carbon chain is thus lengthened by a two carbon unit every cycle.

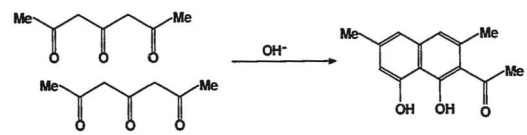
The whole iterative process terminates when the hydrocarbon chain reaches a length specified by a particular enzyme, usually C_{16} or C_{18}, yielding palmitic or stearic acids or their thiol esters. It is believed that as the chain length approaches C_{16}-C_{18}, the active site thiol of some condensing enzymes has a greater affinity for an acetyl-SACP species. Access of acyl substrates bigger than C_{16}-C_{18} to the active site may be hindered due to steric or electronic effects. A recent study showed that some thiolases can control the chain extension by cleaving the fatty acid of a specific carbon chain length from FAS.[18]

Some FASs can utilize a building unit other than acetate, *e.g.*, propionate, isobutyrate, *etc.*, producing naturally occurring fatty acids which possess branched alkyl groups or carbon chains with odd atoms. Methylation of a fatty acid chain by S-adenosyl methionine provides an alternative pathway for generation of methyl branch(es).[8,9]

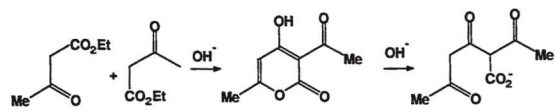
1.3. Biosynthesis of Polyketides[8,9,10,19]

Polyketides are secondary metabolites; that is, they are found in one or a small number of closely related species. Despite the large structural diversity of polyketides, they are derived from small carboxylate units with poly-β-keto acyl chains or their modified forms as intermediates. Plant flavonoids and fungal

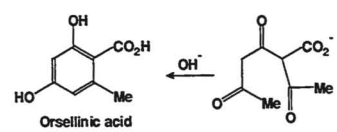
aflatoxins are examples of polyketides. There are hundreds of other polyketides with different structures, which can exhibit antibacterial, antifungal, antitumor or antihelmintic properties.



Diacetyl acetone — Naphthalene derivative



Dehydracetic acid

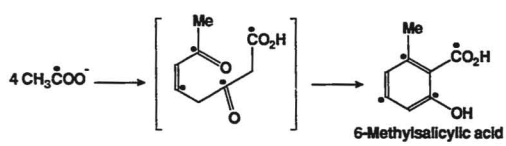


Orsellinic acid

Scheme 1.3.1
Early Experiments in Polyketide Cyclizations.

Some polyketides produced by fungi and bacteria are associated with sporulation or other developmental pathways, while many others have not been found to have any biological activities. The polyketides may be aromatic compounds, polyols, polyenes, polyethers, or macrolides. Some complex polyketides contain structures derived from a mixture of these entities.

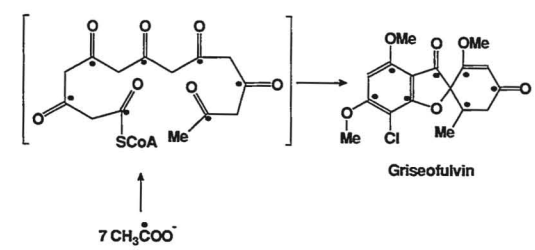
An early experiment involving the *in vitro* cyclization of polyketides was reported by Collie in 1907.[20] He proposed that a similar process may operate *in vivo*. He treated diacetyl acetone with base, and obtained a naphthalene derivative, while cyclization of dehydracetic acid yielded orsellinic acid (a natural polyketide, Scheme 1.3.1). Collie's *acetate hypothesis* received little attention until 1953 when Birch[21,22] established that a wide range of structural types were derived biologically from repeating acetyl-units. He showed in his first example that [1-^{14}C]-acetate was incorporated into 6-methylsalicylic acid during its



6-Methylsalicylic acid

Scheme 1.3.2
Incorporation Pattern of [1-^{14}C] Acetate into 6-Methylsalicylic Acid.

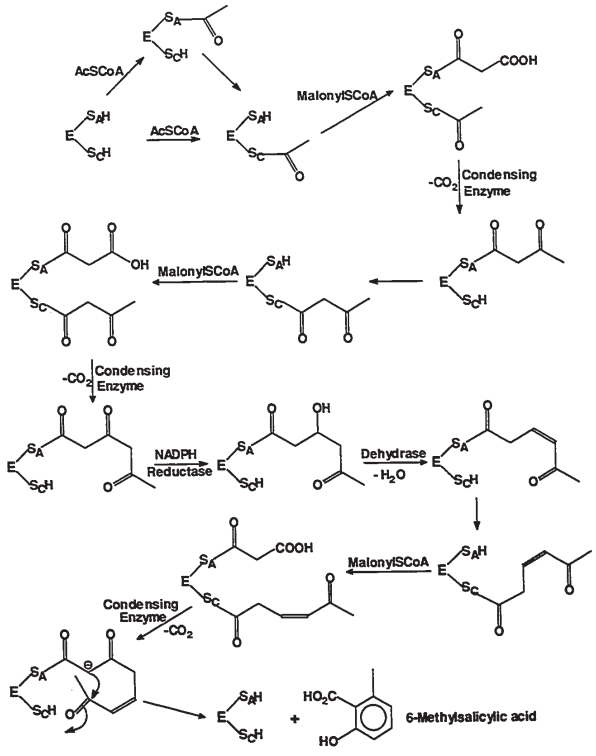
biosynthesis in *Penicillium griseofulvum* organisms as shown in Scheme 1.3.2. Birch also studied a more complicated metabolite, griseofulvin, an antifungal and antibiotic substance isolated from *Penicillium griseofulvum* and obtained the labelling pattern (Scheme 1.3.3). Birch's success in these studies initiated many other studies on polyketide biosynthesis using acetate labelled with ^{14}C, ^{13}C, ^{18}O and ^{2}H.



Griseofulvin

Scheme 1.3.3 Incorporation Pattern of [1-^{14}C] Acetate into Griseofulvin.

Knowledge in the structure elucidation, biosynthesis and bioactivities of polyketides has been growing rapidly for the last three decades. Analogy between FAS and polyketide synthase (PKS) has been well established. Both of FAS and PKS contain ACPSHs and condensing enzymes and uses two distinct thiol groups as active sites for each cycle of the carbon chain elongation. However, the polyketide biosynthesis is still known in much less detail than fatty acid



Scheme 1.3.4 Steps in the Biosynthesis of 6-Methylsalicylic Acid (S_A = Thiol on ACPSH; S_C = Active Site Thiol on Condensing Enzyme)

biosynthesis, due to very few PKSs that are active outside cells and due to the low level of genetic expression of PKS.[8,9]

The growth of a polyketide chain can be exemplified by the biosynthesis of 6-methylsalicylic acid, a small polyketide, as shown in Scheme 1.3.4. The early steps involving binding of and condensation between acetyl and malonyl groups occur in a manner similar to those of fatty acid biosynthesis to generate an acetoacetyl-SACP intermediate. This resulting protein-bound diketide intermediate directly acts as a new starter unit and undergoes a second round malonylation and condensation to afford a triketide chain, whose β-carbonyl group is partially reduced with eventual formation of a *cis*-double bond. One more cycle of the malonylation and condensation leads to formation of an enzyme-bound tetraketide chain. After the chain folds (cyclizes and enolizes) on the enzyme, the final product is released in a stable form.

So far the best known polyketide synthase is the 6-methylsalicylate synthase from *Penicillium patulum*, which has been isolated and investigated.[18] The isolated 6-methylsalicylate synthase can generate one molecule of 6-methylsalicylic acid from one molecule of acetyl-CoA and three of malonyl-CoA in the presence of one molecule of NADPH as coenzyme; no intermediates during the biosynthesis could be detected or characterized. The presence of NADPH is necessary to furnish 6-methylsalicylic acid. Otherwise, triacetic acid

lactone (Fig. 1.3.1) is formed as the sole product. Fatty acid synthase derived from a variety of sources can produce the same lactone in the absence of coenzyme NADPH. So FAS and PKS have similarities in the way they function.

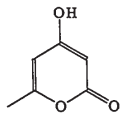


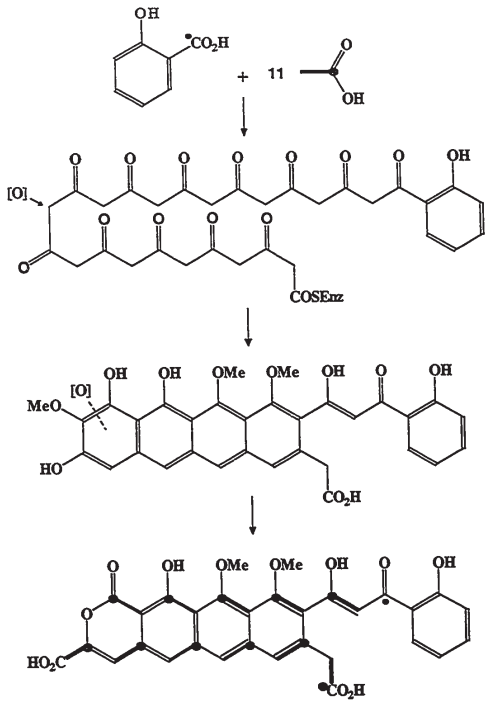
Fig. 1.3.1 Triacetic Acid Lactone

Although some keto functionalities may be reduced to different extents, most or at least some oxygen atoms of β-keto groups from acetate or malonate are retained in the final polyketide metabolites. This provides a clear distinction from fatty acid biosynthesis due to the absence of one or both reductases and dehydrase to completely reduce each β-keto group to methylene. During the growth of a polyketide chain, a new cycle may be initiated with an acyl chain containing a β-keto, β-hydroxy, α,β-ene- or fully reduced β-carbon. A polyketide chain with little or partial reduction can be expected to be a reactive and flexible intermediate. It has been suggested that the growing thioester chain is stabilized by hydrogen bonding to the synthase enzyme, or by chelation of its semi-enolate with a metal ion held by the enzyme.[8] However, the exact nature of the enzyme-bound intermediates still remains unknown. The secondary transformations of the β-keto chain intermediates can proceed in a number of differnt ways and

therefore provide more diversity of structures in the final metabolites.

Polyketide chains are often built biochemically with linear or branched carboxylate units, or carboxylate units with aromatic and aliphatic rings, whereas fatty acids are usually built from acetate units, and only occasionally from propionate, or branched-chain carboxylates. Methylation of polyketide intermediates by S-adenosylmethionine is also much more frequent than that of fatty acid chains. Therefore, more chiral centres are often introduced in polyketide biosynthesis. In addition, carbonyl reduction of β-keto groups to give β-hydroxy groups, which are retained in the final product, may give rise to new chiral centres. The absolute configurations of the resulting chiral centres along a polyketide chain may vary even for identical substituents and depend on the synthase.

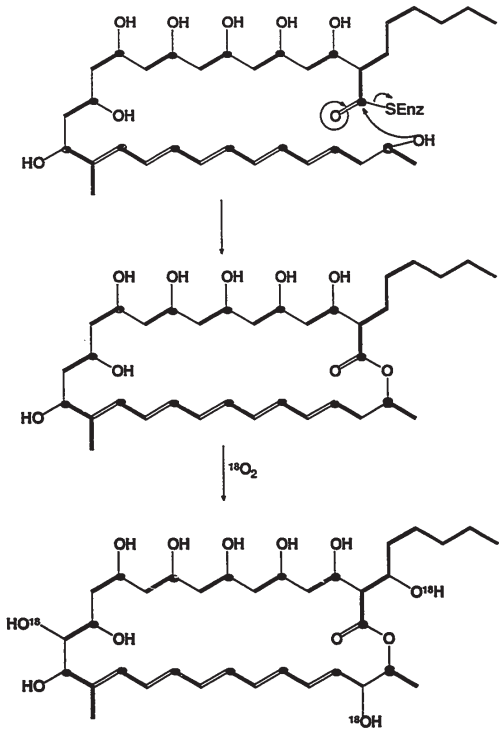
The final stage of polyketide biosynthesis involves several other processes, such as folding, cyclization and lactonization. These modifications are believed to occur while the acyl product still remains attached to the enzyme. After being released from it, the polyketide intermediate may undergo further transformations such as oxidation and formation of an amide bond with an amino acid. This stage on an aromatic PKS always involves intramolecular aldol condensations between carbonyl and active methylene groups. An example of polyketide chain cyclization is found in the biosynthesis of thermorubin, a metabolite of *Thermoactinomyces*



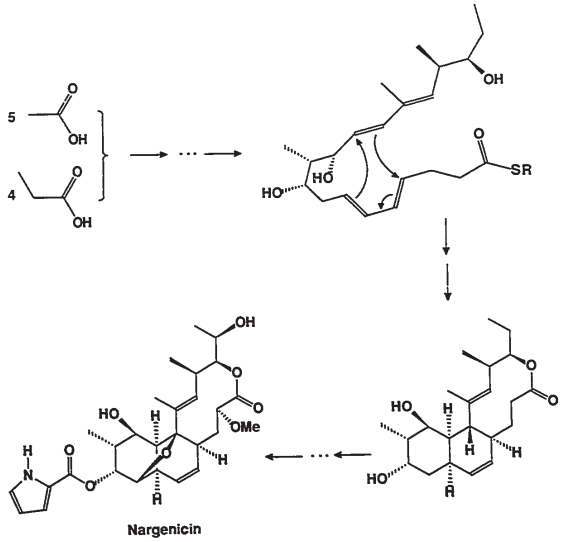
Scheme 1.3.5 Thermorubin

vulgaris. Incorporation studies with ^{13}C-labelled acetate and salicylic acid indicate a pathway in which salicylic acid acts as the starter unit and acetate units are repetitively and sequentially added to the carbonyl terminal by Claisen-type condensations to form a dodecaketide, which cyclizes *via* aldol condensations to furnish four fused aromatic rings. The subsequent oxidative cleavage of the terminal ring as shown in Scheme 1.3.5 gives thermorubin.[24]

An example of a simple lactonization can be seen in the formation of fungichromin, a typical member of the polyene family of antibiotics with a polyol segment, isolated from *Streptomyces cellulosae*. Incorporations of ^{13}C-labelled acetate, propionate and octanoate demonstrated the origin of the carbon skeleton as that shown in Scheme 1.3.6.[25,26] A medium-chain fatty acid unit such as octanoate can be directly utilised by a PKS and incorporated into a polyketide natural product. It was demonstrated that the octanoate is not assembled from acetate units during the fungichromin biosynthesis. The possible source for the octanoate is the degradation of oleate. The lactonization process is accompanied by release of the product from the PKS. Oxidation by molecular oxygen takes place afterwards to produce three more hydroxy groups.



Scheme 1.3.6 Lactonization in the Formation of Fungichromin



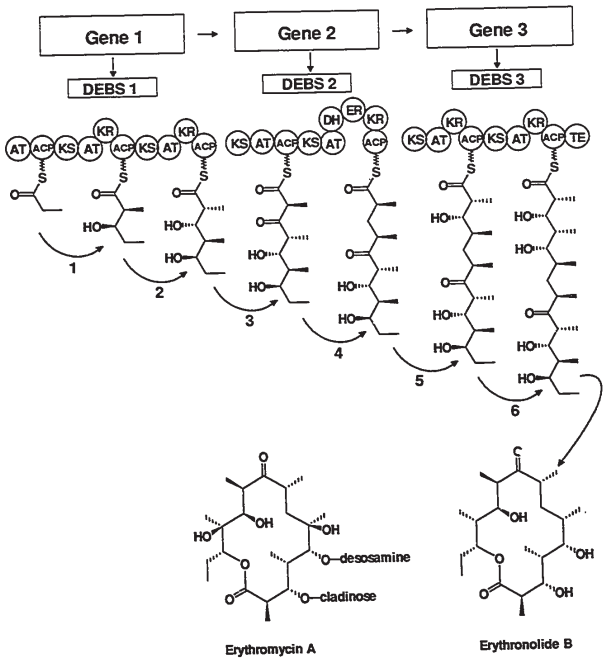
Scheme 1.3.7 Nargenicin Biosynthesis Involving Diels-Alder Cyclization
(Cane, D. E. *et al. J. Am. Chem. Soc.* **1993**, *115*, 527)

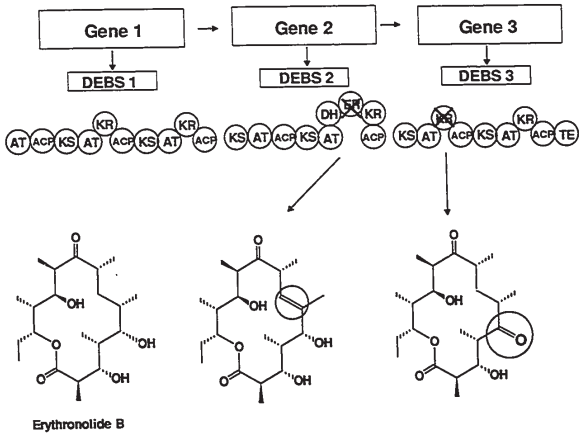
An example of a complex polyketide is nargenicin, a member of a group of naturally occurring reduced alicyclic polyketides containing a characteristic cis-fused octalin ring system.[27] A recent isotope incorporation study supports that the characteristic octalin ring system is generated by an intramolecular Diels-Alder

reaction of a nona-ketide chain, as shown in Scheme 1.3.7.[28]

Remarkable contribution to a better understanding of polyketide biosynthesis has been made by genetic studies of polyketide synthase for the last few years.[29-35] Cloning and sequencing the structural genes of many polyketide-producing strains in the *Actinomycetes* bacteria have been accomplished. The results reveal that there are also two types of PKSs (Type I and Type II), analogous to those of FASs. PKSs assembling aromatic antibiotics (*e.g.*, those of actinorhodin, granaticin and tetracenomycin) are usually emerging as Type II systems;[10,29,36] that is, the gene products are homologous to individual components of a Type II fatty acid synthase. As an exception, 6-methyl-salicylic acid synthase from the fungus *Penicillium patulum* resembles a Type I fatty acid synthase; that is, a single enzyme with two identical multifunctional subunits.[32,37] Another example of Type I polyketide synthase has recently been obtained by genetic studies of the biosynthesis of erythromycin A and its first identifiable intermediate 6-deoxyerythronolide B.[31,39,40] The studies from thorough DNA sequencing of 6-deoxyerythronolide B synthase showed a model of three gene products, DEBS 1, DEBS 2 and DEBS 3, that is, three multifunctional proteins. Each of them apparently catalyzes two of the six cycles of chain extension required to produce 6-deoxyerythronolide B as shown in Scheme 1.3.8. The chain growth follows a basic rule, that is, condensation - oxidation state adjustment - condensation - oxidation state adjustment - and so on.

Scheme 1.3.8 The Organization of the Genes and Enzymes of Erythromycin-Producing Polyketide Synthase in *S. erythraea*
AT = acyltransferase; ACP = acyl carrier protein; KS = β-keto-ACP synthase (condensing enzyme)
KR = β-keto-ACP reductase; DH = dehydratase; ER = enoyl reductase;
TE = thiolesterase (cyclase?). (P. F. Leadlay, *et al. Biochem. Soc. Trans.* 1993, *21*, *218*)





Scheme 1.3.9
Altered Polyketides Produced from Genetic Intervention of Erythromycin-Producing PKS. The Inactivated Domain Is Crossed out. The Differences in the Lactones Produced Are Circled.
(L. Katz and S. Donadio *Ann. Rev. Microbiol.* 1993, *47*, 875)

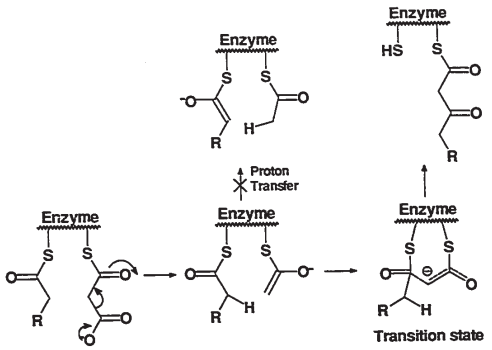
A corresponding active research area in the genetic studies of polyketide biosynthesis is the application of genetic intervention technology to construct hybrid polyketide synthases and the analysis of their metabolic products.[33,41,42,43] For example, a gene strain of 6-deoxyerythronolide B synthase was manipulated

so that the ketoreductase domain in cycle 5 for β-keto reduction was deactivated. As predicted, the major product of the altered synthase was 5-oxo-5,6-dideoxyerythronolide B (Scheme 1.3.9). Similarly, deactivating the enoyl reductase domain for α,β- double bond reduction in cycle 4 led to accumulation of 6,7-anhydroerythromycin C.[29] Some novel aromatic polyketides have also been produced by the functional expression of recombinant PKSs in a specially constructed expression system.[43,44]

1.4 Chemical Similarities Between Fatty Acid and Polyketide Biosynthesis

Malonyl-decarboxylative condensations with a saturated acyl group on a fatty acid synthase and with a poly-β-ketoacyl acyl group on a polyketide synthase are both "intramolecular" type reactions. The synthase complexes hold the acyl or poly-β-ketoacyl and malonyl groups properly close to each other so that after decarboxylation of the malonate to form an enolate, the enolate carbon can easily approach the carbonyl carbon of the other acyl or poly-β-ketoacyl group to form a ring transition state with a new partial carbon-carbon bond, as shown in Scheme 1.4.1.

The decarboxylation catalyzed by both synthases can make a negative charge selectively on the new C_2 unit to be added to the linear chain. This



Scheme 1.4.1 The Enolate Generated by Malonyl-Decarboxylation Acts as an Acyl Acceptor for Intramolecular Acyl Transfer on PKS or FAS.
R = poly-β-ketoacyl, partially reduced poly-β-ketoacyl, or saturated alkyl group.

negative charge is unable to be transferred to other positions in the existing linear chain as the next formation of a new C-C bond is a fast step (Scheme 1.4.1). The two enzyme systems share such an advantage in order to produce regiospecificity and to assure a linear chain growth.

1.5 Biomimetic Chemistry

The word "biomimetic", introduced by R. Breslow in 1972[45], generally refers to any aspect in which a chemical process imitates a biochemical reaction. "Biomimetic chemistry represents the field that attempts to imitate the acceleration and selectivity characteristics of enzyme-catalyzed reactions with enzyme models synthesized in the laboratory."[2] These enzyme models have the advantages of possessing smaller and simpler structures than the corresponding enzymes. Such models can be specially designed and constructed in order to contain one or more features present in the enzymatic system and to mimic some key parameters of enzyme function. That is, biomimetic chemistry with the tools of synthetic chemistry can considerably facilitate the studies of biochemical processes or can provide valuable information to assist in the understanding of biochemical systems.

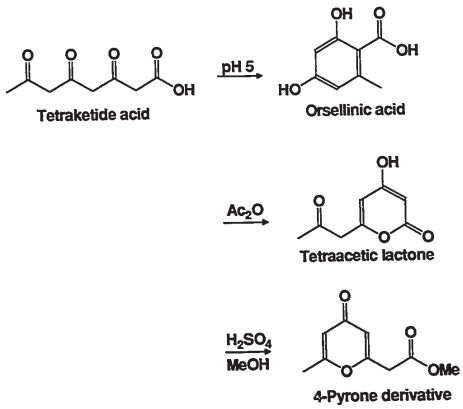
Based on established or proposed biochemical mechanisms, a large number of enzyme models have been developed to demonstrate various enzyme parameters over the last 20 years. Models have been used to show the contribution of intramolecularity to the reaction efficiency of the enzyme, and to demonstrate multifunctional catalysis, the stabilization of tetrahedral intermediates, the participation of stereo-electronic effects, as well as the importance of recognition and binding of the substrate, *etc.*[2]

It should be noted, however, that the large and increasing volume of publications in this area are mainly concentrated on hydrolytic enzyme models, although there are quite a few examples involving co-enzyme models for redox transformations. Molecular models of enzymes[46-49] that have been studied to facilitate formation or cleavage of carbon-carbon bonds are rare, despite the fact that such reactions constitute an important metabolic aspect in biological systems.

1.6 Chemical and Biomimetic Pathway to Make Polyketides

Collie's synthesis of orsellinic acid from ethyl acetoacetate was the first example to exploit the polyketide biosynthetic pathway in chemical synthesis (Scheme 1.3.1). This example mimicked some of biosynthetic features: acetate units as the building material (first to make ethyl acetoacetate). These experiments can also be viewed as crude chemical models of the aromatization processes. But there was little impact of this pioneering work on other chemists until suitable methods such as isotope incorporation and advanced NMR techniques for the study of biochemical pathways became available. Aromatization of linear polycarbonyl chains regained chemists' interests after Birch formulated the polyketide hypothesis in the 1950s. A large volume of

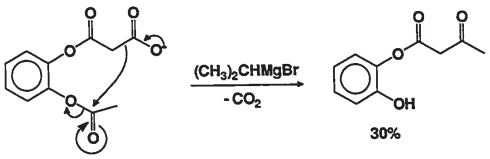
publications in this area appeared in the 1960's and 1970's as reviewed by Harris[48]. For example, the linear tetraketide acid shown in Scheme 1.6.1 was found to undergo an extremely facile aldol cyclization to give orsellinic acid.[50] Tetraacetic lactone was formed from the acid by treatment with acetic anhydride (66%) and a 4-pyrone derivative was obtained in 50% yield by treatment with methanolic H_2SO_4 (Scheme 1.6.1).[51] All these products are naturally occurring



Scheme 1.6.1
Cyclizations of a Tetraketide Acid under Different Conditions.

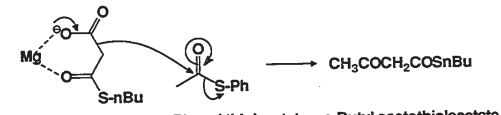
and produced by several fungal systems that normally produce phenolic metabolites.

Another challenging area is the study of chemical models of polyketide and fatty acid synthases, on which linear chains are assembled. Scott *et al.* in 1975 reported catechol as such a model: decarboxylation of catechol acetate malonate by base in the presence of Mg^{2+} afforded catechol monoacetoacetate in 30% yield (Scheme 1.6.2).[46] There have been no further studies reported on this catechol model since then.



Scheme 1.6.2
Catechol as a Template for Condensation between Acyl Units

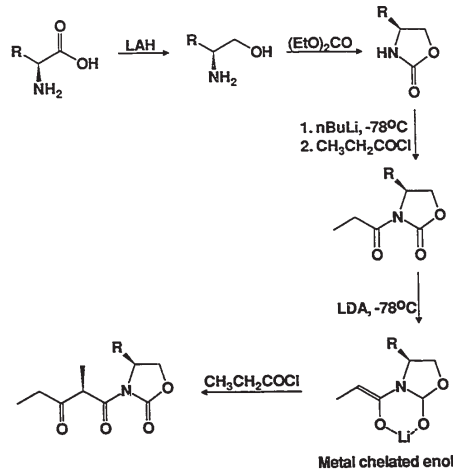
In 1978, Kobuke demonstrated an intermolecular model for decarboxylative acylation of thiolmalonate: n-butyl thiolmalonate was treated with an equivalent of phenyl thiolacetate in the presence of $Mg(OAc)_2$ and imidazole in THF at room temperature for 87 h (Scheme 1.6.3).[47] Under such mild conditions, n-butyl



Scheme 1.6.3 Intermolecular Model of Decarboxylative Condensation

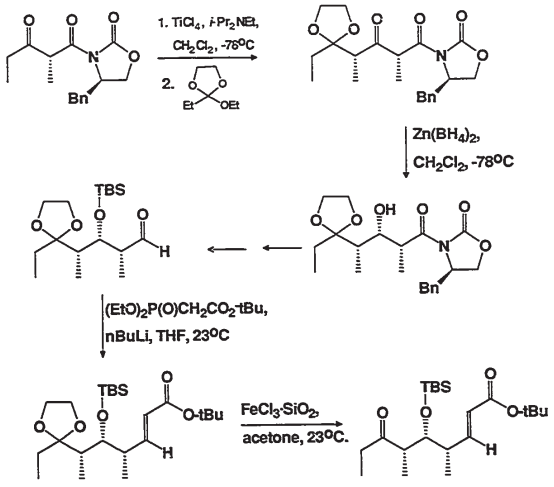
acetothiolacetate was isolated in 60% yield and no self-condensation product (phenyl acetothiolacetate) was observed. Another similar study of intermolecular decarboxylative condensation was also reported.[49]

A recent success in template-directed intermolecular condensation reactions between two acyl units was demonstrated by Evans *et al.*.[52-55] They used chiral oxazolidones as templates, which were derived from enantiomerically pure amino acids, *e.g.*, valine and phenylalanine (Scheme 1.6.4). An acyl group with an α-CH_2 group, *e.g.* propionyl, is first attached to the template via an imide bond. Introduction of a second acyl unit to the α-position of the existing propionyl group on the template can be easiiy realized by treatment with LDA followed by the corresponding acyl chloride at -78°C. The lithium chelation effect in the intermediate enolate can control the direction of the C=C bond and keep it in the same plane of the template ring. The *cis* conformation around the enolate C=C



Scheme 1.6.4 Oxazolidone Templates (R = isopropyl, benzyl, *etc.*) in Stereoselective Synthesis of a Diketide Carbon Chain.

bond is favoured over the *trans*. There is strong steric interaction in the latter between the methyl group on the side chain and the R substituent on the template ring. A electrophile can access the *cis* double bond selectively from the face opposite to the R substituent, producing a β-ketoacyl product with high stereoselectivity (Scheme 1.6.4).



Scheme 1.6.5 Oxazolidone Template Is Used to Assemble the First 3 Acyl Units and Then Allows a Tetraketide Chain to Be Built.
(D.A. Evans, H.P. Howard & D.L. Rieger *J. Am. Chem. Soc.* **1993**, *115*, 11446).

Evans' oxazolidone templates have been used to construct triketide units by introducing a third acyl group at the less substituted position 4 of the 2-methyl-3-oxo-valeryl chain.[54] Further transformations can allow construction of linear

chains equivalent to tetraketide units (Scheme 1.6.5). The oxazolidone templates have proved to be very useful compounds in organic synthesis[55,57] of complicated natural products and in the preparation of oligoketides for biosynthetic studies.[27,59] It should be noted, however, that this sequence of assembly of the first three acyl units proceeds in the opposite direction to the natural one occurring on fatty acid or polyketide synthases. This synthetic pathway can thus be viewed as non-biomimetic.

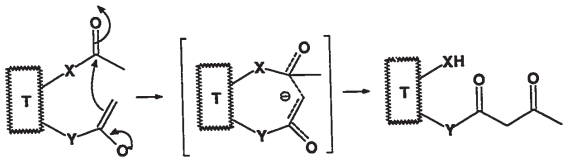
1.7 Objectives of This Research

It still remains an interesting problem to perform repetitive intramolecular acyl transfers on a molecular model to mimic the growth sequence of polyketide or fatty acid chains. The research in this thesis sets out to mimic intramolecular chain growth of fatty acid and polyketide-type compounds on a molecular model of FAS and PKS. The key parameters in the model that were initially targeted are the ability to perform repetitive acyl transfers in an efficient intramolecular manner on the model of the fatty acid and polyketide synthases. A nucleophilic enolate can be envisaged to be generated chemically in different ways, such as deprotonation of an acyl moiety, addition of an anionic species to an α,β-unsaturated system and decarboxylation of a malonyl group. As discussed at the beginning of this chapter, intramolecular reactions are expected to be more

efficient due to the entropy loss in the binding stage. Therefore, to realize repetitive intramolecular condensations between acyl units may provide a synthetically useful method for the construction of carbon chains. Some valuable knowledge may also be obtained from this research in order to build more sophisticated models in the future, which have not only the two binding sites, but some catalytic groups to facilitate the generation of an enolate.

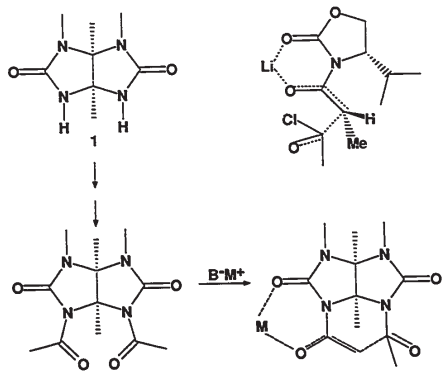
CHAPTER 2. DESIGN OF THE ENZYME MODEL

To mimic the chain growth of fatty acids and polyketides, an enzyme model or a template with two binding sites analogous to the two thiol groups of the synthase is necessary to bind two acyl groups. These two binding sites must be in a proper relative position so that the transition state in Scheme 2.1 can be easily reached. A six-membered cyclic transition state is favoured for such an intramolecular condensation to proceed, although enzyme does not necessarily use such a six-membered ring transition state due to its ability to change conformation during a reaction. A medium sized ring of between seven and ten members is also possible, but the intramolecular condensation could be considerably slowed, and would probably be accompanied by intermolecular condensations. Therefore, very serious consideration of a six member ring transition state was taken in the design of enzyme models.



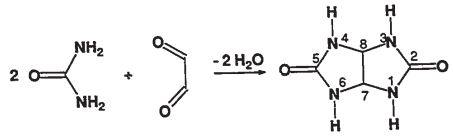
Scheme 2.1 Template-Directed Intramolecular Acyl Transfer (T = Template).

Another consideration is the metal chelation effect present in Evans' oxazolidone system,[52,53] which can control the direction of the C=C bond of an enolate which is bound to the template and thus can control the direction of electrophile access. We wanted to introduce this chelation effect into our bifunctional template in order to facilitate the formation of the new carbon-carbon bond in the condensation between the two acyl units.



Scheme 2.2
A Glycoluril Template and a Possible Metal (M) Chelated Transition State for Its Directed Acyl Transfer (Compared with the Oxazolidone System).

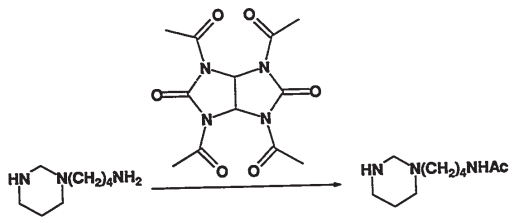
The first model we have designed as an analogue of the synthases of fatty acids and polyketides is compound **1**, a glycoluril derivative. The two equivalent NH groups in compound **1** can be acylated and deacylated to simulate the two active sites of the enzyme. Further, a six-membered ring transition state should be formed after deprotonation at one α-carbon of the two acyl groups situated on the NHs. The compound **1** contains a partial structure analogous to that of oxazolidone and thus the metal chelation effect may force an enolate C=C bond on one of the binding sites towards the other acyl group (Scheme 2.2). A molecular model as well as literature X-ray diffraction studies[73] suggests that such a bicyclo compound can be stable only in the *cis*-fused form of the two rings.



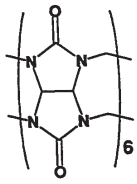
Scheme 2.3 Synthesis of Parent Glycoluril

The glycoluril parent compound has been prepared by condensation of glyoxal with 2 equivalents of urea (Scheme 2.3).[60] The name used for glycoluril in *Chemical Abstracts* is tetrahydro-imidazo[4,5-*d*]imidazole-2,5-(1*H*, 3*H*)-dione using the same numbering system as that in glycoluril, while based on IUPAC

nomenclature, it also can be named 2,4,6,8-tetraazabicyclo[3,3,0]octane-3,7-dione (Fig. 3.1.1). Some glycoluril compounds have been demonstrated to possess a variety of interesting properties. As a useful acylating agent,[61,62] 1,3,4,6-tetraacylglycoluril was developed by Kuhling *et al.* and used by Ganem *et al.* to selectively acylate primary amines over secondary (Scheme 2.4).[63]



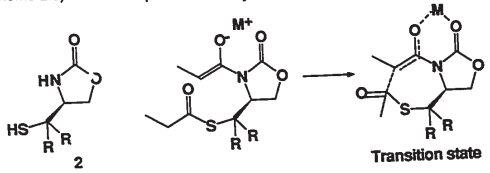
Scheme 2.4 Tetraacetylglycoluril Acts as a Selective Acetylating Agent. (Tice, C. M.; Ganem, B. *J. Org. Chem.* 1983, *48*, 2106)



Scheme 2.5
Cucurbituril, Six Glycoluril Units Connected with Methylene Groups. (Cram, D. *et.al. J. Am. Chem. Soc.* **1982**, *104*, 5826)

The glycoluril skeleton has been widely used to build molecules which possess cavities for host-guest binding studies.[65-75] Some host molecules (*e.g.* cucurbituruil in Scheme 2.5[74]) containing glycoluril units exhibit enzyme-like catalytic activities in their guests' transformations.[67] Rebek and his co-workers have built novel glycoluril derivatives as self-complementary modules and are studying their assembly into host species in the presence of nucleating guests.[75] A large number of other glycoluril derivatives are of commercial value in dyeing[76] and bleaching[77] industries or act as polymer crosslinking agents[78,79] and have been widely documented in hundreds of patents, as exemplified by references 76-79.

We have also designed compounds **2a** (R=H) and **2b** (R=CH_3), bifunctional oxazolidones as molecular models of the PKS and FAS. The SH and NH groups can play the role of the two binding sites; a seven-membered cyclic transition state is expected if the intramolecular condensation takes place (Scheme 2.6). These compounds are very similar to conventional oxazolidones;



Scheme 2.6 Bifunctional Oxazolidone Templates and the Transition State for Their Directed Intramolecular Acyl Transfer (R=H or CH_3).

however, the extra SH group may be used as a potential carrier of the acyl group to be transferred onto the enolate formed on the other NH binding site (or *vice-versa*). These compounds can be prepared in a chirally pure form from amino acids, cysteine or penicillamine. When a propionyl group is to be added to the linear chain, the ensuing intramolecular condensation could potentially result in a stereoselective chain growth with a methyl branch.

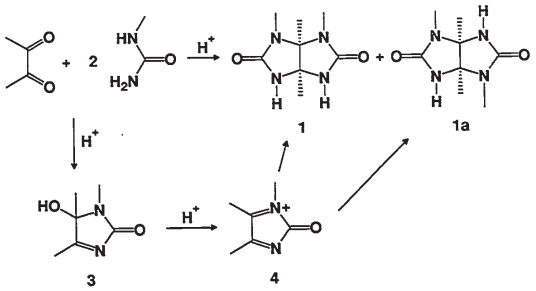
Compound **2b** was synthesized from penicillamine, while Endang Saepudin in our group prepared **2a** from cysteine. Two propionyl groups were introduced onto both **2a** and **2b**. Initial attempts to effect the condensation between the two propionyl groups by a strong base (LDA and *t*-BuOLi) at -78°C were unsuccessful. It was found that one or both propionyl units were removed from the template **2a** or **2b** by base. Because the initial test of the condensation on template **1** was very successful, further investigation of systems **2a** and **2b** in order to find suitable conditions for the condensation was terminated. There is still potential to realize the condensation reactions on templates **2a** and **2b** by varying bases and reaction conditions.

CHAPTER 3. RESULTS AND DISCUSSION

3.1 Synthesis of the Enzyme Model 1

N-Monomethylurea and 2,3-butanedione (diacetyl) were used as starting materials, as reported by Biltz in 1907.[60] These two compounds are cheap and readily available in large quantities. The yellowish 2,3-butanedione liquid is sometimes decomposed or polymerized to form solid material or is shipped in bad quality from suppliers. Commercially available 2,3-butanedione of good quality may be used directly. However, use of freshly distilled 2,3-butanedione can lead to a better yield of the product.

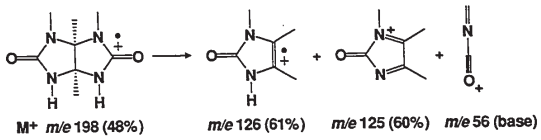
The reaction of diacetyl with two equivalents of monomethylurea is expected to give compound **1** and its isomer **1a** (Scheme 3.1.1). These two isomers should be easily separated due to their different polarity. This reaction was carried out in absolute ethanol at room temperature with several drops of concentrated hydrochloric acid as a catalyst. N-Methylurea was initially suspended in ethanol and the mixture became clear and hot shortly after 2,3-butanedione and hydrochloric acid were added at room temperature. The clear solution remained for about 10 minutes and then extensive precipitation occurred to form the solid mixture of products **1** and **1a**. The mixture was obtained in 51%



Scheme 3.1.1
Synthesis of the Glycoluril Template 1 and Possible Intermediates Involved.

yield. The ^{1}H NMR spectrum shows a ratio **1** to **1a** of 16:15.

The mechanism for this reaction probably involves the formation of monocyclic ring intermediates **3** or **4** (Scheme 3.1.1). Similar intermediates have been previously established to occur in the formation of other glycoluril derivatives.[81,82,83] The C=N bond of **3** or cationic C=N$^+$ double bond of **4** undergoes nucleophilic addition by either of the NH_2 or NHMe groups of the second methylurea molecule. The steric effect of primary and secondary nitrogen atoms is not reflected in the ratio (nearly 1:1) of the two regioisomers **1** and **1a**. The electronic effect or other effects may also be involved in this reaction.



M$^+$ *m/e* 198 (48%) *m/e* 126 (61%) *m/e* 125 (60%) *m/e* 56 (base)

Scheme 3.1.2
Major Products from Molecular Ion Fragmentations of Template 1.

The mixture of **1** and **1a** is insoluble in water or in most organic solvents. Slight solubility was found in methanol and in ethanol, making possible the isolation of **1** by recrystallization. Compound **1a** has much higher solubility than **1** in cold ethanol, from which **1** was recrystallized 3 times and obtained in pure form. No attempt was made at this stage to purify **1a** in the mother liquor containing un-recrystallized **1**.

The proton NMR spectrum of compound **1** shows a single peak of 6 protons at 2.78 ppm for both N-methyl groups, and two singlets at 1.48 and 1.39 ppm for the two bridgehead methyl groups. Compound **1a** exhibits only two singlets at 2.62 and 1.47 ppm (6 protons each) in its ^{1}H NMR spectrum. The IR absorptions of **1** were found at 3282 cm^{-1} for NH and 1683 cm^{-1} for C=O groups. A high abundance of the molecular ion at *m/e* 198 (48%) in the MS of **1** was observed. Another two strong peaks at *m/e* 126 (61%) and 125 (60%) are formed

from loss of a five membered ring and can be accounted for by the fragmentation patterns shown in Scheme 3.1.2.

Single crystals of **1** were obtained as colourless needles and analyzed by X-ray diffractometry. Views of the refined X-ray crystal structure are shown in Fig. 3.1.1 and Fig. 3.1.2. Compound **1** is named 1,4,5,6-tetramethyl-2,4,6,8-tetraazabicyclo[3,3,0]octane-3,7-dione for the purpose of the following discussion of the X-ray diffraction results, based on IUPAC nomenclature. The structural data from the crystallographic study of **1** are listed in Tables 1, 2 and 3. It is clearly seen that the two rings of the bicyclo compound are symmetrical across a mirror plane and *cis* fused to each other, as predicted by molecular modelling. The dihedral angle between the two ring planes is 112°, smaller than that of the parent glycoluril (115°)[84a] due to the presence of two more bridgehead methyl groups. A strong *gauche* interaction between the bridgehead methyl groups C(9) and C(12) is implied by the bond angles C(5)-C(1)-C(9) of 117.3° and C(1)-C(5)-C(12) of 116.9°. The hydrogen atoms of the two bridgehead methyl groups are directed away from each other to minimize the interaction.

The crystals of **1** are very stable to most organic solvents and to temperatures as high as 300°C. The solubilities of **1** are also very low even in polar solvents such as water, methanol and ethanol. In order to account for these features, it was initially suspected that there might be strong intermolecular

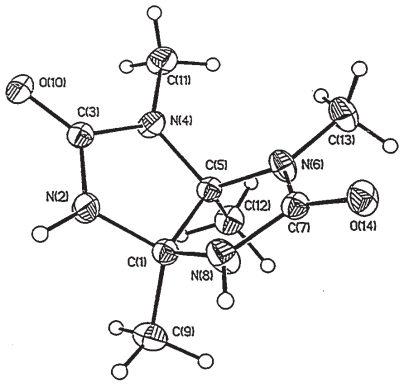


Fig. 3.1.1.
X-Ray Crystal Structure of Template **1**. IUPAC Name Used Here: 1,4,5,6-Tetramethyl-2,4,6,8-tetraazabicyclo[3,3,0]-3,7-dione.

hydrogen bonding in the crystals of **1**, as shown Fig. 3.1.3. Each imide unit might provide one proton donor and one proton acceptor to form eight membered intermolecular rings, as recently reported for the X-ray crystal structure of the parent glycoluril.[84b] Each molecule has two imide units, leading to formation of extensive molecular chains through hydrogen bonding in the single crystals. However, the crystal structure (Fig. 3.1.2) does not show such hydrogen bonds for the intermolecular ring systems and proves that the early speculation is

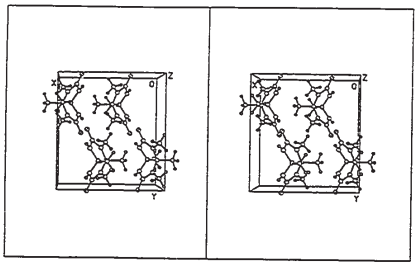


Fig. 3.1.2.
Intermolecular Structure in Crystals of **1**.

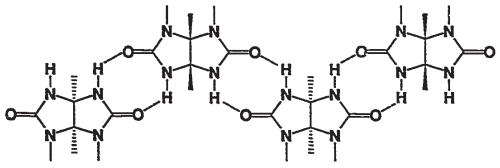


Fig. 3.1.3 The Suspected Intermolecular Hydrogen Bonding of Template 1 Was not Observed in the X-Ray Crystal Structure.

Table 1. Atomic coordinates ($\times 10^4$) and equivalent isotropic displacement coefficients ($Å^2 \times 10^3$) for template 1.

	x	y	z	U(eq)
C(1)	3805(3)	2500	-560(4)	29(1)
N(2)	3026(2)	3573(2)	-408(3)	43(1)
C(3)	3266(2)	4204(2)	1003(3)	30(1)
N(4)	4129(2)	3582(2)	1919(3)	35(1)
C(5)	4599(3)	2500	1106(4)	28(1)
C(9)	4569(5)	2500	-2158(5)	51(2)
O(10)	2776(2)	5162(2)	1401(2)	41(1)
C(11)	4596(3)	4030(3)	3495(3)	46(1)
C(12)	6074(4)	2500	961(5)	41(1)

* Equivalent isotropic U defined as one third of the trace of the orthogonalized U_{ij} tensor

Table 2. Bond lengths (Å) for template 1.

C(1)-N(2)	1.448 (3)	C(1)-C(5)	1.573 (5)
C(1)-C(9)	1.511 (5)	C(1)-N(8)	1.448 (3)
N(2)-C(3)	1.361 (3)	C(3)-N(4)	1.350 (3)
C(3)-O(10)	1.226 (3)	N(4)-C(5)	1.458 (3)
N(4)-C(11)	1.448 (3)	C(5)-C(12)	1.525 (5)
C(5)-N(6)	1.458 (3)		

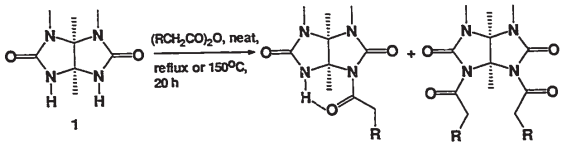
Table 3. Bond angles (°) for template 1.

N(2)-C(1)-C(5)	102.5(2)	N(2)-C(1)-C(9)	111.2(2)
C(5)-C(1)-C(9)	117.3(3)	N(2)-C(1)-N(8)	111.8(3)
C(5)-C(1)-N(8)	102.5(2)	C(9)-C(1)-N(8)	111.2(2)
C(1)-N(2)-C(3)	113.5(2)	N(2)-C(3)-N(4)	108.1(2)
N(2)-C(3)-O(10)	126.6(2)	N(4)-C(3)-O(10)	125.3(2)
C(3)-N(4)-C(5)	113.5(2)	C(3)-N(4)-C(11)	121.5(2)
C(5)-N(4)-C(11)	124.8(2)	C(1)-C(5)-N(4)	102.2(2)
C(1)-C(5)-C(12)	116.9(3)	N(4)-C(5)-C(12)	111.5(2)
C(1)-C(5)-N(6)	102.2(2)	N(4)-C(5)-N(6)	112.0(3)
C(12)-C(5)-N(6)	111.5(2)		

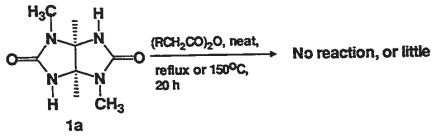
wrong. The true intermolecular structure in the crystals of tetramethylglycoluril is characterized by that each molecule is interacted with four different molecules *via* hydrogen bonding.

3.2 Acylation of 3,4,7,8-Tetramethylglycoluril 1

When **1** was heated at reflux with acetic anhydride for 20 hours under a nitrogen atmosphere, all of the solid starting material dissolved to give a clear solution. After removing excess acetic anhydride, a solid was obtained, which was highly soluble in chloroform. This material was recrystallized from chloroform-ethyl acetate and characterized as the monoacetylated compound **5**, and obtained in 78% yield (Scheme 3.2.1). A trace amount of diacetylated product **6**



R=H	**5** (78%)	**6** (1%)
R=CH_3	**7** (83%)	**8** (9%)
R=C_2H_5	**9** (70%)	**10** (3%)



Scheme 3.2.1 The First Acylations of Template 1 and Its Isomer 1a.

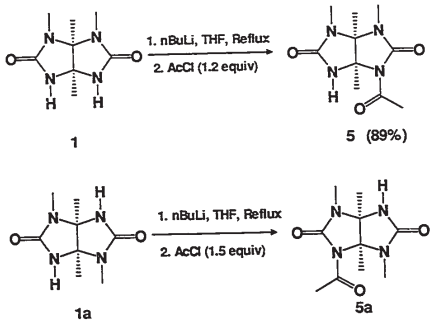
was found in less than 1% yield in this step. This selectivity of acetylation on one NH group of the enzyme model is desired so that two different acyl groups could be bound to this model molecule.

The mixture of **1** and **1a** was also directly used without separation for the acetylation reaction described above. Compound **1a** is stable to these conditions and remains as a solid. After removing acetic anhydride, chloroform was added to the residue. **1a** was then filtered off as a solid, and obtained in pure form after washing with chloroform. The mother liquor was then concentrated and recrystallized to give **5**. The steric effect of the N-methyl group of **1a** may contribute to the difficulty of its acetylation.

The acetylation of **1** largely reduces the intermolecular interactions to afford the asymmetrical product **5**, which is very soluble in chloroform and moderately soluble in THF (~80 mL THF / 1 g **5**). This drastic change in solubility solely as a result of introducing an acetyl group is an interesting phenomenon. It is possible that **5** forms intramolecular hydrogen bonds via a six-membered ring between the acetyl carbonyl oxygen and NH proton, which can extensively reduce its polarity for the strong intermolecular interactions. Therefore, the favourable entropy change can override the unfavourable enthalpy change and largely contribute to the easy dissolution of **5** in chloroform.

In the ^1H NMR spectrum of **5** in $CDCl_3$, a singlet from one NCH_3 group appears at 2.91 ppm, shifting 0.16 ppm down field relative to that of **1**. This singlet is assigned to the methyl group on position 3, closer to the acetyl. Another singlet from one bridgehead methyl group shifts 0.10 ppm from 1.49 to 1.59 ppm due to the acetylation, and is assigned to the methyl at position 7. The resonances of the other two methyl groups at positions 4 and 8 are less affected by mono-acetylation of **1**. The IR spectrum of **5** shows absorptions at 3250 cm^{-1} (NH), and at 1723, 1712 and 1688 cm^{-1} (three C=O groups).

An alternative acylation procedure was developed in order to be used in those cases where the quantity of an acylating agent is limiting. This was a challenging problem due to suitable solvents not being available for dissolving the template **1**. Initially, a suspension of **1** in a large volume of THF was treated with 1 equivalent of n-butyl lithium and the mixture was stirred for up to 5 hours at room temperature. Addition of 1.2 equivalents of acetyl chloride gave no desired product **5** and most of the starting material was recovered. A similarly prepared mixture of **1** and n-butyl lithium in THF was then heated at reflux for 1 hour. Addition of an acyl chloride resulted in a clear solution instantly due to the fast formation of the soluble product (e.g., **5**). Therefore, a complete deprotonation of insoluble **1** by n-butyl lithium can be carried out in THF only at reflux to produce an insoluble intermediate lithium salt, which is quite reactive to acyl chlorides (Scheme 3.2.2). It is not commonly seen in the literature that deprotonation by



Scheme 3.2.2 Deprotonation and Then Acetylation of Template 1 and Its Isomer 1a.

n-butyl lithium proceeds only at reflux. Compound **1a** in THF undergoes deprotonation and acetylation reactions under the same conditions to afford product **5a** (Scheme 3.2.2), which is much less polar than **5** and easily isolated by flash column chromatography.

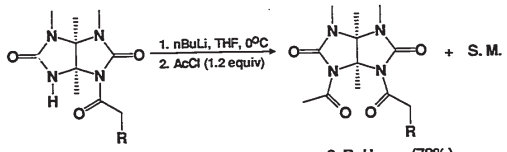
The acetylation procedures described above were used for general acylation with other longer acyl units (Scheme 3.2.1). When propionic anhydride was heated with compound **1** at 150°C for 20 hours under nitrogen, di-propionylated compound **8** was isolated in 8% yield in addition to the mono-propionylated compound **7**, which was obtained in 82% yield. The methylene

protons of **7** form a diastereotopic *ab* system in the ^{1}H NMR spectrum and appear as a multiplet partially overlapped with N-methyl protons. Butyryl derivative **9** was prepared in a similar way in 70% yield with some formation of dibutyryl compound **10** (5%).

3.3 Preparation of Di-Acylated Compounds

The second acyl group was introduced by treatment of a mono-acyl (acetyl, propionyl or butyryl) glycoluril derivative in THF with lithium di-isopropylamide or with *n*-butyl lithium at 0°C followed by addition of a corresponding acyl chloride. This second acylation did not proceed at a temperature lower than -20°C, probably because deprotonation can not take place at such a low temperature. It was found that the acylation takes place exactly at the desired second NH group, instead of at the α-carbon of the existing acyl group (Scheme 3.3.1).

The simplest case is the introduction of a second acetyl group onto the acetyl derivative **5**, affording 1,6-diacetyl-3,4,7,8-tetramethylglycoluril **6** in 78% yield. The starting material **5** was recovered in 10~16% yield; it is more polar than product **6** and is easily separated from the product by flash column chromatography. The ^{1}H NMR spectrum of the product shows 4 singlets in a ratio of 2:2:1:1. There are no absorptions above 3000 cm^{-1} in the IR spectrum of **6**.



5. R=H
7. R=CH_3
9. R=C_2H_5

6. R=H (78%)
11. R=CH_3 (54%)
12. R=C_2H_5 (61%)

Scheme 3.3.1 Second Acylations by Acetyl Chloride.

Single crystals of **6** were obtained as colourless plates by slowly cooling down a saturated hot solution in a mixed solvent of chloroform and ethyl acetate (1:1). The crystal structure from an X-ray diffraction study was obtained (Fig. 3.3.1) and the structural data are listed in Tables 4, 5 and 6. The most interesting feature of the crystal structure is its asymmetry, despite the fact that it contains two identical substructures of N-acetyl-N'-methylurea fused by a C-C bond. The introduction of two identical groups onto the two symmetrical positions of **1** results in loss of its symmetry plane, reflecting strong intramolecular steric interactions. The two bridge head methyl groups are distorted 20° away from the eclipsed conformation which was found in **1**. Another interesting feature is the orientation of the 2 acetyl groups, whose two carbonyl oxygen atoms are forced close to each other and whose two methyl groups are directed away. Such a conformation is probably a combined result of an unfavourable intramolecular interaction

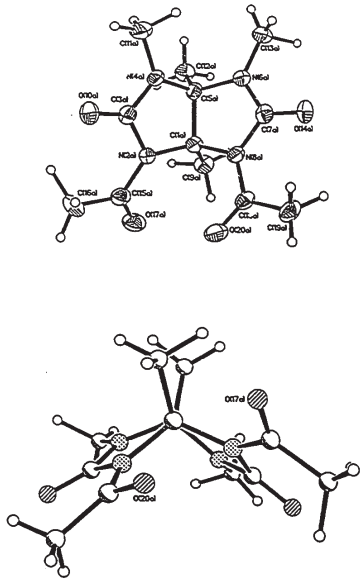


Fig. 3.3.1.
X-Ray Crystal Structure of Compound 6. IUPAC Name Used Here: 2,8-Diacetyl-1,4,5,6-tetramethyl-2,4,6,8-tetraazabicyclo[3,3,0]-3,7-dione.

Table 4. Atomic coordinates (x10^4) and equivalent isotropic displacement coefficients (Å^{2}x10^3) for compound 6.

	x	y	z	U(eq)
C(1A)	2287(6)	7459(4)	4664(3)	37(2)
N(2A)	2676(5)	8757(3)	4559(2)	38(1)
C(3A)	2053(6)	9346(5)	5275(3)	42(2)
N(4A)	1608(5)	8477(3)	5870(2)	41(1)
C(5A)	1910(6)	7227(4)	5624(3)	39(2)
N(6A)	311(5)	6588(4)	5693(2)	44(2)
C(7A)	-517(7)	6691(4)	4981(3)	43(2)
N(8A)	550(5)	7281(3)	4359(2)	38(1)
C(9A)	3617(7)	6529(5)	4307(3)	51(2)
O(10A)	1970(5)	10448(3)	5341(2)	61(2)
C(11A)	1132(8)	8793(5)	6716(3)	61(2)
C(12A)	3317(7)	6518(5)	6101(3)	53(2)
C(13A)	-442(8)	5984(5)	6443(3)	62(2)
O(14A)	-1908(5)	6288(3)	4900(2)	60(1)
C(15A)	3723(7)	9317(5)	3903(3)	51(2)
C(16A)	3763(8)	10704(6)	3856(4)	73(3)
O(17A)	4542(5)	8711(4)	3416(2)	65(2)
C(18A)	110(6)	7657(5)	3559(3)	43(2)
C(19A)	-1642(7)	7477(6)	3299(3)	59(2)
O(20A)	1175(5)	8156(4)	3076(2)	61(1)
C(1B)	2885(6)	7628(4)	9601(3)	36(2)
N(2B)	2516(5)	6355(3)	9514(2)	39(1)
C(3B)	2714(7)	5637(5)	10272(3)	48(2)
N(4B)	2949(6)	6390(4)	10862(2)	49(2)
C(5B)	2811(6)	7695(4)	10566(3)	40(2)
N(6B)	4348(5)	8297(4)	10695(2)	45(2)
C(7B)	5517(7)	8304(4)	10043(3)	41(2)
N(8B)	4735(5)	7837(3)	9381(2)	37(1)
C(9B)	1764(6)	8637(5)	9154(3)	46(2)
O(10B)	2647(6)	4526(3)	10376(2)	81(2)
C(11B)	2964(9)	5919(6)	11739(3)	73(2)
C(12B)	1176(7)	8355(5)	10939(3)	56(2)
C(13B)	4770(7)	8676(6)	11495(3)	63(2)
O(14B)	6958(5)	8659(3)	10025(2)	60(1)
C(15B)	1869(6)	5904(5)	8811(3)	46(2)
C(16B)	2108(9)	4542(6)	8741(4)	84(3)
O(17B)	1194(5)	6585(3)	8289(2)	57(1)
C(18B)	5507(6)	7644(5)	8598(3)	43(2)
C(19B)	7339(7)	7930(6)	8404(3)	62(2)
O(20B)	4682(5)	7241(4)	8087(2)	62(2)

* Equivalent isotropic U defined as one third of the trace of the orthogonalized U_{ij} tensor

Table 5. Bond lengths (Å) for compound 6.

C(1A)-N(2A)	1.462 (6)	C(1A)-C(5A)	1.561 (6)
C(1A)-N(8A)	1.501 (6)	C(1A)-C(9A)	1.522 (7)
N(2A)-C(3A)	1.415 (6)	N(2A)-C(15A)	1.414 (6)
C(3A)-N(4A)	1.336 (6)	C(3A)-O(10A)	1.214 (6)
N(4A)-C(5A)	1.455 (6)	N(4A)-C(11A)	1.458 (6)
C(5A)-N(6A)	1.461 (6)	C(5A)-C(12A)	1.513 (7)
N(6A)-C(7A)	1.348 (7)	N(6A)-C(13A)	1.437 (6)
C(7A)-N(8A)	1.401 (6)	C(7A)-O(14A)	1.215 (7)
N(8A)-C(18A)	1.380 (6)	C(15A)-C(16A)	1.511 (8)
C(15A)-O(17A)	1.197 (7)	C(18A)-C(19A)	1.485 (7)
C(18A)-O(20A)	1.221 (6)	C(1B)-N(2B)	1.459 (6)
C(1B)-C(5B)	1.565 (6)	C(1B)-N(8B)	1.488 (6)
C(1B)-C(9B)	1.520 (6)	N(2B)-C(3B)	1.408 (6)
N(2B)-C(15B)	1.413 (7)	C(3B)-N(4B)	1.342 (7)
C(3B)-O(10B)	1.214 (7)	N(4B)-C(5B)	1.462 (6)
N(4B)-C(11B)	1.467 (6)	C(5B)-N(6B)	1.439 (7)
C(5B)-C(12B)	1.534 (7)	N(6B)-C(7B)	1.345 (6)
N(6B)-C(13B)	1.456 (7)	C(7B)-N(8B)	1.415 (6)
C(7B)-O(14B)	1.209 (7)	N(8B)-C(18B)	1.391 (6)
C(15B)-C(16B)	1.499 (8)	C(15B)-O(17B)	1.199 (6)
C(18B)-C(19B)	1.487 (7)	C(18B)-O(20B)	1.210 (6)

Table 6. Bond angles (°) for compound 6

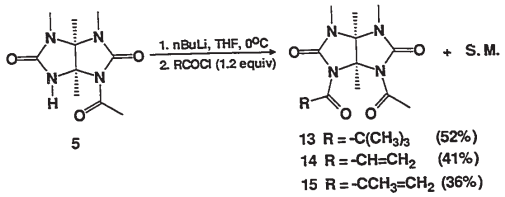
N(2A)-C(1A)-C(5A)	103.0(4)	N(2A)-C(1A)-N(8A)	111.1(3)
C(5A)-C(1A)-N(8A)	100.8(3)	N(2A)-C(1A)-C(9A)	117.6(4)
C(5A)-C(1A)-C(9A)	113.4(4)	N(8A)-C(1A)-C(9A)	109.6(4)
C(1A)-N(2A)-C(3A)	109.8(3)	C(1A)-N(2A)-C(15A)	125.9(4)
C(3A)-N(2A)-C(15A)	123.8(4)	N(2A)-C(3A)-N(4A)	108.0(4)
N(2A)-C(3A)-O(10A)	125.9(4)	N(4A)-C(3A)-O(10A)	126.1(4)
C(3A)-N(4A)-C(5A)	114.0(4)	C(3A)-N(4A)-C(11A)	120.6(4)
C(5A)-N(4A)-C(11A)	124.7(4)	C(1A)-C(5A)-N(4A)	101.7(3)
C(1A)-C(5A)-N(6A)	103.2(4)	N(4A)-C(5A)-N(6A)	111.1(4)
C(1A)-C(5A)-C(12A)	116.3(4)	N(4A)-C(5A)-C(12A)	111.6(4)
N(6A)-C(5A)-C(12A)	112.2(4)	C(5A)-N(6A)-C(7A)	113.3(4)
C(5A)-N(6A)-C(13A)	125.6(4)	C(7A)-N(6A)-C(13A)	120.9(4)
N(6A)-C(7A)-N(8A)	107.8(4)	N(6A)-C(7A)-O(14A)	125.2(4)
N(8A)-C(7A)-O(14A)	127.0(5)	C(1A)-N(8A)-C(7A)	111.4(4)
C(1A)-N(8A)-C(18A)	122.8(4)	C(7A)-N(8A)-C(18A)	125.8(4)
N(2A)-C(15A)-C(16A)	116.9(4)	N(2A)-C(15A)-O(17A)	120.9(5)
C(16A)-C(15A)-O(17A)	122.2(5)	N(8A)-C(18A)-C(19A)	120.2(4)
N(8A)-C(18A)-O(20A)	119.0(4)	C(19A)-C(18A)-O(20A)	120.8(4)
N(2B)-C(1B)-C(5B)	103.2(3)	N(2B)-C(1B)-N(8B)	111.2(3)
C(5B)-C(1B)-N(8B)	100.7(3)	N(2B)-C(1B)-C(9B)	117.4(4)
C(5B)-C(1B)-C(9B)	113.5(4)	N(8B)-C(1B)-C(9B)	109.4(3)
C(1B)-N(2B)-C(3B)	110.2(4)	C(1B)-N(2B)-C(15B)	126.2(4)
C(3B)-N(2B)-C(15B)	123.2(4)	N(2B)-C(3B)-N(4B)	108.5(4)
N(2B)-C(3B)-O(10B)	125.6(5)	N(4B)-C(3B)-O(10B)	125.9(5)
C(3B)-N(4B)-C(5B)	113.4(4)	C(3B)-N(4B)-C(11B)	120.7(4)
C(5B)-N(4B)-C(11B)	124.7(4)	C(1B)-C(5B)-N(4B)	101.6(4)
C(1B)-C(5B)-N(6B)	103.5(3)	N(4B)-C(5B)-N(6B)	111.7(4)
C(1B)-C(5B)-C(12B)	115.3(4)	N(4B)-C(5B)-C(12B)	111.7(4)
N(6B)-C(5B)-C(12B)	112.4(4)	C(5B)-N(6B)-C(7B)	113.8(4)
C(5B)-N(6B)-C(13B)	124.1(4)	C(7B)-N(6B)-C(13B)	121.2(4)
N(6B)-C(7B)-N(8B)	107.2(4)	N(6B)-C(7B)-O(14B)	126.3(5)
N(8B)-C(7B)-O(14B)	126.6(4)	C(1B)-N(8B)-C(7B)	111.4(4)
C(1B)-N(8B)-C(18B)	122.1(4)	C(7B)-N(8B)-C(18B)	126.4(4)
N(2B)-C(15B)-C(16B)	116.8(5)	N(2B)-C(15B)-O(17B)	121.2(5)
C(16B)-C(15B)-O(17B)	122.0(5)	N(8B)-C(18B)-C(19B)	119.5(4)
N(8B)-C(18B)-O(20B)	119.6(4)	C(19B)-C(18B)-O(20B)	120.9(4)

between the two α-methyl groups and the conjugation effect of the two pairs of carbonyl units, each pair connected by a tertiary nitrogen. A structural optimization with the PC-MODEL program gave a similar asymmetrical structure, supporting the idea that intramolecular interactions play a predominant role in the asymmetry and conformation. The orientation of the two acetyl groups revealed in the X-ray crystal structure and PC-MODEL calculation also may represent the predominant one in a solution. The two acetyl methyl groups are nonequivalent, as seen in the crystal structure; however, they were not distinguished by 200 MHz ^{1}H NMR and 50 MHz ^{13}C NMR. Therefore, there may be a fast equilibration between the two conformations in solution. Nevertheless, it is necessary for an intramolecular Claisen-type condensation to take place that one of the acetyl groups rotates upon deprotonation in order for the ionized α-carbon to access and then form a bond to the electrophilic acetyl group. The electrophilic acetyl group also may have to change its orientation in order to provide an optimal angle for the attack of the enolate carbon of the other acetyl group.

The other diacyl compounds were prepared in a similar way and in varying yields. The starting material **5**, **7** or **9** was always partially recovered in each case, usually in between 10-20% yield, even though excess nBuLi or LDA and acyl chloride were sometimes employed. The intramolecular steric strain in the products is probably a major factor causing difficulties for the access of a second acyl group.

1-Acetyl-6-propionyl-3,4,7,8-tetramethylglycoluril **11** was obtained from acetylation of **7** in 54% yield (Scheme 3.3.1).The proton NMR spectrum (200 MHz) shows a singlet of six protons from two N-methyl groups. The carbon-13 NMR spectrum (50 MHz) shows a singlet for the two N-methyl carbons and another singlet for the two urea carbonyl carbons in the two rings. This observation implies that an extra methylene group in the acyl side chain has little impact on the NMR chemical shifts of the proton and carbon atoms other than those of the acyl chain themselves. Similarly, 1-acetyl-6-butyryl-3,4,7,8-tetramethylglycoluril **12** was made in 61% yield from monobutyryl starting material **9**. The two N-methyl groups are 0.004 ppm away in the ^{1}H NMR and 0.04 ppm away from each other in the ^{13}C NMR spectra of **12** (Scheme 3.3.1).

1-Acetyl-6-trimethylacetyl-3,4,7,8-tetramethylglycoluril **13** was prepared in 52% yield by treatment of **5** with nBuLi and trimethylacetyl chloride (Scheme 3.3.2). The ^{1}H NMR chemical shifts of the two N-methyl groups of **13** are different by 0.12 ppm and the corresponding ^{13}C chemical shifts are different by 1.7 ppm, indicating remarkable effects of the bulky *tert*-butyl group of the side chain. Both CI and EI mass spectra of **13** show very weak peaks for the molecular ion at 324 (<1%), but relatively very strong peaks at M+1 (5% and 8%, respectively for CI and EI).



Scheme 3.3.2 Second Acylations of the Glycoluril Template.

1-Acetyl-6-acryloyl-3,4,7,8-tetramethylglycoluril **14** and 1-acetyl-6-methacryloyl-3,4,7,8-tetramethylglycoluril **15** were synthesized from **5** in low yields (41% and 36%, respectively), with formation of some unresolved sticky material probably due to polymerization side reactions (Scheme 3.3.2).

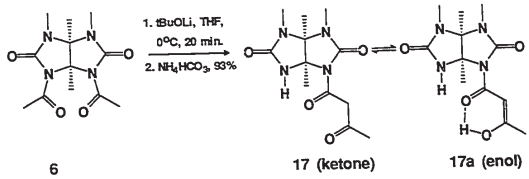
3.4 The Condensation Reaction of the 1,6-Diacetyl Glycoluril Derivative

A variety of conditions were tried in order to effect the intramolecular Claisen-type condensation between the two acetyl groups of **6**, making a new C-C bond and breaking an acetyl-N bond. When *tert*-butyl lithium was used as a base and THF as a solvent, the desired condensation product **17** was obtained in around 10% yield. Lithium diisopropylamide as a base in THF gave this product in less than 50% yield. Compounds **1** and **5** were recovered from the above reactions

even at -78°C; that is, one or both of the acetyl groups was removed from **6** when treated with t-BuLi or LDA. The choice of a quenching agent after the deprotonation and condensation had a great effect on the yield of the product **17**. Solid ammonium bicarbonate was found to be a suitable agent for the quenching. Water appeared to hydrolyse the product and gave a worse yield.

A solution of lithium *tert*-butoxide in THF, freshly prepared from n-butyl lithium and 1.5 equivalent *tert*-butyl alcohol was used to deprotonate one acetyl methyl of the compound **6** at 0°C in THF. After addition of *tert*-BuOLi in 5-10% excess, the reaction was monitored by TLC every five minutes and was found to be complete within 20 minutes. Addition of solid ammonium bicarbonate was followed by stirring the mixture for about 5 hours. Only a single spot of product showed up on a TLC plate at this stage. A yellowish solid product was obtained in 100% yield after removing the solid salt and then the solvent. This crude product was shown to be virtually pure compound **17** by ^{1}H and ^{13}C NMR spectra. A flash column chromatography purification resulted in compound **17** as a white solid in 93% yield (Scheme 3.4.1).

Another less polar fraction from the column purification was identified as **5** (5%), which was probably formed during the work-up as it could not be seen on a TLC plate until after the THF was removed from the reaction mixture. The mechanism for formation of **5** during the condensation process remains unknown.



Scheme 3.4.1
Condensation of Diacetyl Glycoluril Derivative 6 Induced by a Base.

However, the use of water, instead of solid NH_4HCO_3 as a quenching agent after the condensation led to formation of **5** in up to 46% yield and formation of the product **17** in only 39% yield. A subsequent experiment demonstrated that addition of t-BuOLi at 0°C in THF followed by heating the mixture at reflux for up to 5 hours and then quenching with solid ammonium bicarbonate did not lower the product yield. So the product **17** and its deprotonated form are relatively stable to heating. Compound **5** may result from a retro-Claisen type condensation of **17** with an undetermined nucleophile in the case of NH_4HCO_3. Use of water may generate OH^-, which could promote this retro-Claisen condensation reaction.

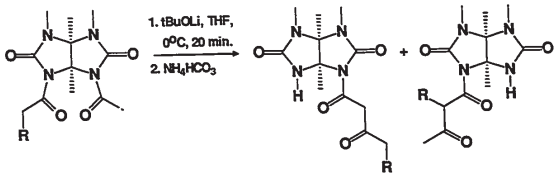
The normal intermolecular Claisen condensation reactions such as those of ethyl acylates, catalyzed with sodium ethoxide, require 8 hours to 3 days to proceed to completion under reflux conditions.[85] Typical yields of the keto ester

products are around 50% and the highest yield of 75% was reported for the ethyl acetoacetate product. In contrast, the condensation reaction of **17** is complete within 20 minutes at 0°C with a little stronger base, lithium *tert*-butoxide, as catalyst. A tremendous efficiency is indicated in this case by a short reaction time and a high yield of condensation product.

The compound **17** was characterized by NMR spectroscopy. Of particular note were the diastereotopic methylene protons between the two carbonyl groups on the side chain. These two protons form an *ab* pattern, and are coupled to each other by 16.4 Hz in the ^{1}H NMR spectrum, a typical value for a geminal coupling constant. Another feature of **17** is the equilibrium between its enol and keto forms. The enol form is seen in the ^{1}H and ^{13}C NMR spectra of **17** as a minor component (10%). The enol hydroxy proton appears at 13.20 ppm and the corresponding olefinic proton at 6.51 ppm in the ^{1}H NMR spectrum. The terminal methyl protons of the enol side chain shift 0.24 ppm upfield to 2.02 ppm from those of the keto form. The FTIR of **17** shows a sharp absorption at 3224 cm^{-1} for the NH group and a very strong and broad absorption composed of several unresolved peaks between 1690 and 1760 cm^{-1} for the carbonyl groups. A weak UV absorption in methanol was observed at a wavelength of 272 nm (ε = 970) and a moderate one at 211 nm (ε = 7600), probably due to the presence of the enol form. Compound **17** is very stable to air and was found to be still pure after standing in air for 4 years at room temperature.

3.5 The Condensations of Other Diacyl Compounds

Treatment of compound **11** with t-BuOLi in THF at 0°C for 20 minutes, followed by quenching with NH_4HCO_3, gave a product as a single spot by TLC. This product was purified on a flash column, resulting in a mixture of **18** and **19** in 81% yield (Scheme 3.5.1). Proton and ^{13}C NMR spectra showed that the compound **18**, 1-(3'-oxovaleryl)-3,4,7,8-tetramethylglycoluril and its enol form dominated in this product mixture. Compound **19**, 1-(2'-methyl-3'-oxobutyryl)-3,4,7,8-tetramethylglycoluril appeared to be present as a minor component. A separation of the two isomers **18** and **19** with a flash column was attempted but unsuccessful. A calculation from the integral of the proton NMR spectrum gave



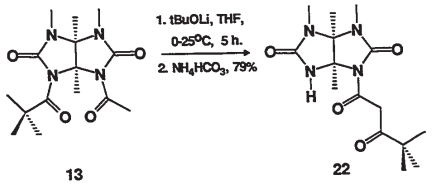
11 R=CH_3 **18** R=CH_3 **19** R=CH_3
12 R=CH_3CH_2 **20** R=CH_3CH_2 **21** R=CH_3CH_2

Scheme 3.5.1 Condensation of Diacyl Glycoluril Derivatives Induced by a Base to Produce 2 Regioisomers in Each Case (18:19=83:17, 20:21=81:19).

a ratio of **18:19** of approximately 83:17. An experiment using LDA as a base gave a mixture of **18** and **19** in 12% yield, which contained more than 30% of the compound **19** as estimated by the proton NMR integrations.

A similar treatment of compound **12** with t-BuOLi for 15 minutes resulted in a mixture of products **20** and **21** in a ratio of 81:19 (by ^1H-NMR integration) and a total yield of 88% (Scheme 3.5.1). This mixture also showed a single spot on a TLC plate and could not be separated by a regular chromatography column.

For the asymmetrical 1-acetyl-6-trimethylacetyl compound **13**, only the acetyl group can be converted to an enolate upon treatment with t-BuOLi. It was suspected that the bulky trimethylacetyl group might have a strong steric effect which would prevent the access of an enolate even by an intramolecular process.

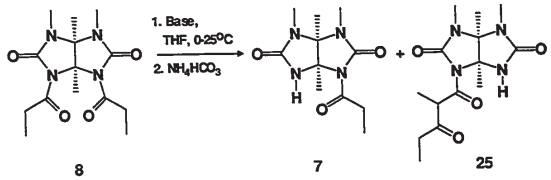


13 **22**

Scheme 3.5.2
The Bulky Trimethylacetyl Group Easily Transferred during Condensation.

It was found, however, that the transfer of the trimethylacetyl group occurred smoothly, affording the product **22** plus its minor enol form in 79% yield, although a longer reaction time of 5 hours was allowed after addition of t-BuOLi and before quenching with ammonium bicarbonate (Scheme 3.5.2).

When lithium *tert*-butoxide was used to induce a condensation between propionyl units in 1,6-dipropionyl-3,4,7,8-tetramethylglycoluril **8**, an inseparable mixture of the condensation product **25** and the monopropionyl compound **7** was obtained (1:1 from ^1H NMR integration). Compound **25** exists as two diastereomers in a ratio of 1:2. A less bulky but more nucleophilic base, lithium *iso*-propoxide, was also tested for the condensation of **8**, producing a mixtur of **25** and **7** in a 1:3 ratio (Scheme 3.5.3). These results show that cleavage of an alkyl group from the template is partially caused by the nucleophilic attack of the base. Further experiments using a more hindered base (*e.g.*, *tert*-AmOLi) to induce the condensation of **8** are currently being carried out.

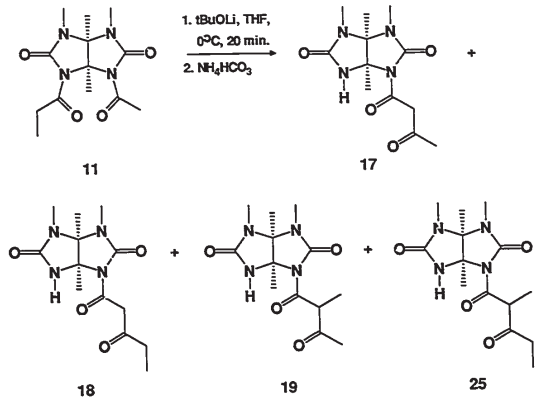


8 **7** **25**

Scheme 3.5.3 Treatment of the Dipropionyl Derivative with tBuOLi or Lithium Propoxide Resulted in a Mixture of Condensation Product and Monopropionylglycoluril.

3.6 Mechanistic Studies on the Template-Directed Condensations

For an intermolecular condensation reaction, the 1-acetyl-6-propionyl glycoluril derivative **11** could have given four possible condensation products **17**, **18**, **19** and **25**, as shown in Scheme 3.6.1. An intramolecular pathway only allows the cross condensation between acetyl and propionyl groups to give compounds **18** and **19** as the rearranged products. No products **17** and **25** were actually



11 **17**

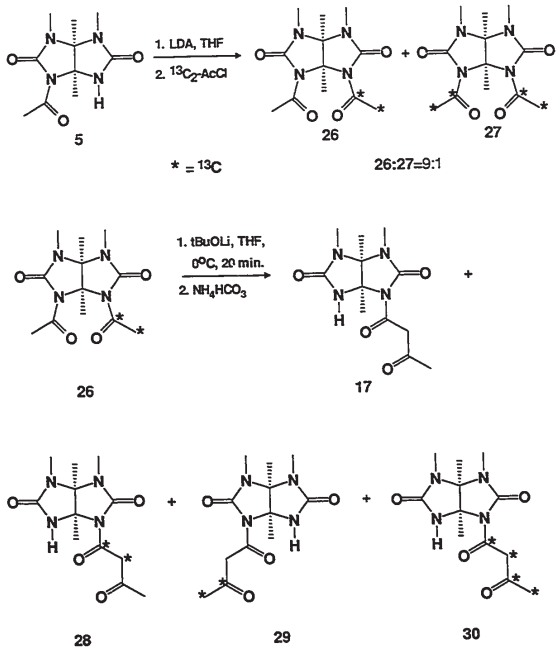
18 **19** **25**

Scheme 3.6.1
Intermolecular Condensation of 11 Would Have Given These 4 Products. However, 17 and 25 Were Actually Not Detected in This Reaction.

detected in the condensation of compound **11**, suggesting that this reaction occurs through an intramolecular mechanism. The condensation reactions of other asymmetrical 1,6-diacyl glycoluril derivatives (e.g., **12** and **13**) led to the same conclusion.

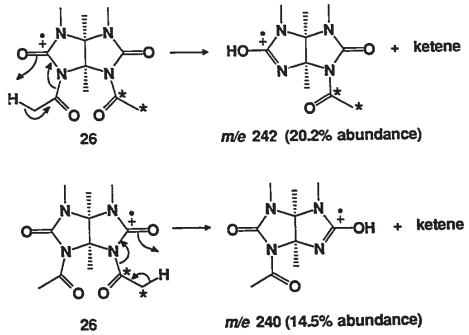
Compared with some regular Claisen condensation reactions, such as that of ethyl acetate with sodium ethoxide,[85] the condensation reactions we studied here have characteristic features of considerably lower reaction temperature, shorter reaction times and higher yields. These observations strongly support the hypothesis that the condensation reactions studied above occur through an intramolecular pathway. The model compound holds the two acyl groups together and a new C-C bond can be easily formed in an intramolecular process as soon as deprotonation occurs on one α-carbon of the two acyl groups. The formation of such a C-C bond probably is much faster for an intramolecular process than for an intermolecular reaction.

Compound **26**, in which one acetyl group is labelled with two ^{13}C atoms, was designed to test further the intramolecularity of acetyl transfers during formation of condensation products. The four possible condensation products **17** (M-2), **28** (M), **29** (M) and **30** (M+2) with different labelling patterns could be detected if intermolecular condensation occurred for **26** (Scheme 3.6.2). Instead, only two, **28** (M) and **29** (M), are possible for an intramolecular mechanism.



Scheme 3.6.2
Intermolecular Condensation of 26 Would Give These Four ^{13}C-Labelled Products. However, 17 Was Actually Not Formed. The Minor Formation of 30 Was due to the Presence of Some Compound 27 in the Starting Material.

These two pathways can be distinguished by detection of the presence of the isotopomers **17** and **30** by mass spectrometry. Unfortunately, it was found that during the preparation of **26** by treatment of **5** with LDA and then $^{13}C_2$-acetyl chloride, the expected product was accompanied by a small amount (16%) of isotopomer **27**, which was labelled with four carbon-13 atoms in both the acetyl groups. Both ^1H NMR integration and the mass spectrum confirmed the presence of **27** in the product **26**. In the EI mass spectrum (Fig. 3.6.1), two peaks at *m/e* 240 and 242 are observed due to the loss of a $^{13}C_2$-ketene molecule and a $^{12}C_2$-ketene molecule, respectively, from the molecular ion of **26** (Scheme 3.6.3). The



Scheme 3.6.3 Molecular Ion Fragmentation of 26 Should Have Given Nearly Equal Abundance for *m/e* 240 and 242. However, *m/e* 242 Is More Intense due to the Presence of a Small Amount of Compound 27.

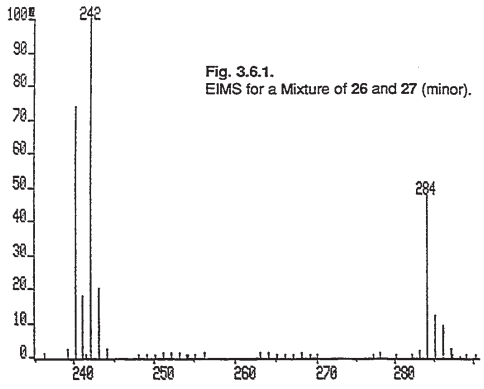


Fig. 3.6.1.
EIMS for a Mixture of **26** and **27** (minor).

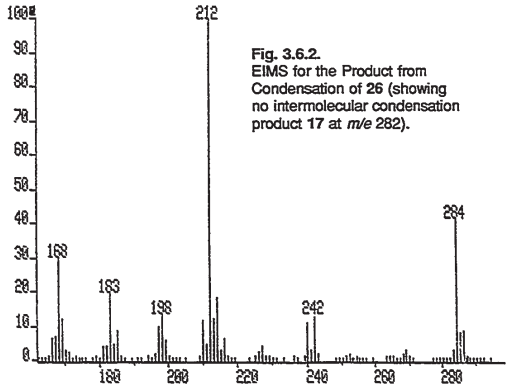
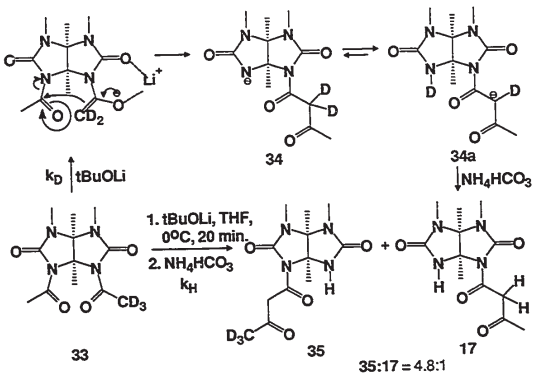


Fig. 3.6.2.
EIMS for the Product from Condensation of **26** (showing no intermolecular condensation product **17** at *m/e* 282).

peak at *m/e* 242 is about 30% higher than *m/e* 240, due to the partial contribution of **27**.

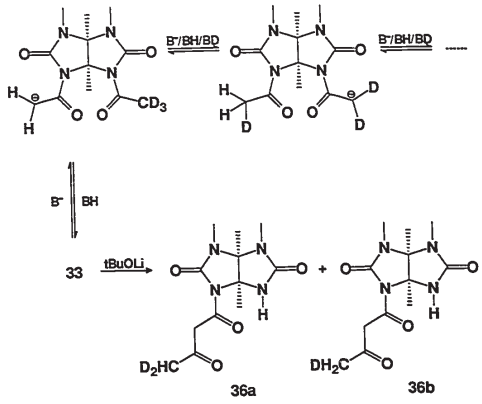
A mixture of **26** and **27** (~9:1) was treated with t-BuOLi according to the standard condensation procedure. A part of the EIMS of the condensation product is shown in Fig. 3.6.2. There is no peak with a meaningful intensity at *m/e* 282 for compound **17**, which could have been formed if an intermolecular acetyl transfer had occurred. Compound **30** was formed, but can be accounted for by an intramolecular condensation of the minor starting material **27**.

In order to determine whether the initial deprotonation is the rate-limiting step for the glycoluril template-directed condensation reactions, a deuterium-labelling experiment was carried out, in which the 1-acetyl-d_3-6-acetyl glycoluril derivative **33** in THF was treated with t-BuOLi (Scheme 3.6.4). Such an experiment is expected to produce unlabelled **17** and labelled **35** according to the standard condensation procedure. Dedeuteration at the labelled acetyl group of **33** by t-BuOLi and then transfer of the unlabelled acetyl group generates an intermediate **34**. The two methylene deuterium atoms between the two carbonyl groups of **34** are easily exchanged with the protons of ammonium bicarbonate due to their acidity to give **17**. The ratio of **17**:**35** was obtained from ^{1}H NMR integrations of the product mixture, and found to be 1:4.8. A strong singlet in the ^{2}H NMR spectrum at 2.20 ppm indicates the presence of a CD_3 group for the



Scheme 3.6.4
A Deuterium-Labelling Experiment Gave a Primary Isotope Effect of 4.8 due to the Slow Cleavage of the C-H Bond by Base.

keto form of **35** and another small singlet at 1.96 ppm is assigned to the enol form. These results can be explained by a slow deprotonation of one acetyl group and then a fast transfer of the other electrophilic acetyl group, whose CH_3 or CD_3 group remains unaffected in the final product. If the deprotonation were a fast step, the negative charge would have migrated between acetyl methyl groups to give a more complex labelling pattern, including labelled products **36a** and **36b** (Scheme 3.6.5), which were not detected by ^{1}H or ^{2}H NMR. The ratio of **17**:**35**



Scheme 3.6.5 For a Fast Deprotonation-Protonation Equilibration of 33, Compounds 36a and 36b Would Dominate the Condensation Products. However, 36a and 36b Were Actually Not Detected.

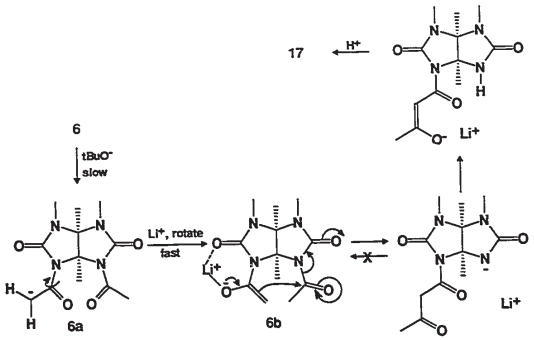
of 1:4.8 shows a primary isotopic effect of 4.8 due to the rate-limiting cleavage of C-H *versus* C-D bonds.

Furthermore, the stronger base LDA was used to perform the same deuterium-labelling experiment. In this case, cleavage of a C-H or C-D bond was expected to be faster and hence less selective than when t-BuOLi was used. The

product ratio **17**:**35** was found to be 1:1.6 and the yield of product mixture was only 46%.

As discussed earlier, the X-ray crystal structure shows that the two acetyl methyl groups of **6** are directed away from each other. Such an orientation is probably true in solution in order to minimize the intramolecular interaction. Rotation of the enolate generated by deprotonation of **6** is necessary for its negatively charged carbon to access the electrophilic acetyl carbonyl carbon. The rotation and the nucleophilic attack proceed very fast relative to the initial cleavage of a C-H bond. The rotation process is probably facilitated by the chelation effect of lithium cation (Scheme 3.6.6). The next acetyl transfer to accomplish the key step of the efficient condensation can result in a tremendous release of the intramolecular strain between the two acetyl units. As a consequence of the steric effects, the reverse intramolecular acyl transfer from carbon to nitrigen is unlikely to occur (Scheme 3.6.6).

Compound **6** upon deprotonation was expected to have a strong tendency for the negatively charged oxygen of the enolate directly to attack the electrophilic acyl group to provide the *O*-acylated product. The conformation of **6** shows that each oxygen atom of the side chains is much closer to the other acyl carbonyl carbon than each α-carbon and the resulting enolate would need little rotation for the *O*-acylation process. However, the *O*-acylation product was not detected at



Scheme 3.6.6 The Mechanism of Template-Directed Condensation Involves a Slow Deprotonation and a Fast Acyl Transfer. The Acyl Transfer Step May Not Be Reversible due to the Large Intramolecular Strain in 6a and 6b.

all in the condensation reactions. The absence of the *O*-acylation could be explained by chelation of the lithium counterion in the putative enolate intermediate.

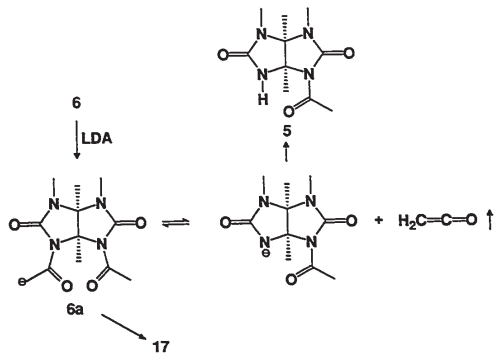
In order to gain further insight into the metal chelation effect, Louise Edwards, a postdoctoral fellow in our group during 1992-1993, undertook an investigation using different bases in the condensation reaction of compound

12 (Table 7). When sodium *tert*-butoxide was used as base, the condensation product was obtained in only 56% yield with a ratio of **20:21** of 60:40. The results from potassium *tert*-butoxide and lithium *tert*-butoxide in the presence of crown ethers were anticipated to be similar to that when sodium *tert*-butoxide was used as base. The yields and the product ratios of **20:21** in these two cases were actually found to be much higher. Use of t-AmOLi as base resulted in the best regioselectivity (**20:21**=86:14). These results are difficult to interpret in terms of metal chelation effects and the chelation patterns may be more complicated than that expected earlier, probably due to the multiple heteroatoms in the template system.

Table 7. The Condensations of **12** Induced by Different Bases

BASE	ADDITIVE	YIELD	Ratio **20:21**
LiOtBu		88%	81:19
NaOtBu		56%	60:40
KOtBu	18-Crown-6	80%	67:33
LiOtBu	12-Crown-4	80%	75:25
LiOtAm		80%	86:14

When a stronger base is used to cleave the α-C-H bond of **6**, the following rotation process of the enolate becomes relatively slower. A stronger base may also pass more energy to an enolate during the C-H bond cleavage. These factors may considerably increase the risk for the deprotonated acetyl group to be cleaved from the template, resulting in low yields of the condensed product as observed in the cases of LDA and t-BuLi. But perhaps the most important aspect is that the entropy effect provides a basis for the possible loss of the enolate from the template. That one of the acetyl groups falls off upon formation



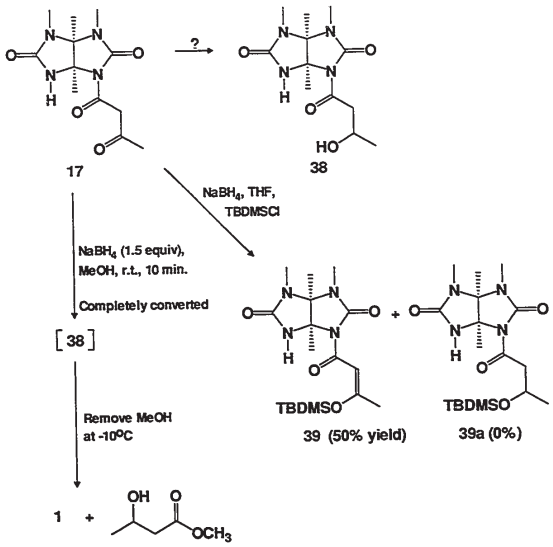
Scheme 3.6.7 A Possible Mechanism for the Extensive Formation of 5 when 6 Was Treated with LDA. Loss of a Ketene Molecule May Be Favored due to the Steric Effect in 6a and due to Faster Deprotonation by LDA.

of an enolate provides an alternative pathway to release the intramolecular strain and results in a favourable entropy increase. The contribution from this alternative pathway may get more important if the generated enolate has very high energy or is not in the right orientation for the condensation and cannot adjust its orientation in a very short time.

Ketene may be generated as a result of enolate loss from the template. Ketene is a very reactive electrophile and its generation may be a reversible process (Scheme 3.6.7). However, its reactivity may be largely offset by the entropy effect due to the large intramolecular strain and by its low concentration in solution. Further work is needed to prove the ketene formation and to account for the low yields of the condensations when a stronger base such as LDA is used.

3.7 Selective β-Carbonyl Reduction of Compound 17

Conversions of **17** are necessary prior to the next cycle of acylation and intramolecular condensation to make a lengthened carbon chain. A selective reduction of the β-carbonyl group of **17** was anticipated to be easily realized to afford β-hydroxybutyryl compound **38**. However, achieving such a reduction in practice required an investigation of a series of different solvents such as acetic



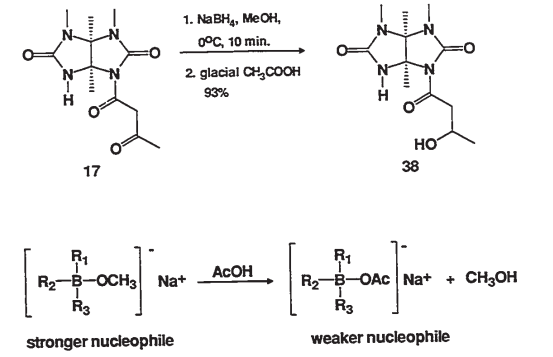
Scheme 3.7.1 Treatment of 17 with $NaBH_4$ under Different Conditions.

acid, *iso*-propanol, ethanol, THF or mixtures of THF with alcohols, as well as different reducing agents such as $NaBH_4$, $NaBH_3CN$, L-Selectride and $NaBH(OCH_3)_3$. Usually, the yield of β-hydroxybutyryl product **38** was around 40%

or lower and the starting material was always partially recovered. If an excess of reducing agent was used, both the starting material and the product were observed to disappear. In the presence of *tert*-butyldimethylsilyl chloride, the reduction of **17** with $NaBH_4$ in THF led to formation of β-silyl enol ether **39** in 50% yield, suggesting that the enolization of **17** is the major cause of the difficulty in its reduction (Scheme 3.7.1).

When monitored by a TLC plate, it was found that the compound **17** was completely converted to the desired product **38** within 10 minutes when 1.5 equivalent $NaBH_4$ was slowly added to a methanol solution of **17** in an ice-water bath. Unfortunately, upon removal of the methanol solvent even at -10°C, none of the desired product **38** was found, but rather methyl β-hydroxybutyrate and template **1** were isolated (Scheme 3.7.1). It was also found that **38** is relatively stable to pure methanol. Therefore, the methoxide group from the generated boron methoxide can act as a nucleophile and undergo nucleophilic attack on the desired product **38** and destroy it.

Acetic acid was found to be an efficient reagent to convert the boron methoxide rapidly to the much weaker nucleophile, boron acetate. Therefore, as soon as the reduction of **17** with $NaBH_4$ in methanol was complete when monitored by TLC, glacial acetic acid was added and the product **38** was isolated in 83-93% yield (Scheme 3.7.2).

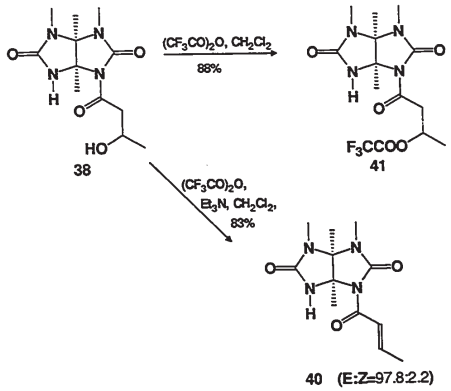


Scheme 3.7.2 Addition of AcOH in Time Can Protect the Reduction Product of 37 from Nucleophilic Attack by Boron Methoxide.

Compound **38** is much more polar than **17** and is easily separated from it by flash column chromatography. Both ^1H and ^{13}C NMR spectra of **38** showed a mixture of two diastereomers. The diastereoselectivity of the reduction was difficult to determine from integration of the 500 MHz ^1H NMR spectrum but appeared not to be remarkable. There were strong IR absorptions for OH and NH groups. EIMS indicated a strong tendency to lose a molecule of water from the molecular ion of **38** and similar abundances of *m/e* 266 and molecular ion at *m/e* 284 were observed.

3.8 Dehydration of 1-(β-Hydroxybutyryl)-3,4,7,8-tetramethylglycoluril 38

Elimination of a molecule of water from **38** is expected to produce the α,β-unsaturated crotonyl glycoluril derivative **40**. Initial attempts to use acids such as *p*-TsOH to induce such an elimination gave low yields (~10%) of **40** with recovery of most of the starting material. The *trans* to *cis* ratio around the C=C bond of **40** was shown to be higher than 99:1 from ^1H NMR integrations. Remarkably, **38** was found to react with neat trifluoroacetic acid to form ester **41**, which was later



Scheme 3.8.1
Dehydration of 38 by Treatment with Acid Anhydride and Base.

prepared from trifluoroacetic anhydride in 88% yield. Compound **41** is not stable to air at room temperature and easily loses its trifluoroacetyl group with recovery of **38**. Addition of triethylamine directly to a mixture of trifluoroacetic anhydride and **38** in dichloromethane at room temperature and then heating at reflux for 20 minutes afforded the desired product **40** in 83% yield with an E:Z ratio of 97.8:2.2 (Scheme 3.8.1).[86]

Compound **40** has a strong UV absorption at λ_{max} = 229 nm (ε = 12,000) for the conjugated crotonyl imide group. IR absorptions at 1667, 1627, 919 and 962 cm^{-1} are an indication of the presence of the C=C bond and two olefinic C-H bonds. In addition, the NH group absorbs at 3310 cm^{-1} and the C=O groups absorb at 1722 cm^{-1}. The base peak on the EI mass spectrum of **40** is from its molecular ion (266), indicating strong stability of the molecular ion due to the conjugation effect. The two olefinic protons are *trans* to each other and coupled by 15.3 Hz. A NOE experiment showed that the α-proton has a strong interaction with the side chain methyl group and not with the β-proton.

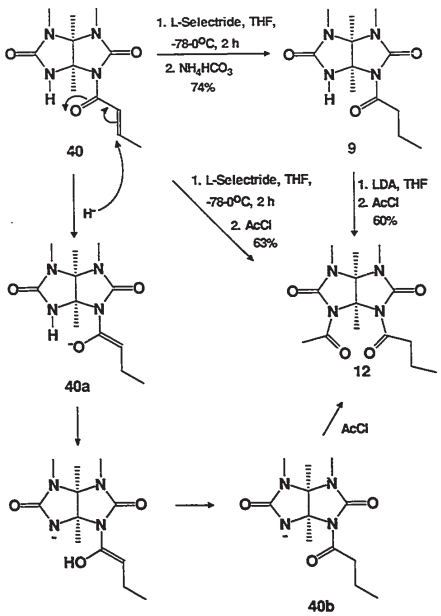
Compound **40** was also made by heating the template **1** in THF with n-butyl lithium at reflux and then treating the mixture with crotonyl chloride, but the E:Z ratio was only 93:7 (^{1}H NMR integrations).

3.9 Conversions of 1-Crotonyl-3,4,7,8-tetramethylglycoluril 40 and a Second Cycle of Condensation

Several synthetic methods have been developed to induce hydride addition in a 1,4 fashion to α,β-unsaturated carbonyl compounds. For example, it has been demonstrated that rhodium(I) complexes catalyze the 1,4-addition of silicon hydrides[87,88] and catecholborane[89] to enones and enoates.

We have investigated several reagents in order to reduce the C=C double bond of the crotonyl glycoluril derivative **40** and the best results were obtained by using L-selectride as a reducing agent. L-selectride was reported in the reduction of C=C bonds in uracil ring and in orotic acid derivatives[90]. This reagent allowed a simple procedure without use of a catalyst and gave the saturated butyryl derivative **9** in 74% yield (Fig. 3.9.1). The ^{1}H and ^{13}C NMR spectra of **9** obtained here are identical to those obtained previously by a direct butyrylation of template **1**. Compound **9** obtained here was further acetylated to give **12** (60%), whose NMR spectra are also exactly the same as those previously obtained.

A better overall yield of **12** (63%) can be obtained directly from **40** by reduction with 1 equivalent of L-selectride followed by quenching with AcCl (Scheme 3.9.1). Therefore, the NH proton in **40a**, an intermediate from the



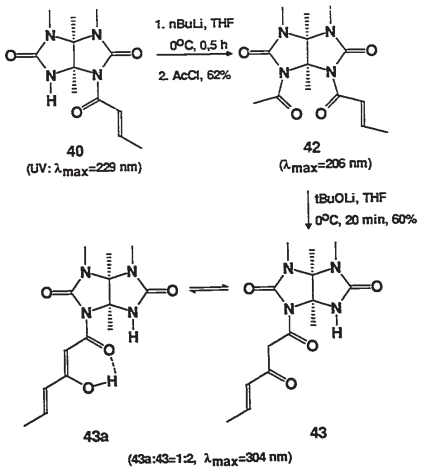
Scheme 3.9.1
Hydride Conjugate Addition and then Acetylation to Give 12, Which Can Undergo a Second Round of Intramolecular Condensation.

hydride conjugate addition on the double bond of **40**, can be transferred to the α-position of the acyl chain to produce **40b**. The following acetylation occurs on the negatively charged nitrogen atom.

Since the condensation of **12** was also previously demonstrated to give hexanoyl derivative **20** as the major product, this process constitutes to our knowledge the first example of the formation of a fatty-acid-like carbon chain by using a template-directed approach to add acyl units repetitively. Further conversions of **20** should not be a challenging problem, and could be effected by use of the developed procedures, namely reduction with $NaBH_4$, dehydration with $(F_3CCO)_2O$ / Et_3N, reduction with L-Selectride, acetylation and condensation.

3.10. Repetitive Condensations to Make Unsaturated Carbon Chains

In order to test the generality of repetitive acyl transfers on the glycoluril template **1**, we next investigated the chain extension of the crotonate derivative **40**. Acetylation of **40** gave **42** (62%) with 12% starting material recovered, using the general procedure to make diacylated compounds (Scheme 3.10.1). Compound **42** has a UV absorption at λ_{max} = 206 nm (ε = 11,000), remarkably different from that of **40** (λ_{max} = 229 nm, ε = 12,000). This wavelength difference of 23 nm is probably a result of intramolecular interaction between crotonyl and

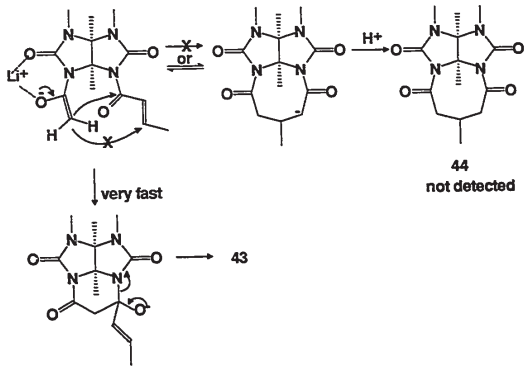


Scheme 3.10.1
Acetylation of the Crotonyl Derivative and a Second Round Condensation to Make an Unsaturated Chain with 6 Carbon Atoms.

acetyl groups in **42**, which forces members of the crotonyl imide unit out of the plane of the N-C=O unit of the glycoluril ring. Therefore a fully effective conjugation can not be obtained. However, the abundance of molecular ion of **42** in the EI mass spectrum is 44%, the second highest peak. The partial conjugation

may still be a factor in stabilizing the molecular ion and contributing to their high abundance.

Treatment of **42** in THF with t-BuOLi produced compound **43** (60%) which contains a six carbon chain (Scheme 3.10.1). Another two minor fractions from the column chromatographic purification of **43** were obtained, but could not be characterized due to their very complicated ^{1}H and ^{13}C NMR spectra.



Scheme 3.10.2
Product from Intramolecular Conjugate Addition of an Intermediate Enolate to α,β-Unsaturated Side Chain Was Not Observed.

Interestingly, several other possible condensation products derived from transfer of the acetyl group were not detected and this reaction proceeded exclusively with the transfer of the crotonyl group to give the desired linear chain. Conjugate 1,4-additions of enolates to α,β-unsaturated carbonyl compounds are very common reactions in most organic chemistry textbooks,[91] but this was not the case when a crotonyl group and an enolate are bound to **1**. No conjugate addition product **44** was observed, probably as a result of the special molecular orientation and steric effect preventing formation of an 8 membered ring intermediate (Scheme 3.10.2).

Compound **43** exists with its enol isomer **43a** in a 2:1 ratio, as determined by ^{1}H NMR integrations. This ratio shows that the enol form **43a** is considerably more stable than that of β-oxobutyryl glycoluril derivative **17**, probably due to the conjugation effect of the two C=C bonds in **43a**. An absorption in the UV spectrum of **43** was observed at λ_{max} = 304 nm (ε = 3,500), which is probably due to the presence of **43a**. A 500 MHz NMR COSY-90 experiment (Fig. 3.10.1) shows the presence of two uncorrelated components (**43** and **43a**).

Reduction of **43** is challenging due to its more stable enol form and due to two possible 1,2- and 1,4- hydride additions to the conjugate ketone. The procedure for the β-carbonyl reduction of **17** was simply used here for reduction of **43** to give product **45** in 44% yield with 35% recovery of the starting material

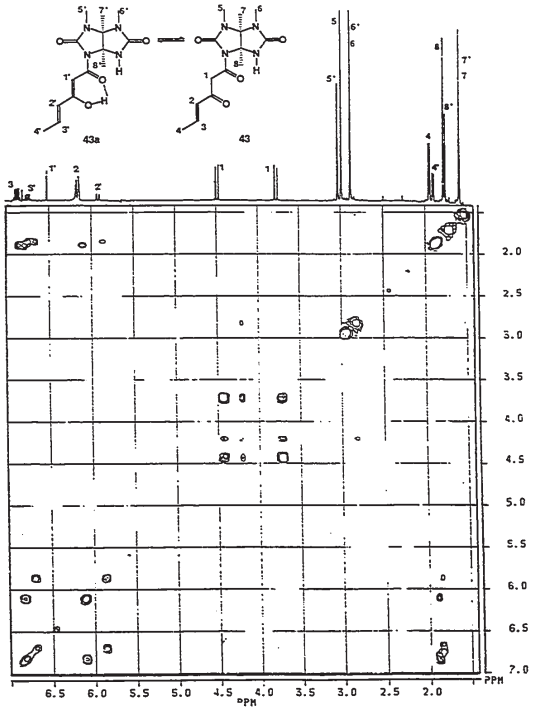
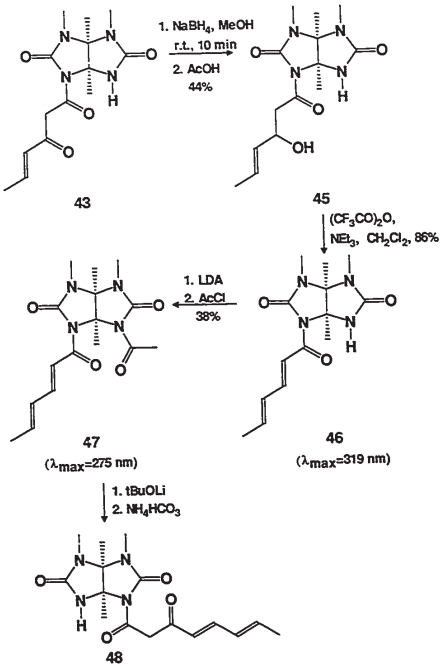


Fig. 3.10.1 A 500 MHz NMR COSY-90 Spectrum of 43 & 43a

43 (Scheme 3.10.3). The 500 MHz ^{1}H NMR spectrum of **45** (Fig. 3.10.2) shows two diastereomers and a trace amount of impurity, which seems to be derived from loss of unsaturation from **45**. This reduction was not further optimized and there is still potential to improve the reduction as some catalysts (*e.g.* lanthanoid chlorides)[92] can catalyze selective 1,2-reduction of conjugate carbonyl compounds.

Elimination of water from **45** was similar to that from compound **38**, yielding **46** (86%). Although a satisfactory elemental analysis and a high resolution mass spectrum of **46** were obtained, minor impurities were found in the ^{1}H and ^{13}C NMR spectra. A very strong peak (36%, second to base peak *m/e* 125) for molecular ion was observed in the EI mass spectrum as a result of conjugate stabilization of the molecular radical cation. A strong UV absorption of **46** at λ_{max} = 319 nm (ε = 26,000) also indicates an extensive conjugated system in **46**.

The next round of acetylation gave **47** in only 38% yield with 45% recovery of **46**. The α-proton resonance of compound **47** appears as a doublet at 6.97 ppm, the β-proton as a doublet of doublets at 7.37 ppm, the γ-proton as a triplet at 6.26 ppm with fine structure due to the long range coupling with the side chain methyl group and the δ-proton as a doublet of quartets at 6.15 ppm (Fig 3.10.3). The stereo structure around the two C=C bonds in **47** was confirmed by NOE



Scheme 3.10.3
Conversions of 6 Carbon Chain Derivatives and a Third Round Condensation to Make an 8 Carbon Chain Product.

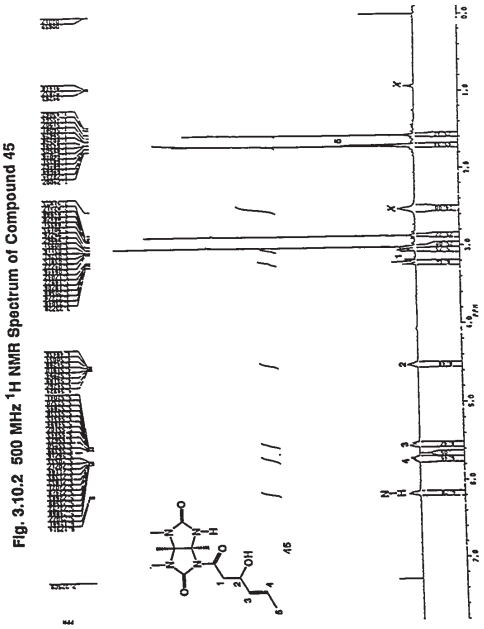


Fig. 3.10.2 500 MHz ^{1}H NMR Spectrum of Compound 45

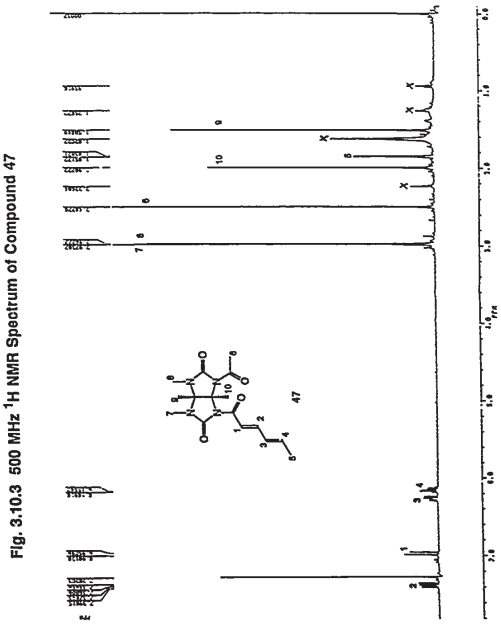


Fig. 3.10.3 500 MHz ^{1}H NMR Spectrum of Compound 47

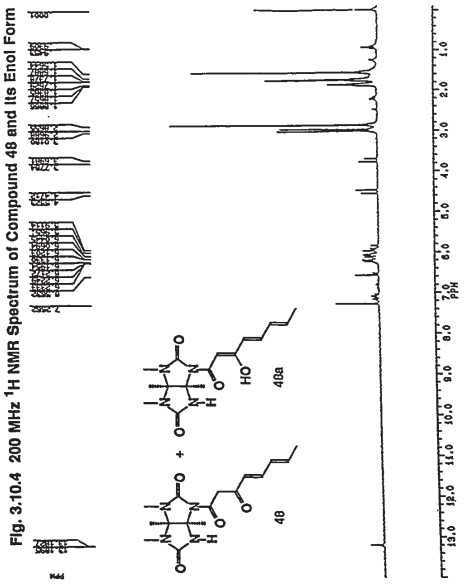


Fig. 3.10.4 200 MHz ^{1}H NMR Spectrum of Compound 48 and its Enol Form

experiments: saturation of the resonances at the α-, and β-protons of the side chain gave, respectively, resonance enhancements of the γ- and δ-protons. This reaction was performed only once and could be further optimized. However, as discussed previously, there is a strong intramolecular interaction when two acyl groups are introduced onto template **1**. The larger the first acyl group on **1**, the more difficult the introduction of the second acetyl group, as seen from the acetylations of **5** (78%), **9** (61%) and **46** (38%). The UV absorption of **47** moves to λ_{max} = 275 nm (ε = 9,300), 44 nm shorter than that of **46**. The conjugation of the hexadienoyl group in **47** is probably more affected than that of the crotonyl group in **42** by the interaction with the acetyl group.

The third round of condensation proceeded in 60% yield, giving compound **48** with an 8-carbon chain (Scheme 3.10.3). The ^{1}H NMR spectrum of **48** (Fig. 3.10.4) looks very complicated due to the presence of a mixture of keto and enol forms (4:3) and some impurity. For example, signals at 0.94 ppm may represent a minor undesired compound derived from loss or partial loss of the unsaturation from **48**. The major signals for **48** and its enol form can be clearly seen in the ^{1}H NMR spectrum. Three UV absorptions of **48** were observed at λ = 319, 280 and 215 nm (ε = 10,800, 11,600 and 11,500, respectively).

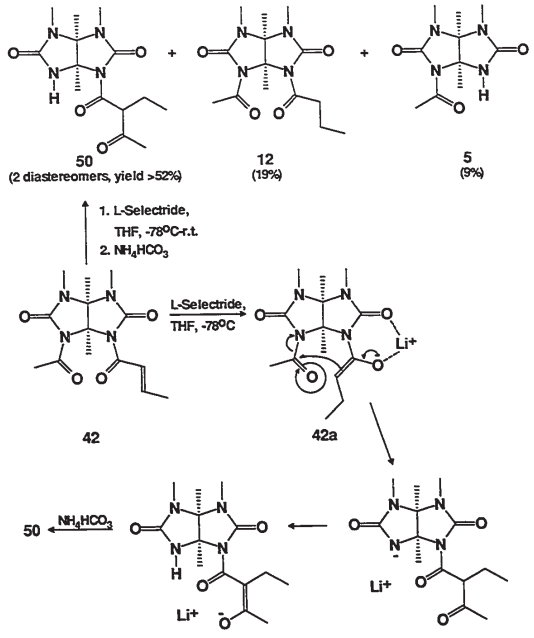
Most of the unsaturated compounds studied above were found to be stable, but not sufficiently so for prolonged standing in air at room temperature.

It is recommended that each unsaturated compound be converted within two weeks after being prepared, or stored under nitrogen in the refrigerator. The C=C double bonds were considerably affected when the compounds were reserved for longer than a month in air, especially for those with multiple C=C double bonds. The ^{1}H NMR integrations of the unsaturated side chains of such samples were largely reduced, relative to those of the other signals on the rings, while signals representing the undesired component (*e.g.*, at 0.94 ppm in the ^{1}H NMR spectrum of **48**) were increased.

3.11. Condensations Induced by Conjugate 1,4-Hydride Additions

As discussed earlier, the enolate formation from diacylglycolurils is a slow step, followed by a fast acyl transfer to achieve an intramolecular condensation. Deprotonation occurs preferably on the more acidic and less hindered position, as reflected by the condensed product ratio.

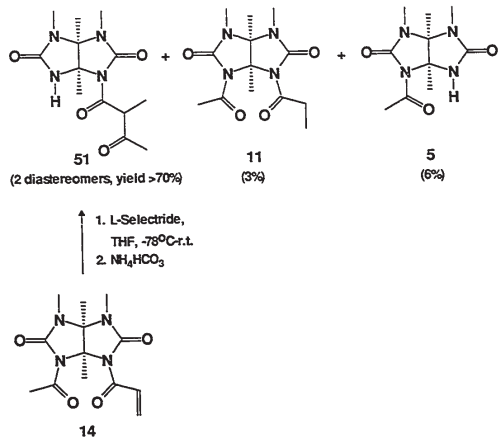
The regioselectivity of condensation may be controlled if an enolate can be selectively generated on a desired position other than the more acidic and less hindered one. A good hint for an alternative method of generation of enolates was given by the L-Selectride reduction of 1-crotonyl-3,4,7,8-tetramethylglycoluril **40**. Structure **40a** in Scheme 3.9.1 would be an interesting



Scheme 3.11.1
Treatment of Acetyl Crotonyl Glycoluril Derivative with L-Selectride.

intermediate if there were one more intact acyl group to replace the NH hydrogen. Such an ideal intermediate could be generated by L-Selectride treatment of **42** (Scheme 3.11.1). In fact, the condensation product from the L-Selectride reduction of **42** was found to be solely that derived from acetyl transfer to the α-position of the butyryl side chain and was obtained as a mixture of two diastereomers **50a** and **50b** (**50a**:**50b** = 1.5:1) with a total yield higher than 52%. The side products were found to be reduced but uncondensed compound **12** (19%) and acetyl glycoluril derivative **5** (9%). Compounds **12**, **50a**, and **50b** proved difficult to separate. However, repeated flash column chromatographic purification allowed an estimation of the condensation product yield by ^{1}H NMR integrations. The more polar isomer **50b** was partially separated in a fairly pure form. This reaction was performed only once and could have been optimized. However, we became more interested in investigating a similar reaction of the acryloyl acetyl derivative **14**.

An acryloyl unit upon reduction of the C=C bond may act as an equivalent of a propionyl unit, which is a building block in many natural polyketides. We wished to see whether acryloyl units can be used to build carbon chains by repetitive hydride-reductive condensations. The L-Selectride reduction of **14** was carried out at -78°C for 3 hours and then the mixture was quenched with NH_4HCO_3 (Scheme 3.11.2). The subsequent filtration was very slow even with the aid of celite due to some sticky material, which was probably formed from L-



Scheme 3.11.2
Treatment of Acetyl Acryloyl Glycoluril Derivative with L-Selectride.

Selectride or side polymerization of acryloyl derivative.

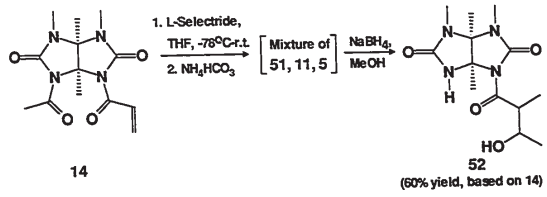
The condensation product **51**, consisting of two diastereomers **51a** (less polar) and **51b** (more polar), was mostly separated from the side product **5** by flash column chromatography and was obtained in 70% yield (purity > 97%). As

some of **51a** was still not isolated from **5** and was not counted in the yield, the true yield for the formation of **51** could be higher. **51a** and **51b** were partially separated from each other to give two fairly pure single diastereomers. The diastereomer ratio of **51a**:**51b** was initially shown to be 2.1:1 by ^{1}H NMR analysis of the crude reduction mixture. When either of the single diastereomers **51a** or **51b** was treated with potassium *tert*-butoxide and then quenched with glacial acetic acid, a similar ratio (2.1:1 - 2.2:1) of **51a**:**51b** was obtained.

The NMR spectra of **51a** and **51b** showed none of their corresponding enol forms. Treatment of **51a** in $CDCl_3$ with one equivalent of trifluoroacetic acid for 5 days led to complete recovery of only **51a** without epimerization. D_2O was used to treat **51b** in $CDCl_3$ for 10 days and no D-H exchange at the α-position of **51b** was observed, while the α-protons of acetoacetylglycoluril **17** were completely exchanged with D_2O within 24 hours. These observations demonstrated the rather low kinetic acidity of the β-keto imide **51**, as observed and discussed earlier by Evans *et al.* in oxazolidone systems and other *N,N*-dialkylamides derived from α-substituted β-keto acids.[53] The low kinetic acidity allows a partial separation of the two diastereomers **51a** and **51b** and their ratio is determined by kinetic protonation of the enolate.

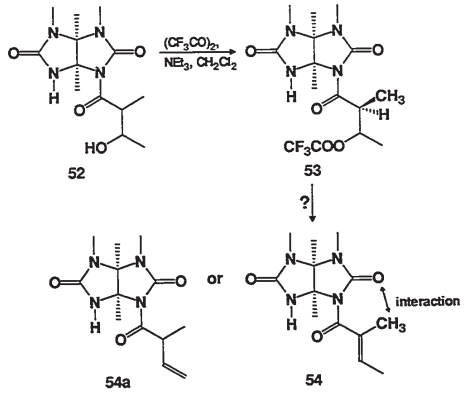
Because of the difficulty in isolation of **51** from the side product **5**, the product mixture from L-Selectride reduction was filtered briefly through a flash

column without separation and then directly further reduced with $NaBH_4$ (Scheme 3.11.3). The product is much more polar and easily purified by flash column chromatography. The 500 MHz ^{1}H NMR spectrum of the product reveals the presence of 4 diastereomers of β-hydroxy compound **52**. For example, there are sixteen peaks in the region 1.0-1.3 ppm for the two methyl groups on the side chain, each methyl of the four isomers being a distinct doublet. No attempt was made to separate these stereo isomers. EIMS shows that molecular ion of **52** at *m/e* 298 are not stable and a relatively very strong peak appears at *m/e* 280 due to elimination of a molecule of water from **52**.



Scheme 3.11.3
An Unseparated Mixture from Treatment of Acetyl Acryloyl Glycoluril Derivative with L-Selectride Was Directly Further Reduced with $NaBH_4$.

However, a further transformation of **52** in order to eliminate water by treatment of **52** with trifluoroacetic (TFA) anhydride and triethylamine was not



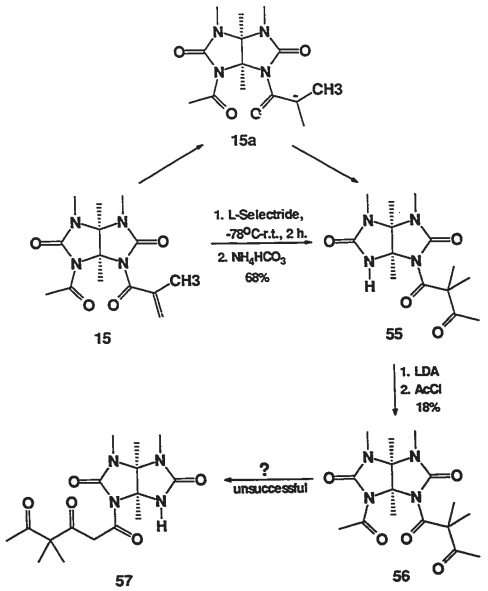
Scheme 3.11.4 Unsuccessful Elimination of Water from Compound 52

successful. The TFA ester derivative **53** was isolated instead. Compound **53** was then treated with different bases (*e.g.*, pyridine, K_2CO_3 and *t*-BuOK) even under heating conditions and none of the desired elimination product **54** or its isomer **54a** was formed (Scheme 3.11.4). The starting material **53** was recovered under mild conditions and destroyed or converted to **52** under severe conditions. Compared with the dehydration reaction of compound **38**, the steric effect due to the α-methyl substituent on the side chain of **53** may prevent the access of a

base, especially a bulky one, to the α-proton. The steric effect may also cause a drastic decrease in the kinetic acidity of the α-proton. In one stable conformation of **53** (Scheme 3.11.4), the side chain α-hydrogen (H_α) is predisposed to lie nearly orthogonal to the conjugate system of the imide carbonyl unit due to the minimization of nonbonding interactions between template, α-methyl, and TFA substituents. Accordingly, allylic (1,3) interactions between either the TFA or the α-methyl group and the template ring carbonyl oxygen can hinder in development of those transition states wherein the carbonyl function is stereoelectronically disposed to contribute to H_α acidification. These arguments were used by Evans *et al.* to account for the low kinetic acidity of α-substituted β-keto imides of oxazolidones.[53]

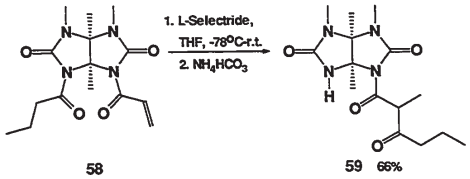
The allylic (1,3) strain mentioned above can reach a maximum in the elimination product **54** (Scheme 3.11.4). It was suspected that the difficulties encountered in pyrolysis of **53** to produce a C=C double bond could be a result of instability in product **54** due to the α-methyl substituent. In order to examine the effect of this α-methyl group, the preparation of the similar compound **15** was attempted by a direct methacryloylation of **5**. Such an attempt was surprisingly successful and **15** was made in 36% yield (Scheme 3.3.2). The steric effect may be a factor contributing to the low yield, but is not large enough to prevent formation of the product. Therefore, how to convert **52** or **53** to product **54** is still an open question but was not further investigated.

Conjugate hydride reduction of **15** provided another interesting reaction. The intermediate enolate **15a** in Scheme 3.11.5 is more hindered, more basic and much less nucleophilic than enolates such as **42a** in Scheme 3.11.1. Despite these disadvantages, a fast acetyl transfer is still favoured to provide the condensation product **55** in 68% yield, which was easily separated by flash column chromatography. A direct acetylation on the NH group of **55** should be feasible due to the absence of more acidic protons between the 1,3-dicarbonyl groups of the side chain. But the acetylation product **56** was obtained in a very low yield (18%). Further attempts to induce condensation of **56** with *t*-BuOLi, LDA and *n*-BuLi were not successful. Compound **56** is very stable to one equivalent of base and was mostly recovered even after 24 hours. If a larger quantity of base was used, **56** was partially converted back to **55** with the acetyl group missing. The steric effect may be the reason for prevention of the intramolecular acyl transfer. However, condensation of compound **13**, which is also expected to have strong steric effects, gave a good yield (79%) of trimethylacetyl-transferred product **22**. Another possible reason is that the γ-protons on the dicarbonyl side chain of **56** are more acidic than the α-protons of the acetyl imide and thus the enolate may be formed on the terminal of the dicarbonyl side chain. However, a second equivalent of base destroyed **56** and converted it to **55** and the mechanism for the acetyl group missing is still unknown.



Scheme 3.11.5 A Hindered Carbanion from Hydride Reduction of a Methacryloyl Derivative Can Be a Very Good Nucleophile.

The intramolecular condensations studied above involve those of diacyl glycoluril derivatives in which at least one acyl group is acetyl. The acetyl group is very small and has a steric advantage to act both as an electrophile or as a nucleophile upon deprotonation. The base-induced condensation of dipropionyl derivative **8**, which does not possess an acetyl group, gave considerably lower yield of condensation product. Compound **58**, an acryloyl butyryl derivative was designed in order to test the generality of the L-Selectride reduction-condensations when a larger saturated acyl unit than the acetyl group is the one to be transferred. By following the standard procedure described above, **58** was treated with one equivalent of L-Selectride and the condensation product **59** was obtained in more than 66% yield (Scheme 3.11.6). This yield is much higher than that of the condensation of **8** induced by t-BuOLi, meaning that 1,4-hydride addition of acryloyl group is a better way to generate an enolate on the template. The product also comprised two diastereomers (2.2:1 ratio) due to the exclusive butyryl group transfer to the α-position of the latent propionyl



58 **59** 66%

Scheme 3.11.6 An Acyl Group Bigger than Acetyl Can Be Condensed with an Enolate Formed by Hydride Reduction of Acryloyl Side Chain.

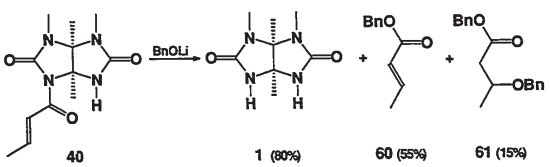
group. This example demonstrates the feasibility of performing a wide range of L-Selectride reduction-condensations between acyl units and an α,β-unsaturated group.

3.12. Removal of Synthesized Carbon Chains from the Template

The products from efficient condensations of two acyl units bound to the bifunctional template **1** can be transformed to allow further acylation, regioselective condensation and further transformations, while the template remains intact, mimicking the head-to-tail assembly sequence of acyl units in the biosynthesis of fatty acids and polyketide-type natural products. These encouraging results demonstrate that this strategy may be a useful synthetic method when selective Claisen condensations could lead to useful intermediates.

A synthesized carbon chain from intramolecular condensations of acyl units on **1** may have to be removed from the template before being used or further transformed, while the recovered template can be recycled. In the previous study of the β-carbonyl reduction of **17** with $NaBH_4$ in MeOH, it was found that the side chain was easily cleaved from the template to give methyl β-hydroxybutyrate if AcOH was not added in time (Scheme 3.7.1). This previously undesired process may provide a useful reaction for the template removal, although this was not further investigated.

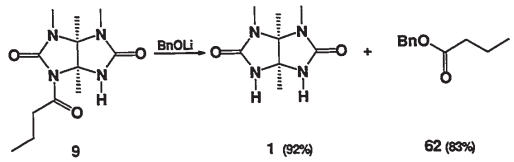
As the benzyl group is a good protecting group and can be easily removed by deprotection, lithium benzyloxide was tested to displace the template **1** from a synthesized acyl glycoluril derivative. Lithium benzyloxide has been previously used by Evans *et al.* to remove synthesized carbon chains from the oxazolidone template.[52,93] 1-Crotonyl-3,4,7,8-tetramethylglycoluril **40**, prepared by modifications (reduction, dehydration) of the first round intramolecular condensation product **17**, was treated with one equivalent of BnOLi (1.5 equivalent of BnOH was used to prepare BnOLi) in THF for 12 hours at room temperature (Scheme 3.12.1). The template **1** was recovered from this mixture in 80% yield. Two other products were benzyl crotonate **60** (55% yield) and benzyl β-benzyloxybutyrate **61** (15% yield). There may be an equilibration between **60** and **61** due to the 1,4-addition and α,β-elimination catalyzed by a base.



40 **1** (80%) **60** (55%) **61** (15%)

Scheme 3.12.1 Removal of a Synthesized Crotonyl Chain from the Template.

A further experiment to displace template **1** with benzyloxide was demonstrated with 1-butyryl-3,4,7,8-tetramethylglycoluril **9** derived from L-Selectride reduction of **40**. Template **1** was recovered in this case in 92% yield and benzyl butyrate **62** isolated in 83% yield (Scheme 3.12.2). These experiments indicate the ability to remove synthesized intermediates from the template and provide further support for the usefulness of the glycoluril template-directed condensation reactions.



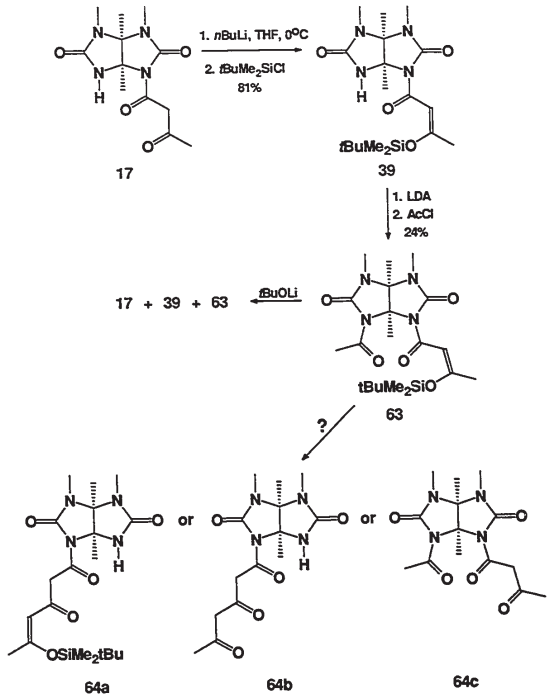
9 **1** (92%) **62** (83%)

Scheme 3.12.2 Removal of a Synthesized Butyryl Chain from the Template.

3.13. Other Experiments

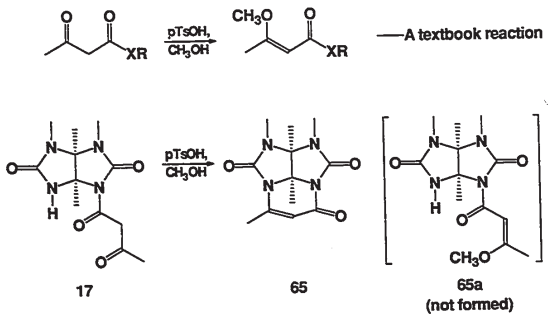
It is interesting and challenging to realize repetitive intramolecular condensations on a molecular template at different oxidation states to generate carbon chains with variable functionalities such as polyketones, polyols or polyenes. As several major steps had been developed to assemble fatty acid and polyene chains on **1**, efforts were made to explore the assembly of carbon chains with polycarbonyl and polyol functionalities. Protection of the β-carbonyl group in **17** is a necessary step for a further acetylation on its NH group. The reaction of **17** with NaH (or *n*-BuLi) and *t*-BuMe$_2$SiCl provided the protected product **39** in 81% yield, which was acetylated on the free NH group to give the diacyl derivative **63** in only 24% yield (Scheme 3.13.1). Compound **39** was partially converted back to **17** during acetylation and lost both of the protecting silyl and acetyl groups. Treatment of **63** in THF with *t*-BuOLi led to recovery of **17**, **39** and **63** without detection of any of the desired condensation product **64a**. Other possible products during the induction of condensation such as **64b** and **64c** were also not found.

Usually, the β-keto carbonyl group of 1,3-dicarbonyl compounds can be protected by acid-catalyzed formation of an enol ether with an alcohol (Scheme 3.13.2).[94] Such a reaction was attempted with **17** by treatment with a catalytic



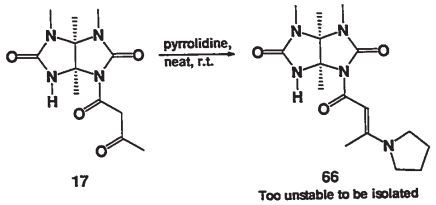
Scheme 3.13.1 Unsuccessful Attempts to Make a Triketide Chain with 3 Carbonyl Groups. None of Compounds 64a, 64b or 64c Was Detected.

amount of *p*-toluene sulphonic acid in methanol, but the only detected product was **65**; formation of the six membered ring occurs due to internal nucleophilic attack of the NH on the β-carbonyl group (Scheme 3.13.2). The desired product **65a** was not formed. Methylation of **17**, by treatment with *t*-BuOLi and methyl iodide, gave no reaction for several hours and produced an un-resolvable mixture after stirring over night, although a similar textbook reaction can lead to methylation at the β-carbon.



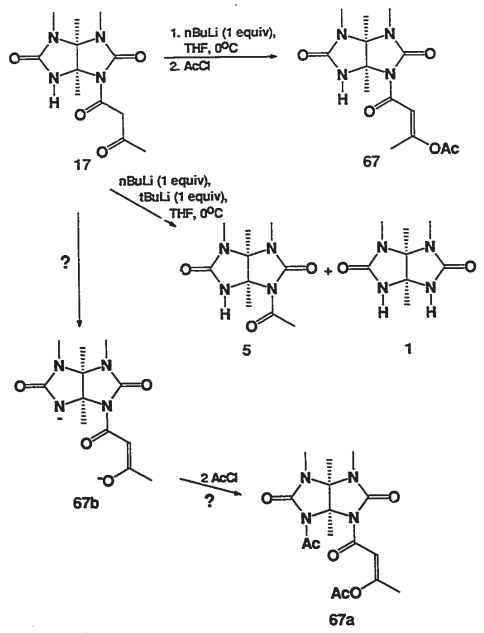
Scheme 3.13.2 An Unsuccessful Attempt to Protect the β-Carbonyl Group by Making a β-Enol Methyl Ether. Only Cyclized Product 65 Was Formed.

When **17** was stirred with neat pyrrolidine, the formation of an enamine product **66** seemed to proceed very efficiently (Scheme 3.12.3). Unfortunately, the product was too unstable to be isolated from the mixture. A column chromatographic purification of crude **66** led to complete recovery of **17**. A direct acetylation of the crude, dry compound **66** also gave only **17** without formation of the desired product.



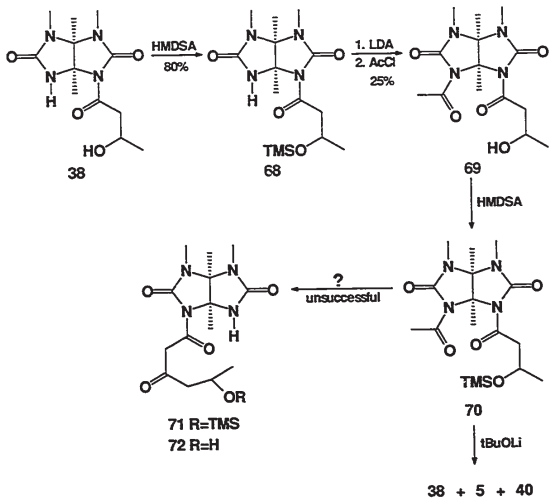
Scheme 3.13.3 A β-Enamine Product Was Observed but not Isolated.

Compound **67** was easily made by treatment of **17** with *n*-BuLi and AcCl at 0°C. But **17** was found to be destroyed by 2 equivalents of butyl lithium at 0°C to afford decomposed products **5** and **1**. When **17** was treated with 2 equivalents of *n*-BuLi and then AcCl at -20°C, the product was also **67** and expected product **67a** from acetylation of a dianion intermediate **67b** was not obtained (Scheme 3.12.4). The dianion intermediate **67b** might not have been formed.



Scheme 3.13.4 A Preparation of 67a Was Unsuccessfully Attempted by Generating Dianion 67b with 2 Equivalents of Base.

In an effort to protect the hydroxy group, the β-hydroxybutyryl glycoluril derivative **38** was stirred with hexamethyldisilazane[95,96] and the protected product **68** was isolated in 80% yield (Scheme 3.12.5). When **68** was treated with LDA and AcCl, the acylated product **69**, with loss of the protecting TMS group, was obtained in 25% yield. Treatment of **69** or its protected form **70** with *t*-BuOLi gave a complicated mixture of **38**, **5** and **40**. The desired condensation products **71** and **72** were not detected.



Scheme 3.13.5
An Unsuccessful Attempt to Make a Triketide Chain with Hydroxy Groups.

CHAPTER 4. SUMMARY

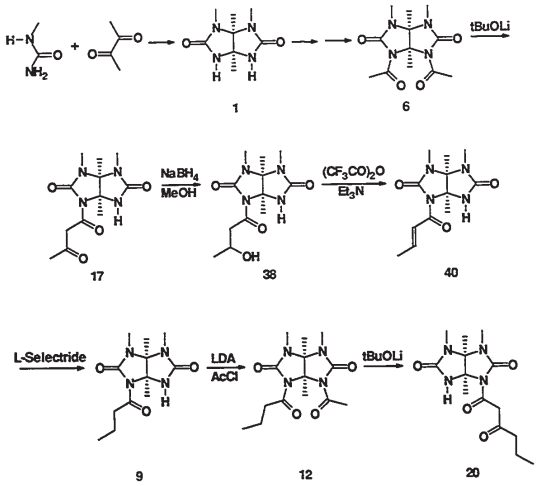
The major work accomplished in this project is summarized in Schemes 4.1, 4.2 and 4.3.

1. A molecular template, 3,4,7,8-tetramethylglycoluril **1** with two NH groups as two acyl binding sites, has been synthesized and isolated (Scheme 4.1).

2. Selective monoacylation allows preparation of symmetrical or asymmetrical diacyl products in good yields.

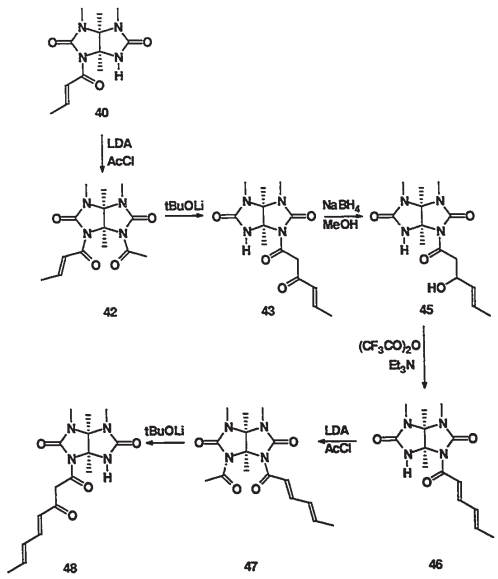
3. Condensations between two acyl units bound to template **1** have been shown to be very efficient and successful, proceeding under mild conditions in high yields. When the two acyl units are different, a fair degree of regioselectivity has been demonstrated, favouring deprotonation on the acyl group with the less substituted α-position and then transfer of the other acyl group.

4. Intramolecularity in the condensations between acyl units on template **1** has been established. The mechanism involves slow cleavage of an α-C-H bond by the base, followed by a fast intramolecular acyl transfer to the resulting enolate.



Scheme 4.1
A Summary of Steps in the Assembly of Short Fatty Acid Chains Using Template 1.

5. Efficient transformations of the first round condensation products such as β-ketobutyryl derivative **17** have been developed to give compounds with a lengthened, saturated chain. After a second acetylation, the system undergoes a second round of condensation (Scheme 4.1). During the study of these two cycles of condensation, some basic problems have been solved in order to

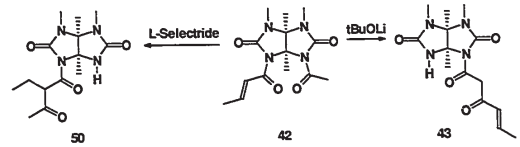


Scheme 4.2 A Summary of Steps in the Assembly of Unsaturated Carbon Chains Using the Glycoluril Template 1.

assemble various fatty acids from acetate units.

6. An unsaturated chain of eight carbons from four acetyl units has been assembled on the template **1** by three repetitive condensations (Scheme 4.2).

7. An alternative method for generation of an enolate for the intramolecular condensation has been realized by a hydride 1,4-addition of L-Selectride to a conjugated acyl unit. This method provides reversal of the regioselectivity, when compared with the condensations induced by deprotonation where acetyl is one of the two acyl groups on the template **1**, as shown in Scheme 4.3.



Scheme 4.3 An Example of L-Selectride Reduction-Condensations Directed by the Glycoluril Template 1 to Give a Reverse Regioselectivity, Compared with a Base Deprotonation-Induced Condensation.

8. Despite the tremendous success of using glycoluril derivative **1** as a template to allow efficient condensations between two acyl units, template **1** has been found to be a good but not a perfect molecular model of the fatty acid or polyketide synthase. The major disadvantage is the steric effect in the binding

region, which limits the preparation of its diacyl derivatives especially if one of the acyl groups is large. On the other hand, enzyme-catalytic generation of enolates and stereoselective condensations are not considered in our glycoluril system. The key parameters we tested are the efficiency of intramolecular condensation due to the initial binding, repetitivity and sequence of adding acyl units to make carbon chains. This research provides some great experience for the design of more sophisticated models of fatty acid and polyketide synthases.

9. Successful removal of a synthesized carbon chain from the template with lithium benzyloxide makes this methodology ready to be used in the preparation of some useful intermediates, especially in the preparation of isotopically labelled precursors for biosynthetic studies as well as in synthesis of useful natural products.

As a final conclusion, the molecular template 3,4,7,8-tetramethyl-glycoluril allows efficient intramolecular condensations between two acyl units bound to it. The condensation products, after modifications such as reduction and dehydration, allowed further acylation and condensations, mimicking the assembly sequence of carbon chains taking place on fatty acid or polyketide synthase.

CHAPTER 5. EXPERIMENTAL SECTION

General

Melting points are uncorrected. Proton and carbon-13 NMR spectra were obtained on Bruker AC 200, Bruker AC 300 or Bruker AC 500 spectrometers. Proton chemical shifts refer to TMS and those of ^{13}C to $CDCl_3$. The infrared (IR) spectra were recorded on a Perkin-Elmer 283 instrument and FTIR spectra on a Bio-Rad SPC 3200. Ultra violet (UV) spectra were run on a Perkin-Elmer Lambda 9 UV/VIS/NIR spectrophotometer. Mass spectra were recorded on a VG analytical ZAB-E machine. Microanalyses were performed by Guelph Chemical Laboratory Ltd., Guelph, Ontario. Flash column chromatography was performed with Kieselgel 60 (230-400 mesh ASTM). THF was freshly distilled under nitrogen protection from potassium/benzophenone, diisopropylamine from calcium hydride and *tert*-butanol from sodium.

Synthesis of 3,4,7,8-Tetramethylglycoluril (1)

To a stirred suspension of N-methylurea (38.0 g, 0.514 mole) in absolute ethanol (65 mL) was added concentrated hydrochloric acid (15 drops) and 2,3-butanedione (22.5 mL, 22.5 g, 0.260 mole). After stirring for 5 minutes, the solution clarified and after 15 minutes it became hot (exothermic). A precipitate

appeared in about 20 min. The mixture was cooled in a refrigerator after stirring for another hour. The crystals were collected, washed with cooled ethanol and ether, and dried in air and then under vacuum. Total crude product was 26 g (51%) as a 1 : 1 mixture of two regioisomers **1** and **1a**. The crude product was recrystallized twice in ethanol (~60 mL ethanol/1 g solid). The desired isomer **1** was obtained as colourless crystals (21%). m.p. (EtOH) >300°C; ^1H NMR (CD_3OD, 200 MHz) δ 2.77 (s, 6H), 1.48 (s, 3H), 1.39 (s, 3H); ^{13}C NMR (CD_3OD, 50 MHz) δ 161.0 (C=O), 83.1, 74.7, 26.8, 22.1, 15.9; FTIR (KBr pellet, cm^{-1}) 3282 (NH), 2920 (C-H), 1683 (C=O), 1506, 1441, 1414, 1389, 1263, 1230, 1120, 1087, 1003, 963, 768, 721; EIMS m/e 198(M^+), 183, 140, 126, 125, 111, 85, 65 (base); HREIMS. Calcd for $C_8H_{14}N_4O_2$: 198.1117. Found: 198.1121.

Isolation of 1,4,7,8-Tetramethylglycoluril (1a):

A mixture of **1** and **1a** (2.0 g, 1:1) was heated at reflux with acetic anhydride (20 mL) for 20 h. Compound **1** was completely converted and dissolved. After cooling down to room temperature, the solid material was collected and washed with $CHCl_3$, THF and cooled ethanol. Compound **1a** was obtained in a yield of 810 mg (81% based on the true quantity of **1a** in the starting material. ^1H NMR (CD_3OD, 200 MHz) δ 2.61 (s, 6H), 1.40 (s, 6H); HREIMS. Calcd for $C_8H_{14}N_4O_2$: 198.1117. Found: 198.1111.

General Procedure for Monoacylation of Glycoluril Template 1

Method *a*:

A mixture of recrystallized template **1** and carboxylic anhydride (10 mL/1g **1**) was heated at 150-160°C overnight under nitrogen. After ~20 h, all the starting material dissolved (the isomer **1a** is not reactive and remains as a solid if any is present in the starting material). The majority of the carboxylic anhydride was removed on the rotary evaporator under vacuum. The residue was cooled and chloroform (10 mL/1g S.M.) was added. Solid material was filtered off to give a clear filtrate, which was concentrated to remove most of the solvent. To this liquid residue (if there is any solid, add minimum amount of $CHCl_3$ to dissolve it) was added ethyl acetate (10 mL/1g S.M.) and the mixture was cooled in the refrigerator overnight to induce crystallization. The crystals were collected, washed with diethyl ether and recrystallised from chloroform and ethyl acetate (dissolved in a minimum amount of $CHCl_3$ and precipitated with EtOAc).

Method *b*:

To a stirred suspension of fine powdered template **1** in dry THF (200 mL/1 g **1**) under nitrogen was added 1.1 equiv of n-BuLi at room temperature. The mixture was then heated at reflux for 2 h. After cooling down to near room temperature, 1.5 equiv. of acyl chloride was added. The solution clarifies in seconds if the acyl chloride is acetyl or propionyl chloride. The mixture was heated at reflux for another half hour. Unreacted solid material was removed by

filtration, and the filtrate was concentrated to give crude solid product. A recrystallization as described in Method *a* was carried out to purify the product.

1-Acetyl-3,4,7,8-tetramethylglycoluril (5):

Compound **1** (2 g, 0.01 mole) was heated with acetic anhydride (20 mL) to give **5** (1.89 g, 78%) from Method *a*. The same in THF (200 mL) was treated with nBuLi (7.0 mL, 0.011 mole, 1.6 M) according to Method *b* to give **5** (2.15 g, 89%) as a white solid. m.p. ($CHCl_3$+AcOEt) 178~180°C; ^1H NMR ($CDCl_3$-TMS, 200 MHz) δ 6.00 (s, 1H, NH), 3.02 (s, 3H, NCH_3), 2.87 (s, 3H, NCH_3), 2.48 (s, 3H, $-COCH_3$), 1.69 (s, 3H, $-CH_3$ bridgehead), 1.56 (s, 3H, $-CH_3$ bridgehead); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 171.0, 157.2, 153.0, 78.5, 76.4, 27.0, 26.3, 24.8, 19.5, 15.6; FTIR (KBr pellet, cm^{-1}) 3250 (NH), 2980 (CH), 2940 (CH), 1723(C=O), 1689 (C=O), 1487, 1416, 1326, 1117, 943; EIMS *m/e* 240 (M^+), 225, 210, 198, 183, 168, 156, 140, 125 (base); HREIMS. Calcd for $C_{10}H_{16}N_4O_3$: 240.1222. Found: 240.1231. Anal. Calcd for $C_{10}H_{16}N_4O_3$: C, 49.99; H, 6.71; N, 23.32. Found: C, 50.27; H, 6.85; N, 23.16.

1-Acetyl-3,6,7,8-tetramethylglycoluril (5a):

Isolated initially as a minor and more polar product in preparation of **5** due to presence of **1a** in starting material. Purified by flash column chromatography (eluting solvent $CHCl_3$:CH_3OH=99:1). **5a** was also prepared by acetylation of **1a** (510 mg, 2.57 mmole) according to Method *b* (310 mg, 50%). ^1H NMR ($CDCl_3$,

200 MHz) δ 6.19 (s, 1H), 2.98, 2.85, 2.51, 1.84 and 1.51 (5 singlets for 5 methyl groups).

1-Propionyl-3,4,7,8-tetramethylglycoluril (7):

Compound **1** (2 g, 0.01 mole) was heated with propionic anhydride (20 mL) according to Method *a*. The product was purified by flash column chromatography (2% MeOH in $CHCl_3$) to give **7** (2.14 g, 83%) as a white solid. The 1,6-dipropionyl compound **8** was obtained in 9% yield; For **7**, m. p. (from $CHCl_3$) 160~162°C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 6.29 (s, 1H, NH), 3.01 (s, 3H, NCH_3), 2.87 (s, 3H, NCH_3), 3.0 ~2.74 (m, 2H, CH_2), 1.70 (s, 3H, CH_3 bridgehead), 1.58 (s, 3H, CH_3 bridgehead), 1.11 (t, 3H, CH_3-propionyl, J = 7.3 Hz); ^{13}C NMR ($CDCl_3$ 50 MHz) 174.5, 157.0, 152.7, 78.4, 77.2, 29.6, 26.7, 26.1, 19.3, 15.4, 7.9; IR (KBr pellet, cm^{-1}) 3265 (NH), 3108, 3000, 2942, 1730 (C=O), 1718 (C=O), 1685 (C=O), 1461, 1411; EIMS *m/e* 254 (M^+), 197, 180, 168, 156, 140, 125 (base); HREIMS. Calcd for $C_{11}H_{18}N_4O_3$: 254.1379. Found: 254.1370.

1,6-Dipropionyl-3,4,7,8-tetramethylglycoluril (8):

Isolated in the preparation of **7**. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.00 (dq, 2H, J = 7.2 Hz, 17.7 Hz), 2.96 (s, 6H), 2.76 (dq, 2H, J = 7.2 Hz, 17.7 Hz), 1.95 (s, 3H), 1.49 (s, 3H), 1.13 (t, J = 7.4 Hz); EIMS *m/e* 310 (M^+), 254, 225, 199, 180, 168, 156, 141, 125 (base), 83, 56; HREIMS. Calcd for $C_{14}H_{22}N_4O_4$: 310.1641. Found: 310.1653.

1-Butyryl-3,4,7,8-tetramethylglycoluril (9):

Compound **1** (2.6 g, 0.013 mole) was heated with butyric anhydride (20 mL) according to Method *a*. The product was purified by flash column chromatography (2% MeOH in $CHCl_3$) to give **9** (2.48 g, 70%) as a white solid. The 1,6-dibutyryl compound **10** was obtained in 5% yield; For **9**, m.p. ($CHCl_3$) 125~126°C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 6.01 (s, 1H, NH), 3.01 and 2.87 (s, 3H, 2 NCH_3), 2.96~2.75 (m, 2H, CH_2), 1.68 and 1.56 (s, 3H, 2 CH_3), 1.70~1.50 (m, 2H, CH_2), 0.96 (t, 3H, CH_3, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 174.1, 157.2, 153.0, 78.6, 76.5, 38.2, 27.1, 26.4, 19.6, 17.7, 15.7, 13.6; IR (KBr pellet, cm^{-1}) 3252 (NH), 2998, 2956, 2875, 1710 (C=O), 1689 (C=O), 1458, 1408; EIMS *m/e* 268 (M^+), 253, 240, 211, 199, 183, 168, 156, 141, 125 (base); HREIMS. Calcd for $C_{12}H_{20}N_4O_3$: 268.1535. Found: 268.1526. Anal. Calcd for $C_{12}H_{20}N_4O_3$: C, 53.71; H, 7.51; N, 20.88. Found: C, 53.63; H, 7.22; N, 20.64.

General Preparation of Diacylated Compounds

One equiv of n-BuLi in hexane or freshly prepared LDA in THF was added to a stirred solution of monoacylated template in THF (~80 mL/g starting material) at 0°C. After stirring for 30 min., an acyl chloride in 20-50% excess was added. After being stirred for another 1 h at room temperature, this solution was concentrated to remove most of the THF. To the liquid residue, chloroform (~30 mL/g S.M.) was added, and the mixture was stirred and then cooled. The

resulting solid was removed by filtration and the solvent was removed from the filtrate to give a crude product, which was separated to give the pure diacyl product and the minor starting material by flash column chromatography (1% methanol in chloroform).

1,6-Diacetyl-3,4,7,8-tetramethylglycoluril (6):

1-Acetyl-3,4,7,8-tetramethylglycoluril **5** (910 mg, 3.79 mmole) was treated with n-BuLi (2.5 mL, 4 mmole, 1.6 M) and then AcCl (0.4 mL, 5.6 mmole) to produce **6** (834 mg, 78%, white solid) with partial recovery of **5** (17%); m.p. 194~196°C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.97 (s, 6H, 2 CH_3), 2.48 (s, 6H, 2 CH_3), 1.95 (s, 3H, CH_3 bridgehead), 1.51 (s, 3H, CH_3 bridgehead); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 170.5, 153.1, 80.4, 77.3; 26.7, 26.0, 19.0, 14.5; IR (KBr pellet, cm^{-1}) 2975, 2920, 1705 (C=O), 1688 (C=O), 1399, 1358, 1314, 1248, 1082, 1035, 994, 930, 869, 744, 696; EIMS *m/e* 282 (M^+), 240, 225, 210, 198, 183, 168, 156, 141, 125 (base); HREIMS. Calcd for $C_{12}H_{18}N_4O_4$: 282.1328. Found: 282.1336. See the X-ray crystal structure in Fig. 3.3.1.

1-(Acetyl-d_3)-6-acetyl-3,4,7,8-tetramethylglycoluril (33)

Compound **5** (1.18 g, 4.92 mmole) was treated with n-BuLi (3.0 mL, 4.8 mmole, 1.6 M) and then AcCl-d_3 (0.35 mL, 4.72 mmole) to produce **33** (0.948 g, 68%, white solid). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.98 (s, 6H), 2.47 (s, 3H), 1.95 (s, 3H), 1.53 (s, 3H); ^{2}H NMR ($CHCl_3$, 77 MHz, reference $CDCl_3$ 7.248): 2.43 (s);

^{13}C NMR ($CDCl_3$, 200 MHz) δ 170.1, 152.8, 80.1, 77.6, 26.6, 25.9, 18.7, 14.2 (^{13}C NMR signal from CD_3 was too weak to be observed); EIMS *m/e*: 285 (M^+). HREIMS. Calcd for $C_{12}D_3H_{15}N_4O_4$: 285.1513. Found: 285.1515.

1-(Acetyl-$^{13}C_2$)-6-acetyl-3,4,7,8-tetramethylglycoluril (26)

Compound **5** (1.53 g, 6.38 mmole) was treated with n-BuLi (4.3 mL, 6.40 mmole, 1.49 M) and then AcCl-$^{13}C_2$ (0.6 g, 7.45 mmole) to produce **26** (1.44 g, 80%, white solid). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.99 (s, 6H), 2.47 (s & dd, 6H, $^1J_{CH}$ = 130 Hz, $^2J_{CH}$ = 6.8 Hz; integration ratio of s : dd = 1 : 1.25); 1.95 (s, 3H); 1.55 (s, 3H). ^{13}C NMR ($CDCl_3$, 200 MHz) δ 170.0 (d, enhanced, $^1J_{CC}$ = 52.4 Hz), 170.0, 152.7, 80.0, 77.5, 25.7 (d, enhanced, $^1J_{CC}$ = 52.2 Hz), 25.7, 18.6, 14.1; EIMS *m/e* (abundance %): 286 (3.2, containing 4 carbon-13 atoms), 285 (4), 284 (10.4), 242 (20.2), 240 (14.5), 198 (12.8), 168 (20), 156 (31.2), 141 (48), 125 (100); HREIMS. Calcd for $C_{10}{}^{13}C_2H_{18}N_4O_4$: 284.1396. Found: 284.1385.

1-Acetyl-6-propionyl-3,4,7,8-tetramethylglycoluril (11):

1-Propionyl-3,4,7,8-tetramethylglycoluril **7** (2.52 g, 9.92) was treated with nBuLi (8.0 mL, 10.4 mmole, 1.3 M) and then AcCl (0.75 mL, 15.5 mmole) to produce **11** (1.59 g, 54%) with partial recovery of **7** (19%); m.p. 180~182°C; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.08~2.70 (m, 2H, CH_2), 2.99 and 2.98 (s, 3H, NCH_3), 2.47 (s, 3H, CH_3), 1.95 and 1.54 (s, 3H, 2 CH_3 bridgehead), 1.13 (t, 3H, CH_3, J=7.3 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 174.1, 170.1, 152.8 (2C=O), 80.2, 77.6,

30.8, 26.4 ($2CH_3$-N), 25.8, 18.8, 14.2, 8.2; IR (KBr pellet, cm^{-1}) 2975, 2942, 1758 (C=O), 1722 (C=O), 1445, 1411; EIMS *m/e* 296 (M^+), 254, 241, 225, 199, 183, 168, 156, 141, 125 (base), 86; HREIMS. Calcd for $C_{13}H_{20}N_4O_4$: 296.1484. Found: 296.1497. Anal. calc. for $C_{13}H_{20}N_4O_4$, C, 52.96; H, 6.80; N, 18.91. Found: C, 52.83; H, 6.60; N, 19.26.

1-Acetyl-6-butyryl-3,4,7,8-tetramethylglycoluril (12):

1-Butyryl-3,4,7,8-tetramethylglycoluril **9** (1.20 g, 4.48 mmole) was treated with nBuLi (2.8 mL, 4.48 mmole) and then AcCl (0.5 mL, 7.6 mmole) to produce **12** (0.85 g, 61%) with partial recovery of **9** (20%); m.p. (chloroform) 130~132°C; ^1H NMR ($CDCl_3$, 200 MHz) δ 2.99 (s, 6H, 2 NCH_3), 2.97~2.66 (m, 2H, -CH_2), 2.47 (s, 3H, CH_3), 1.95 and 1.55 (s, 3H, 2 CH_3 bridgehead), 1.75~1.59 (m, 2H, CH_2), 0.95 (t, 3H, CH_3, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 173.1, 170.1, 152.71, 152.66, 80.1, 77.5, 39.1, 26.4, 26.3, 25.8, 18.7, 17.4, 14.1, 13.3; IR (KBr pellet, cm^{-1}) 2980, 2945, 1756, 1735, 1722, 1410; EIMS *m/e* 310 (M^+), 282, 268, 241, 199, 183, 168, 156, 141, 125(base); HREIMS. Calcd for $C_{14}H_{22}N_4O_4$: 310.1641. Found: 310.1633. Anal. Calcd: C, 54.18; H, 7.15; N, 18.06. Found: C, 54.05; H, 7.07; N, 17.81.

1-Acetyl-6-trimethylacetyl-3,4,7,8-tetramethylglycoluril (13):

1-Acetyl-3,4,7,8-tetramethylglycoluril **5** (550 mg, 2.3 mmole) was treated with nBuLi (1.5 mL, 2.4 mmole, 1.6 M) and then trimethylacetyl chloride (0.350

mL, 2.8 mmole) according to the standard procedure, yieiding 273 mg (52%) of the desired product **13**. m.p. (EtOAc): 180-182°C; ^1H NMR ($CDCl_3$, 200 MHz) δ 3.02 (s, 3H), 2.90 (s, 3H), 2.50 (s, 3H), 1.91 (s, 3H), 1.56 (s, 3H), 1.32 (s, 9H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 183.3, 170.5, 153.6, 152.8, 80.5, 78.4, 43.7, 27.2, 27.0, 26.9, 25.2, 19.5, 15.4; FTIR (KBr pellet, cm^{-1}) 2985, 2924, 1748, 1733, 1710, 1690, 1449, 1411, 1386, 1327, 1274, 1229, 1113, 1093, 945, 886, 811, 760, 740, 683, 603, 587; EIMS *m/e* 325 (M^++1), 269, 211, 225, 198, 168, 141, 126 (base), 125.

1-Acetyl-6-acryloyl-3,4,7,8-tetramethylglycoluril (14):

1-Acetyl-3,4,7,8-tetramethylglycoluril **5** (9.6 g, 0.04 mole) was treated with nBuLi (25 mL, 0.04 mole, 1.6 M) and then acryloyl chloride (4.5 mL, 0.055 mole) according to the standard procedure to give the desired product **14** (4.9 g, 41%, polymerization is probably the side reaction); ^1H NMR ($CDCl_3$, 200 MHz) δ 7.23 (dd, 1H, J = 16.9 Hz, 10.3 Hz), 6.45 (dd, 1H, J = 17.0 Hz, 1.9 Hz), 5.78 (dd, 1H, J = 10.3 Hz, 1.8 Hz), 2.98 (s, 6H), 2.49 (s, 3H), 1.99 (s, 3H), 1.53 (s, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 170.4, 165.5, 153.0, 130.4, 129.7, 80.5, 77.9, 26.8, 26.7, 19.0, 14.6; FTIR (KBr pellet, cm^{-1}), 3033, 2943, 1754, 1729, 1706, 1619, 1452, 1399, 1338; UV (MeOH): λ_{max} = 226 nm, ε = 9,930; EIMS *m/e* 294 (M^+), 252, 168, 125 (base); Anal. Calcd for $C_{13}H_{18}N_4O_4$: C, 53.05; H, 6.16; N, 19.04. Found: C, 53.14; H, 6.00; N, 19.10.

1-Acetyl-6-methacryloyl-3,4,7,8-tetramethylglycoluril (15):

1-Acetyl-3,4,7,8-tetramethylglycoluril **5** (1.35 g, 5.6 mmole) was treated with nBuLi (3.5 mL, 5.6 mmole, 1.6 M) and then methacryloyl chloride (0.65 mL, 6.65 mmole) according to the standard procedure, to give the desired product **15** (0.63 g, 36%); ^1H NMR ($CDCl_3$, 200 MHz) δ 5.40 (s, 2H), 3.00 (s, 3H), 2.87 (s, 3H), 2.44 (s, 3H), 1.95 (s, 3H), 1.89 (s, 3H), 1.53 (s, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 170.7, 169.8, 152.7, 152.4, 141.4, 121.7, 79.7, 77.9, 26.7, 26.5, 24.9, 18.8, 18.2, 14.9; EIMS *m/e* 308 (M^+), 266, 168, 142, 126 (base); HREIMS. Calcd for $C_{14}H_{20}N_4O_4$: 308.1485. Found: 308.1485.

1-Acryloyl-6-butyryl-3,4,7,8-tetramethylglycoluril (58):

1-Butyryl-3,4,7,8-tetramethylglycoluril **9** (0.728 g, 2.72 mmole) was treated with nBuLi (1.7 mL, 2.72 mmole, 1.6 M) and then acryloyl chloride (0.27 mL) according to the standard procedure to give the desired product **58** (0.312 g, 36%); m.p. (chloroform + ether), 143-145°C; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.19 (dd, 1H, J = 10.4 Hz, 17.0 Hz), 6.44 (dd, 1H, J = 1.8 Hz, 17.0 Hz), 5.76 (dd, 1H, J = 1.8 Hz, 10.3 Hz), 2.98 (s, 3H), 2.96 (s, 3H), 3.01~2.71 (m, 2H), 1.99 (s, 3H), 1.70~1.60 (m, 2H), 1.51(s, 3H), 0.95 (t, 3H, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.6, 165.6, 153.1, 130.6, 129.6, 80.7, 78.0, 39.4, 26.8 ($2CH_3N$), 19.2, 17.9, 14.7, 13.6; FTIR (KBr pellet, cm^{-1}): 2965, 2935, 1754, 1731, 1706, 1401; UV (MeOH): λ_{max} = 223.6 nm, ε = 11,700; EIMS *m/e* 322 (M^+), 294, 253, 179, 125 (base); Anal. Calcd for $C_{15}H_{22}N_4O_4$: C, 55.88; H, 6.88; N, 17.38. Found: C,

56.00; H, 6.95; N, 17.43.

The General Procedure for Condensations to Make Diketide Compounds

A solution of 1.1 equiv of t-BuOLi was prepared by adding 1.5 equiv of dry t-BuOH and then 1.1 equiv of n-BuLi in hexane to THF(~3 mL/mmole n-BuLi) with stirring at 0°C. This solution was stirred at room temperature for 5-10 min and then added to a vigorously stirred solution of 1.0 equiv of starting diacylated template in THF (80 mL/g S.M.) at 0°C. After 20 min, the reaction was complete by TLC and a large excess of ammonium bicarbonate powder was added and the mixture was stirred for 5 h. The solid was filtered off through Celite and the filtrate was concentrated on the rotary evaporator to give a crude product, which was purified by flash column chromatography (methanol : chloroform = 1 : 98). White solid products were obtained.

1-(3'-Oxobutyryl)-3,4,7,8-tetramethylglycoluril (17):

Compound **6** (0.364 g, 1.29 mmole) was treated with lithium t-butoxide to give **17** (0.339 g, 93%); m.p. (chloroform) 154~156°C; ^1H NMR ($CDCl_3$, 200 MHz) δ 13.45 (s, 0.09H, enol OH), 6.51 (s, 0.09H, olefinic proton for enol), 5.95 (s, 1H, NH), 4.31 (d, 0.91H, CH_2, ^2J = 16.4 Hz), 3.59 (d, 0.91H, CH_2, ^2J = 16.4 Hz), 3.01 (s, 0.27H, enol NCH_3), 2.98 (s, 2.73H, NCH_3) 2.86 (s, 3H, NCH_3), 2.25 (s, 2.73H, CH_3CO), 2.02 (s, 0.27H, CH_3 for enol side chain), 1.74 and 1.56 (s, 3H, 2 CH_3-bridgehead); ^{13}C NMR ($CDCl_3$, 50 MHz, for keto form only) δ 201.3,

167.1, 157.1, 152.8, 78.7, 76.6, 52.2, 30.0, 27.1, 26.4, 19.2, 15.7; FTIR (KBr pellet, cm^{-1}) 3224 (NH), 3095, 2931, 1690-1760 (C=O, very strong and broad), 1500, 1415, 1394, 1385, 1341, 1312, 1264, 1215, 1167, 1108, 1088, 998, 970, 901, 789, 761, 652, 591, 544; UV (MeOH): λ_{max} = 272.4 nm (ε = 970), λ = 211.0 nm (ε = 7600); EIMS *m/e* 282 (M^+), 267, 240, 210, 183, 156, 140, 125; HREIMS. Calcd for $C_{12}H_{18}N_4H_4$: 282.1328. Found: 282.1319. Anal. Calcd for $C_{12}H_{18}N_4H_4$: C, 51.05; H, 6.43; N, 19.85. Found: C, 50.90; H, 6.35; N, 19.67.

1-(4'-*d*$_3$-3'-Oxobutyryl)-3,4,7,8-tetramethylglycoluril (35):

Compound **33** (310 mg, 1.09 mmole) was treated with tBuOLi to give **35** (247 mg, 80%), which contained 17% nondeuterated product **17**; ^1H NMR ($CDCl_3$, 500 MHz) δ 5.97 (s, 1H, NH), 4.30 (d, 1H, ^2J = 16.4 Hz), 3.60 (d, 1H, ^2J = 16.5 Hz), 2.98 (s, 3H), 2.86 (s, 3H), 2.25 (s, 0.52H), 1.74 (s, 3H), 1.56 (s, 3H); ^2H NMR ($CHCl_3$, 77 MHz) δ 2.20 (s); EIMS *m/e* 285 (M^+), 282, 267, 241, 213, 210, 125 (base); HREIMS. Calcd for $C_{12}D_3H_{15}N_4O_4$: 285.1513. Found: 285.1512.

1-(1',2'-$^{13}C_2$-3'-Oxobutyryl)-3,4,7,8-tetramethylglycoluril (28), 1-(3',4'-$^{13}C_2$-3'-oxobutyryl)-3,4,7,8-tetramethylglycoluril (29) and **1-(1',2',3',4'-$^{13}C_4$-3'-oxobutyryl)-3,4,7,8-tetramethylglycoluril (30)**:

This mixture was obtained in a yield of 0.304 g (87%) from condensation of **26** (0.35 g); ^1H NMR ($CDCl_3$, 200 MHz) δ 5.96 (s, 1H, NH), 4.69~3.21 (m, 2H), 2.98 (s, 3H), 2.86 (s, 3H), 2.25 (s & dd, 3H, $^1J_{CH}$ = 128.0 Hz, 6.1 Hz, integration

ratio of s : dd = 1 : 1.27), 1.74 (s, 3H), 1.56 (s, 3H); EIMS *m/e* (abundance %): 286 (3.2), 285 (3.2), 284 (9.6), 242 (4), 240 (3.2), 212 (17.6), 140 (24), 125 (100); HREIMS. Calcd for $C_{10}{}^{13}C_2H_{18}N_4O_4$ of **28** or **29**: 284.1396. Found: 284.1385.

1-(3'-Oxovaleryl)-3,4,7,8-tetramethylglycoluril (18):

Compound **11** (350 mg, 1.18 mmole) was used to give a inseparable mixture of **18** and **19** (284 mg, 81%), which contained 19% of 3'-oxo-2'-methylbutyryl compound **19**. For **18**, m.p. ($CHCl_3$) 148~152°C; ^1H NMR ($CDCl_3$, 200 MHz) δ 5.99 (s, 1H, NH), 4.33 (d, 1H, CH_2, ^2J = 16.4 Hz), 3.56 (d, 1H, CH_2, ^2J = 16.4 Hz), 2.99 and 2.86 (s, 3H, 2 NCH_3), 2.54 (q, 2H, CH_2, J = 7.2 Hz), 1.75 and 1.56 (s, 3H, 2 CH_3 bridgehead), 1.08 (t, 3H, CH_3, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 204.4, 167.4, 157.3, 152.8, 78.8, 76.7, 51.2, 35.9, 27.1, 26.4, 19.3, 15.7, 7.4; IR (KBr, cm^{-1}) 3230 (NH), 2991, 2940, 1743 (C=O), 1717 (C=O), 1689 (C=O), 1457, 1410; EIMS *m/e* 296 (M^+), 267, 254, 240, 224, 168, 140, 125, 97, 86, 70; HREIMS. Calcd for $C_{13}H_{20}N_4O_4$: 296.1484. Found: 296.1482.

1-(3'-Oxohexanoyl)-3,4,7,8-tetramethylglycoluril (20):

Compound **12** (270 mg, 0.87 mmole) was used to give a inseparable mixture of **20** and **21** (237 mg, 88%), which contained 17% of 3'-oxo-2-ethylbutyryl compound **21**. For **20**, m.p. ($CHCl_3$) 119-121°C; ^1H NMR ($CDCl_3$, 200 MHz) δ 6.12 (s, 1H, NH), 4.32 (d, 1H, CH_2, ^2J = 16.4 Hz), 3.57 (d, 1H, CH_2, ^2J = 16.4 Hz), 2.97 and 2.86 (s, 3H, 2 NCH_3), 2.61~2.46 (m, 2H, CH_2), 1.75 and

1.57 (s, 3H, 2 CH_3), 1.71~1.60 (m, 2H, CH_2), 0.93 (t, 3H, CH_3, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 203.7, 167.3, 157.1, 152.7, 78.6, 76.5, 51.4, 44.6, 27.0, 26.3, 19.2, 16.7, 15.6, 13.5; IR (KBr pellet, cm^{-1}) 3255 (NH), 2962, 2941, 1745 (C=O), 1725 (C=O), 1692 (C=O), 1466, 1413; EIMS *m/e* 310 (M^+), 282, 267, 240, 199, 183, 125; HREIMS. Calcd for $C_{14}H_{22}N_4O_4$: 310.1641. Found: 310.1637.

1-(3'-Oxo-4',4'-dimethylvaleryl)-3,4,7,8-tetramethylglycoluril (22):

Compound **13** (100 mg, 0.308 mmole) in THF was treated with t-BuOLi at 0°C by following the standard procedure, but the reaction was allowed to proceed for 5 hours (maybe not necessary), producing the condensation product **22** (79 mg, 79%). ^1H NMR ($CDCl_3$, 200 MHz) 13.26 (s, enol OH), 6.64 (s, olefinic H of enol), 6.04 (s, 1H, NH), 4.57 (d, 1H, ^2J = -16.6 Hz), 3.68 (d, 1H, ^2J = -16.6 Hz), 3.02 (s, enol NCH_3), 2.97 (s, 3H), 2.86 (s, 3H), 1.76 (s, 3H), 1.57 (s, 3H), 1.20 (s, -tBu for β-ketone form), 1.19 (s, -tBu for enol form); ^{13}C NMR ($CDCl_3$, 50 MHz) 209.9 (β-C=O), 189.8 (weak, for enol β-carbon), 171.6 (weak, enol), 168.4, 157.2, 152.6, 86.6 (enol α-carbon), 78.6, 76.6, 46.6, 44.3, 27.4, 27.1, 26.5, 26.4, 19.9 (enol), 19.2, 15.6; FTIR (KBr pellet, cm^{-1}) 3268 (NH), 3104, 2961, 2924, 1737, 1711, 1692, 1610 (weak), 1491, 1451, 1410, 1333, 1176, 1112, 1064, 760, 696, 664; EIMS *m/e* 325 (M^++1), 267, 143, 125 (base); HREIMS. Calcd for $C_{15}H_{25}N_4O_4$ (M+1$^+$): 325.1876. Found: 325.1851.

1-(3'-Oxo-2'-methylvaleryl)-3,4,7,8-tetramethylglycoluril (25):

Method a: Compound **8** (102 mg, 0.33 mmole) in THF (10 mL) was treated with t-BuOLi at 0°C by following the standard procedure, but the reaction was allowed to proceed for 2 hours (maybe not necessary). A fraction from flash column chromatography was obtained to be a 1:1 mixture of the condensation product **25** and the monopropionyl compound **7** (total 62 mg). Compound **8** (21 mg) was recovered here.

Method b: Compound **8** (128 mg, 0.41 mmole) in THF (10 mL) was treated with lithium *iso*-propoxide at 0°C by following *Method a*. A 1:3 mixture of the condensation product **25** and the monopropionyl compound **7** (total 58 mg) was obtained with recovery of **8** (14 mg).

Compound **25** containing **7** was obtained as 2 diastereomers (**25a**, **25b**) in a 2:1 ratio (NMR integration). The more polar diastereomer **25b** was partially isolated in a pure form in a second round column purification. For **25a**: ^1H NMR ($CDCl_3$, 200 MHz) δ 6.01 (s, 1H, NH), 4.47 (q, 1H, J = 7.2 Hz), 2.95 (s, 3H), 2.85 (s, 3H), 2.75-2.53 (m, 2H), 1.75 (s, 3H), 1.55 (s, 3H), 1.33 (d, 3H, J = 7.3 Hz), 1.07 (t, 3H, J = 7.2 Hz). For **25b**: ^1H NMR ($CDCl_3$, 200 MHz) δ 6.01 (s, 1H, NH), 4.59 (q, 1H, J = 7.2 Hz), 2.98 (s, 3H), 2.88 (s, 3H), 2.59-2.46 (m, 2H), 1.68 (s, 3H), 1.57 (s, 3H), 1.34 (d, 3H, J = 7.2 Hz), 1.05 (t, 3H, J = 7.2 Hz).

Reduction of β-Keto Acyl Compounds with $NaBH_4$

To a solution of the β-ketoacyl compound in MeOH (35 mL/g) was added

sodium borohydride (1.5-1.8 equiv) at 0°C. This mixture was stirred for 10 min at room temperature and 10 mL glacial acetic acid was then added immediately. The rotary evaporator was used to remove the solvents (both of MeOH and AcOH) to give a solid mixture, to which was added 40 mL dichloromethane. The mixture was stirred for 5 min and solid material was filtered off. The filtrate was concentrated and purified through a flash column (MeOH : $CHCl_3$ = 2 : 98).

1-(β-Hydroxybutyryl)-3,4,7,8-tetramethylglycoluril (38):

Compound **17** (1.06 g, 3.76 mmole) was treated with $NaBH_4$ (0.21 g, 5.55 mmole). The desired product was obtained in a yield of 0.985 mg (93%) as a mixture of 2 diastereomers. ^1H NMR ($CDCl_3$, 200 MHz) δ 6.19 (s, 1H, NH), 4.24 (m, 10 peaks, 1H, CH), 3.27-2.80 (m, 2H, CH_2), 3.01 (s, 3H, NCH_3), 2.87 (s, 3H, NCH_3) 1.71 and 1.56 (s, 3H, 2 CH_3), 1.26 (d, 3H, CH_3, ^3J = 6.3 Hz); ^{13}C ($CDCl_3$, 50 MHz, features for 2 diastereomers) δ 173.6, 157.1, 152.9, 78.7, 76.7, 64.1, 44.7, 27.1, 26.4, 22.4, 19.7, 15.5; EIMS *m/e* 284 (M^+), 284, 266, 240, 156, 140, 126 (base), 111, 86; HREIMS. Calcd for $C_{12}H_{20}N_4O_4$: 284.1485. Found: 284.1492. Anal. Calcd for $C_{12}H_{20}N_4O_4$: C, 50.69; H, 7.09; N, 19.71. Found: C, 50.42; H, 7.10; N, 19.65.

Dehydration to Make 1-Crotonyl-3,4,7,8-tetramethylglycoluril (40):

1-(β-hydroxybutyryl)-3,4,7,8-tetramethylglycoluril **38** (640 mg, 2.25 mmole) was dissolved in 15 mL CH_2Cl_2, followed by addition of 3 equiv of trifluoroacetic

anhydride at 23°C and stirring for 1 h. A solution of Et_3N (2 equiv.) in 5 mL CH_2Cl_2 was added dropwise and 10 min later, 2 mL Et_3N was added. The mixture was heated at reflux for 20 min. All liquid was removed on the rotatory evaporator and the residue was purified by flash column chromatography (MeOH : $CHCl_3$ = 1 : 99). The desired product was obtained in a yield of 500 mg (83%) with E : Z ratio = 97.4 : 2.6. ^1H NMR ($CDCl_3$, 200 MHz) δ 7.27 (d, 1H, shows fine structure due to the long range coupling with Me, CH, ^3J = 15.2 Hz, ^4J = 1.4 Hz), 7.08 (dq, 1H, CH, ^3J = 15.2 Hz, 6.4 Hz), 6.13 (s, 1H, NH), 3.02 and 2.87 (s, 3H, N-CH_3), 1.93 (dd, 3H, CH_3, ^3J = 6.5 Hz, ^4J = 1.3 Hz), 1.71 and 1.57 (s, 3H, 2 CH_3s); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 165.2, 157.1, 152.6, 144.8, 123.0, 78.2, 76.2, 26.7, 26.0, 19.3, 18.0, 15.3; FTIR (KBr pellet, cm^{-1}) 3310 (NH), 2990, 1722 (C=O), 1667 (C=O), 1627; UV (CH_3OH): λ_{max}=229 nm, ε=12 K; EIMS *m/e* 266 (M^+, base), 251, 192, 168, 142, 125; Anal. Calcd for $C_{12}H_{18}N_4O_3$: C, 54.12; H, 6.81; N, 21.04. Found: C, 53.92; H, 6.78; N, 20.79.

1-Acetyl-6-crotonyl-3,4,7,8-tetramethylglycoluril (42):

Compound **40** (700 mg, 2.63 mmole) was treated with nBuLi (1.5 mL, 2.4 mmole, 1.6 M) and then acetyl chloride (0.3 mL) according to the procedure used for making **6**; The title product was purified with a flash column ($CHCl_3$ as eluting solvent) and obtained in a yield of 502 mg (62%, with 12% recovery of starting material); ^1H NMR ($CDCl_3$, 500 MHz) δ 7.09~7.02 (m, 1H, CH), 6.96 (d, 1H, CH, ^3J = 15.3 Hz), 2.95 (s, 6H, NCH_3), 2.48 (s, 3H, CH_3), 1.98 and 1.47 (s, 3H, 2

CH_3), 1.91 (d, crotonyl-CH_3, ^3J = 6.6 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 170.5, 165.6, 153.2, 153.1, 144.6, 124.9, 80.5, 77.8, 26.7 (2CH_3N), 26.0, 19.1, 18.3, 14.6; FTIR (KBr pellet, cm^{-1}) 2939, 1749 (C=O), 1722 (C=O), 1703 (C=O), 1637 (C=O), 1408, 1341; UV (CH_3OH): λ_{max}=206 nm, ε=11 K; EIMS *m/e* 308 (M^+), 280, 241, 192, 167, 124, 125 (base); Anal. Calcd for $C_{14}H_{20}N_4O_4$: C, 54.53; H, 6.54; N, 18.17. Found: C, 54.48; H, 6.88; N, 18.06.

1-(3'-Oxo-4'-hexenoyl)-3,4,7,8-tetramethylglycoluril (43):

The starting material **42** (220 mg, 0.71 mmole) was treated with t-BuOLi at 0°C in the same way as described for preparation of **17** to give **43** (132 mg, 60%); m.p. 147~150°C (from $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz, shows 37% enol form) δ 13.2(s, 1H, enol-OH), 6.96(dq, 1H, CH, J = 6.7 Hz, 15.7 Hz), 6.74 (dq, 1H, CH, J = 6.6 Hz, 15.8 Hz), 6.51(s, 1H, CH), 6.16~6.12 (m, 2H, NH & CH), 5.90 (d, 1H, CH, J = 15.3 Hz), 4.47 (d, 1H, CH_2, J = 16.2 Hz), 3.75 (d, 1H, CH_2, J = 16.2 Hz), 3.0 (s, 3H, enol-NCH_3), 2.97 (s, 3H, NCH_3), 2.86 (s, NCH_3 for both ketone and enol), 1.93 (dd, 3H, ketone C<u>H</u>$_3$CH=, J = 6.9 Hz, 1.7 Hz), 1.88 (dd, 3H, enol C<u>H</u>$_3$CH=, J = 7.0 Hz, 1.6 Hz), 1.76 (s, 3H, CH_3-bridgehead), 1.74 (s, 3H, enol CH_3-bridgehead), 1.56 (s, 3H, Bridgehead CH_3 of ketone & enol); ^{13}C NMR ($CDCl_3$, 50 MHz, shows a mixture of keto and enol isomers) δ 192.9, 172.6, 171.5, 167.8, 157.2, 152.8, 144.2, 137.3, 131.2, 126.6, 90.8, 78.7, 78.6, 76.8, 76.7, 49.0, 27.1, 26.4, 20.0, 19.3, 18.3, 15.7; FTIR (KBr pellet, cm^{-1}) 3223, 3094, 2948, 1741, 1717, 1690, 1668; UV (CH_3OH): λ_{max}=304 nm, ε=3.5 K; EIMS *m/e*

308 (M^+), 236, 125 (base); HREIMS Calcd for $C_{14}H_{20}N_4O_4$: 308.1485. Found: 308.1453. Anal. Calcd for $C_{14}H_{20}N_4O_4$: C, 54.53; H, 6.54; N, 18.17. Found: C, 53.97; H, 6.63; N, 17.78.

1-(3'-Hydroxy-4'-hexenoyl)-3,4,7,8-tetramethylglycoluril (45):

Compound **43** (540 mg, 1.75 mmole) was reduced with $NaBH_4$ (110 mg) in MeOH by using the previously described general reduction procedure to give **45** (240 mg, 44%) with 51% recovery of starting material. ^1H NMR ($CDCl_3$, 200 MHz, shows 2 diastereomers) δ 6.12 & 6.10 (s, 1H, NH), 5.76~5.71 and 5.58~5.53 (m, 2H, HC=CH), 4.4~4.6 (m, 1H, CH), 3.30~3.00 (m, 2H, CH_2), 3.02 (s, 3H, NCH_3), 2.87 (s, 3H, NCH_3), 1.71 and 1.57 (s, 3H, 2 CH_3), 1.69(d, 3H, CH_3, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 173.0, 157.1, 152.1, 132.1, 127.1, 78.7, 76.7, 68.8, 43.5, 27.1, 26.4, 19.7, 19.6, 17.6, 15.7; FTIR (KBr pellet, cm^{-1}) 3390 (OH), 3322 (NH), 1721 (C=O); EIMS *m/e* 310 (M^+), 292, 240, 199, 125 (base); HREIMS Calcd for $C_{14}H_{22}N_4O_4$ 310.1641. Found: 310.1654.

1-(2',4'-Hexadienoyl)-3,4,7,8-tetramethylglycoluril (46):

Compound **45** (194 mg, 0.626 mmole) was treated with trifluoroacetic anhydride (0.25 mL) and triethyl amine (0.1 mL and then 2 mL), using the procedure for making **40** to give **46** (157 mg, 86%). ^1H NMR (CD_2Cl_2, 200 MHz) δ 7.37 (dd, 1H, J = 9.4 Hz, 15.5 Hz), 7.23 (d, 1H, J = 15.1 Hz), 6.39~6.12 (m, 2H), 2.99 (s, 3H), 2.82 (s, 3H), 1.86 (d, 3H, J = 5.6 Hz), 1.68 (s, 3H), 1.54 (s,

3H); ^{13}C NMR (CD_2Cl_2, 50 MHz) δ 166.4, 157.6, 153.3, 145.5, 140.4, 130.8, 120.4, 78.9, 77.1, 27.3, 26.5, 20.0, 18.9, 15.9; FTIR (KBr Pellet, cm^{-1}) 3289, 3089, 2915, 1731, 1712, 1668, 1597; UV (MeOH): λ_{max}=319 nm, ε=26 K; EIMS *m/e*: 292(M^+), 218, 168, 125 (base); Anal. Calcd for $C_{14}H_{20}N_4O_3$: C, 57.52; H, 6.90; N, 19.16. Found: C, 57.41; H, 6.98; N, 18.81.

1-Acetyl-6-(2',4'-hexadienoyl)-3,4,7,8-tetramethylglycoluril (47):

Compound **46** (160 mg, 0.54 mmole) in THF (30 mL) was treated with 1.1 equiv of LDA and AcCl (0.05 mL, 0.7 mmole) using the procedure for making **6** to give **47** (68 mg, 38%) with 45% recovery of starting material. ^1H NMR ($CDCl_3$, 500 MHz) δ 7.37 (dd, 1H, *J* = 10.9 Hz, 15.0 Hz), 6.97 (d, 1H, *J* = 15.1 Hz), 6.26 (t, 1H, *J* = 11.4 Hz, 14.9 Hz), 6.15 (dq, 1H, *J* = 6.6 Hz, 14.8 Hz), 2.97 (s, 3H), 2.96 (s, 3H), 2.49 (s, 3H), 1.99 (s, 3H), 1.84 (d, 3H, *J* = 6.8 Hz), 1.51 (s, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 170.6, 166.1, 153.2, 145.3, 139.8, 130.5, 121.1 80.7, 77.8, 26.9, 26.7, 26.2, 19.2, 18.7, 14.7; FTIR (KBr Pellet, cm^{-1}) 3447 (NH), 2939, 1735 (C=O), 1702 (C=O); UV (MeOH): λ_{max}=275.4 nm, ε=9.3 K; EIMS *m/e*: 334 (M^+), 293, 241, 149, 125 (base); HREIMS Calcd for $C_{16}H_{22}N_4O_4$: 334.1641. Found: 334.1646.

1-(3'-Oxo-4',6'-octadienoyl)-3,4,7,8-tetramethylglycoluril (48):

Compound **47** (40 mg, 0.12 mmole) was treated with *tert*-BuOLi using the procedure for making **17** to give **48** (24 mg, 60%); ^1H NMR ($CDCl_3$, 500 MHz,

shows 46% enol) δ 13.18 (s, 1H, enol-OH), 7.15~5.83 (m, 4H, Olefinic Hs), 6.56 (s, 1H, enol -α-H), 4.51 (d, 1H, *J* = 16.1 Hz), 3.74 (d, 1H, *J* = 16.1 Hz), 3.02 (s, 3H, enol-NCH_3), 2.97 (s, 3H, NCH_3), 2.86 (s, 3H, NCH_3 for both enol & ketone), 1.87 (d, 3H, *J* = 5.0 Hz), 1.85 (d, 3H, *J* = 6.7 Hz), 1.76 (s, 3H), 1.74 (s, 3H), 1.56 (s, 3H); ^{13}C NMR ($CDCl_3$, 200 MHz) δ 193.3, 172.9, 167.9, 157.2, 152.6, 144.3, 141.4, 138.5, 137.4, 130.6, 130.1, 126.6, 123.7, 91.9, 78.7, 77.7, 49.3, 27.1, 26.4, 20.0, 19.3, 18.8, 15.7; FTIR (KBr, cm^{-1}) 3426 (NH), 2927, 1719 (C=O); UV (MeOH): λ = 319, 280 and 215 nm, ε = 10,800, 11,600 and 11,500, respectively; EIMS *m/e*: 334 (M^+), 319, 260, 198, 125, 83 (base); HREIMS Calcd for $C_{16}H_{22}N_4O_4$: 334.1641. Found: 334.1633.

Olefinic reduction of 40 to make 1-butyryl-3,4,7,8-tetramethylglycoluril (9):

The crotonyl compound **40** (220 mg, 0.82 mmole) was dissolved in 100 mL dry THF under N_2 and cooled in an acetone-dry ice bath. L-Selectride (0.82 mL, 0.82 mmole, 1 M in THF) was added through a syringe with stirring. The mixture was allowed to reach room temperature over 2 h. NH_4HCO_3 was added as a quenching agent and the mixture was stirred for another 4 h. The solid was filtered off through Celite and the filtrate concentrated and purified by flash column chromatography ($CHCl_3$ as eluting solvent). The desired product was obtained in a yield of 164 mg (74%), and showed exactly the same analytical features as those of the compound made from the direct butyrylation of template **1.**

Olefinic reduction of 40 and *in situ* acetylation to make 1-butyryl-6-acetyl-3,4,7,8-tetramethylglycoluril (12):

By using the same procedure as described above for L-Selectride reduction of crotonyl compound **40** (100 mg, 0.385 mmole), acetyl chloride (0.05 mL) was used to quench the reaction instead of NH_4HCO_3. And the Celite filtration was not necessary. The desired product was obtained in a yield of 73 mg (63%), and showed exactly the same analytical features as those of the compound made from the direct acetylation of **9.**

General Procedure for L-Selectride Reduction-Condensation Reactions

To a solution of the diacylated compound (in which at least one of the acyl groups is α,β-unsaturated) in dry THF (150 mL/1g starting material) with stirring and under protection of N_2, was added 1 equiv of L-selectride at -78°C. The mixture was allowed to reach room temperature in 2 h. NH_4HCO_3 was added as a quencher and stirred for another ~8 h. The solid was found to be difficult to remove by filtration and the filtration was done slowly with the aid of Celite. The filtrate was concentrated and purified by flash column chromatography ($CHCl_3$ as eluting solvent). The column purification must be performed with great care in order to give satisfactory resolution.

L-Selectride reduction of 14 to make 51:

The starting material **14** (1.21 g, 4.1 mmole) in THF was treated with L-

Selectride. The ratio of 2 diastereomeric products was determined to be 2.1 : 1 by ^1H NMR analysis of the crude reaction mixture. Five fractions were collected from flash column chromatography. The first fraction with the largest R_f was identified as reduced but uncondensed compound **11** (3%). The second fraction of products was obtained in a yield of 132 mg, and was estimated by ^1H NMR to contain 53% of monoacetyl compound **5** and 47% of the less polar single diastereomer of **1-(2'-methyl-3'-oxobutyryl)-3,4,7,8-tetramethyl-glycoluril 51a.** The third fraction (340 mg) contained **5** (6%) and **51a** (94%). The forth fraction (460 mg) contained the 2 diastereomers of **51** in a 1:1 ratio. The fifth fraction of 56 mg contains the more polar diastereomer **51b** (97%) and the less polar isomer **51a** (3%). The total yield of condensation product is more than 70%. The two pure single diastereomers can be obtained by recrystallization of fraction 3 and fraction 5, respectively.

The **less polar diastereomer 51a**: ^1H NMR ($CDCl_3$, 200 MHz) δ 5.94 (s, 1H, NH), 4.48 (q, 1H, *J* = 7.3 Hz), 2.96 (s, 3H), 2.86 (s, 3H), 2.29 (s, 3H), 1.74 (s, 3H), 1.55 (s, 3H), 1.34 (d, 3H, *J* = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 205.9, 170.5, 157.2, 153.0, 78.9, 76.9, 53.8, 28.2, 27.0, 26.4, 19.0, 15.7, 12.0; FTIR (KBr, cm^{-1}) 3346 (NH) 3000, 2940, 1721 (C=O), 1689 (C=O), 1489, 1457, 1411, 1353, 1309, 1245, 1160, 1110; EIMS *m/e* 296 (M^+), 254, 222, 199, 180, 156, 140, 125 (base); HREIMS. Calcd for $C_{13}H_{20}N_4O_4$: 296.1485. Found: 296.1495.

The **more polar diastereomer 51b**: ^1H NMR ($CDCl_3$, 200 MHz) δ 6.05(s,

1H, NH), 4.60 (q, 1H, J = 7.2 Hz), 2.99 (s, 3H), 2.89 (s, 3H), 2.22 (s, 3H), 1.70 (s, 3H), 1.57 (s, 3H), 1.35 (d, 3H, J = 7.2 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 204.2, 171.1, 157.0, 153.0, 78.7, 76.6, 53.6, 28.3, 27.1, 26.6, 19.9, 15.8, 12.5; FTIR (KBr pellet, cm^{-1}) 3319 (NH), 2989, 1720 (C=O), 1698 (C=O), 1490, 1412, 1302, 1112, 1089, 938, 762; EIMS *m/e* 297 (M^++1), 254, 125 (base); HREIMS. Calcd for $C_{13}H_{20}N_4O_4$: 296.1485. Found: 296.1488.

1-(2'-Methyl-3'-hydroxybutyryl)-3,4,7,8-tetramethylglycoluril (52):

A mixture (total 351 mg) of the 2 diastereomeric (2.1 : 1) 1-(2'-methyl-3'-oxobutyryl)-3, 4, 7, 8-tetramethyl-glycolurils **51a** and **51b** containing monoacetyl compound **5** (~23%) was treated with $NaBH_4$ (67 mg) using the procedure for $NaBH_4$ reduction of 1-(3'-oxobutyryl)-3,4,7,8-tetramethylglycoluril **17**. The desired title product was obtained in a yield of 210 mg (60% based on the total mass of the starting mixture) and easily separated from monoacetyl compound **5**. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.28, 6.16, 6.12 and 6.11 (singlets, total 1H, NH for 4 diastereomers), 4.10~3.65 (m, 2H), 2.99 (s, 3H), 2.84 (s, 3H), 1.68, 1.67, 1.66 and 1.65 (singlets, total 3H), 1.53 (s, 3H), 1.23~1.08 (16 peaks, 6H, for 2 methyl groups of side chain); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 177.8, 177.4, 177.2, 157.4, 157.3, 157.2, 153.3, 153.2, 78.5, 77.2, 76.8, 71.0, 70.0, 68.6, 67.4, 45.6, 43.6, 43.5, 27.2, 26.4, 21.5, 20.9, 20.6, 19.7, 19.6, 19.5, 19.1, 15.8, 15.7, 14.8, 14.4, 10.9, 10.3; EIMS *m/e* 299 (M^++1), 280 (M^+-H_2O), 254, 125 (base). HREIMS. Calcd for $C_{13}H_{23}N_4O_4$ (M^++1): 299.1719. Found: 299.1704.

L-Selectride reduction of 42 to make 1-(2'-ethyl-3'-oxobutyryl)-3,4,7,8-tetramethylglycoluril (50):

Compound **42** (377 mg, 1.22 mmole) was treated with L-Selectride (1.22 mL, 1.22 mmole, 1 M). The ratio of 2 diastereomeric products was determined to be 1.6:1 by ^{1}H NMR of the crude reaction mixture. The first fraction with the largest R_f was identified as reduced but uncondensed compound **12** (71 mg, 19%). The second fraction was the monoacetyl compound **5** (26 mg, 9%). The third contains the major less polar diastereomer of the product (**50a**) containing a trace of monoacetyl compound and of the more polar diastereomer, which underwent a repeated column chromatographic separation to remove most monoacetyl compound. The fourth fraction is the more polar diastereomer **50b** in pure form. The total condensation product yield was 197 mg (52%). The 2 diastereomers were partially separated. For the **less polar diastereomer 50a**: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 5.97(s, 1H), 4.42 (dd, 1H, J = 9.0 Hz, 3.9 Hz), 2.97 (s, 3H), 2.86 (s, 3H), 2.28 (s, 3H), 1.97~1.90 (m, 2H), 1.73 (s, 3H), 1.55 (s, 3H), 1.00 (t, 3H, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 205.1, 169.8, 157.2, 153.0, 78.7, 76.9, 60.8, 29.0, 27.1, 26.4, 20.6, 19.1, 15.7, 12.7. **For the more polar diastereomer 50b**: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 6.04 (s, 1H, NH), 4.58 (dd, 1H, J = 8.0 Hz, 5.3 Hz), 2.99 (s, 3H), 2.88 (s, 3H), 2.21 (s, 3H), 1.99~1.81 (m, 2H), 1.71 (s, 3H), 1.57 (s, 3H), 0.97 (t, 3H, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 203.4, 170.5, 156.9, 153.0, 78.6, 76.6, 60.6, 28.9, 27.1, 26.6, 21.4, 19.9, 15.9, 12.4; EIMS *m/e* 311 (M^++1), 310 (M^+), 268, 125 (base); HREIMS. Calcd for

$C_{14}H_{22}N_4O_4$: 310.1641. Found: 310.1627.

L-Selectride reduction-condensation to make 1-(2',2'-dimethyl-3'-oxobutyryl)-3,4,7,8-tetramethylglycoluril (55):

Compound **15** (148 mg, 0.48 mmole) was treated with L-Selectride (0.48 mL, 0.48 mmole, 1 M) using the general procedure to give **55** (101 mg, 68%); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 6.02 (s, 1H, NH), 2.95, 2.86, 2.21, 1.72, 1.55, 1.47 and 1.37 (7 singlets for methyl groups); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 207.5, 173.8, 157.1, 152.3, 78.7, 77.2, 57.7, 27.0, 26.4, 25.0, 23.5, 22.5, 19.1, 15.7; EIMS *m/e* 311 (M^++1), 268, 199, 194, 125 (base); Anal. Calcd for $C_{14}H_{22}N_4O_4$: C, 54.18; H, 7.14; N, 18.05. Found, C, 54.05; H, 7.09; N, 17.98.

1-Acetyl-6-(2',2'-dimethyl-3'-oxobutyryl)-3,4,7,8-tetramethylglycoluril (56):

The starting material **55** (62 mg, 0.20 mmole) was treated with nBuLi (0.13 mL, 0.208 mmole) and AcCl (0.024 mL, 0.34 mmole) according to the general preparation of diacylated Compounds. Compound **56** was isolated in a yield of 13 mg (18%) and **55** was recovered in 50% yield. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.97, 2.90, 2.50, 2.18, 1.98, 1.51, 1.47, 1.39 (singlets for 8 methyl groups); ^{13}C NMR ($CDCl_3$, 50 Hz) δ 207.3, 173.2, 170.5, 153.1, 152.8, 81.2, 78.9, 59.1, 26.8, 26.7, 26.0, 25.2, 24.3, 22.5, 19.0, 14.8; EIMS *m/e* 353 (M^+), 310, 268, 241, 199, 125 (base); HREIMS. Calcd for $C_{16}H_{25}N_4O_5$: 353.1825. Found: 353.1823.

L-Selectride reduction-condensation to make 1-(2'-methyl-3'-oxohexanoyl)-3,4,7,8-tetramethylglycoluril (59):

The starting material **58** (168 mg, 0.52 mmole) led to a column-purified condensation mixture 126 mg (containing 15 mg of monobutyryl compound **5** by ^{1}H NMR). The yield for the condensation product was estimated to be 66% (diastereomeric ratio 1 : 2.1). The 2 diastereomers were partially separated in a second round of column separation. For the **less polar diastereomer 59a**: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 5.96 (s, 1H, NH), 4.46 (q, 1H, J = 7.4 Hz), 2.95 (s, 3H), 2.85 (s, 3H), 2.68 & 2.54 (2 dt, AB pattern, 2H, J = 7.1 Hz, -17.8 Hz), 1.74 (s, 3H), 1.65~1.57 (m, 2H), 1.33 (d, 3H, J = 7.3 Hz), 0.92 (t, 3H, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 208.2, 170.8, 157.3, 152.9, 78.8, 77.2, 53.4, 42.5, 27.0, 26.4, 19.0, 16.7, 15.7, 13.7, 12.3. For the **more polar diastereomer 59b**: m. p. (chloroform + ether) 134~136°C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.00 (s, 1H), 4.60 (q, 1H, J = 7.2 Hz), 2.98 (s, 3H), 2.87 (s, 3H), 2.54 & 2.45 (2 dt, AB pattern, 2H, J = 7.4 Hz, -17.5 Hz, 7.2 Hz), 1.69 (s, 3H), 1.65~1.53 (m, 2H), 1.57 (s, 3H), 1.34 (d, 3H, J = 7.2 Hz), 0.90 (t, 3H, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 206.1, 171.5, 157.0, 152.9, 78.6, 76.6, 53.1, 42.7, 27.1, 26.5, 19.8, 16.9, 15.9, 13.6, 12.6; EIMS *m/e* 324 (M^+), 281, 254, 199, 180, 152, 125 (base). Anal. Calcd for $C_{15}H_{24}N_4O_4$: C, 55.54; H, 7.46; N, 17.28. Found: C, 55.79; H, 7.42; N, 17.44.

Removal of a Synthesized Carbon Chain from Template 1

Lithium benzyloxide was prepared by addition of 1 equiv n-butyl lithium to

1.5 equiv benzyl alcohol in THF (~8 ml/mmole). To a solution of compound **9** (100 mg, 0.37 mmole) or **40** (165 mg, 0.62 mmole) in THF (20 ml) was added the prepared lithium benzyloxide (1 equiv) at room temperature. The mixture was stirred for 12 hours and 2 equivalents of glacial acetic acid was added. THF was removed on the rotary evaporator and $CHCl_3$ was added. The mixture was stirred for a further 0.5 hours. Solid product was collected by filtration, washed with $CHCl_3$ and THF and dried in air for 2 days. The solid product was found to be pure template **1** and obtained in yields 68 mg (92%) and 69 mg (80%), respectively for **9** and **40**. The filtrate was concentrated and purified with a flash column (hexane as eluting solvent).

Benzyl crotonate (60): Obtained in a yield of 43 mg (55%) from **40**; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.30 (m, 5H), 7.03 (dq, 1H, J = 15.5 Hz, 6.9 Hz), 5.89 (dq, 1H, J = 15.6 Hz, 1.5 Hz), 5.17 (s, 2H), 1.88 (dd, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 166.3, 145.1, 136.1, 128.5, 128.1 (remarkably high), 122.4, 65.9, 18.0; FTIR (neat, cm^{-1}) 3034, 2947, 1721, 1658, 1263, 1174, 1103, 1015, 969, 838, 738, 698; EIMS *m/e* 176 (M^+), 158, 149, 131, 107, 91 (base), 77, 69, 65; HREIMS. Calcd for $C_{11}H_{12}O_2$: 176.0837. Found: 176.0818.

Benzyl β-benzyloxybutyrate (61): obtained in a yield 19 mg (15%) from **40**; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.33 (m, 10H), 5.13 (s, 4H), 4.06 (m, 1H), 2.70 (dd, 1H, J = 15.1 Hz, 7.4 Hz), 2.49 (dd, 1H, J = 15.0 Hz, 5.6 Hz), 1.26 (d, 3H, J = 6.2 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 171.3, 135.9, 128.5, 128.3, 128.2, 127.6, 127.5, 127.0, 72.0, 70.9, 66.3, 42.1, 19.8.

Benzyl butyrate (62): obtained in a yield of 55 mg (83%) from **9**; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.36 (m, 5H), 5.11 (s, 2H), 2.33 (t, 2H, J = 7.4 Hz), 1.67 (sextet, 2H, J = 7.4 Hz), 0.94 (t, 3H, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 173.4, 136.1, 128.5 (2C ?), 128.1 (2C ?), 66.0, 36.1, 18.4, 13.6; EIMS *m/e* 178 (M^+), 108, 91 (base), 71, 43; HREIMS. Calcd for $C_{11}H_{14}O_2$: 178.0994. Found: 178.1003.

1-(3'-*tert*-Butyldimethylsilyloxycrotonyl)-3,4,7,8-tetramethylglycoluril (39):

Method a: To a stirred solution of compound **17** (60 mg, 0.21 mmole) in THF (25 mL) was added $NaBH_4$ (8 mg, 0.21 mmole) and $tBu(Me)_2SiCl$ (35 mg, 0.23mmole) at 0°C. The mixture was stirred for 10 hours at room temperature. THF was removed with a rotary evaporator and the mixture residue was separated by flash column chromatography (eluting solvent: $CHCl_3$:EtOAc = 90:10). Product **39** was obtained in a yield of 42 mg (50%).

Method b: To a stirred solution of compound **17** (360 mg, 1.28 mmole) in THF (40 mL) was added n-butyl lithium (0.8 mL, 1.28 mmole, 1.6 M) at 0°C. After stirring for 20 minutes, $tBu(Me)_2SiCl$ (335 mg, 2.56 mmole, 2 equiv) was added. The mixture was stirred for 12 hours at room temperature. THF was removed with a rotary evaporator and the mixture residue was separated by flash column chromatography (eluting solvent: $CHCl_3$:EtOAc = 90:10). Product **39** was obtained in a yield of 413 mg (81%).

^{1}H NMR ($CDCl_3$, 200 MHz) δ 6.70 (s, 1H), 6.12 (s, 1H), 3.00(s, 3H), 2.86

(s, 3H), 2.28 (s, 3H), 1.71 (s, 3H), 1.54 (s, 3H), 0.94 (s, 9H), 0.28 (s, 6H).

1-Acetyl-6-(3'-*tert*-butyldimethylsilyloxycrotonyl)-3,4,7,8-tetramethylglycoluril (63):

Compound **39** (102 mg, 0.26 mmole) was acetylated by following the general preparation of diacyl glycoluril compounds to afford product **63** in a yield of 28 mg (24%) after purification with a flash column (eluting solvent: $CHCl_3$:EtOAc = 90:10). ^{1}H NMR ($CHCl_3$, 200 MHz) δ 6.32 (s, 1H), 2.96 (s, 6H), 2.49 (s, 3H), 2.28 (s, 3H), 1.98 (s, 3H), 1.49 (s, 3H), 0.95 (s, 9H), 0.28 (s, 3H), 0.26 (s, 3H).

Compound 65:

A solution of **17** (100 mg, 0.355 mmole) in methanol (10 mL) in the presence of a catalytic amount of *p*-toluenesulfonic acid was stirred at room temperature for 5 hours. The solvent was then removed on the rotary evaporator and the residue was purified by flash column chromatography (eluting solvent $CHCl_3$:CH_3OH = 99:1). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 5.51 (s, 1H), 3.00, 2.97, 2.40, 1.71, 1.64 (5 singlets for 5 methyl groups).

1-(3'-Acetoxycrotonyl)-3,4,7,8-tetramethylglycoluril (67):

To a stirred solution of compound **17** (400 mg, 1.42 mmole) in THF (50 mL) was added 1 equiv n-butyl lithium at 0°C. After stirring for 20 minutes, acetyl

chloride (2 equiv) was added. The mixture was stirred for 2 hours at room temperature. THF was removed on the rotary evaporator and the residue was separated by flash column chromatography (eluting solvent: $CHCl_3$:MeOH = 99:1). The yield for product **67** was 59%. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.03 (s, 1H), 6.05 (s, 1H), 3.00, 2.86, 2.22, 2.05, 1.68 and 1.59 (6 singlets for 6 methyl groups).

1-(3'-Trimethylsilyloxybutyryl)-3,4,7,8-tetramethylglycoluril (68):

To a stirred solution of **38** (130 mg, 0.458 mmole) in dichloromethane (50 mL) was added 5 equivalents of hexamethyldisilazane at room temperature. The mixture was then heated at reflux for 18 hours. The solvent was removed on the rotary evaporator and the residue was separated by flash column chromatography (eluting solvent: $CHCl_3$:MeOH = 99:1). Product **68** was obtained in a yield of 131 mg (80%) yield. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 6.06 and 6.03 (1H, NH for 2 diastereomers), 4.40-4.20 (m, 1H), 3.30-2.70 (m, 2H), 3.01 (s, 3H), 2.86 (s, 3H), 1.69 (s, 3H), 1.56 (s, 3H), 1.21 (2 doublets for 2 diastereomers, 3H, J = 6.1 Hz, 6.1 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 171.7, 157.0, 152.8, 78.4, 77.2, 65.0, 45.9, 27.0, 26.3, 23.9, 19.6, 15.7; EIMS *m/e* 356 (M^+), 341, 284, 268, 240, 198, 160, 126 (base); HREIMS. Calcd for $C_{15}H_{28}N_4O_4Si$: 356.1880. Found: 356.1877.

1-Acetyl-6-(3'-hydroxybutyryl)-3,4,7,8-tetramethylglycoluril (69):

The starting material **68** (70 mg, 0.20 mmole) in THF was treated with LDA and AcCl according to the general procedure for the preparation of di-acylglycoluril compounds and **69** was obtained from a flash column separation of the product mixture in a yield of 16 mg (25%). ^{1}H NMR ($CDCl_3$, 200 MHz) δ 6.06 (s, 1H), 4.24 (m, 1H), 3.24 (dd, 1H, *J* = 17.2 Hz, 2.8 Hz), 3.01 (s, 3H), 2.87 (s, 3H), 2.85 (dd, 1H, *J* = 17.2 Hz, 9.0 Hz), 1.71 (s, 3H), 1.57 (s, 3H), 1.25 (d, 3H, *J* = 6.4 Hz); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 170.7, 153.0, 80.5, 77.5, 64.0, 46.6, 26.9, 26.8, 22.1, 19.2, 14.6; CIMS *m/e* 344 (M^++18), 327 (M^++1, base), 309, 241, 213, 169.

CHAPTER 6. REFERENCES

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