

**Synthesis of Azatriquinacene**  
**and**  
**Backbone Modified DNA using Transition**  
**Metal Catalysis**

*by*

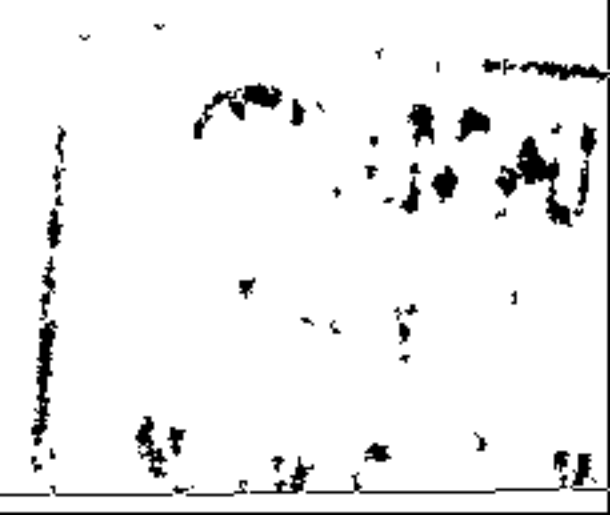
**Manuel de Lera Ruiz**



A Thesis submitted to the University of Nottingham

for the degree of Doctor of Philosophy

October 2001



*To my wonderful parents*

# DECLARATION

I declare that the substance of this Thesis has not been submitted, nor is concurrently being submitted, in candidature for any other degree. I also declare that the work embodied in this Thesis is the result of my own investigations. Where the work of other investigators has been used, this has been fully acknowledged in the text.

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Manuel de Lera Ruiz

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Christopher J. Hayes

# ACKNOWLEDGEMENTS

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I thank the University of Nottingham for financial support.

Finally, I would like to thank my wife and all my family and friends for their support throughout the last three years.



# ABBREVIATIONS

A	adenine
AcOEt	ethyl acetate
aq	aqueous
Arg	arginine
B	base
BBN	borabicyclononane
Bn	benzyl
Boc	<i>tert</i> -butoxy carbamate
BtOH	benzotriazole
Bu	<i>n</i> -butyl
<sup>t</sup> Bu	<i>tert</i> -butyl
C	cytosine
conc	concentrated
Cys	cysteine
dba	dibenzylidene acetone
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCM	dichloromethane
DMAP	4-dimethylaminopyridine
DMF	<i>N,N</i> -dimethylformamide
DMT	dimethoxytrityl
DNA	deoxyribonucleic acid
DIAD	diisopropylazodicarboxylate
DIPEA	diisopropylethylamine

dppf	1,1'- <i>bis</i> (diphenyl-phosphino)ferrocene
EDC	1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide
EDTA	ethylenediaminetetraacetic acid
eq	equivalent
Et	ethyl
Fmoc	9-fluorenylmethyloxycarbonyl
G	guanine
Glu	glutamic acid
HRMS	high resolution mass spectrometry
KHMDS	potassium <i>bis</i> (trimethylsilyl)amide
L	ligand
LAH	lithium aluminium hydride
Lys	lysine
M	metal
Me	methyl
MeOH	methyl alcohol
mRNA	messenger ribonucleic acid
MS	mass spectrometry
N-base	nitrogenated base
NBS	<i>N</i> -bromosuccinimide
NIS	<i>N</i> -iodosuccinimide
NMM	<i>N</i> -methyldmorpholine
nmr	nuclear magnetic resonance
nOe	nuclear Overhauser effect
OAc	acetate

P	protecting group
Ph	phenyl
<sup>i</sup> Pr	isopropyl
RNA	ribonucleic acid
RT	room temperature
SM	starting material
T	thymidine
TBAF	tetrabutylammonium fluoride
TBS	<i>t</i> -butyldimethylsilyl
TBDPS	<i>t</i> -butyldiphenylsilyl
TEA	triethylamine
Tf	trifluoromethanesulfonyl
Tf <sub>2</sub> O	trifluoromethanesulfonic anhydride
TFP	<i>tri</i> -2-furyl phosphine
THF	tetrahydrofuran
tRNA	transference ribonucleic acid
Δ	heat

# **Part I**

## **SYNTHESIS OF AZATRIQUINACENE**

**Supervised by Dr Mark Mascal**

## Abstract

- Azatriquinacene (10-Azatricyclo[5.2.1.0<sup>1,10</sup>]deca-2,5,8-triene) and azatriquinadiene (10-Azatricyclo[5.2.1.0<sup>1,10</sup>]deca-2,8-diene) have been synthesised in respectively eight and seven steps from pyrrole.<sup>27</sup> Woodward dimerisation<sup>4</sup> of azatriquinacene has been attempted although no evidence of diazadodecahedrane has yet been found.
- A unique nonacyclic species (10-Azatricyclo[5.2.1.0<sup>1,10</sup>]-2,9-bis[1-azatricyclo[5.2.1.0<sup>1,10</sup>]decane]dec-1-ene) has been obtained by trimerisation of an enamine.<sup>19</sup> Its structure and extraordinarily high basicity (pK<sub>a</sub> 25.1) make it a new class of “proton sponge”.
- During this study thirteen new substituted azatriquinanes and four new substituted azabicycles have been synthesised, and nine crystal structures have been solved, providing valuable insights into the chemistry and structure of this novel heterocyclic system.

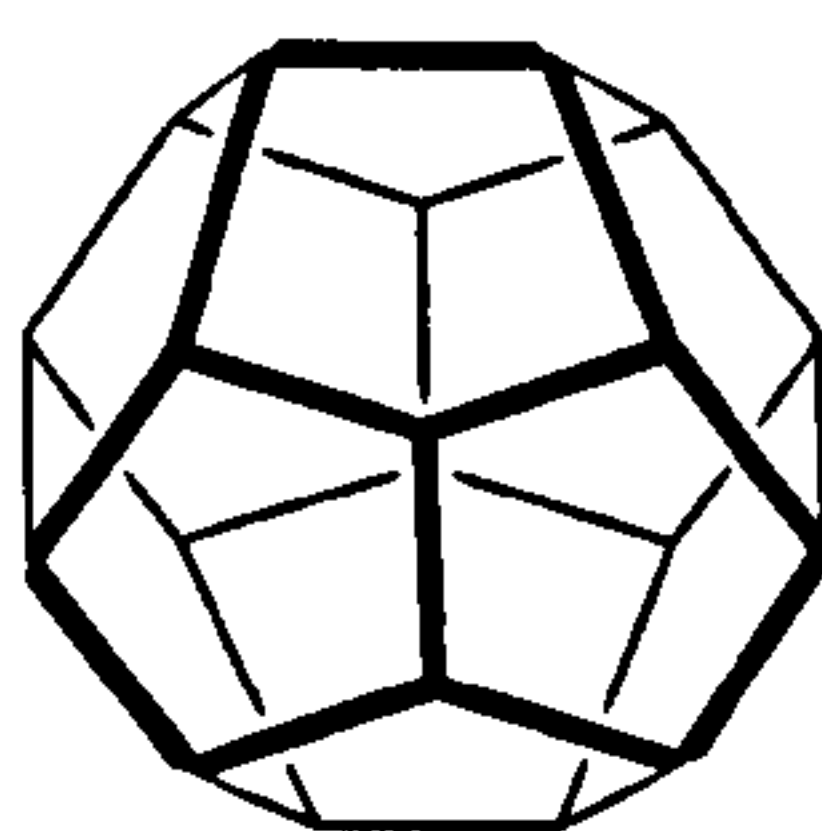
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# 1. Introduction

## 1.1 Dodecahedrane

A great deal of effort has been focused on the synthesis of dodecahedrane **1** during the past three decades<sup>1</sup> due to its symmetric and beautiful structure. It comprises 12 five-membered rings, formed from 20 identical  $sp^3$ -hybridised carbon atoms with ideal tetrahedral character, generating an impressive level of symmetry ( $I_h$ ).



**1**

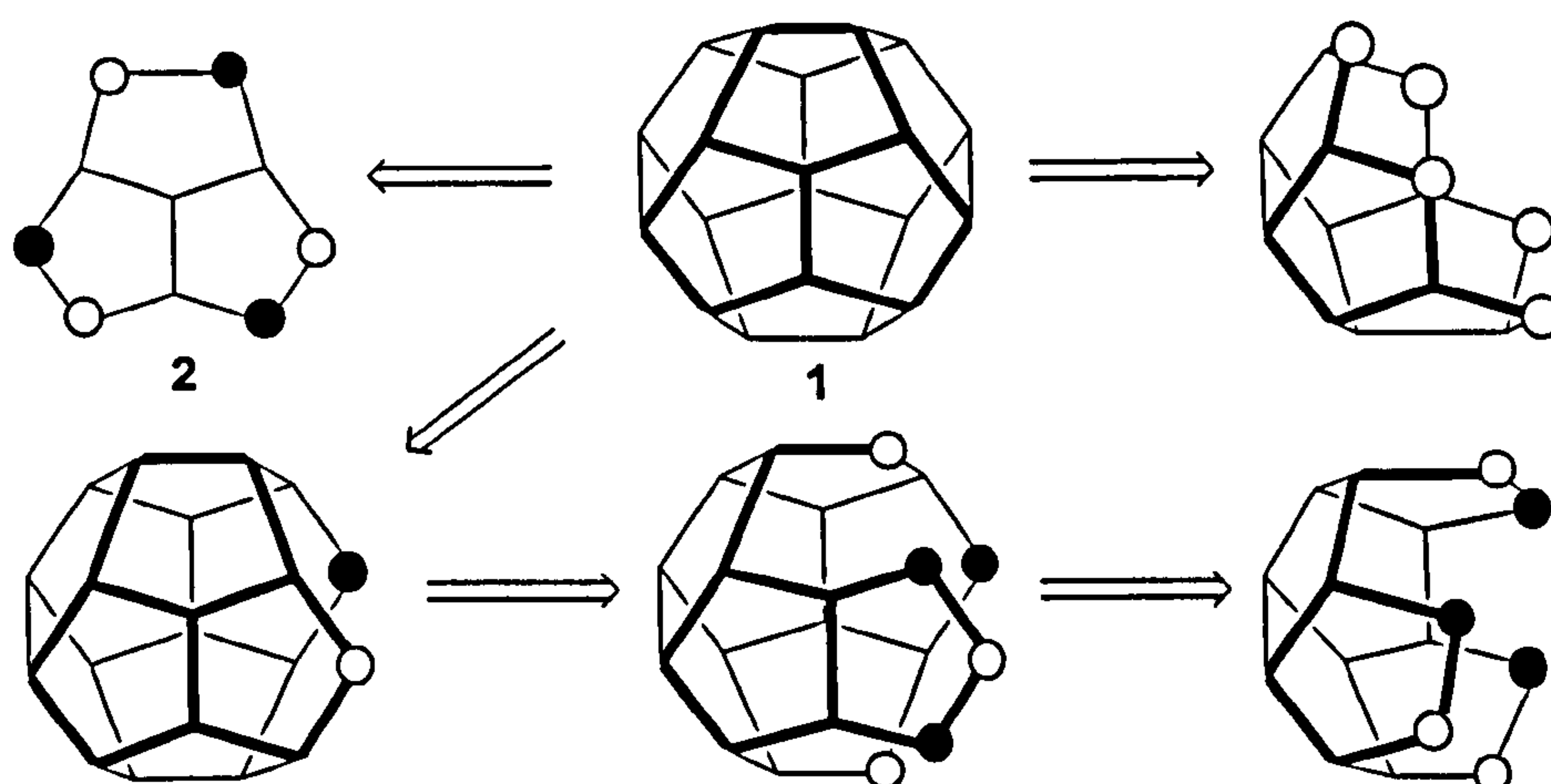
The possibility of including an atom or ion inside the dodecahedrane cavity has been investigated computationally<sup>2</sup> and it has been proposed that this process is energetically unfavourable except perhaps in the case of  $Be^{2+}$ .<sup>3</sup>

## 1.2 Retrosynthetic analysis of dodecahedrane construction

Many ways exist in principle to plan a dodecahedrane total synthesis, and the number of potential functional groups that could be used is very large. It is important to know in this synthesis that every new carbon incorporated into the structure has the hydrogen atom specifically situated at the molecular exterior.

Scheme 1 illustrates three possible routes to **1** in which the white circles represent the sites where functionality is necessary, while the black circles indicate the location of complementary functional groups. For a triquinane structure, such as **2**, the second structural component is an identical tricyclic  $C_{10}$  molecule and in this case it is very important to analyse the steric and entropic factors, due to the congested approach between the two triquinane units.

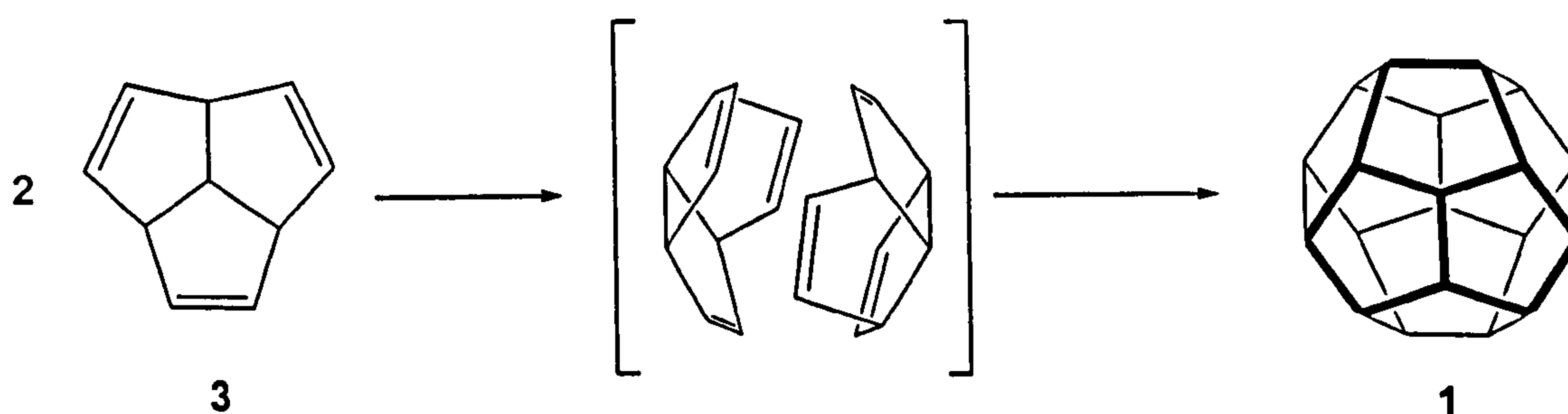




Scheme 1

### 1.3 Dimerisation schemes

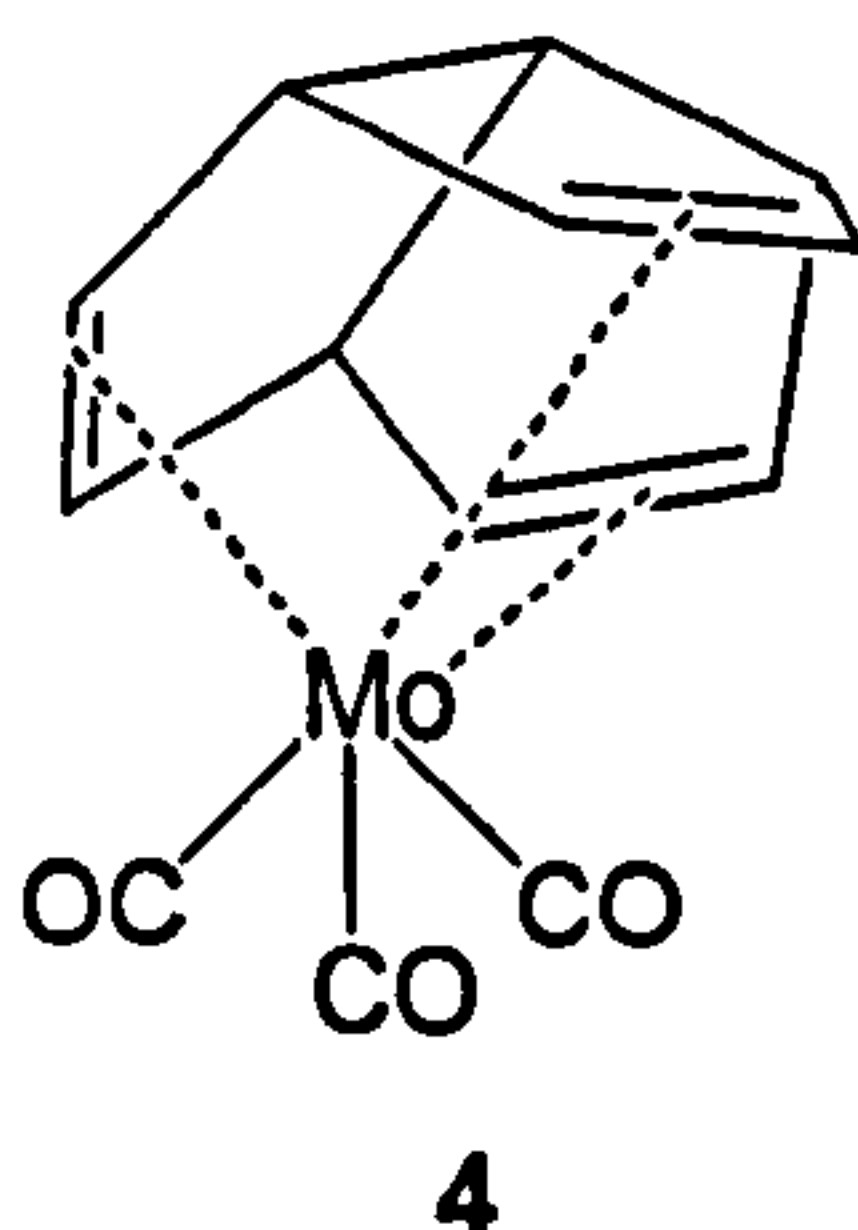
A photochemical  $[2+2+2+2+2+2]\pi$  cycloaddition reaction is a theoretically allowed process. Woodward synthesised triquinacene **3**, a tricyclic hydrocarbon  $C_{10}H_{10}$ , with the aim of dimerising it to form dodecahedrane **1**. This strategy, developed independently by both Woodward<sup>4</sup> and Jacobson<sup>5</sup> more than 30 years ago, remains to be realised (Scheme 2). The overall process is expected to be exothermic ( $\Delta H = -97$  kcal/mol) as a result of the formation of six C-C  $\sigma$  bonds and loss of six C-C  $\pi$  bonds. However, this cycloaddition has not been achieved thermally, photochemically, by means of transition-metal catalysis or using high pressure.



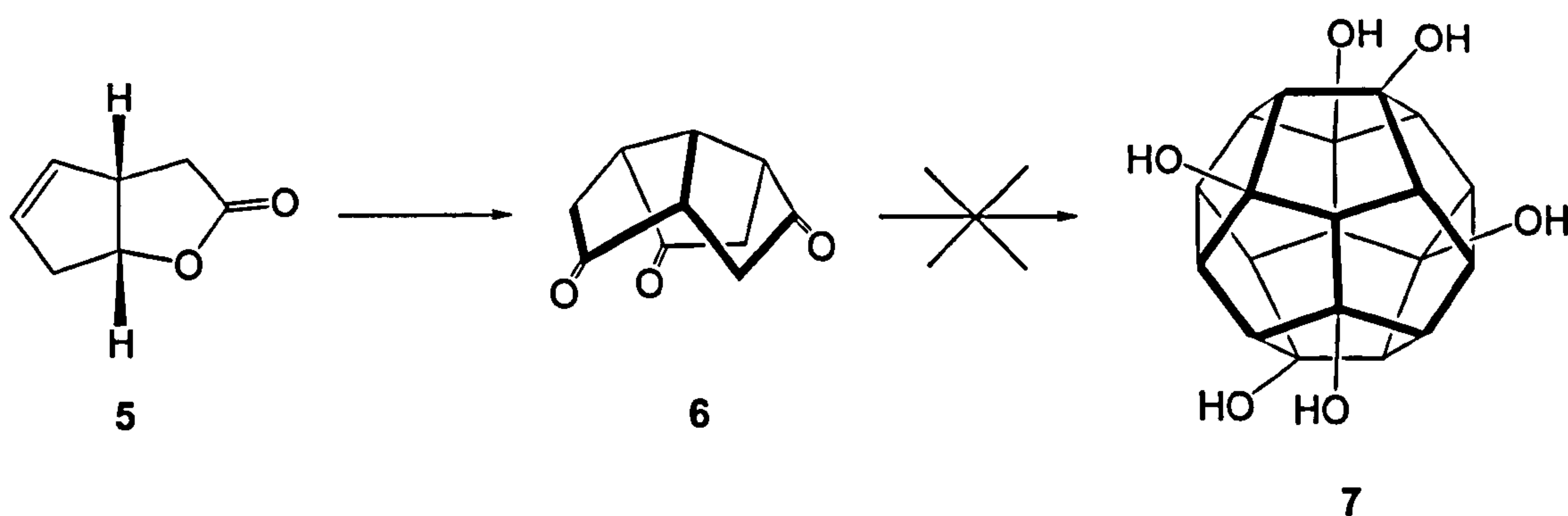
Scheme 2



Although triquinacene **3** has been shown to react with  $\text{Mo}(\text{CO})_6$  to give **4** and with  $(\text{CH}_3\text{CN})_3\text{W}(\text{CO})_3$  to give the tricarbonyl tungsten analogue,<sup>6</sup> the small size of the dodecahedrane cavity prevents the dimerisation of such molecules.

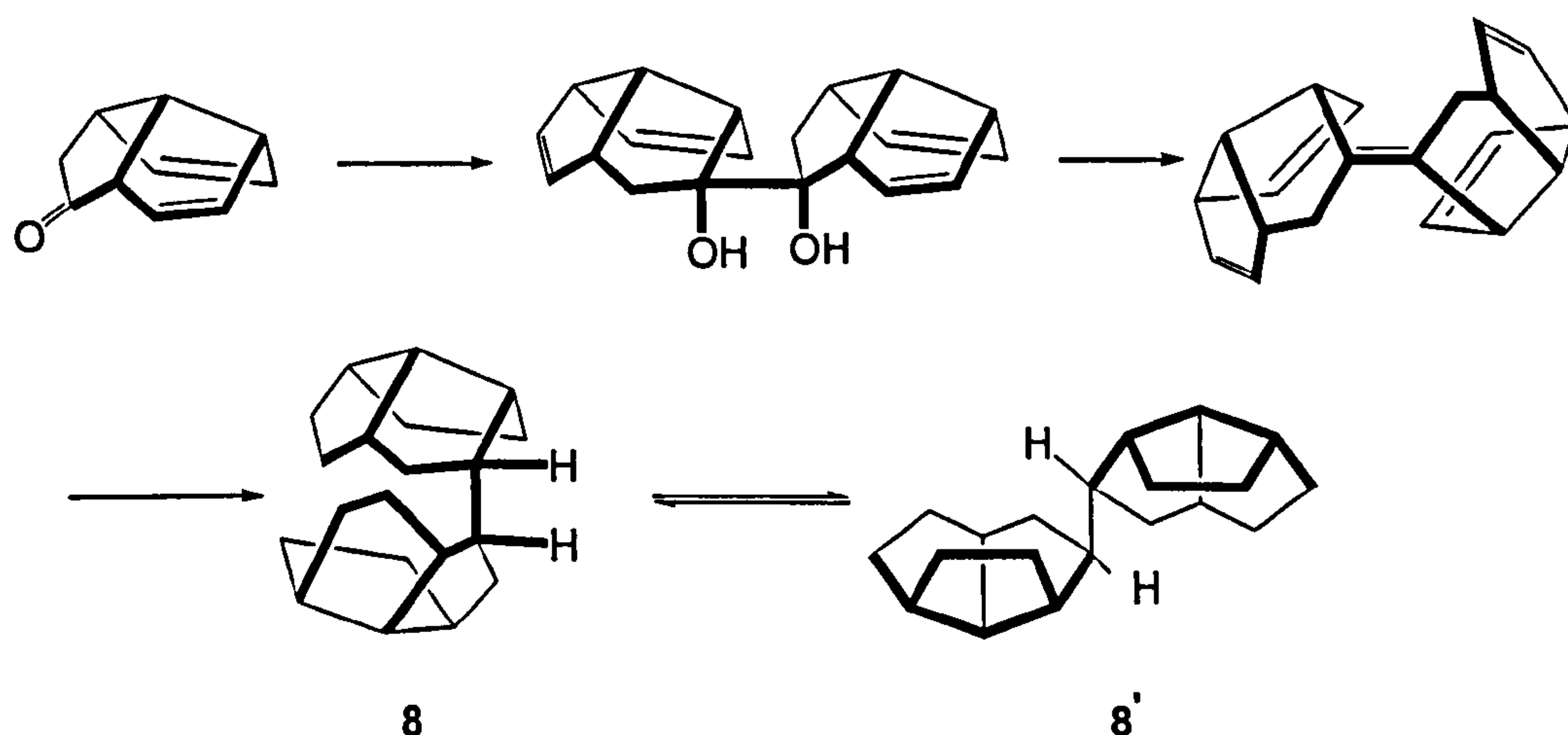


In a different approach to the synthesis of a dodecahedrane, Serratosa prepared the known  $\text{C}_3$ -triketone **6** in an enantioselective manner starting from the optically pure lactone **5** (Scheme 3).<sup>7</sup> This achievement represented the first assignment of the absolute configuration of an optically active triquinacene. In the dimerisation of triketone **6**, three regioselective aldol condensations have to occur, however the reaction did not give dodecahedrane **7** due to the multiple condensation possibilities.



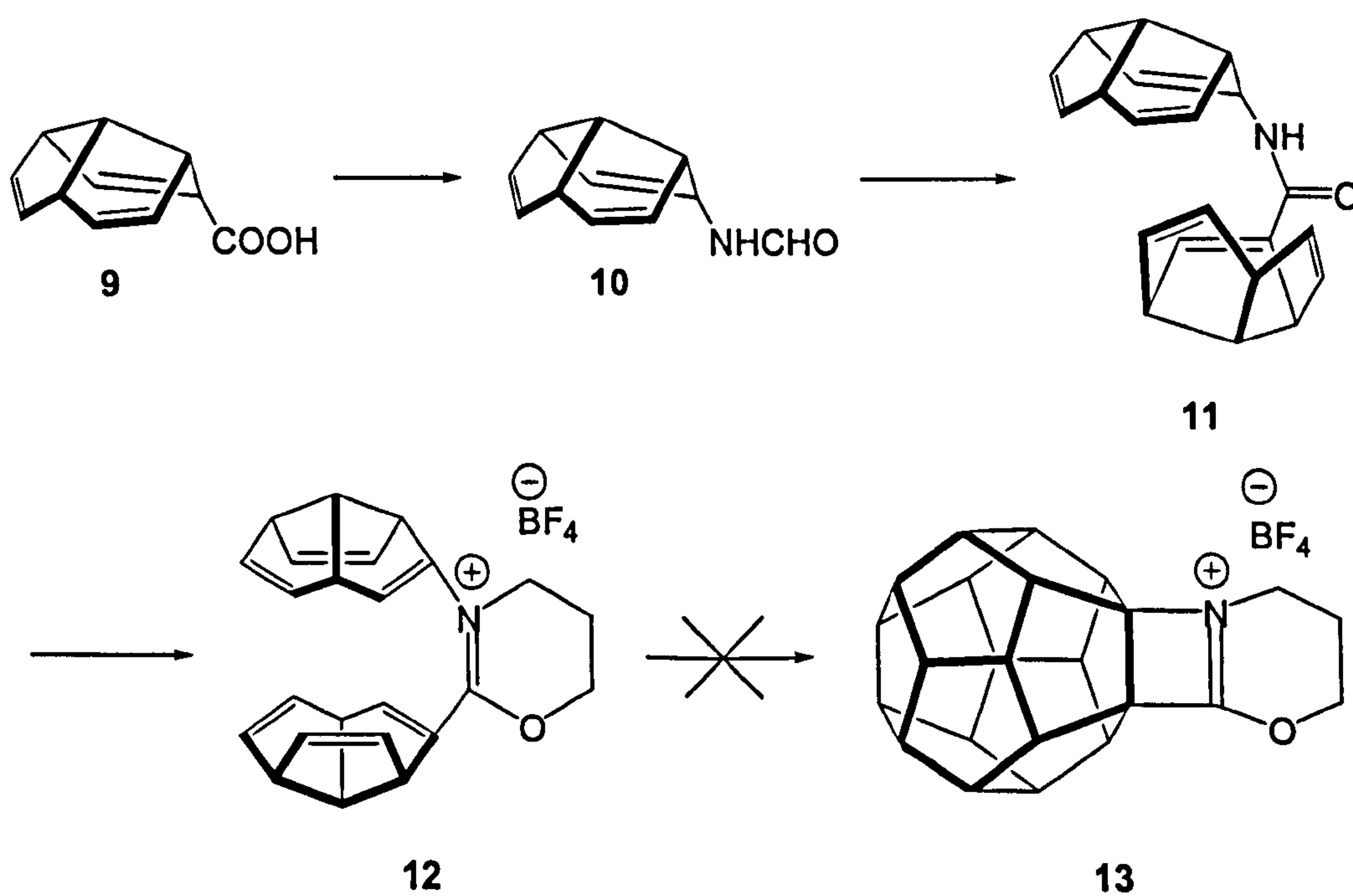
Scheme 3

Scheme 4 shows another strategy for the synthesis of **1** using the triquinane dimer **8**. In this case, the idea was to try a dehydrogenative closure of **8**, but it has not succeeded, perhaps because the conformation **8'** is strongly favoured (as shown by X-ray analysis).<sup>8</sup>



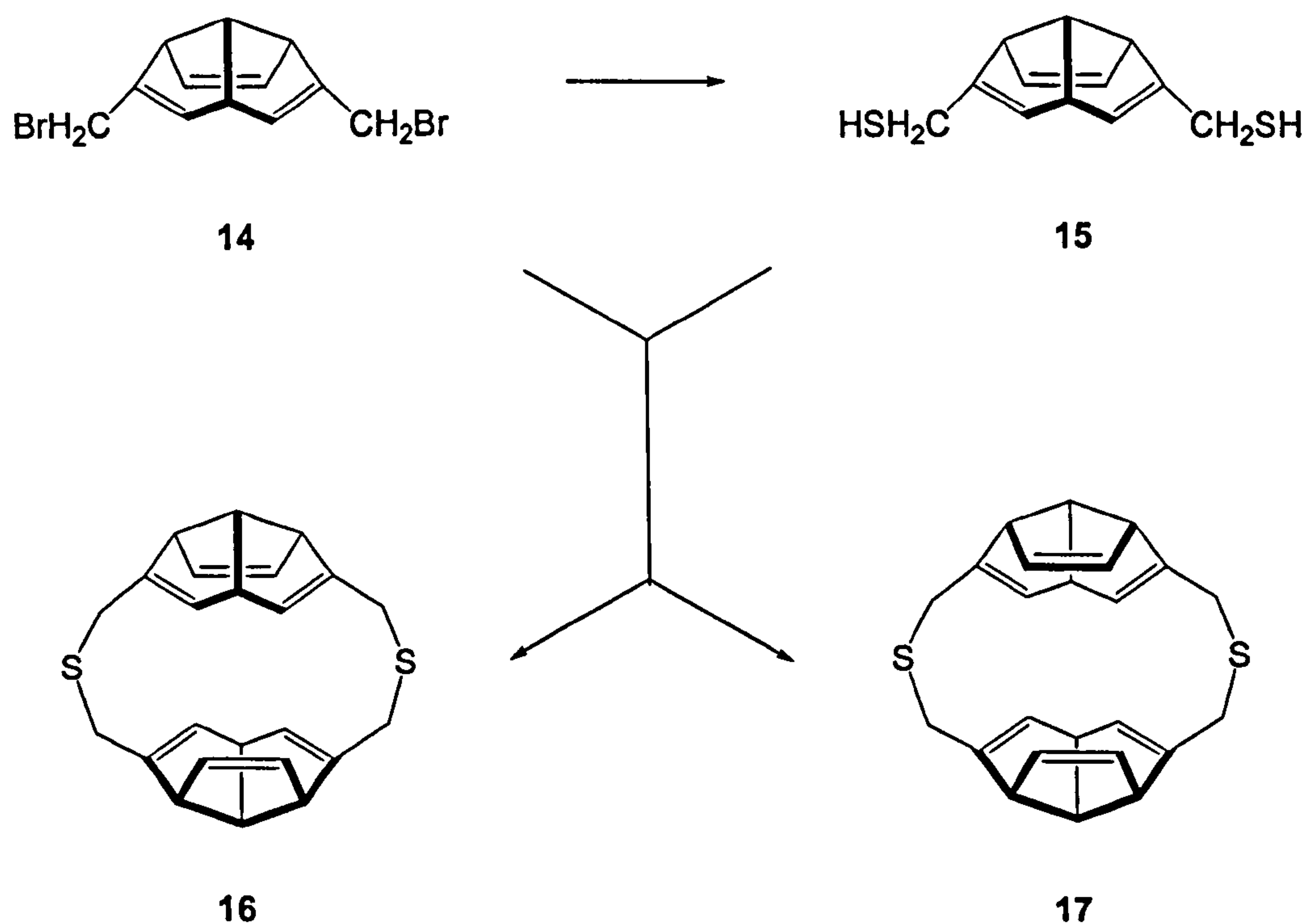
Scheme 4

To avoid the problem mentioned above a triquinane dimer **12** was synthesised with the appropriate reactive conformation. Deslongchamps *et al.*<sup>9</sup> prepared (+)-triquinacene-2-carboxylic acid **9** which was transformed into the (+)-2-formamido derivative **10**. Condensation of **10** with the acid chloride of (+)-**9** gave the secondary amide **11** which was converted into the cyclic imidate salt **12**. However cyclisation of **12** to the dodecahedrane **13** was not successful (Scheme 5).



Scheme 5

Coupling of dibromide **14** with dimercaptan **15** gave a mixture of the *anti*- and *syn*-triquinacenophanes **16** and **17**. These isomers were separated chromatographically and their identities established by X-ray crystal structure. Although cyclisation of a molecule such as **16** appears possible on the basis of molecular modelling, again this process has not been observed (Scheme 6).<sup>10</sup>



Scheme 6

Jayaraman *et al.*<sup>11</sup> have also attempted the synthesis of dodecahedrane **1** by dimerisation of triquinacene **3** under high pressure. Triquinacene **3** is a liquid at room temperature but freezes to a clear crystalline solid at about 18°C. The molecules in the crystal are positioned in layers perpendicular to the *c*-axis, and contacts between molecules are top-to-top (convex faces) and bottom-to-bottom (concave faces) in alternate layers (Figure 1). The idea was that by the application of high pressure to a triquinacene crystal the two molecules could move the small distance required for proper overlap. However, compression of triquinacene to nearly 20 GPa in a diamond

cell and exposure to 248 nm and 308 nm ultraviolet radiation at 5 GPa failed to produce any detectable dodecahedrane 1.

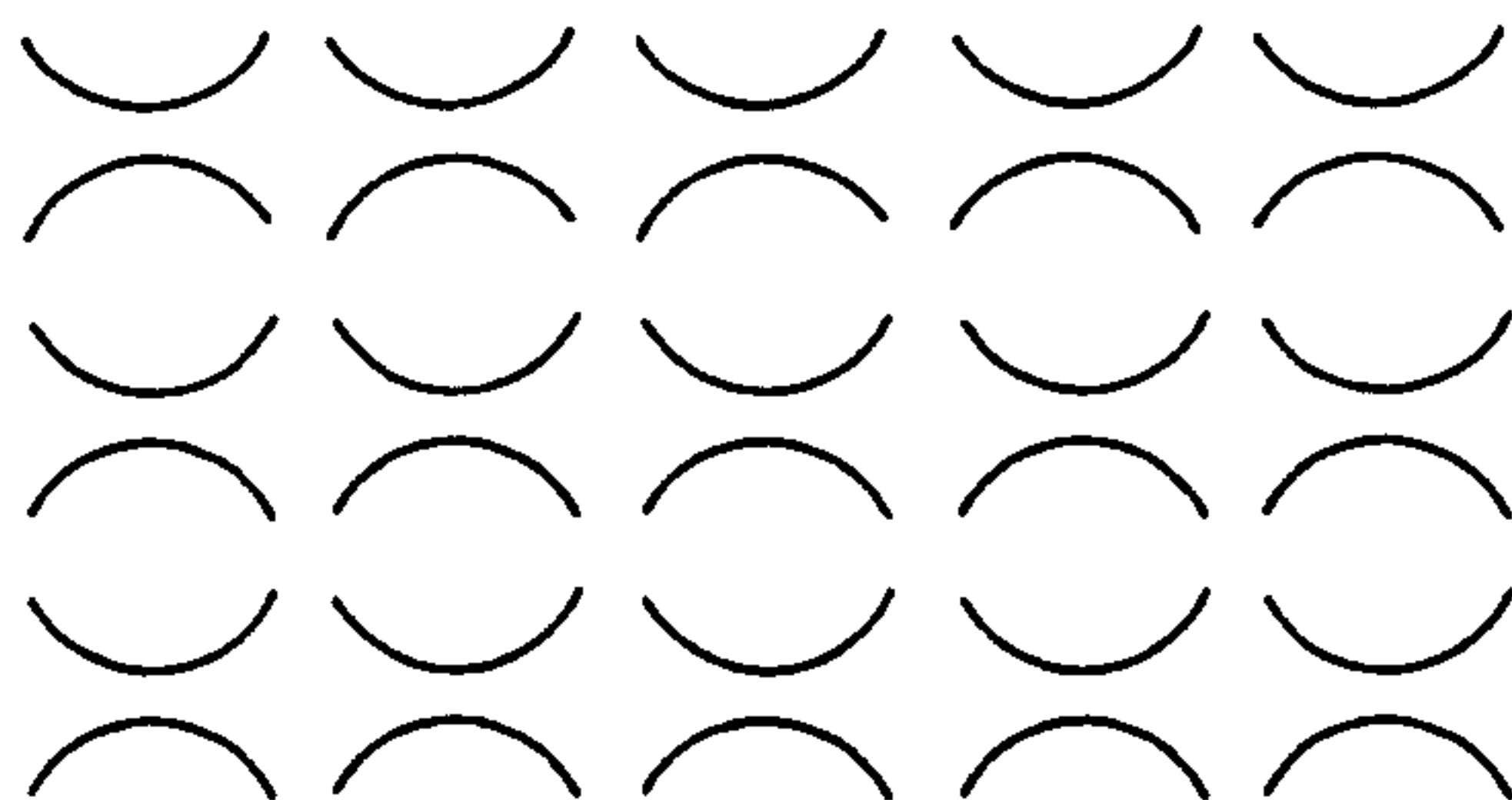
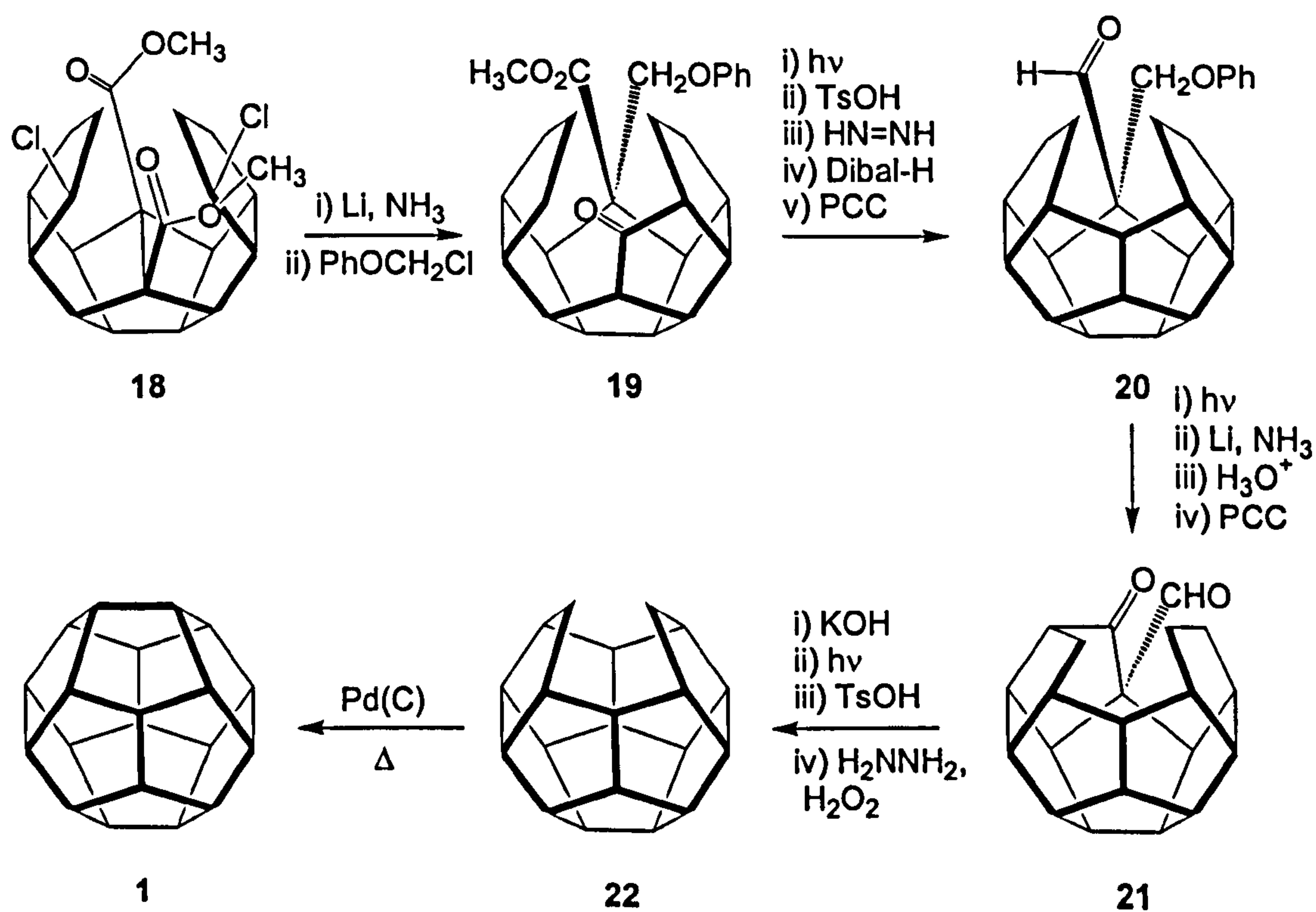


Figure 1

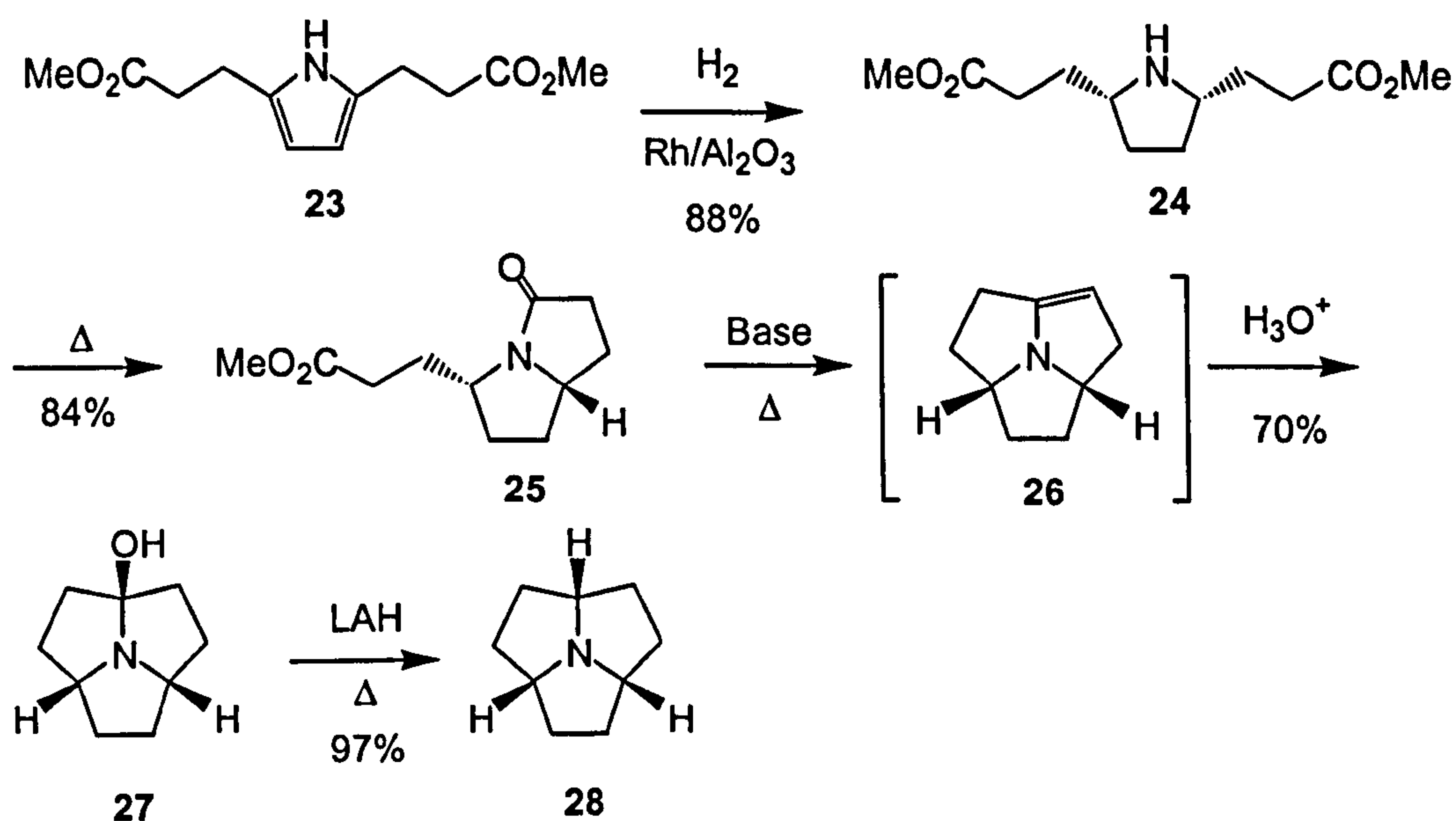
Dodecahedrane 1 was synthesised by Paquette *et al.*<sup>12</sup> in 1982 in 23 steps, but a direct synthesis from triquinacene has not as yet been realised. The key steps of the synthesis of dodecahedrane 1 are presented in Scheme 7.



Scheme 7

## 1.4 Synthesis of azatriquinane: an approach to the synthesis of azatriquinacene

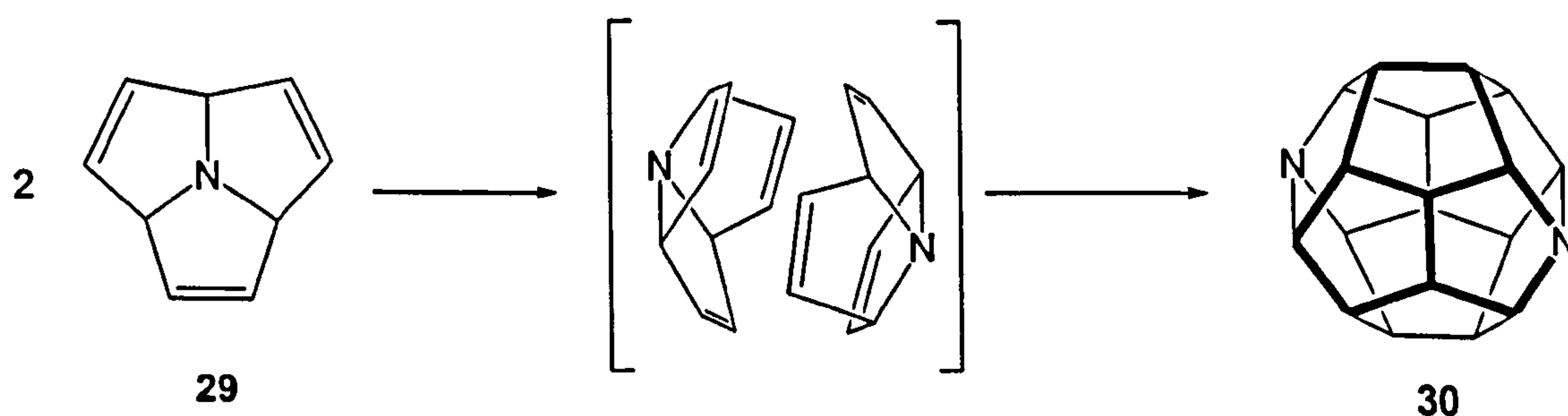
It could be possible to use, in all the strategies mentioned above, triquinane analogues such as azatriquinanes. The parent compound, azatriquinane **28** was prepared by Mascall and coworkers in 1996.<sup>13</sup> This synthesis, presented in Scheme 8, also provides an approach to the synthesis of azatriquinacene **29**.



**Scheme 8. Synthesis of azatriquinane.**

## 1.5 Aims and objectives

The main target of this project is the synthesis of azatriquinacene **29**, which is a potential precursor to 1,16-diazadodecahedrane **30**. If triene **29** could be prepared the Woodward dimerisation strategy could be re-examined (Scheme 9).



### Scheme 9



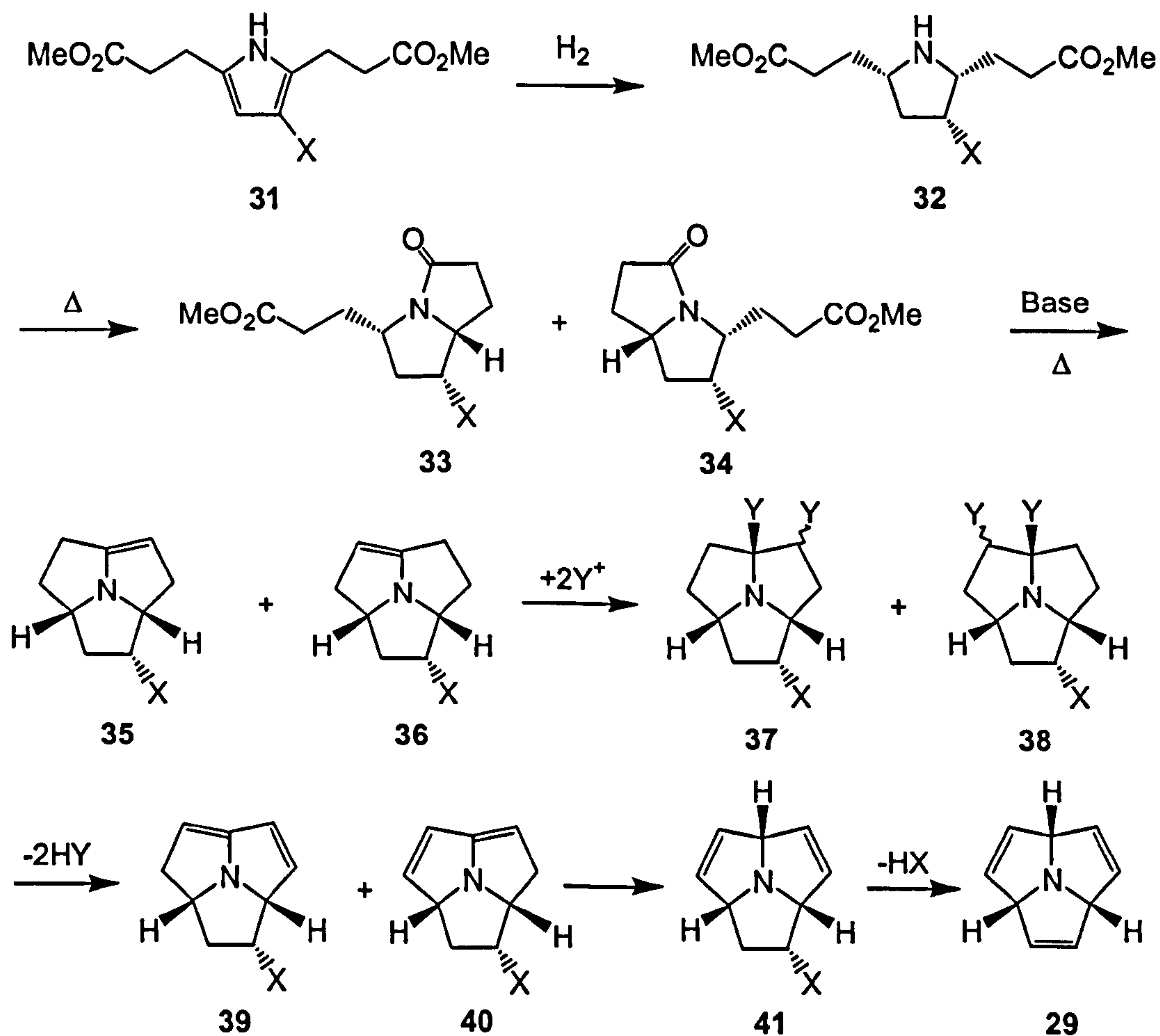
There are two main differences between **3** and **29** which could favour the dimerisation of the aza-analogue **29**:

- (i) The aza-analogue **29** would be expected to possess some aqueous solubility, particularly as the hydrochloride. When substances with non-polar regions are dissolved in water, they tend to associate so as to diminish the hydrocarbon-water interfacial area. Thus, by attempting the dimerisation of the aza-analogue **29** in aqueous solution it may adopt the “reactive conformation” in which the concave *endo* faces of two molecules are in close contact. A similar phenomenon has already been described for [4+2] cycloaddition reactions, where substantial acceleration of cycloaddition rates in aqueous verses organic media were observed.<sup>14</sup>
- (ii) There is a possibility that the incorporation of either an amine or quaternary ammonium centre, could favourably affect the electronic properties of the triquinane relative to **3** and facilitate dimerisation.

## 2. Results and discussion

### 2.1 Enamine 26 reactivity: a key compound in this study

With the synthesis of azatriquinane 28 in hand, a possible route to azatriquinacene 29 based on a substitution-elimination strategy was proposed as presented in the following scheme:



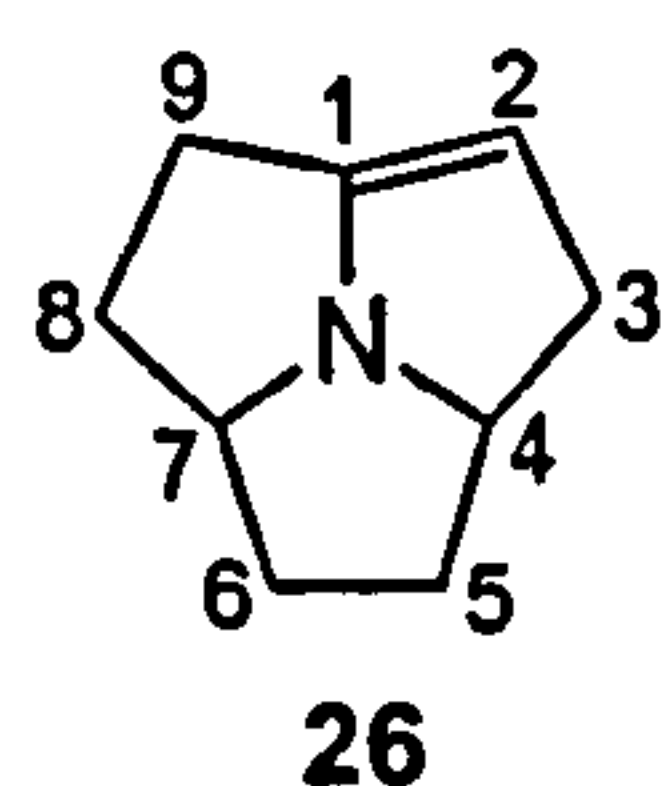
Scheme 10

This synthesis starts with pyrroles in which the 3-substituent could be eliminated to give the bottom double bond. Up to the formation of the enamines 35 and 36 this synthesis is analogous to that of azatriquinane itself (Scheme 8). The two top double

bonds could be introduced by disubstitution of the azatricycles **35** and **36**, bis-elimination and isomerisation.

For the purpose of the disubstitution of the double bond in **35** and **36**, model reactions involving **26** were studied.

The X-ray crystal structure of azatriquinane<sup>13</sup> shows that these molecules have the



expected convex geometry due to the rigid tricyclic structure. Therefore,

the double bond of **26** could be considered part of an enamine or as a

bridgehead alkene. Experimentally, it has been observed that the

nitrogen in crystalline enamines varies from tetrahedral ( $sp^3$  hybridized) to planar ( $sp^2$  hybridized) geometry.<sup>15</sup> It would be conformationally unfavourable for the nitrogen in **26** to adopt  $sp^2$  hybridization due to the rigid nature of the tricyclic framework. So it was supposed the double bond would behave like a bridgehead alkene rather than an enamine.

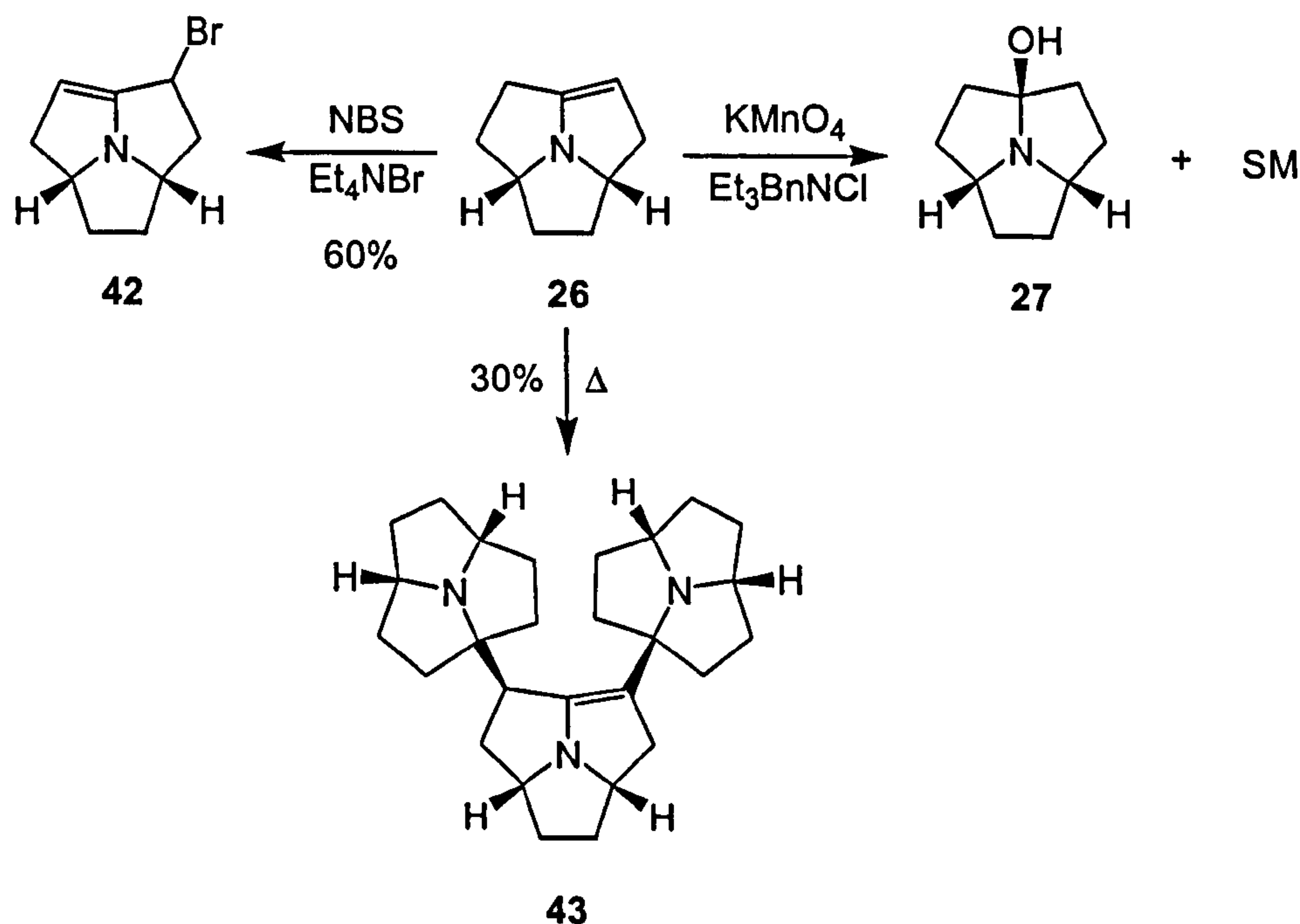
Two different reactions were attempted: a dibromination and a dihydroxylation of the double bond. The dibromination using *N*-bromosuccinimide-tetraethylammonium bromide<sup>16</sup> gave the monobrominated product **42** (Scheme 11) and the dihydroxylation reaction was attempted using potassium permanganate and triethylbenzylammonium chloride,<sup>17</sup> giving a mixture of starting material and the hemiaminal **27** (Scheme 11).

In earlier attempts to synthesise **26**, vigorous agitation with water during work up gave only hemiaminal **27** suggesting that **26** is water sensitive and was partially hydrolysed in the work up. Attempts to dehydrate hemiaminal **27** to obtain **26** using aluminum trichloride, phosphorus pentoxide, neutral alumina or boron trifluoride etherate<sup>18</sup> failed. Compound **26** is synthesized by a cyclisation/distillation method<sup>13</sup> and it was possible to avoid the hemiaminal formation by introducing dry ethyl ether and freshly



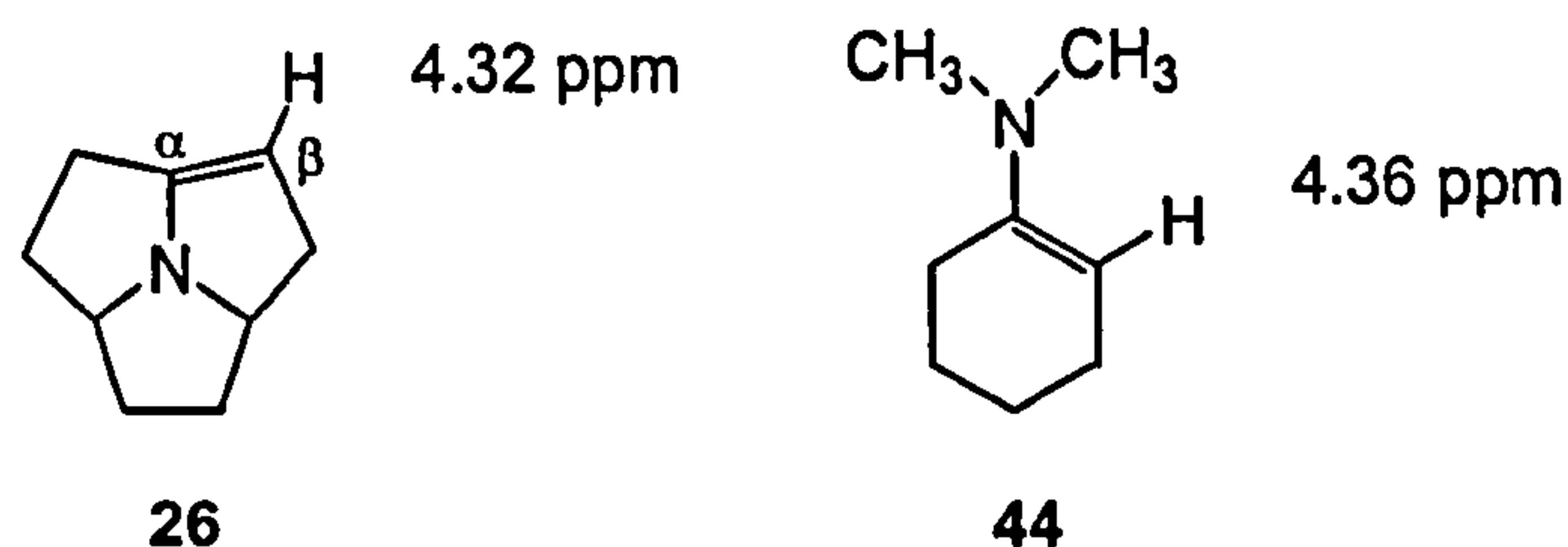
activated powdered 4 Å molecular sieves in the collection flask to maintain anhydrous conditions and eliminating the aqueous work up.

An attempt to further purify **26** by short path distillation *in vacuo* gave mostly the compound **43** (Scheme 11),<sup>19</sup> the structure and interesting features of which will be discussed in detail in Section 2.2.



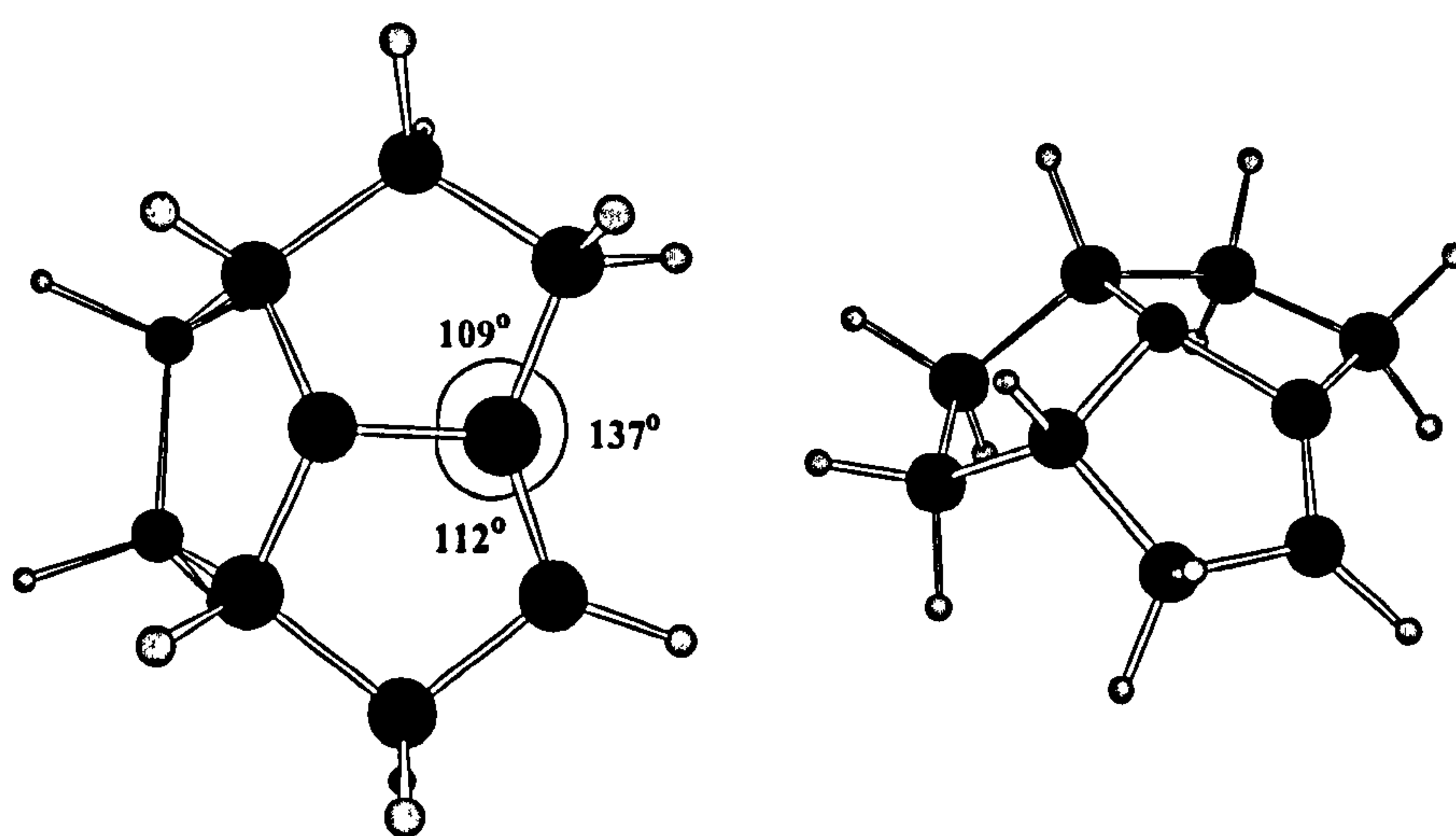
Scheme 11

The product obtained in the bromination of **26**, its unsuccessful dihydroxylation, its hydrolysis to give hemiaminal **27** and the formation of trimer **43** all suggested that this compound behaves as an enamine and not as a bridgehead double bond as it was assumed at the beginning. The enamine character of **26** is also supported by a characteristic high field chemical shift of the olefinic proton in  $^1\text{H}$ -NMR spectrum (4.32 ppm) and the C2 in  $^{13}\text{C}$ -NMR spectrum (93.2 ppm). It also shows a characteristic strong absorption at  $1660\text{ cm}^{-1}$  in the infrared spectrum.



**Figure 2.** Comparison of the  $^1\text{H}$ -NMR chemical shift of the vinylic protons in enamine 26 and the classical enamine 44.

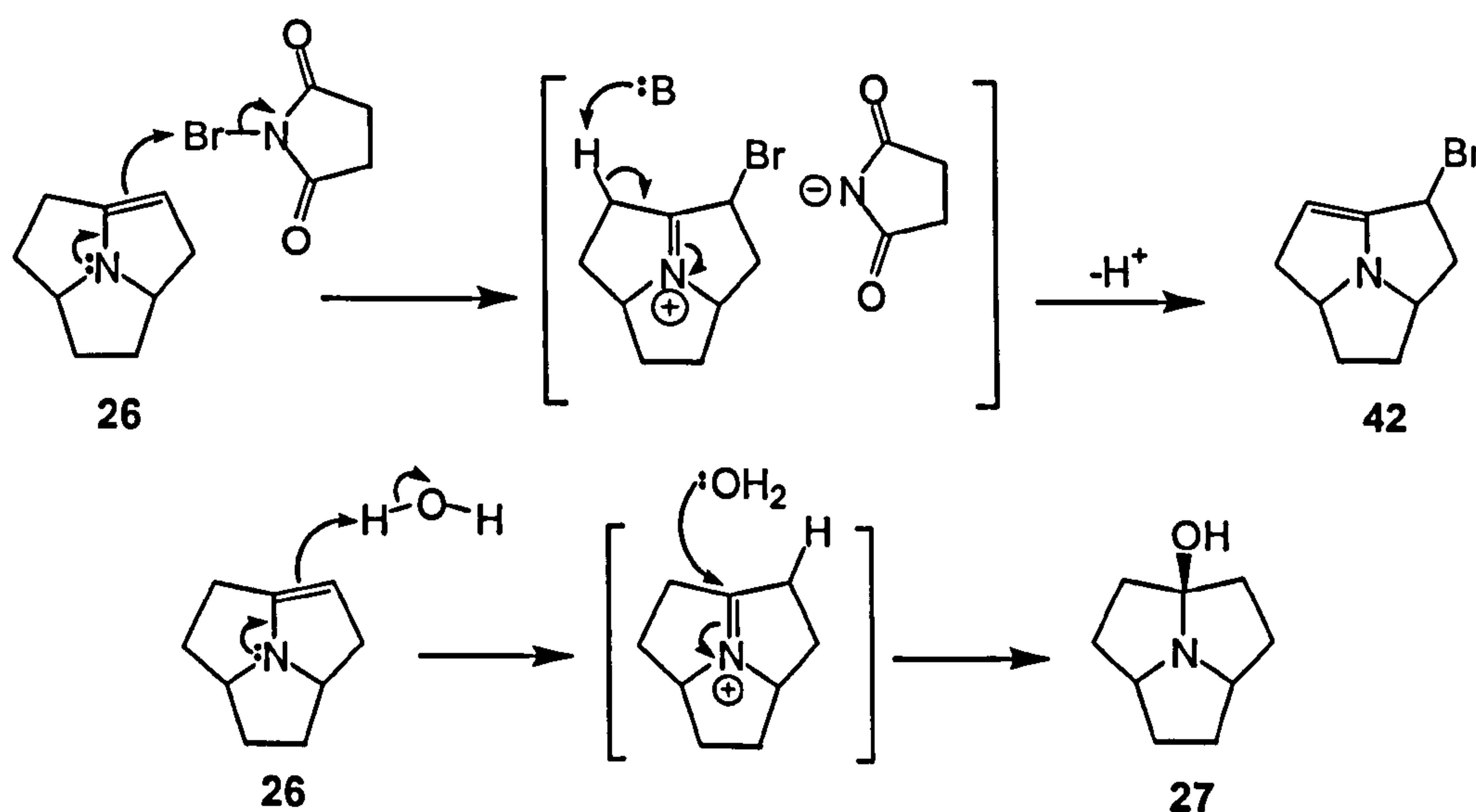
A correlation exists between enamine reactivity and  $^1\text{H}$ -NMR chemical shift: the greater the degree of  $p$ - $\pi$  overlap, the greater the electron density at the  $\beta$ -carbon atom, and consequently, the greater the magnetic shielding of the vinylic proton.<sup>15</sup> The reactivity of an enamine depends on the degree of substitution at the  $\alpha$ - and  $\beta$ -positions. Alkyl substituents at C- $\alpha$  increase the electron density and reactivity at C- $\beta$  by hyperconjugative and inductive effects, while substitution at C- $\beta$  decreases the reactivity at this position due to steric and electronic effects.<sup>15</sup>



**Figure 3.** A) Bond angle values around C1 of 26. B) View of the convex geometry of 26. Structure obtained using semiempirical calculations which were performed with the PM3 method implemented in Spartan 4.0.

In the case of enamine **26**, it is substituted at C- $\alpha$  and mono-substituted at C- $\beta$  which renders it highly reactive. For **26** the chemical shift of the vinylic proton is 4.32 ppm, which is a significantly high field shift characteristic of a reactive enamine. For example, a classical enamine as **44** has a chemical shift for the vinylic proton of 4.36 ppm as shown Figure 2. The high reactivity could also be attributed to the location of the double bond at a strained bridgehead position in which the bond angles: N-C1-C9, N-C1-C2 and C9-C1-C2 have unusual values (Figure 3).

If compound **26** is thus considered as an enamine, it is possible to explain the results obtained above. Bromoenamine **42** is the result of the bromination of the enamine **26** with N-bromosuccinimide and the hemiaminal **27** formation is the consequence of enamine hydrolysis which does not require the presence of acid due to its high reactivity (Scheme 12). The formation of the species **43** is another consequence of the enamine character of **26** and the mechanism for its trimerisation will be discussed in the next section.



Scheme 12

## 2.2 Trimer

The solid residue from the enamine **26** after heating was purified by column chromatography to afford the nonacyclic structure **43** in 30% isolated yield.<sup>19</sup> Crystals of it were grown by diffusion of ether into a dichloromethane solution of **43** and the X-ray crystal structure of this compound is shown in Figure 4.

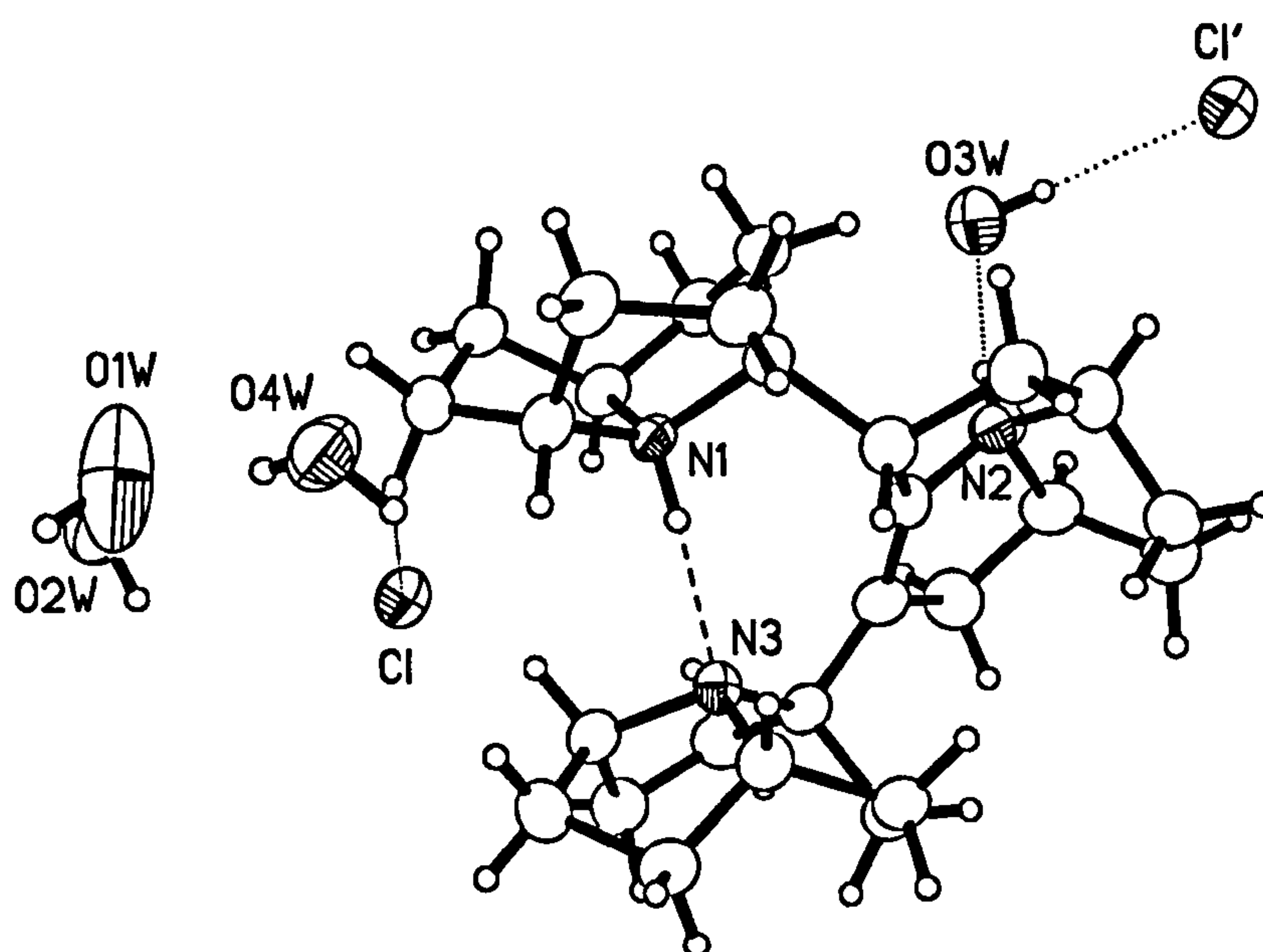
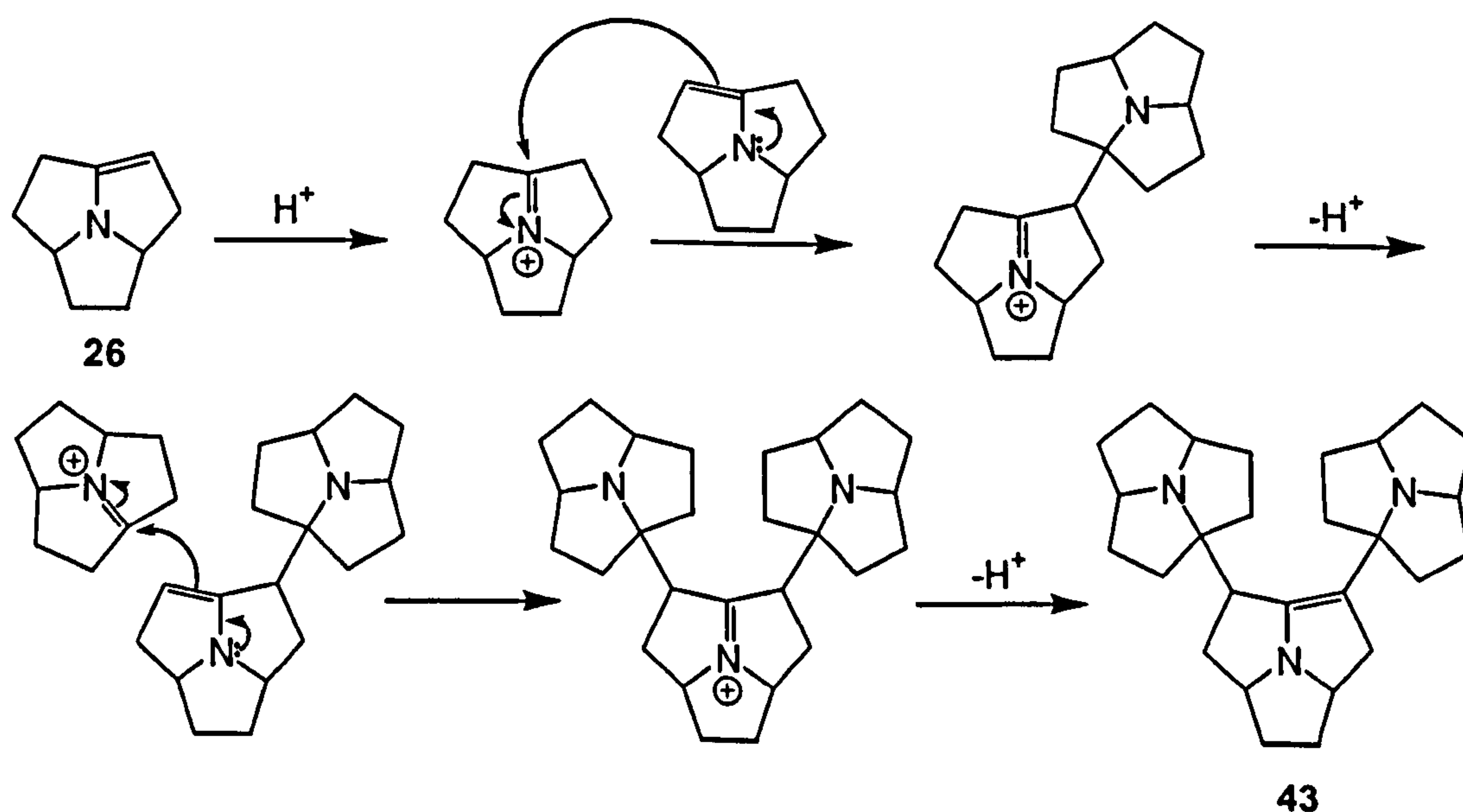


Figure 4. X-ray crystal structure of **43** ( $\text{C}_{27}\text{H}_{40}\text{N}_3\text{Cl} \times 4\text{H}_2\text{O}$ ).



Scheme 13

In Scheme 13 is shown a possible mechanism of the trimer 43 formation. The crude enamine 26 was contaminated with dichloromethane and the small amount of HCl present in this solvent could act as a catalytic proton source. To test this hypothesis, the formation of the trimer was attempted with crude enamine 26 which came from an ethereal solution. By heating 26 at 140°C no reaction took place until two drops of dichloromethane were added.

The exotic species 43 has three tricycles, three basic centres and an enamine moiety. In the crystal structure (Figure 4) the most interesting feature is the conformation of the two terminal azatricycles due to an intramolecular hydrogen bond between the proton on N1 and the N3. Nitrogens N1 and N3 face each other separated by a distance of 2.745 Å with a N1-N1H-N3 angle of 177° and this indicates the existence of a strong intramolecular hydrogen bond.

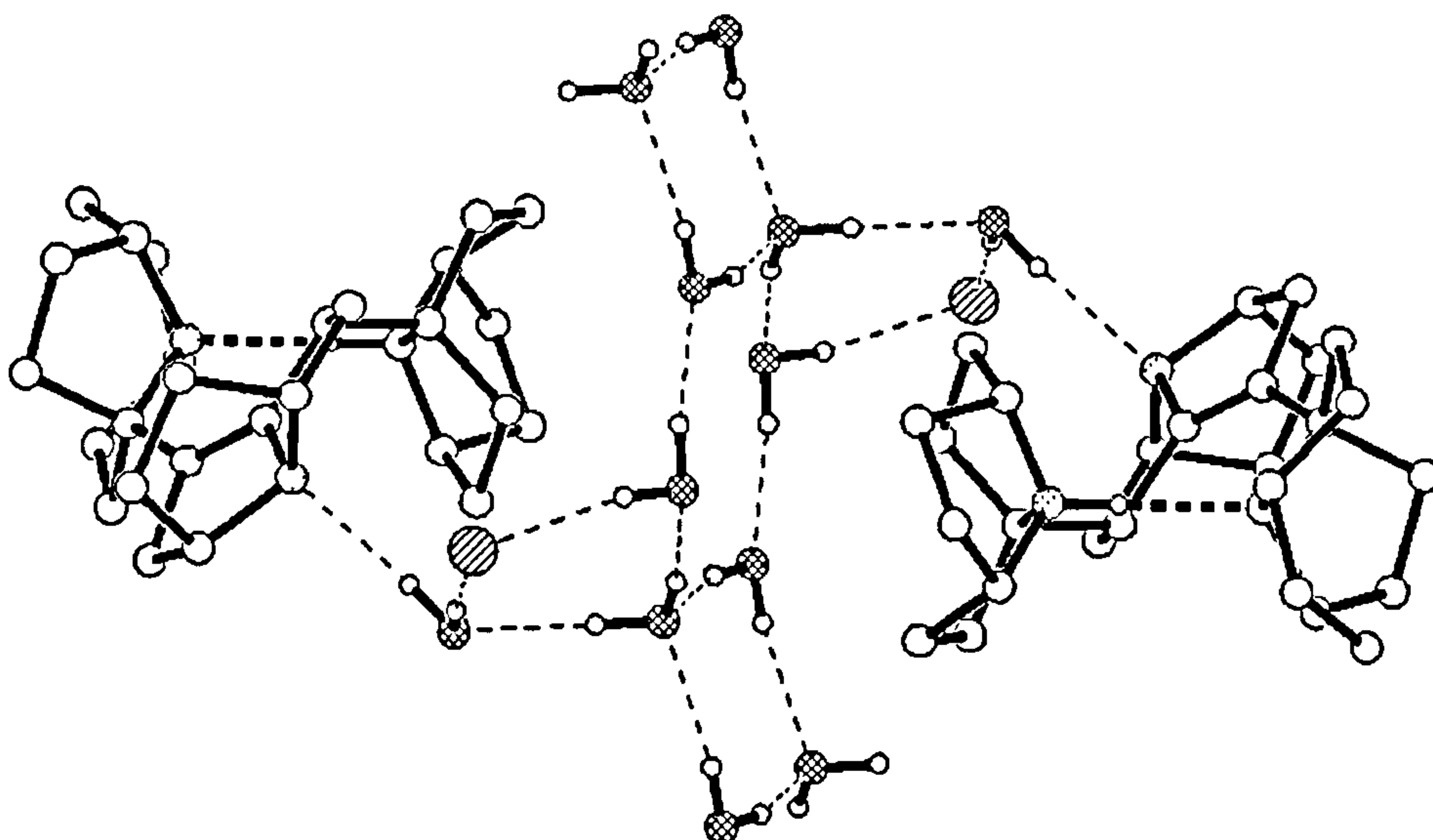


Figure 5. X-ray structure of 43.

Another view of the trimer 43 is presented in Figure 5 which shows the hydrogen bonds between the nitrogens, chloride ions and water molecules in the crystal. In the middle of it is observed an infinite column of hydrogen bonded water molecules. The



molecules of **43** are linked to these columns by hydrogen bonds *via* water molecules and chloride ions.

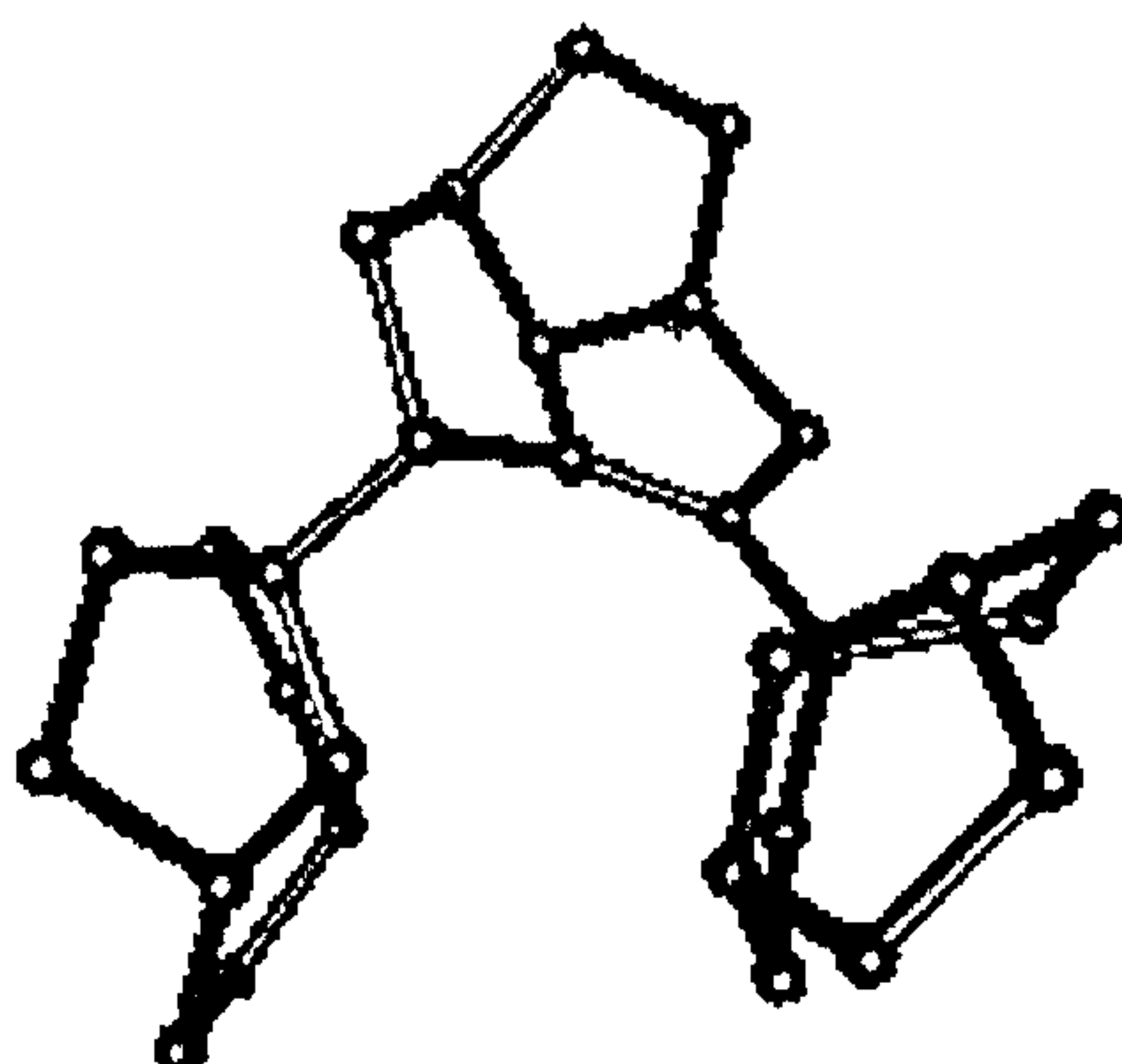
An attempt was made to abstract the proton on N1 of trimer **43** using the same methodology that was successful in the deprotonation of azatriquinane salts (KOH 2 M). However, the deprotonation was not possible using this base probably due to an increase in the  $pK_a$  of **43** by stabilisation of the protonated form by the high quality intramolecular hydrogen bond between the proton on N1 and the N3. This fact makes **43** a new class of “proton sponge”.

By comparison of the  $^1\text{H}$ -NMR of the trimer **43** with those of **26** and azatriquinane **28**, the five signals between 4.12 ppm and 3.44 ppm have been assigned as being the six protons  $\alpha$  to nitrogen. The N1 proton was successfully abstracted using KHMDS and this fact was supported by a characteristic high field shift of the protons  $\alpha$  to nitrogen in the  $^1\text{H}$ -NMR spectrum. However, re-protonation by atmospheric water was observed suggesting that compound **43** had an extraordinary basicity.

An investigation of the basicity of **43** was thus undertaken using both chemical and computational approaches. The experimental value of the  $pK_a$  of **43** using potentiometric titration in acetonitrile solution, and its protonation energy ( $\Delta E_{\text{prot}}$ ) using the HF/6-31G(d)//HF/6-31G(d) theoretical model, were calculated by L. Chmurszynski *et al.* The  $pK_a$  in acetonitrile was experimentally determined to be 25.1 which showed that the molecule was in fact highly basic, about seven orders of magnitude more than 1,8-*bis*(dimethylamino)naphthalene (“proton sponge”) and 4-dimethylaminopyridine (DMAP). This result was supported by the calculation of its theoretical  $pK_a$  value which was 26.0.<sup>19</sup>

The extraordinary basicity of **43** is attributed to the relief of lone pair repulsion between the nitrogens N1 and N3 in enforced proximity (Figure 6). The establishment

of a high quality H-bond on protonation, and the extensive resonance stabilisation of the  $\text{HB}^+$  ion can also be the responsible facts of the high  $\text{pK}_a$  as happens in other known proton sponges.<sup>20</sup>



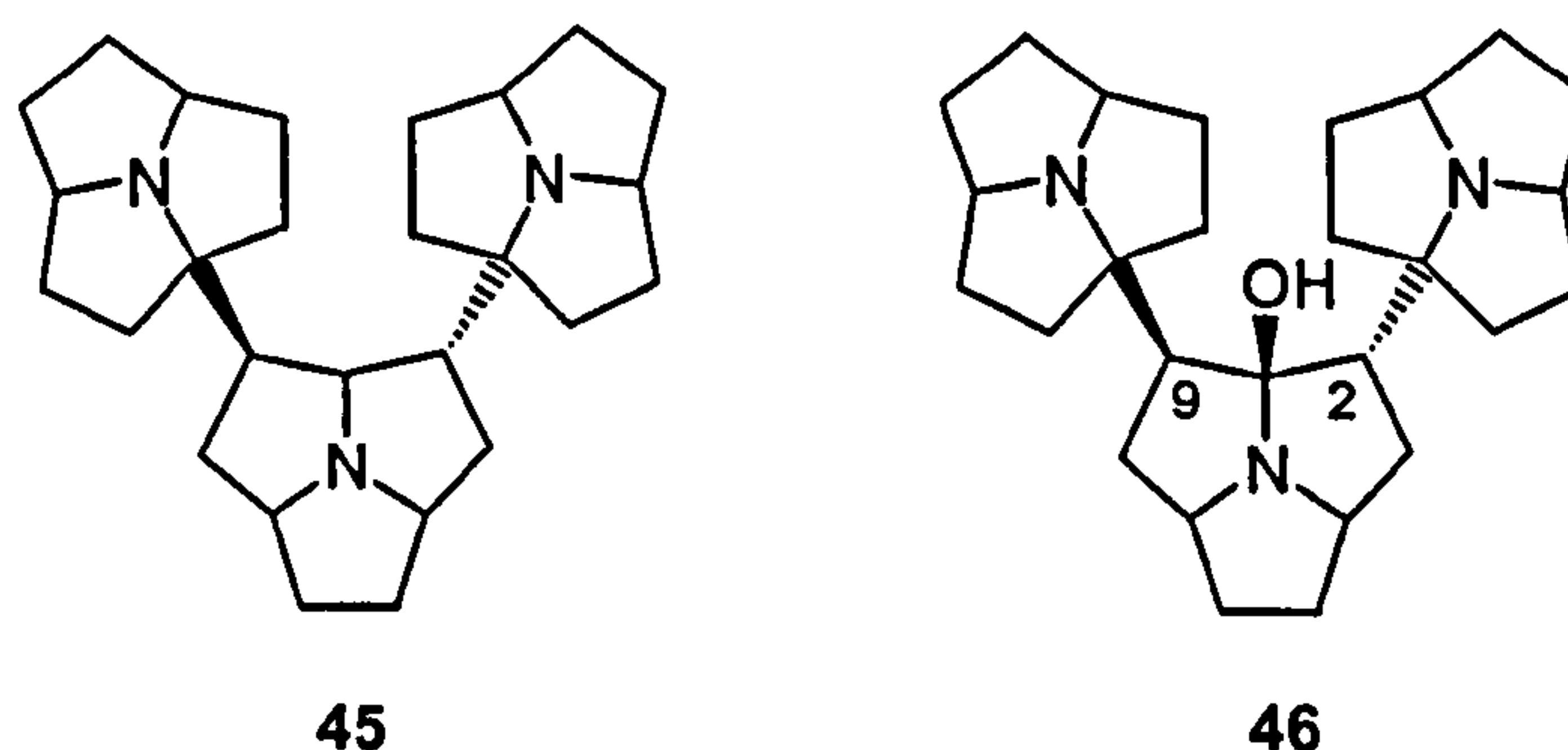
**Figure 6.** Possible conformation of trimer **43** in the deprotonated state. The semiempirical QM calculations were performed with the PM3 method as implemented in Spartan 4.0.

To investigate the possibility of different conformations of the two terminal tricycles of trimer **43**, crystallisation experiments using the deprotonated trimer were attempted but unfortunately, due to its solubility in apolar solvents it was not possible to grow them under similar conditions to those used with the di-protonated trimer. Other attempts to grow crystals of **43** were made without success.

With the idea mentioned above of obtaining a different conformation of trimer **43**, a protonation reaction was carried out. Due to the positive charges on the N1 and N3 a conformation in which the two terminal azatricycles are not facing each other could be obtained induced by similar electrostatic effects as in the deprotonated state. Therefore, the protonation was attempted treating the trimer with trifluoroacetic acid to afford a compound in which a downfield shift of the protons  $\alpha$  to nitrogen in the  $^1\text{H}$ -NMR spectrum indicated a possible protonation. Unfortunately, the conformation of

this species was not determined because crystals of this compound again could not be grown.

The reduction of the double bond in **43** was attempted with the aim of synthesising the saturated trimer **45**. Catalytic hydrogenation,<sup>21</sup> sodium borohydride in acid,<sup>22</sup> sodium cyanoborohydride in acid<sup>23</sup> and formic acid<sup>24</sup> were all tried but unfortunately, in all the cases starting material was recovered. Catalytic hydrogenation failed, probably due to steric considerations. Poor reactivity of the enamine moiety in the trimer due to the bulky substituent in the  $\beta$ -carbon, could be the reason for the resistance to reduction in the case of the hydride reactions.<sup>15</sup>



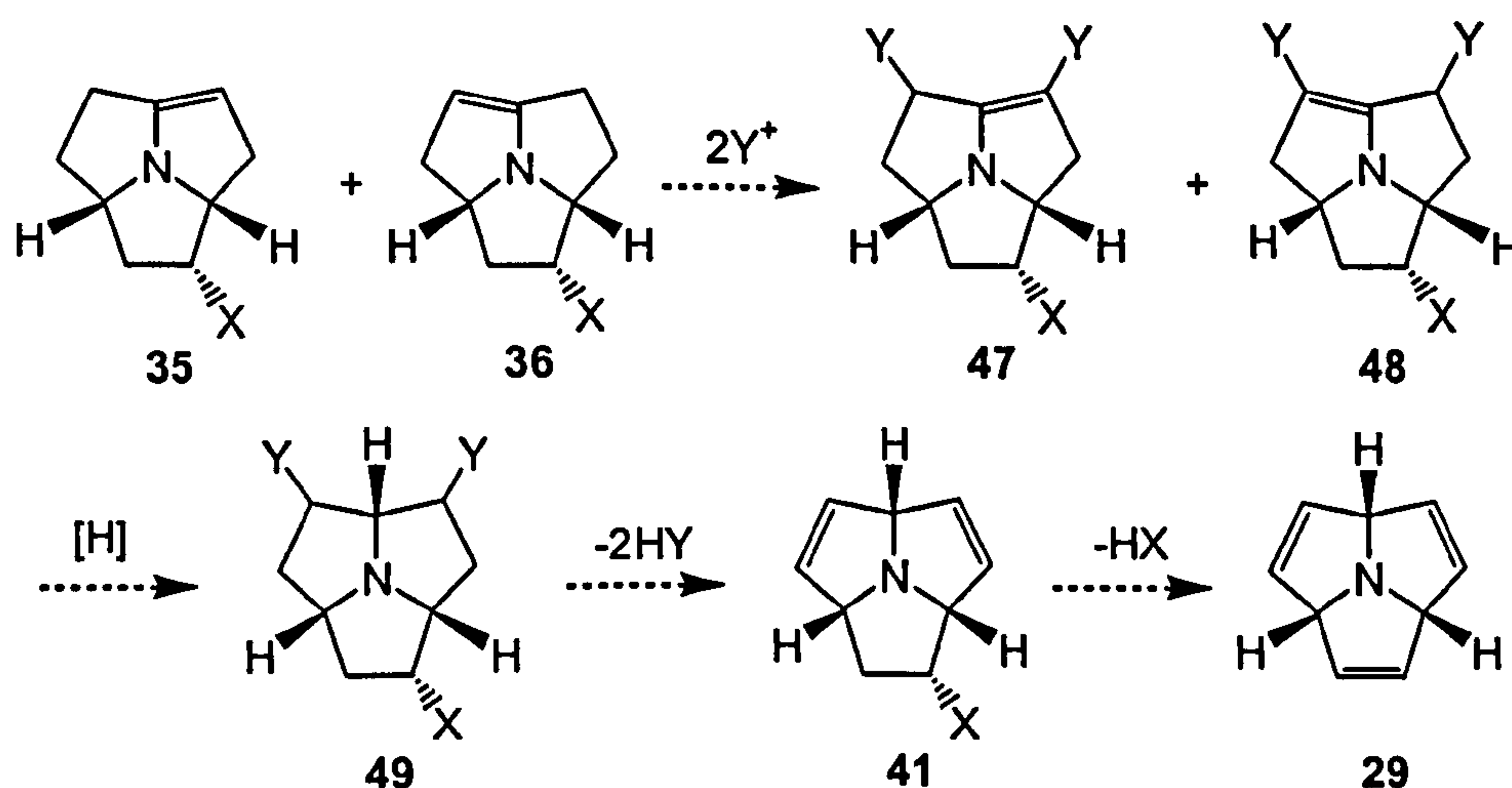
An asymmetric structure was formed by hydrolysis with aq HCl to afford the *trans*-trimer-hemiaminal **46** in which the protonation took place on the less sterically hindered *exo* face. The proposed structure is supported by the peak in the mass spectrum of 424 and the *trans*-stereochemistry suggested by the presence of the two different multiplets at 3.00 ppm and at 2.88 ppm corresponding to the protons on the C2 and C9 of the central azatricycle.



## 2.3 Synthesis of azatriquinadiene

As was seen in the substitution-elimination strategy in Section 2.1, compound **26** should be considered an enamine. So, it was proposed that it might be possible to functionalise the 2- and 9-positions of the azatriquinane system by taking advantage of its enamine character. Then, by reduction and *bis*-elimination, two of the three double bonds of azatriquinacene could be introduced.

The following substitution-elimination strategy was therefore proposed:

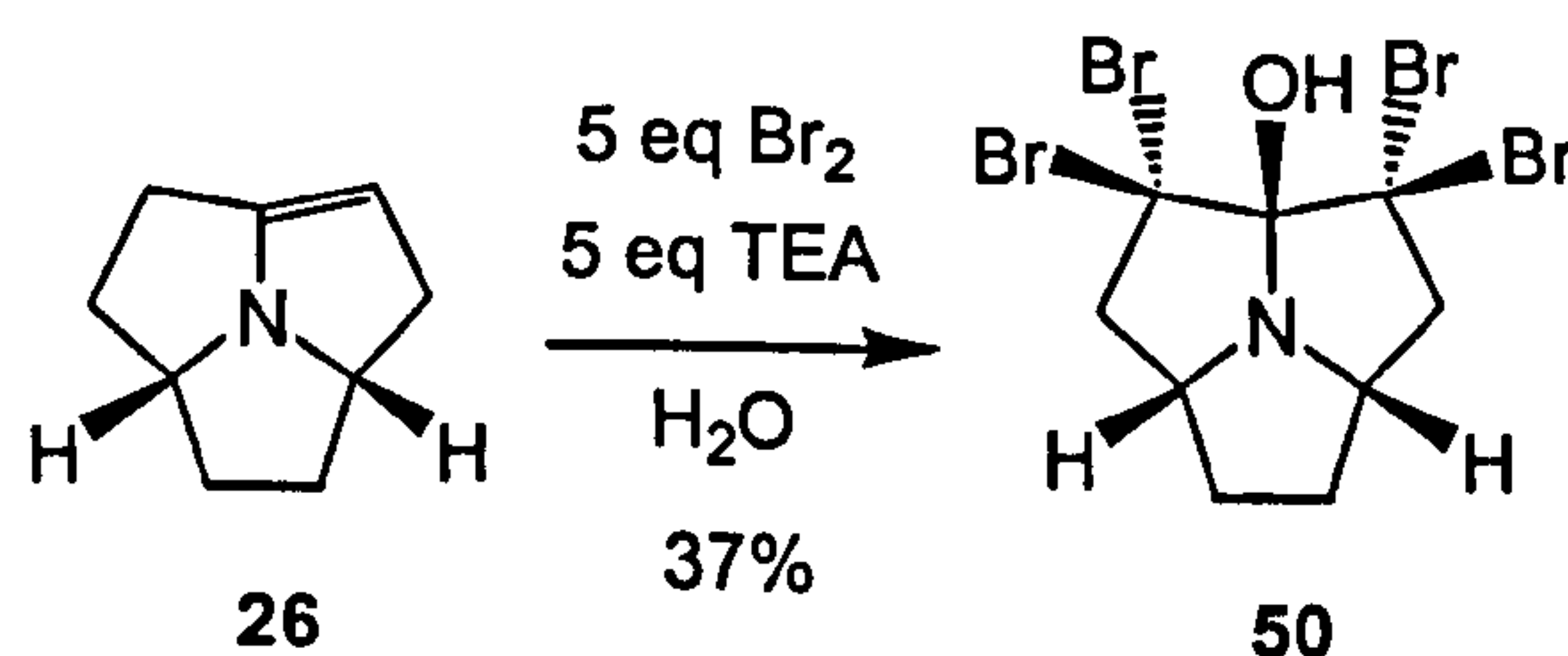


Scheme 14

It was decided to carry out model reactions using the readily available enamine **26** to examine the possibility of forming the C2-C3 and C8-C9 double bonds.

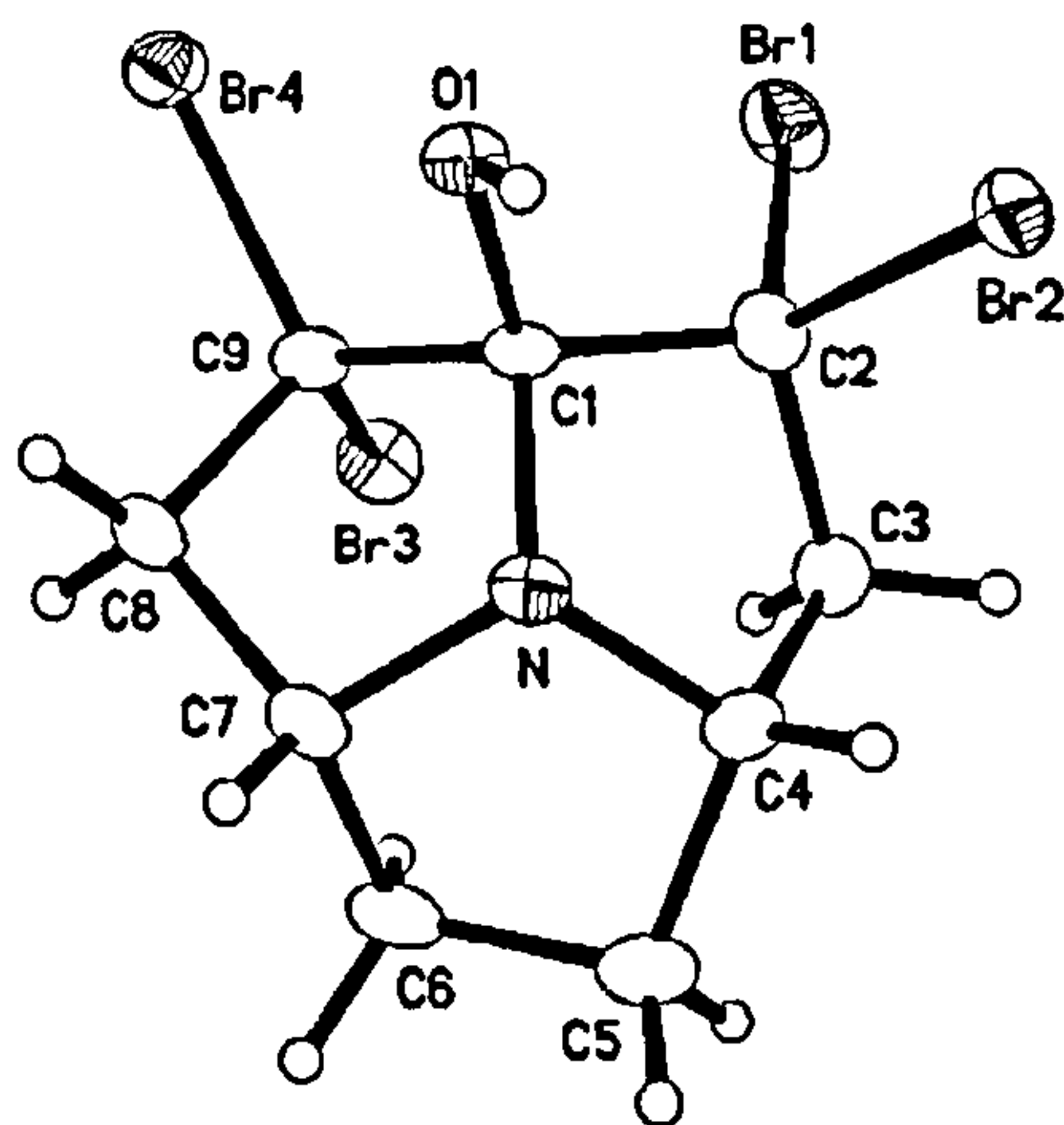
Three different electrophiles were examined: methylchloroformate ( $Y=CO_2Me$ ), paraformaldehyde ( $Y=CH_2OH$ ), and bromine ( $Y=Br$ ).<sup>25</sup> These reagents were chosen due to the possibility of performing an elimination with consequent alkene formation. This could be achieved by oxidative decarboxylation<sup>26</sup> in the first two cases or elimination of HBr in the case of bromine.

Treatment of enamine **26** with methylchloroformate gave a mixture of products in which trimer **43** was the mayor compound formed in this reaction. When paraformaldehyde was used as the electrophile a mixture of products was again obtained which was not possible to purify. Pleasingly, using bromine a molecule substituted at the 2- and 9-positions was obtained. Furthermore, treatment of enamine **26** with two equivalents of bromine gave not dibromoazatriquinane, but a tetrabrominated derivative (Scheme 15).<sup>27</sup> Due to the high reactivity of molecular bromine, it was impossible to stop the bromination at the dibromo stage and, although only two equivalents of bromine were used, the tetrabromo product **50** was obtained. When four equivalents of bromine were used **50** was formed in 37% yield. The hydroxy group results from the hydrolysis of the iminium salt during aqueous work-up.



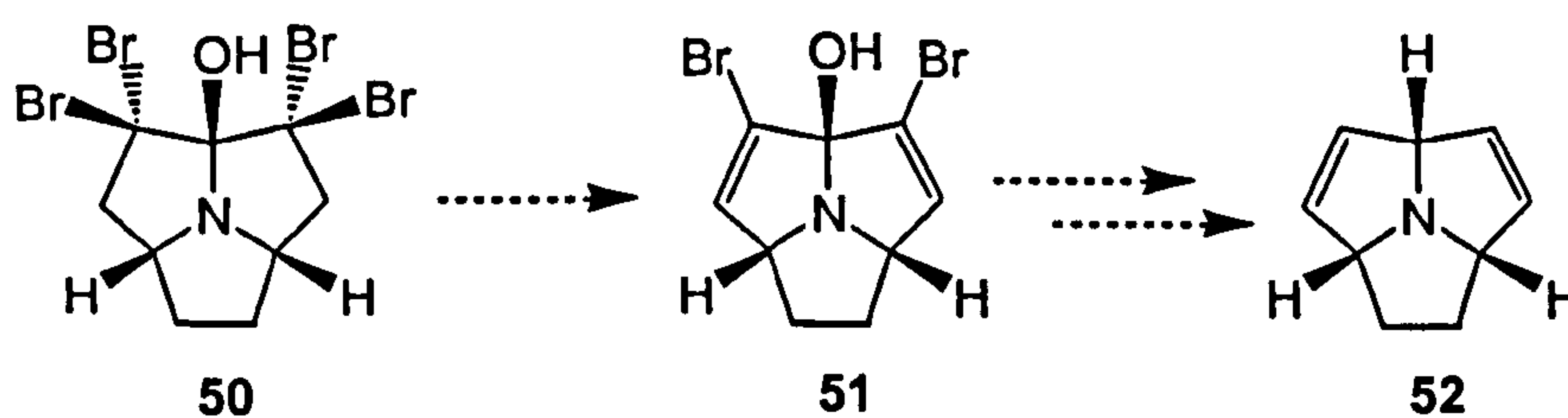
Scheme 15

The tetrabromo compound **50** is a highly crystalline solid and single crystals were obtained by slow evaporation of dichloromethane. Interestingly, the X-ray crystal structure of this compound (Figure 7) shows that the molecule is asymmetric in the solid state. Its convex geometry is deformed due to steric requirements of the bromines which instead of adopting an eclipsed conformation, they are offset with respect to each other, giving a highly strained azatriquinane structure.



**Figure 7. X-ray crystal structure of 50.**

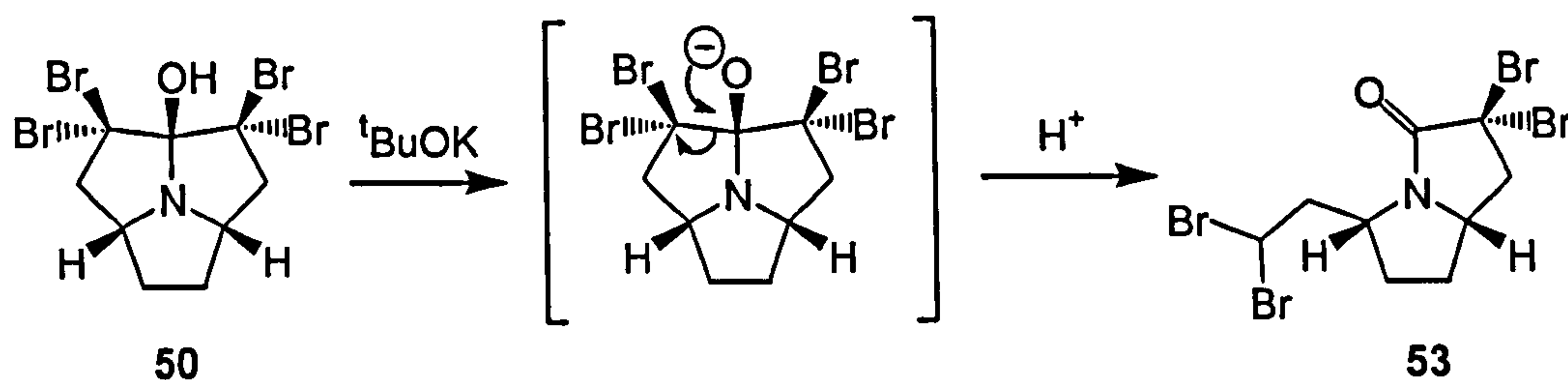
Tetrabromide **50** is a possible precursor of azatriquinadiene **52** *via bis*-elimination and reduction of the resulting vinylbromides and the hemiaminal function, therefore the strategy outlined in Scheme 16 was examined.



### Scheme 16

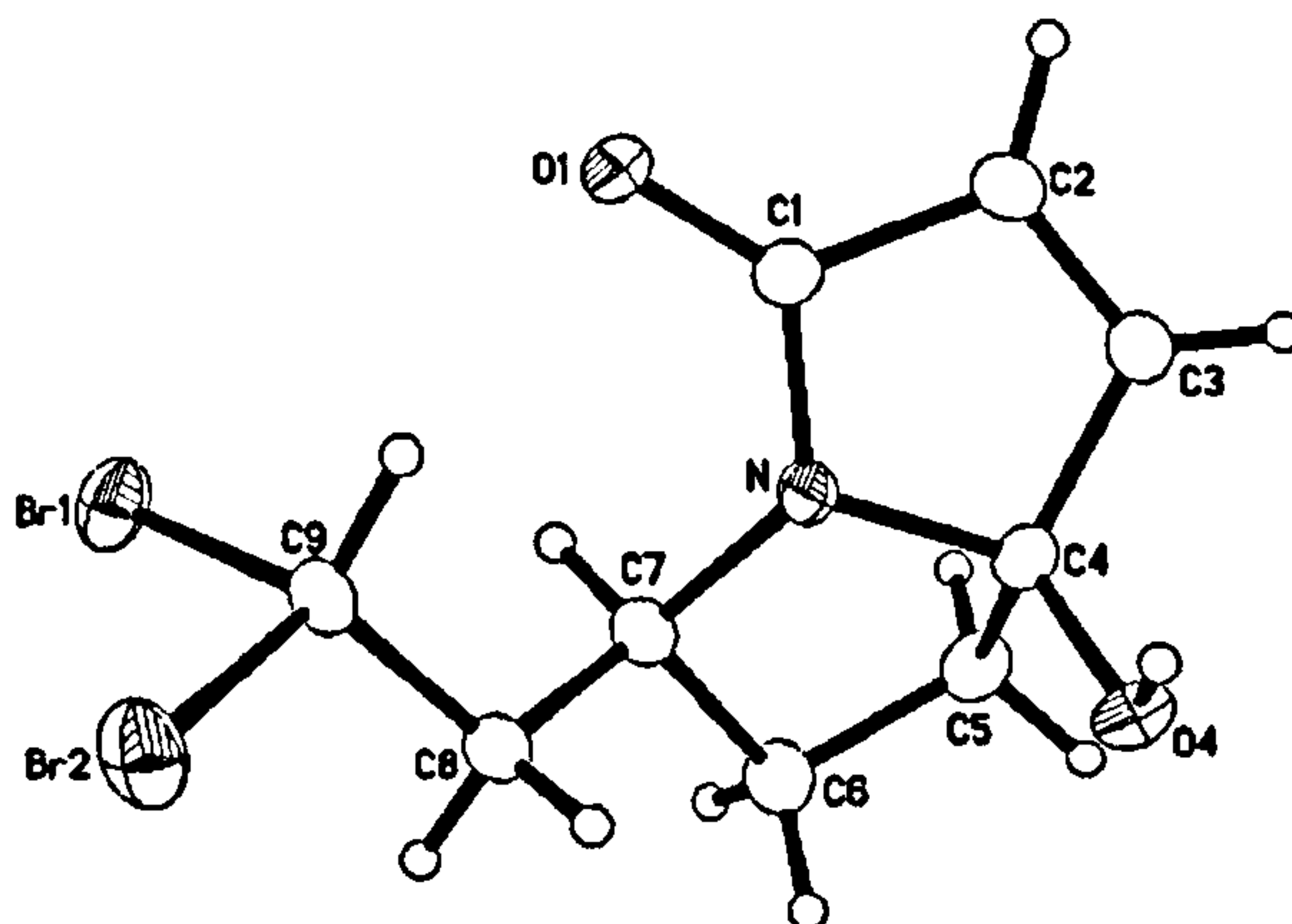
### 2.3.1 Studies on bis-elimination from tetrabromide 50

The first attempt at *bis*-elimination was made using potassium *tert*-butoxide in THF at 0°C which gave a crystalline solid. <sup>1</sup>H-NMR did not show the presence of olefinic protons and surprisingly, a strong band characteristic of a carbonyl group appeared in the infrared spectrum proving that the azatricycle was opened to give bicycle **53**. This compound was obtained in 59% yield and a possible mechanism for this ring opening is presented in the Scheme 17.<sup>28</sup>

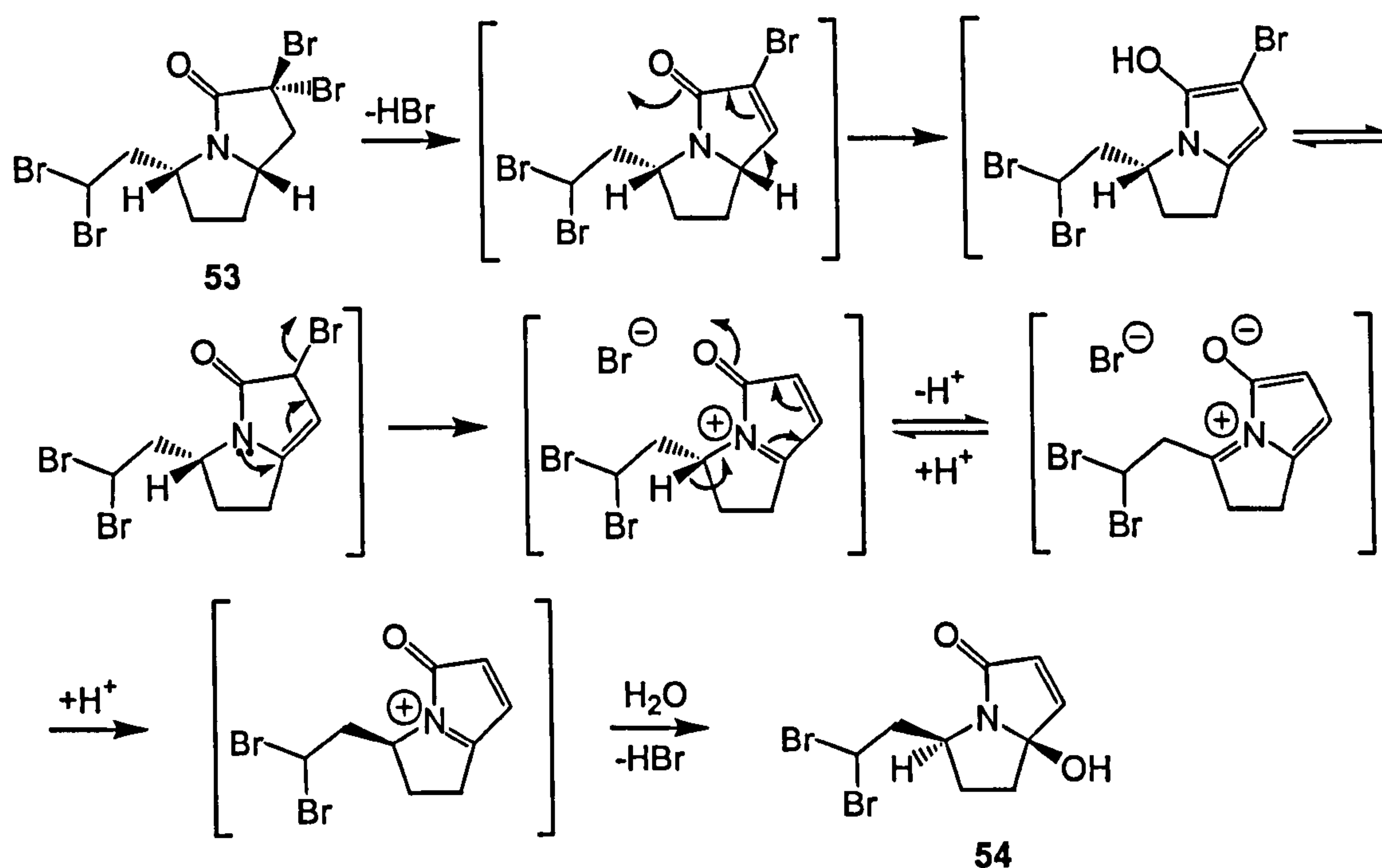


Scheme 17

After this unsuccessful experiment, the elimination was attempted with DBU, a base commonly used for the elimination of HBr.<sup>29</sup> Thus, treatment of the tetrabromo compound **50** with DBU in THF at room temperature led mainly to decomposition plus a small amount of the bicycle **54** (4% yield) whose structure was determined by X-ray crystallography (Figure 8).<sup>28</sup>

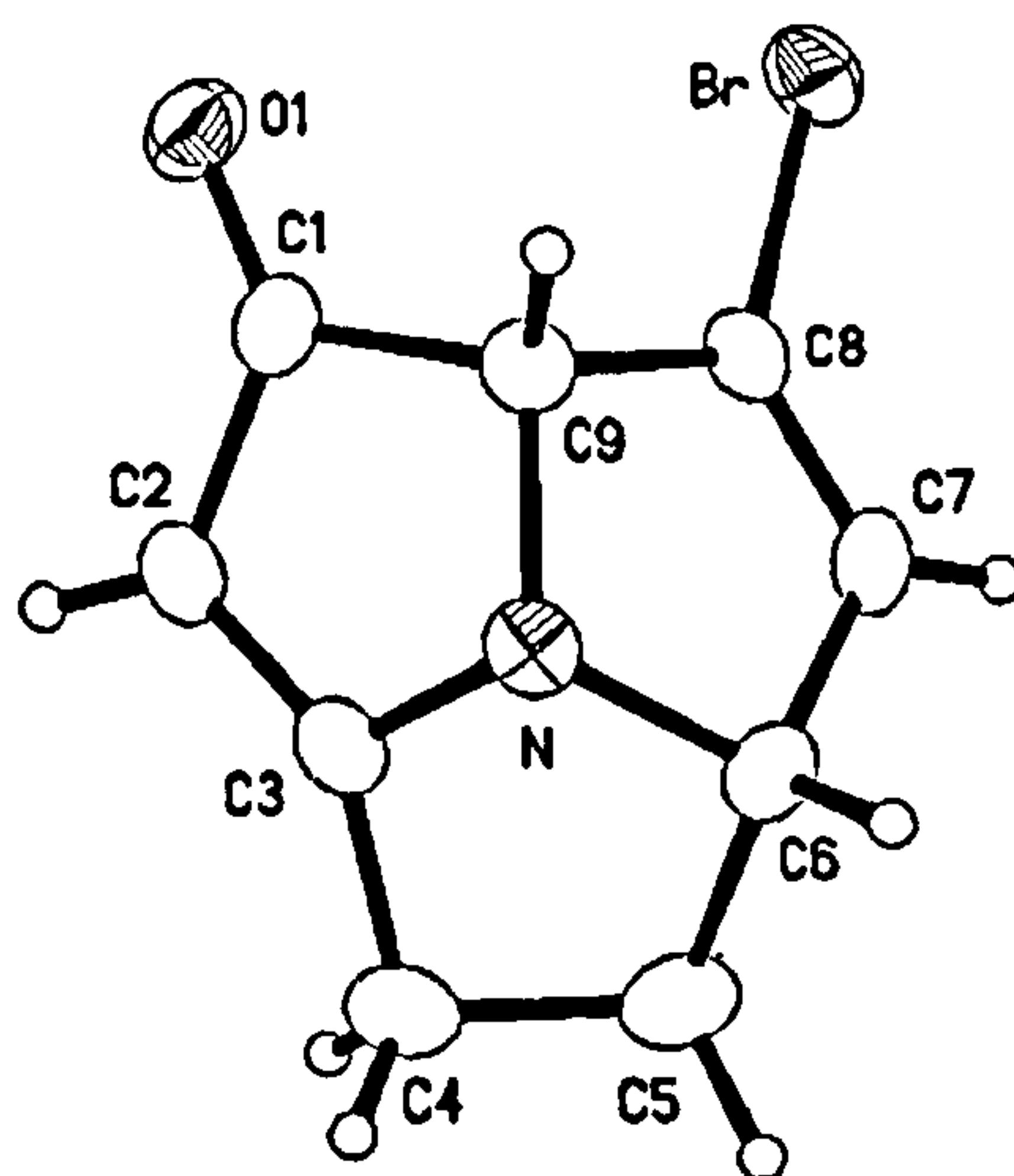
Figure 8. X-ray crystal structure of **54**.

A probable mechanism for this reaction, starting from the product of the first ring opening **53** is shown in Scheme 18. In this case two subsequent eliminations of HBr have taken place after the ring opening, followed by attack on the resulting iminium salt by water.



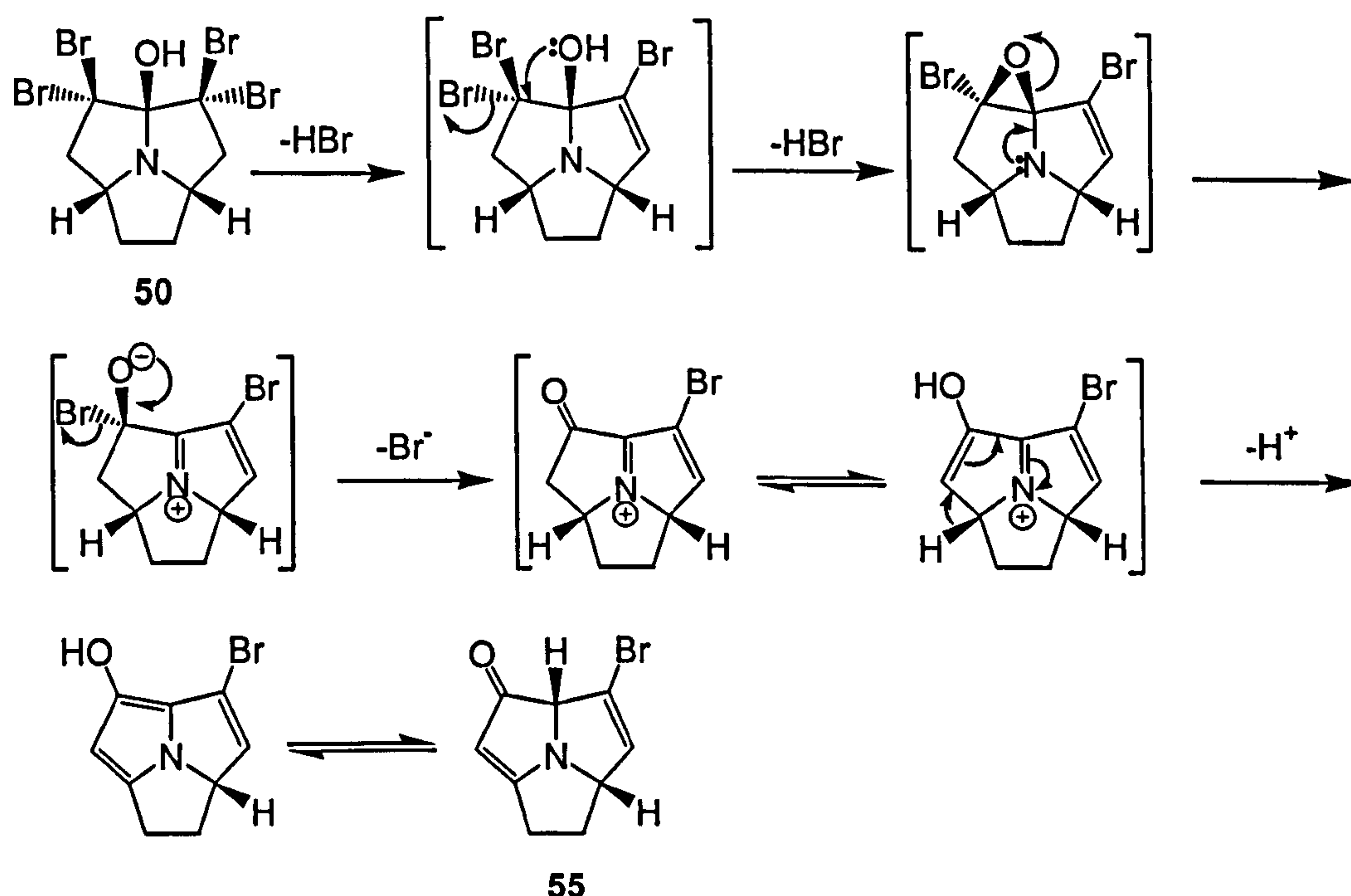
Scheme 18

Attempts at elimination using DBU at lower temperatures ( $0^\circ\text{C}$  and  $-78^\circ\text{C}$ ) afforded yet another new compound **55** in 23% yield, in which the azatricyclic structure is maintained.<sup>28</sup> It was possible to grow single crystals of this compound and its X-ray crystal structure is presented in Figure 9.

Figure 9. X-ray crystal structure of **55**.



In Scheme 19 a possible mechanism for the formation of **55** is proposed. An elimination of HBr takes place during the reaction to give an alkene, then the oxygen is transposed from the bridgehead position to the adjacent carbon *via* an intramolecular nucleophilic displacement followed by ring opening of the resulting epoxide. The carbonyl group is formed by the displacement of a third bromide, and finally, the enaminone moiety is obtained by the loss of a proton. It is important to mention that a number of variations in the sequence of events are possible.



Scheme 19

The enaminone moiety in **55** displays interesting reactivity which makes it a possible precursor for the synthesis of the triene **29**, as will be discussed in Section 2.4.2.

The lack of success in these *bis*-elimination reactions could be attributed to the strained nature of the tetrabromo **50** in which the presence of four bulky bromines makes it a highly strained tricyclic structure which readily undergoes various rearrangements and/or eliminations simply by treatment with base. It was thus decided to attempt the synthesis of a less strained molecule.

One possibility was iodination of enamine **26** instead of bromination. In this case, due to the greater size of the iodine atom in comparison with bromine, the halogenation might stop at the diiodinated state. The iodination of the enamine **26** was attempted using the same conditions as for the bromination but unfortunately, the reaction gave a complex mixture and no diiodinated compound could be detected by mass spectrometry.

The next reaction attempted was the substitution of the bromines in **50** with chlorines with the aim of reducing the strain in the system as a result of the smaller size of chlorine. Thus, heating tetrabromide **50** in a 1:1 mixture of acetonitrile and aqueous saturated NaCl,<sup>29</sup> resulted in no halogen exchange, but another new product **56** was obtained in 30% yield.<sup>28</sup> Single crystals of this compound were grown and its X-ray structure is presented in Figure 10.

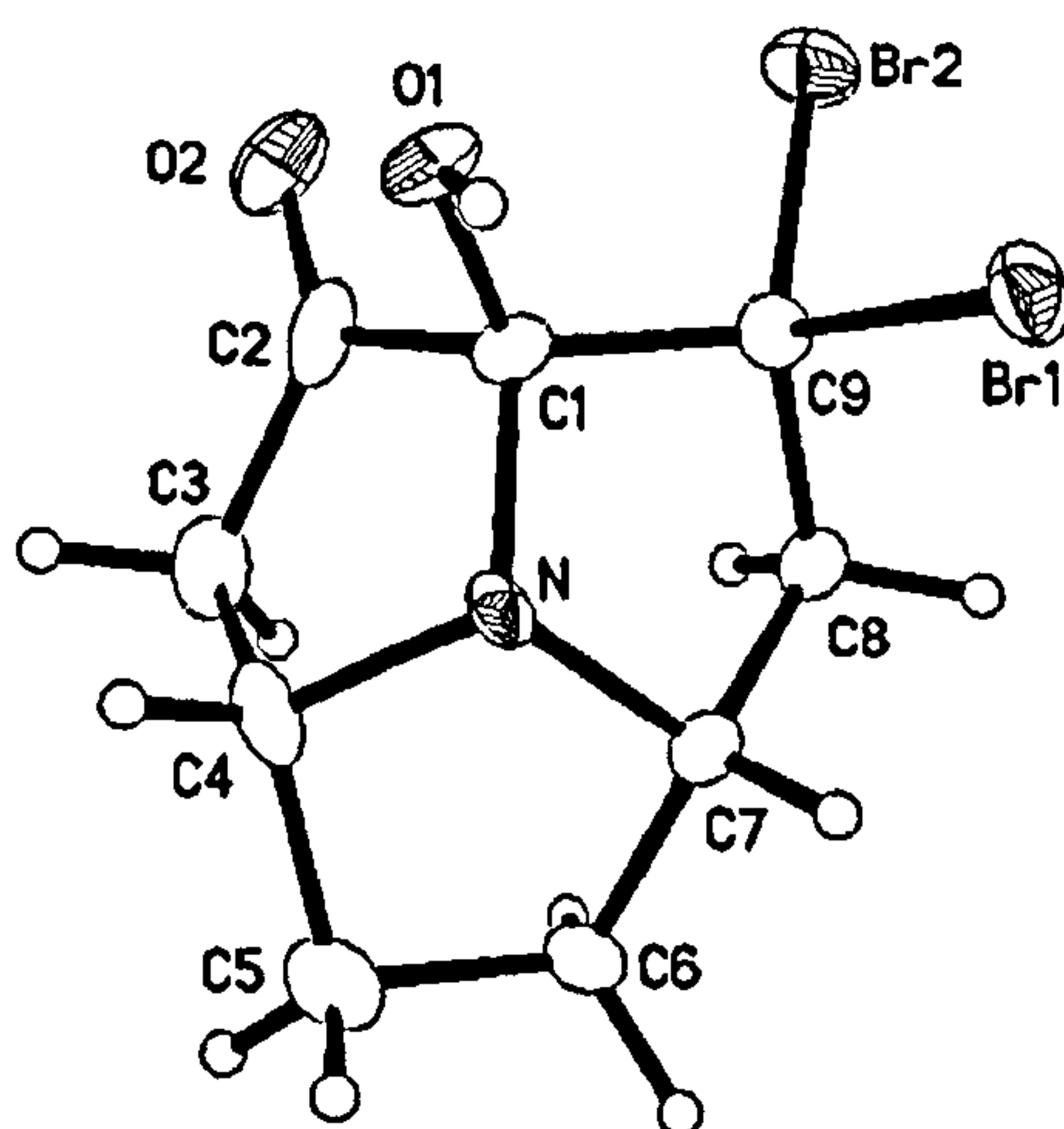
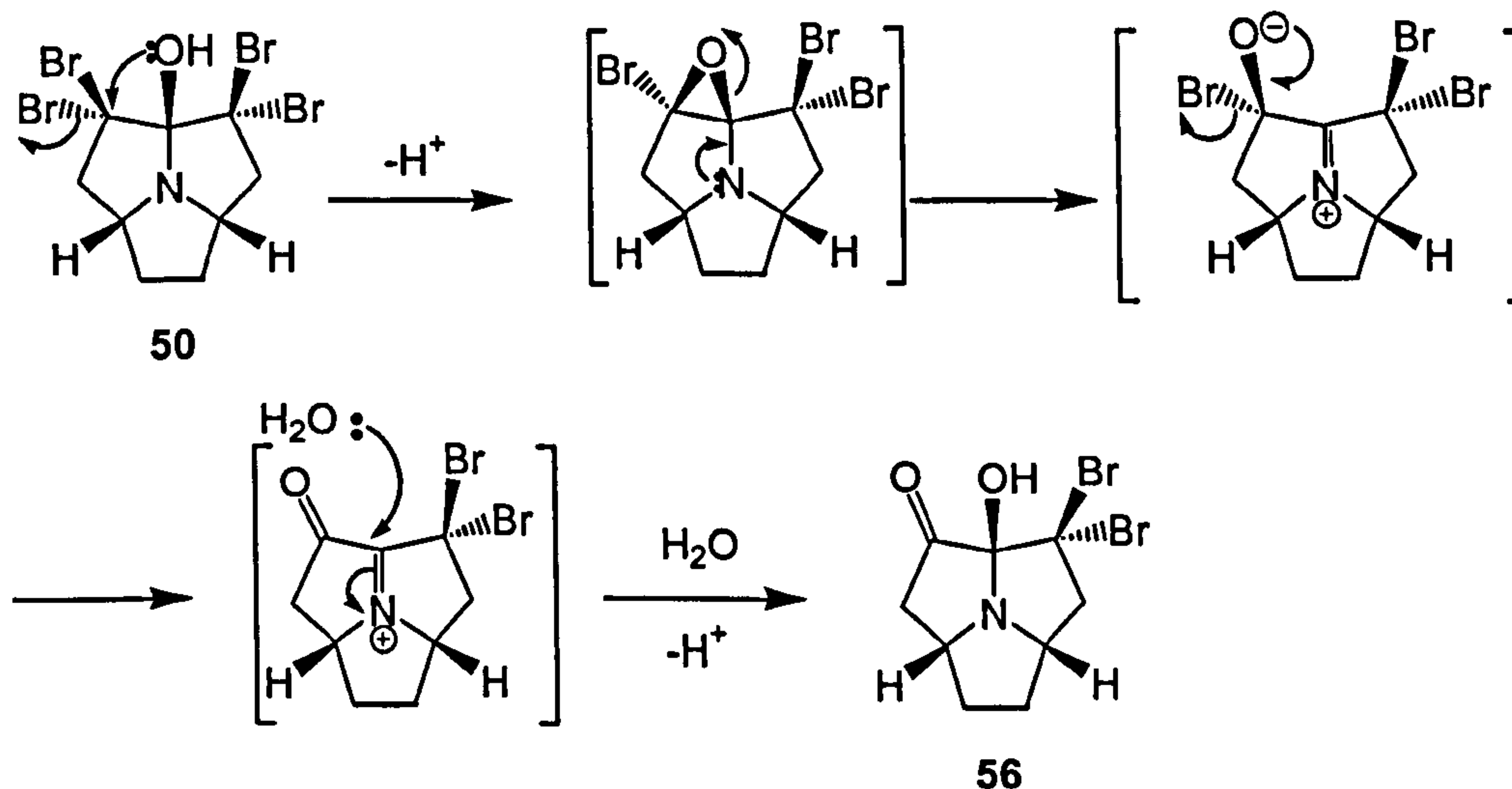


Figure 10. X-ray crystal structure of **56**.

In this case the same transposition of the oxygen appears to have taken place as in the formation of enaminone **55**. However, due to the presence of water in the reaction, the iminium salt is hydrolysed without further rearrangement to give **56** (Scheme 20).



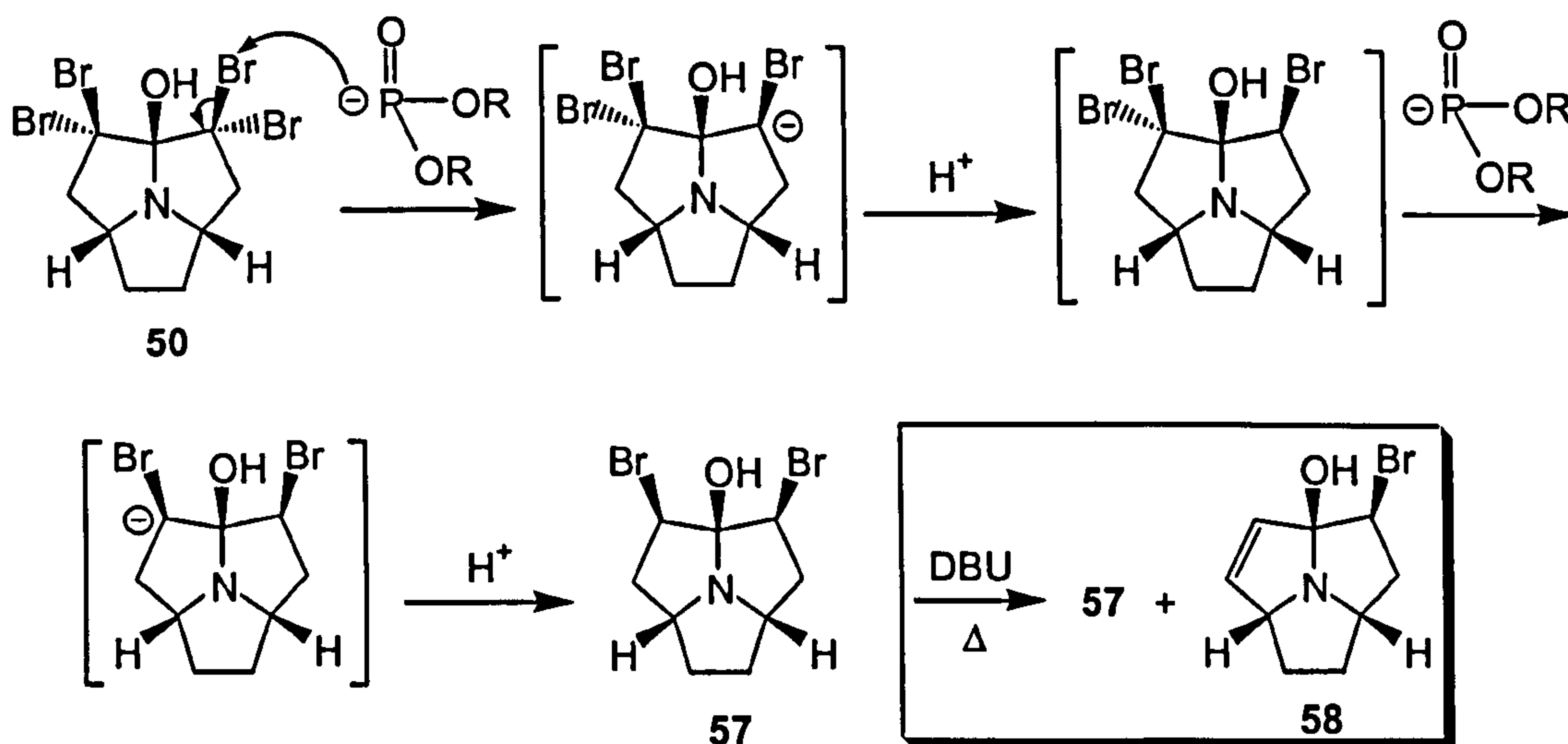
Scheme 20

Another attempt to diminish the strain in the tetrabromide **50** was by reduction of the two *gem*-dibromo moieties to give dibromoazatriquinane **57** using diphenyl phosphite and triethylamine, a method which is used for the reduction of *gem*-dibromocyclopropanes.<sup>30</sup> This reaction afforded **57** in 38% yield, the mass spectrum of which indicated the presence of two bromines in the molecule. The <sup>1</sup>H-NMR and <sup>13</sup>C-NMR spectra of this product showed that it was symmetrical suggesting a *syn*-disposition of the two bromines. The *exo* stereochemistry assigned to the two bromines in **57** is based on the probable mechanism of this reaction. The formation of the anions is responsible for the final stereochemistry of the molecule in which an *exo* disposition of the bromines is more stable than the *endo* orientation due to steric requirements (Scheme 21).

The above hypothesis was supported by the fact that, on treating the dibromo compound **57** with DBU in THF, it was necessary to heat at reflux overnight to achieve any elimination. A mixture of starting material and the monoeliminated product **58** was obtained. The difficulties with this elimination are attributed to the fact that the base has to abstract a proton from the more sterically hindered *endo* face due



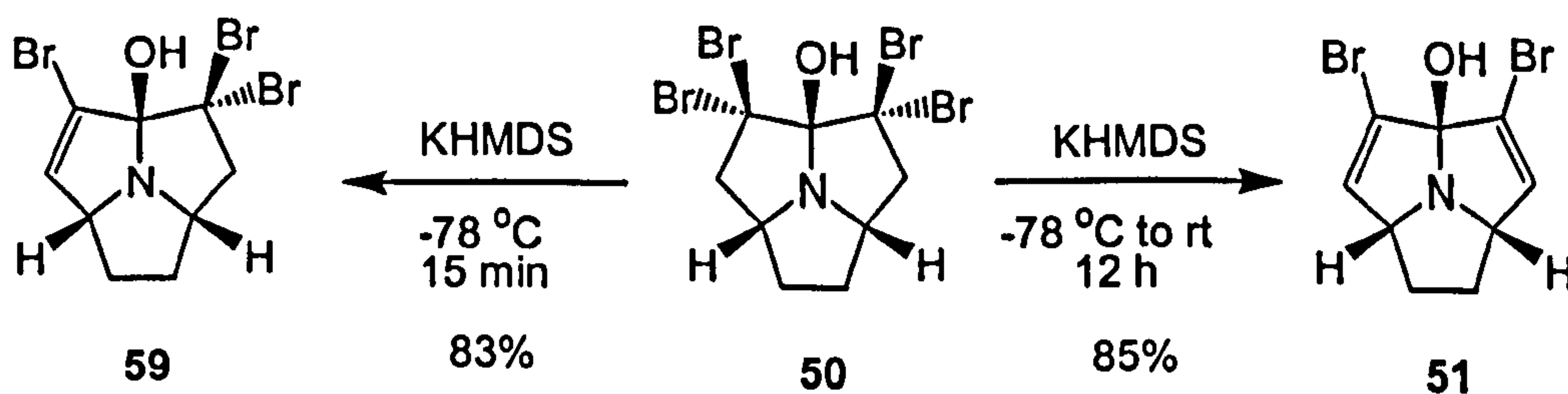
to the *exo* disposition of the bromines. In the case of the formation of enaminone **55**, one elimination was observed with DBU at 0°C and this is probably due to the fact that, in that case, the abstraction took place from the less sterically hindered *exo* face.



Scheme 21

Due to the difficulties encountered in *bis*-elimination with **57**, we continued to investigate the elimination of HBr with the tetrabromo compound **50** using other bases.

The elimination of HBr from **50**, without any further reaction, was finally achieved using KHMDS. It is noteworthy that it was possible to obtain the monoelimination product **59** or the *bis*-elimination product **51** in 83% and 85% yields, respectively, using different conditions (Scheme 22). It was possible to grow crystals of both elimination products and their X-ray crystal structures are presented in Figure 11.<sup>28</sup>



Scheme 22

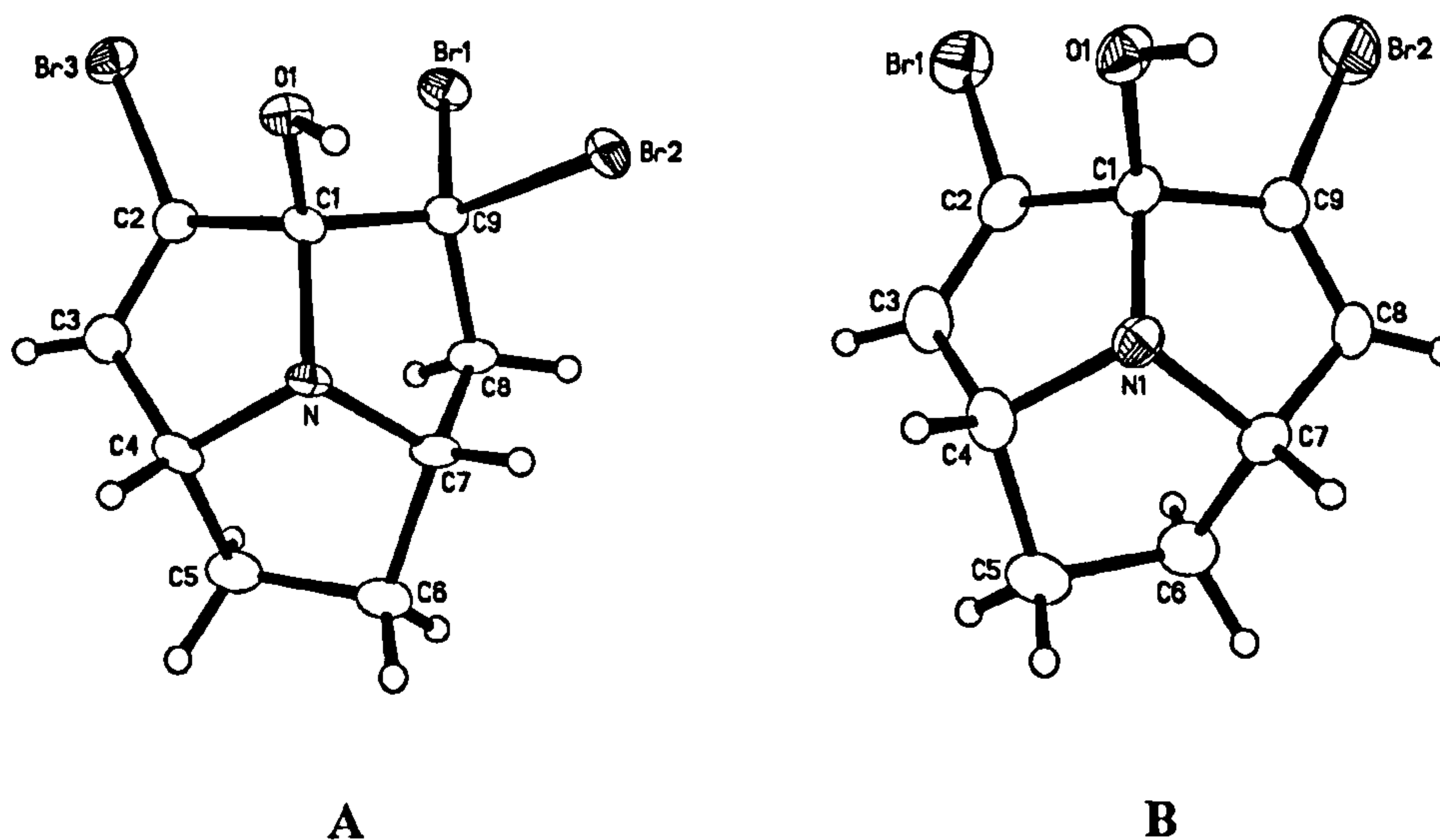


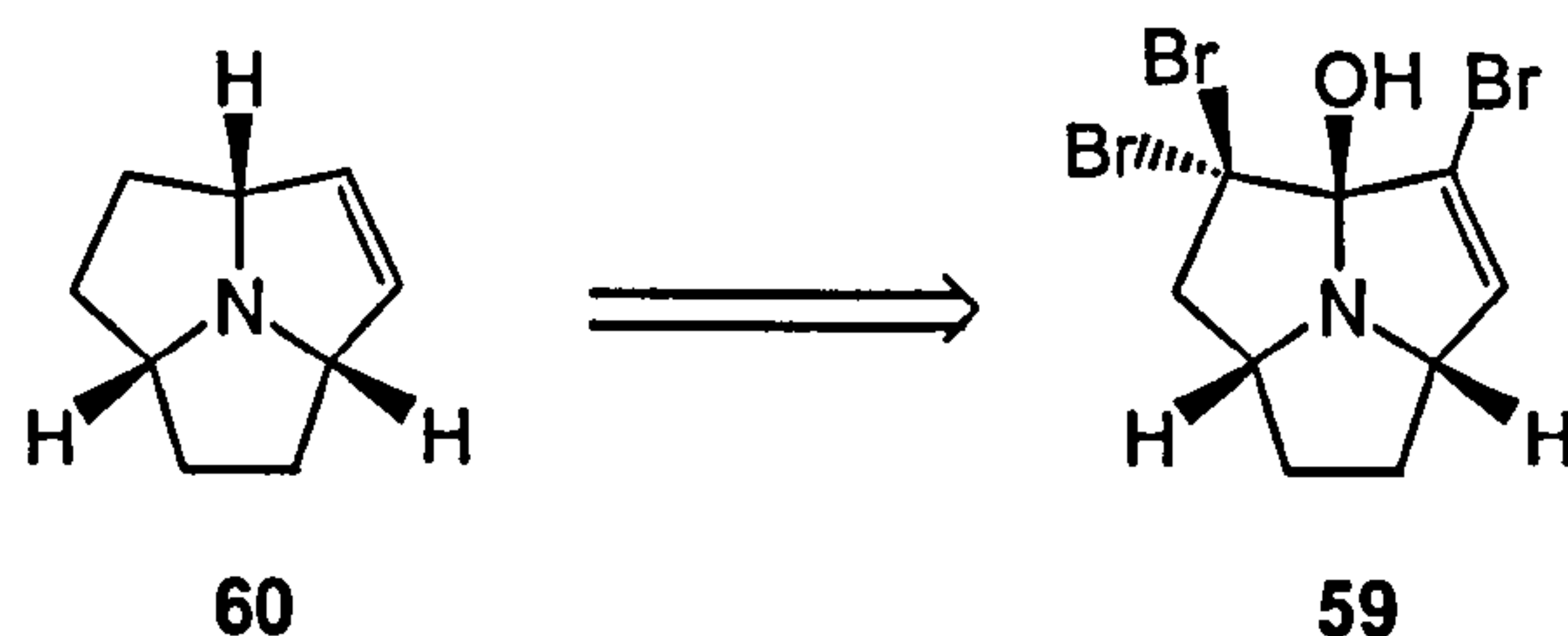
Figure 11. A) X-ray crystal structure of 59. B) X-ray crystal structure of 51.

A possible explanation for the different rates of the first and second eliminations is that, due to the deformation of the convex geometry of tetrabromo compound 50, each side of the molecule has only one antiperiplanar disposition of atoms for the elimination of HBr. However, on one side the elimination of HBr is strongly favoured due to the base abstracting a proton from the less sterically hindered *exo* face compared to the other side of the molecule, where the base has to abstract a proton from the more hindered *endo* face.

The crystal structure of 59 (Figure 11A) shows that there is not an antiperiplanar disposition close to  $180^\circ$  between either of the two protons at C8 with Br1 or Br2. This could explain why the second loss of HBr whether the base approaches *via* the *endo* or *exo* face, requires longer reaction times and higher temperatures. Thus, on treating the tetrabromo compound 50 with KHMDS at  $-78^\circ\text{C}$ , an elimination of HBr takes place within 15 min but it is necessary to maintain these basic conditions for 12 h and also allow the mixture to warm up to room temperature to achieve the second elimination.

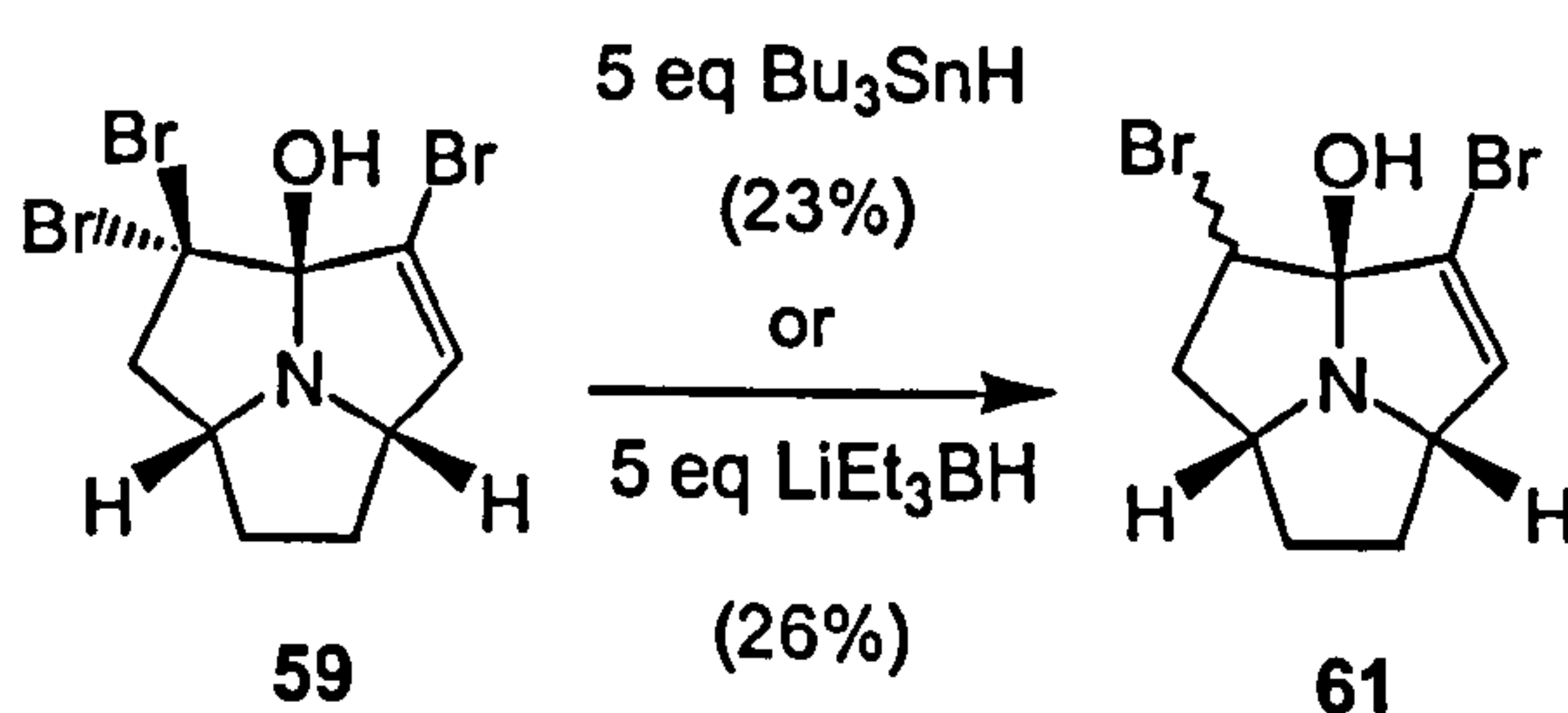
### 2.3.2 Approaches to the synthesis of monoene 60

With **59** in hand the synthesis of **60** was examined (Scheme 23).



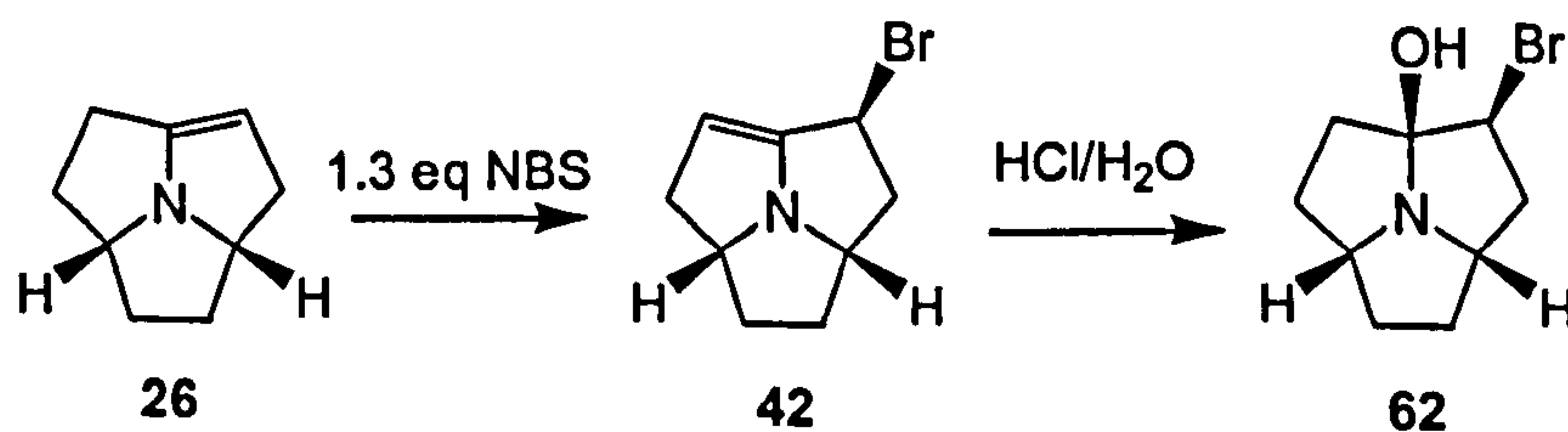
Scheme 23

The reduction of the *gem*-dibromo moiety was attempted using tributyltin hydride<sup>7</sup> and superhydride.<sup>8</sup> In both cases compound **61** was obtained, illustrating the difficulty in reducing the two bromines in C9 (Scheme 24).



Scheme 24

As seen in Section 2.1, bromination of the enamine **26** with NBS gave **42**. It was possible to get the monobromo compound **62** exposing **42** to hydrolysis conditions (Scheme 25).



11% over two steps

Scheme 25

Attempts to eliminate HBr from compound **62** towards the synthesis of **60** using <sup>t</sup>BuOK, KHMDS and NaH failed, giving recovered starting material. This difficulty was attributed to the *exo* stereochemistry of the bromine atom which forces the base to abstract a proton from the more sterically hindered *endo* face (Figure 12).<sup>28</sup>

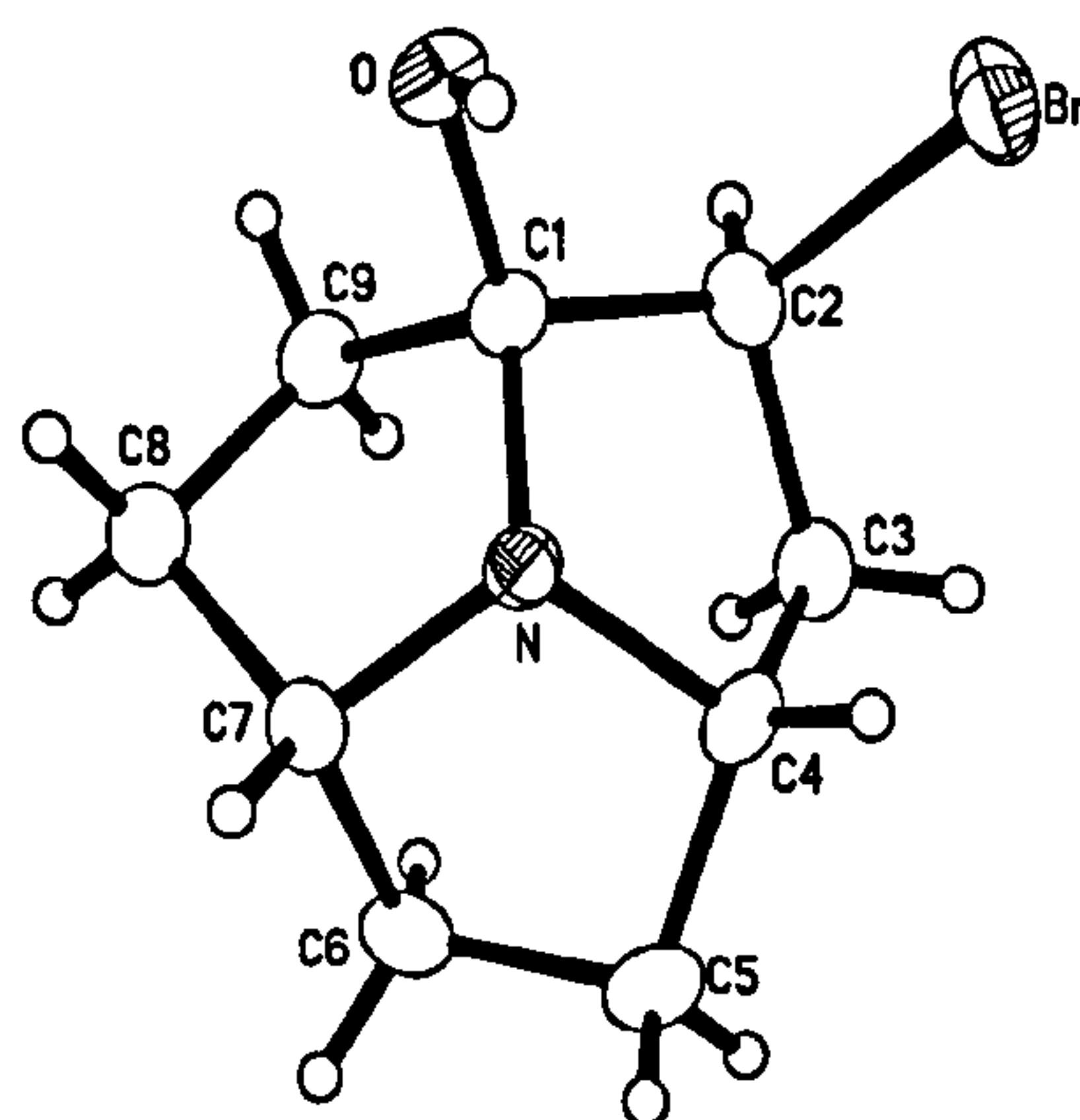
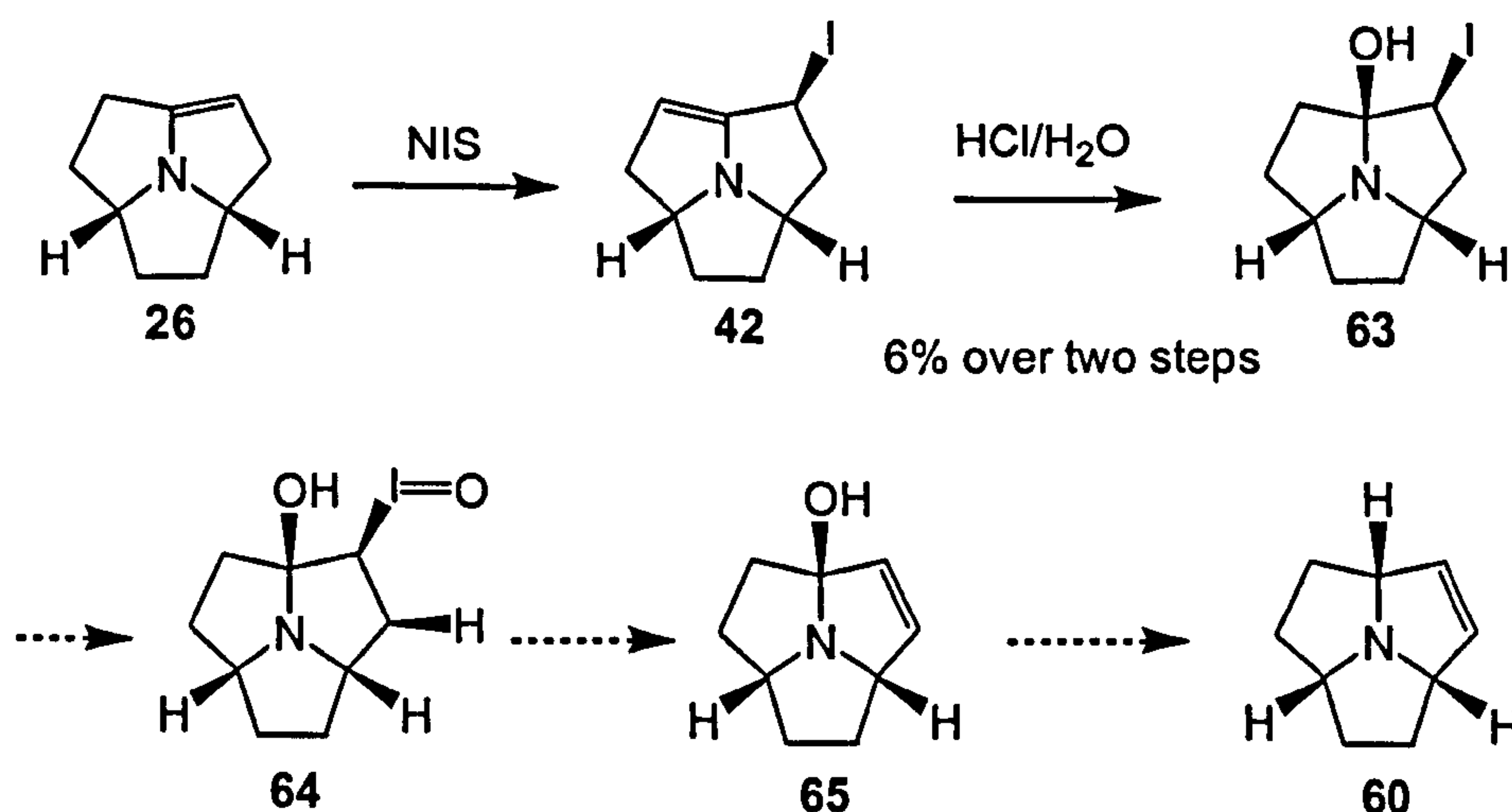


Figure 12. X-ray crystal structure of **62**.

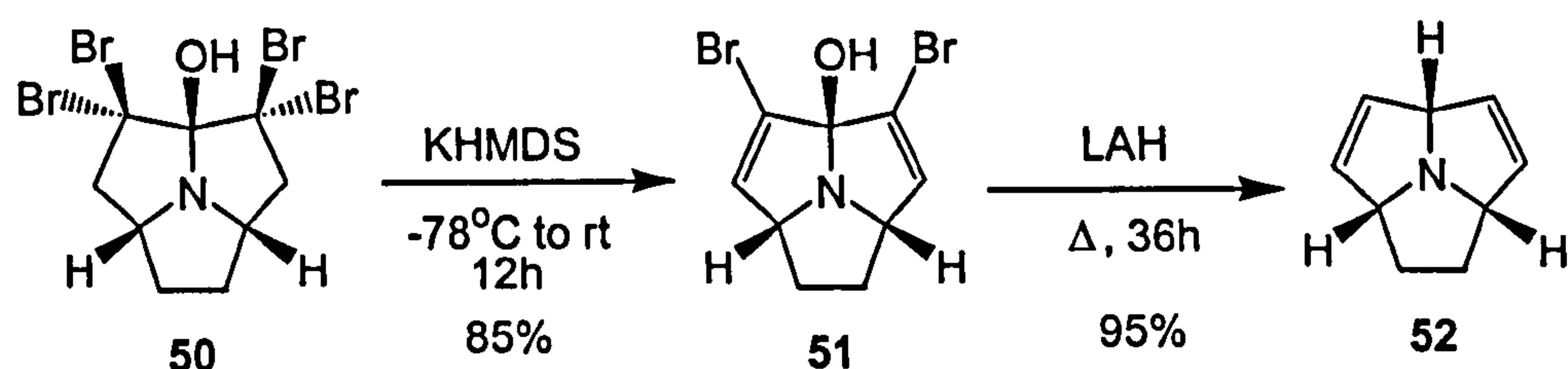
The iodo-analogue compound **63** was synthesised with the aim of introducing the double bond of **60**. Based on literature precedents,<sup>33</sup> a *syn*-elimination of HIO could take place after oxidation of the iodine atom (Scheme 26). Unfortunately, the elimination did not take place and only starting material was recovered.



Scheme 26

### 2.3.3 Synthesis of azatriquinadiene **52** by reduction of **51**

The reduction of **51** to synthesise the diene **52** was attempted and pleasingly, using the conditions for the reduction of the hemiaminal OH with  $\text{LiAlH}_4$ ,<sup>13</sup> it was also possible to reduce both vinyl bromides in the same step, affording diene **52** in almost quantitative yield.<sup>27</sup> The final steps in the synthesis of azatriquinadiene **52** are presented in Scheme 27.



Scheme 27

The free base of the diene **52** is a volatile oily solid which was isolated and purified as its trifluoroacetate salt. Its structure was supported by the presence of two double doublets at 5.82 ppm and 5.69 ppm in the  $^1\text{H}$ -NMR spectrum, corresponding to the four olefinic protons in which the major coupling constant (6.3 Hz) is characteristic of that of the olefinic proton coupling constants in cyclopentene derivatives. The presence of a singlet at 5.46 ppm confirmed the reduction of the hemiaminal OH. Its  $^{13}\text{C}$ -NMR spectrum has five peaks, supporting the presence of a mirror plane in the molecule in which the peaks at 127.9 ppm and 127.8 ppm correspond to the olefinic carbons.

### 2.3.4 Hydrogen bonded dimers

Figure 13 shows that in the solid state, the four structures shown below, the tetrabromo, tribromo, dibromo and monobromo compounds **50**, **59**, **51** and **62**,



respectively, form dimers *via* two intermolecular hydrogen bonds between the proton of the hemiaminal OH of one molecule and the nitrogen of the other.

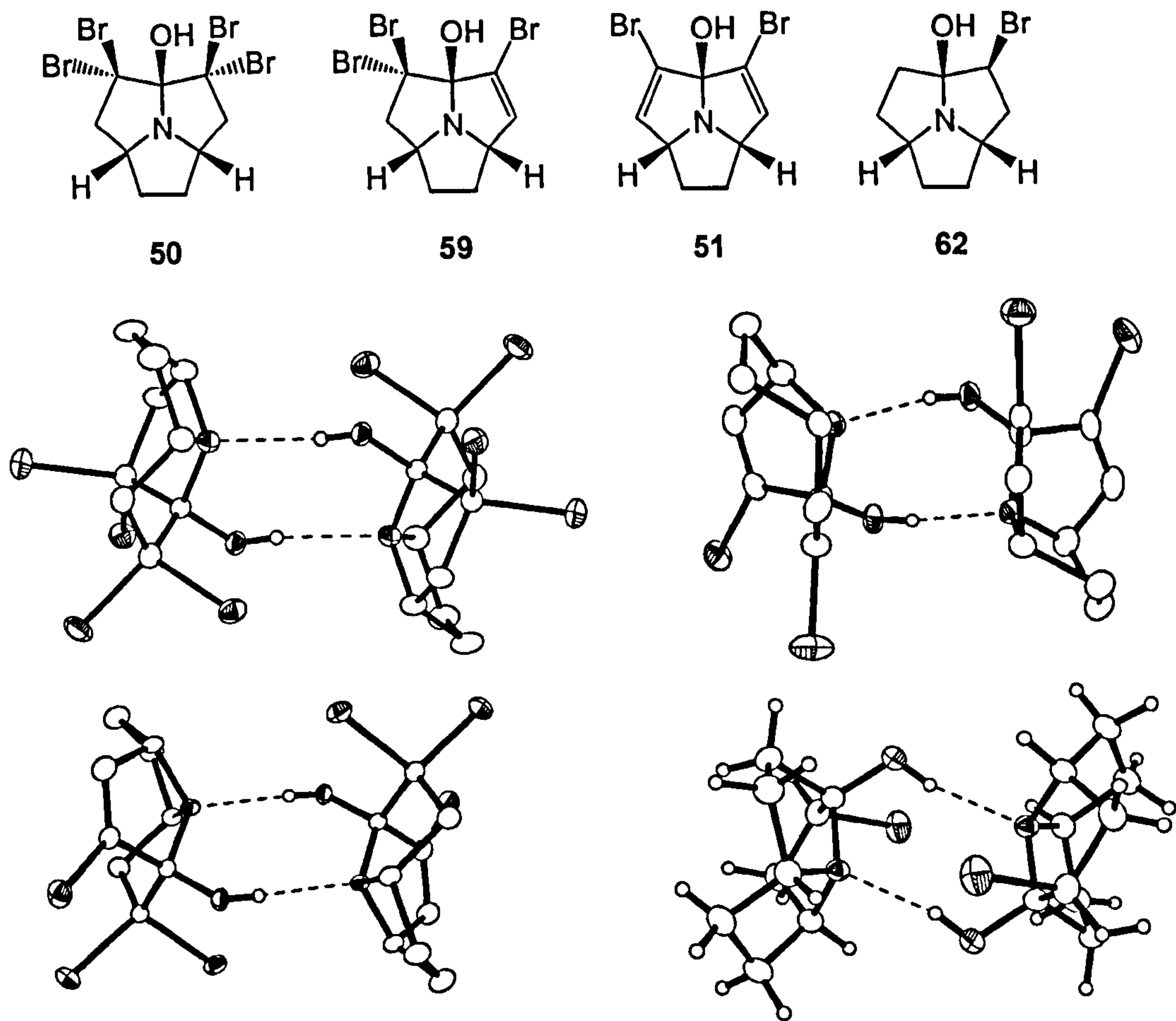


Figure 13. Top left: tetrabromo 50, bottom left: tribromo 59, top right: dibromo 51, bottom right: monobromo 62.

The distances and angles of the intermolecular hydrogen bonds are shown in Table 1.

	O-H...N (Å)	O...N (Å)	O-H..N angle (°)
Tetrabromo 50	2.08	2.89	161.1
Tribromo 59	2.00	2.80	159.4
Dibromo 51	2.01, 1.99 <sup>a</sup>	2.85, 2.82 <sup>a</sup>	172.8, 169.0 <sup>a</sup>
Monobromo 62	1.99	2.80	163.0

Table 1. Distances and angles of the intermolecular hydrogen bonds of dimers 50, 59, 51 and 62. <sup>a</sup> Two values are shown due to its asymmetric character.

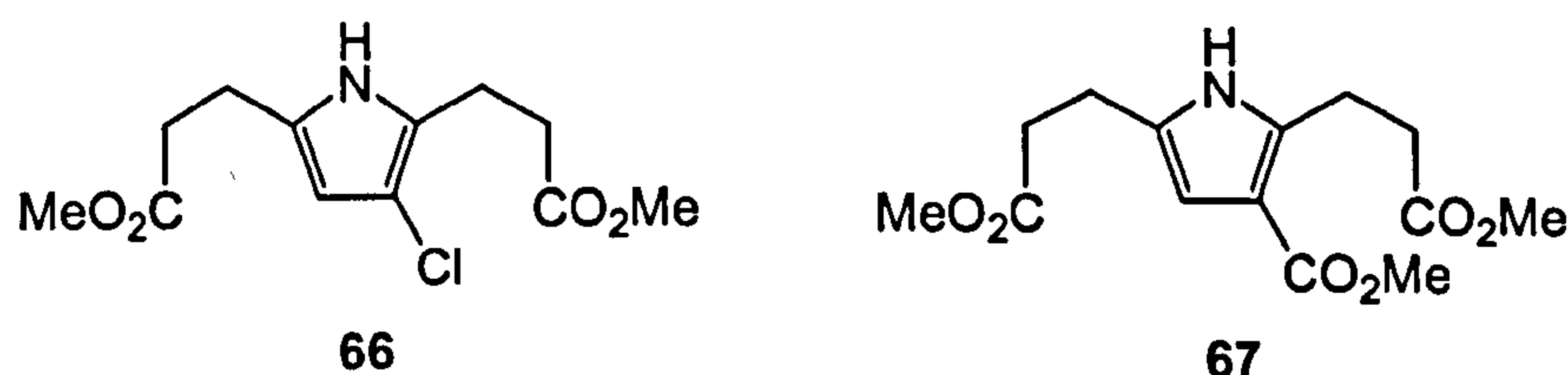


## 2.4 Synthesis of azatriquinacene

### 2.4.1 Substitution-elimination strategy: attempts to introduce the bottom double bond

With the synthesis of diene **52** in hand, we hoped to employ the strategy outlined in Section 2.3 to introduce the bottom double bond of azatriquinacene **29**.

One possibility we have already seen is to start with a substituent on the 3-position of pyrrole which is capable of undergoing elimination later in the synthesis. Thus, hydrogenation of 2,3,5-trisubstituted pyrroles should give 2,3,5-trisubstituted pyrrolidines with the three substituents on the same side. It is also necessary that the substituent at the 3-position may not be removed under the hydrogenation conditions and for this purpose, the following 3-substituted pyrroles were synthesised:



Chloropyrrole **66** was prepared by reacting dimethyl-1*H*-pyrrole-2,5-dipropanoate **23** with *N*-chlorosuccinimide in chloroform. The 3-methoxycarbonyl analogue **67** was obtained using methylchloroformate and catalytic aluminium chloride.

An attempted hydrogenation of 3-chloropyrrole **66** showed that hydrogenolysis of the halogen unfortunately occurred faster than hydrogenation of the pyrrole ring to give dimethyl-*cis*-2,5-pyrrolidine dipropanoate **24**.

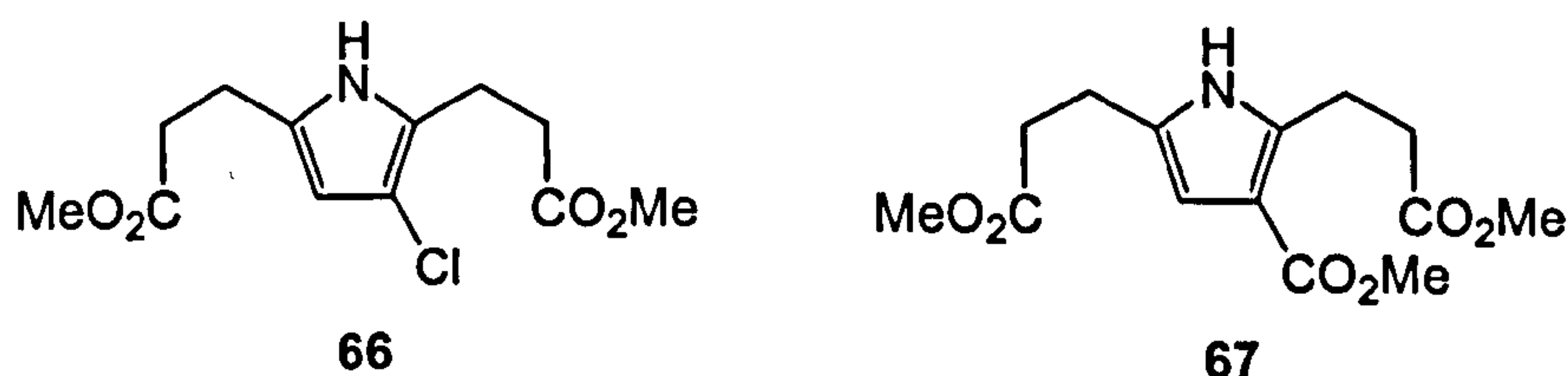
The hydrogenation of the 3-methoxycarbonyl analogue was also attempted, but was not achieved possibly due to the electron withdrawing nature of the ester at the 3-position. Various attempts to hydrogenate **67** by increasing the hydrogen pressure,

## 2.4 Synthesis of azatriquinacene

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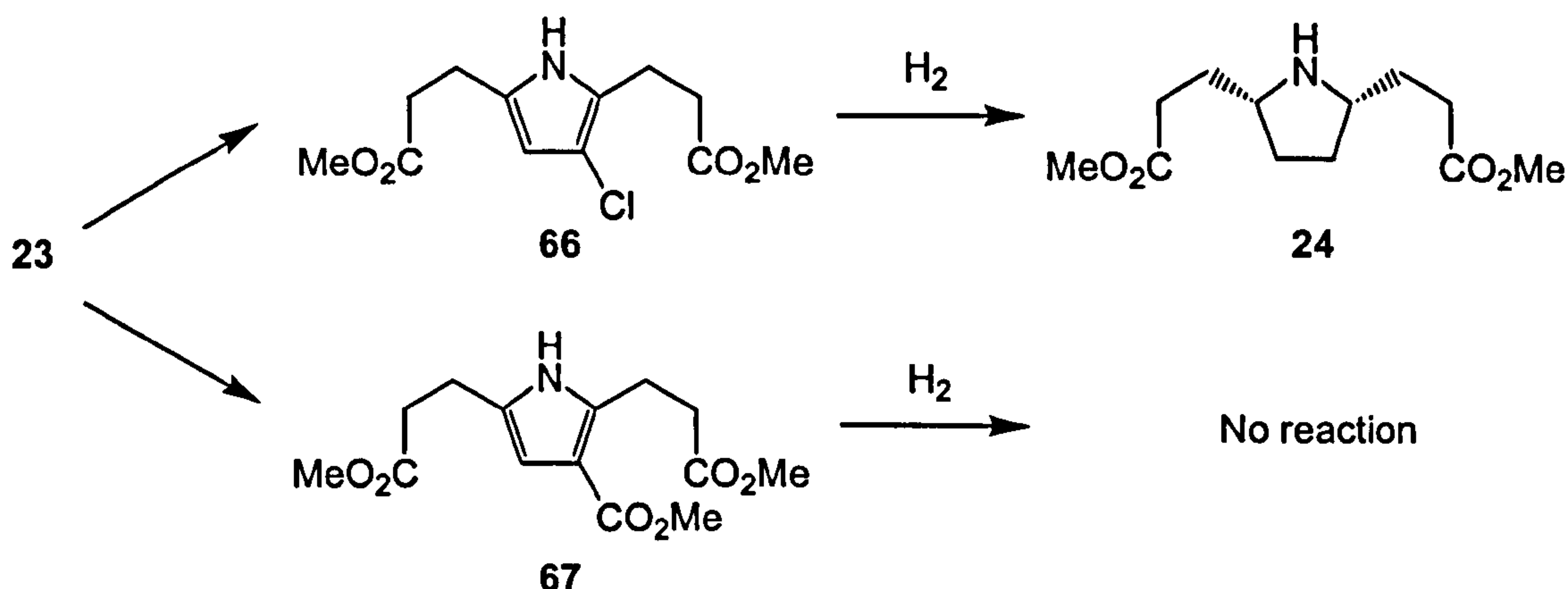
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The hydrogenation of the 3-methoxycarbonyl analogue was also attempted, but was not achieved possibly due to the electron withdrawing nature of the ester at the 3-position. Various attempts to hydrogenate **67** by increasing the hydrogen pressure,

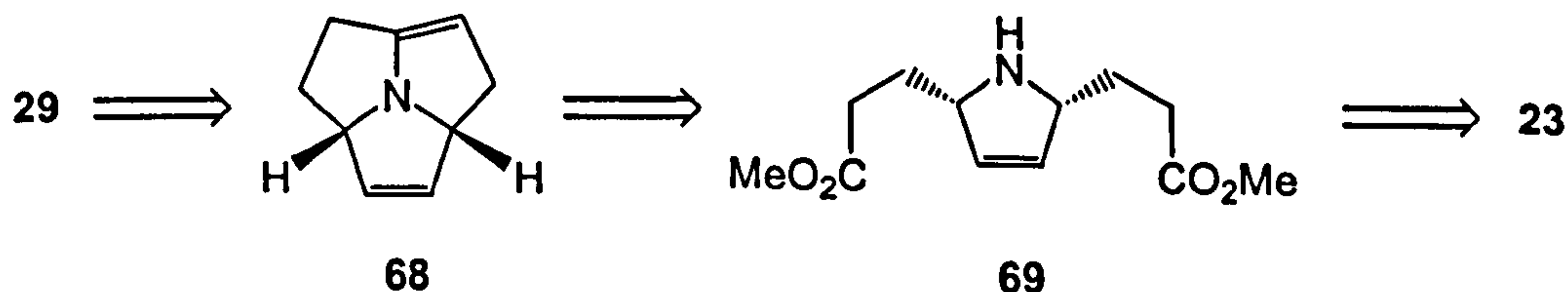
temperature, amount of catalyst, and also using other conditions ( $\text{PtO}_2 / \text{AcOH}$ ) were all unsuccessful.

The synthesis of the two different pyrroles and the results of their respective hydrogenation attempts are presented in Scheme 28.



Scheme 28

The synthesis of 3-pyrroline 69 was attempted by reduction of pyrrole 23 with the idea of synthesising a cyclisation precursor which contains a bottom double bond instead of a substituent. The 3-pyrroline 69 should cyclise to give enamine 68 which after bromination, elimination and reduction by the same protocol used for the synthesis of azatriquinadiene 52 (Section 2.3) might give azatriquinacene 29 (Scheme 29).



Scheme 29

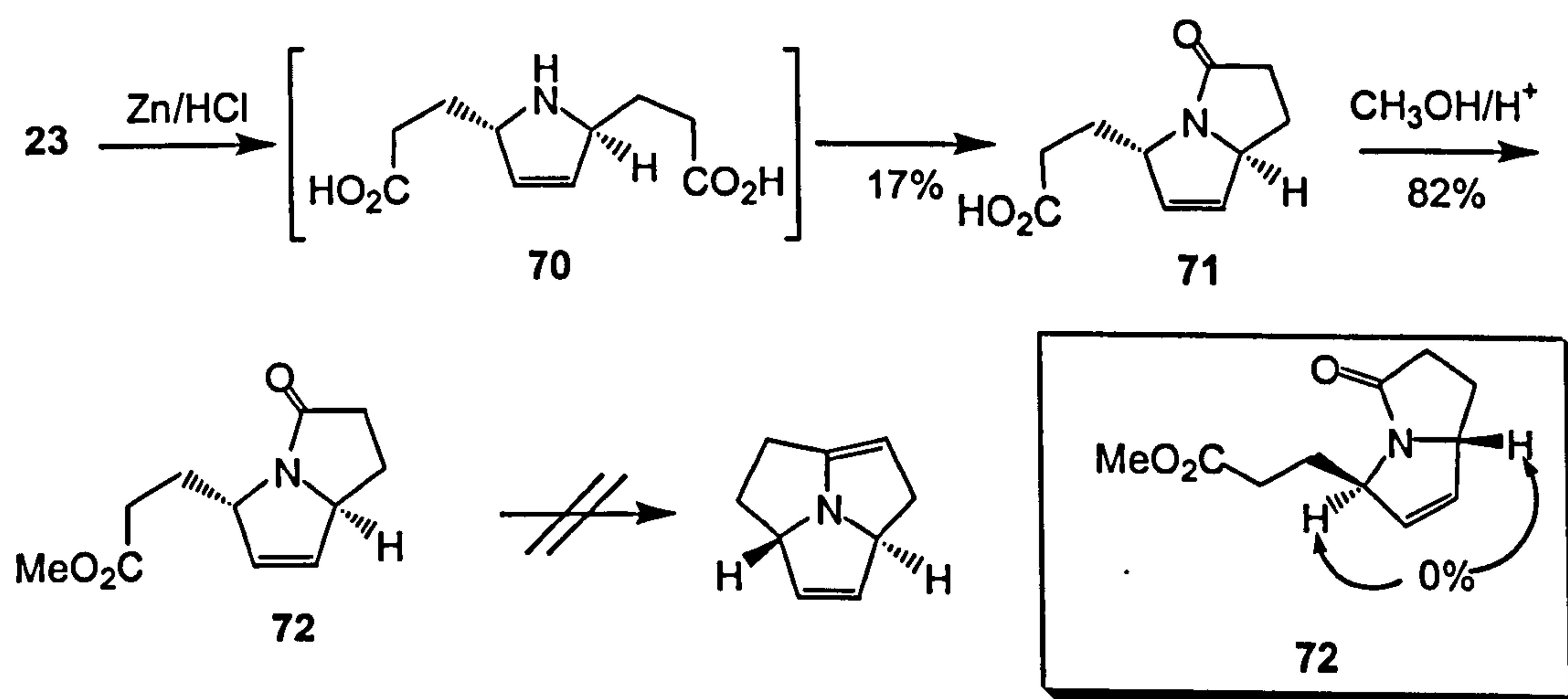
Thus, treating pyrrole 23 with Zn and  $\text{HCl}$ ,<sup>35</sup> the bicycle 71 was obtained (Scheme 30).

Surprisingly, the first cyclisation took place *in situ* without heating whereas in the case



of the pyrrolidine **24**, heating at reflux for 10 days in toluene was necessary to achieve the same cyclisation. This first cyclisation could be promoted by the activation of the ester under the acidic reaction conditions or due to an enhanced nucleophilic character of the nitrogen in **70** relative to **24**.

Acid catalysed esterification of **71** with methanol provides **72**, however, when the second cyclisation was attempted, some decomposition occurred and only starting material was isolated from the reaction. To explain this result, it has been assumed that the pyrrolizinone **72** has *trans*-stereochemistry and this fact has been reinforced by the absence of an NOE interaction between the protons shown in Scheme 30. Due to the *cis,cis,cis*-geometry of the azatriquinane structure it is necessary to use pyrrolizinones with a *cis*-stereochemistry as the precursors, to achieve the second cyclisation.

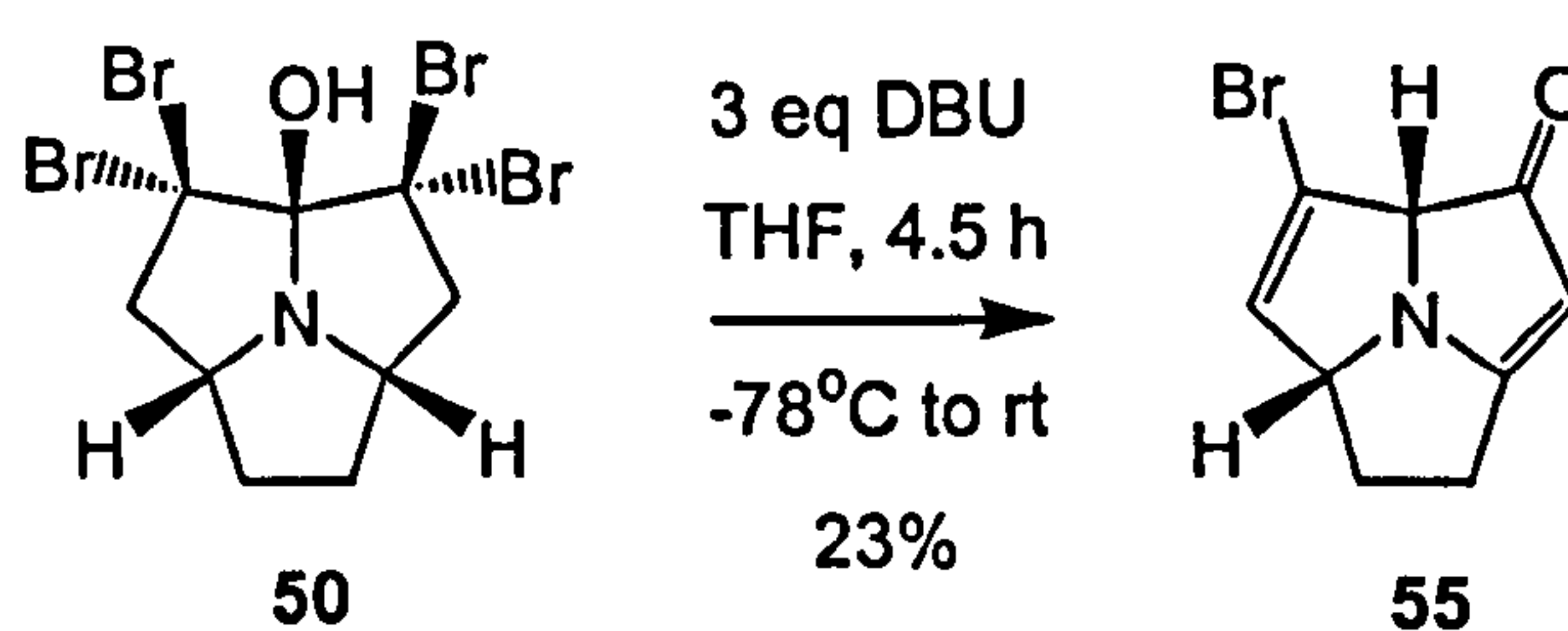


Scheme 30

#### 2.4.2 New strategies using enaminone **55**

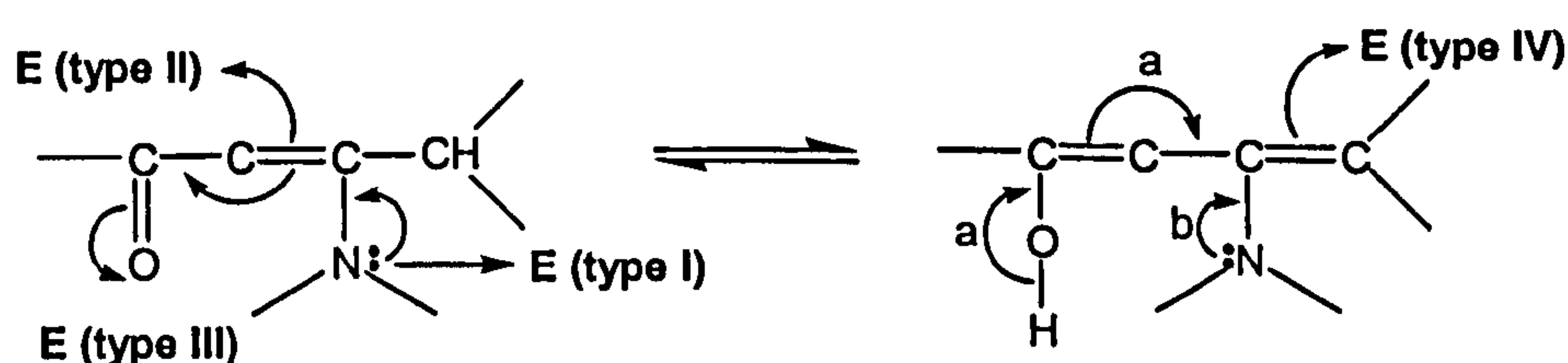
After failing to introduce the bottom double bond, we started to consider the enaminone **55** as a precursor to azatriquinacene **29**.

As seen in Section 2.3, treating tetrabromide **50** with DBU at 0°C gave **55** (Scheme 31).



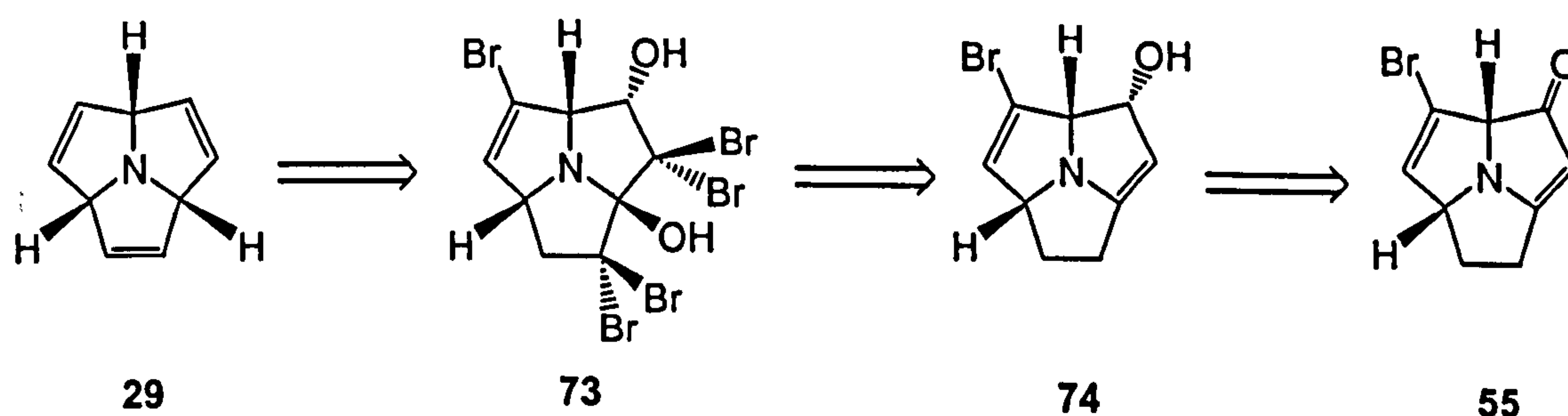
Scheme 31

The enaminone moiety displays very interesting reactivity. The nucleophilic reactions of conjugated enaminones are classified into the four types shown in Scheme 32.<sup>36</sup>



Scheme 32

Using type II reactivity it might be possible to introduce four bromine atoms in the enaminone **55** as with the enamine **26**. In this case it is probably necessary to reduce the carbonyl group of the enaminone to remove its conjugation with the double bond (Scheme 33).

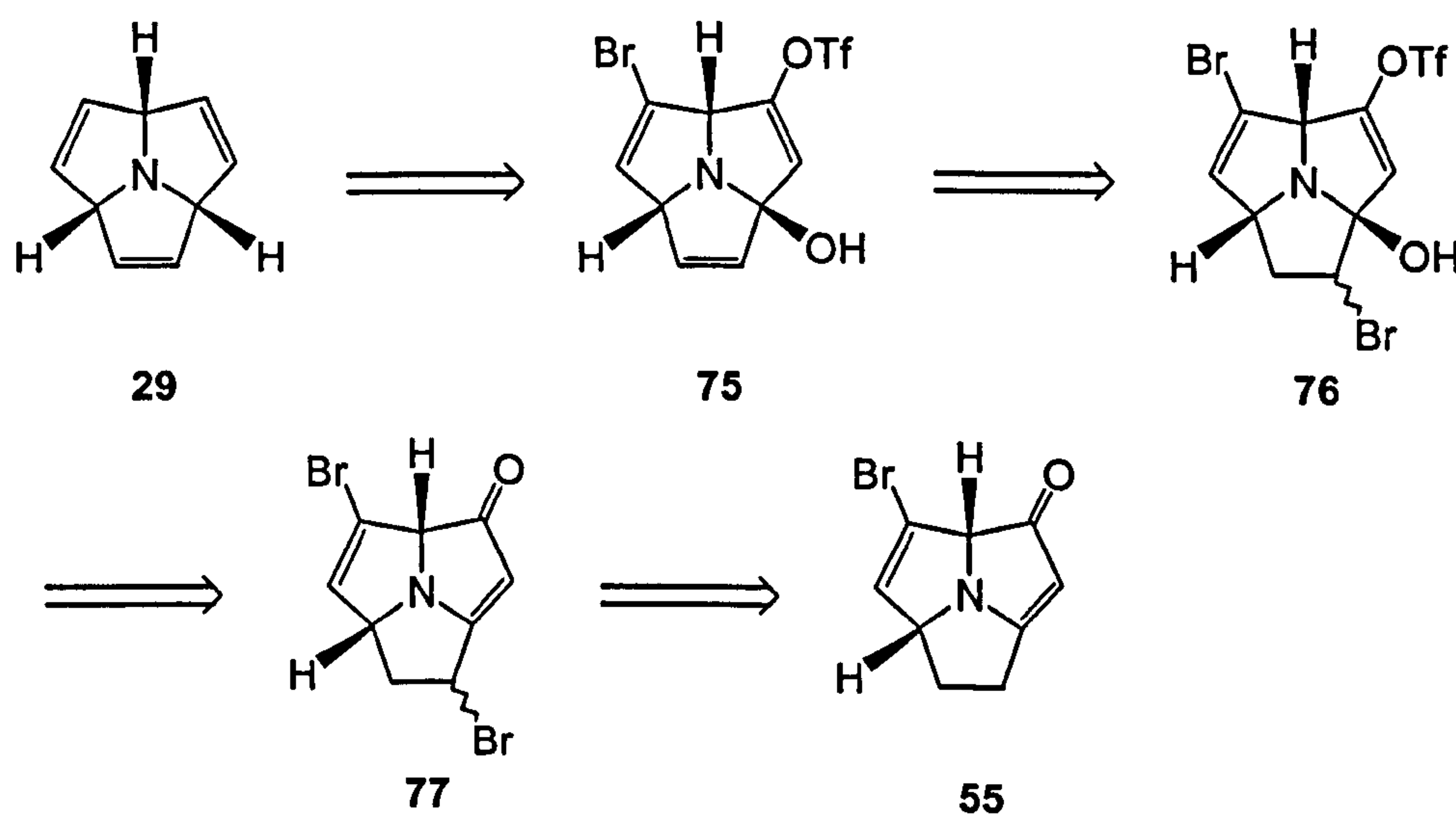


Scheme 33

It was possible to selectively reduce the carbonyl group of **55** by treatment with sodium borohydride<sup>37</sup> to obtain the enaminol **74**. As seen in Section 2.1, the chemical shift of the vinylic proton of the parent enamine **26** in the <sup>1</sup>H-NMR is 4.32 ppm. The

chemical shift of the analogous proton in the enaminol **74** is 4.58 ppm which suggested enamine character. This slight downfield shift also indicated that **74** may be less reactive than **26**. The bromination of the enaminol **74** was attempted using the same conditions as those used with enamine **26**. However, the reaction afforded starting material and a complex mixture of brominated products, despite longer reaction periods compared with the enamine **26**.

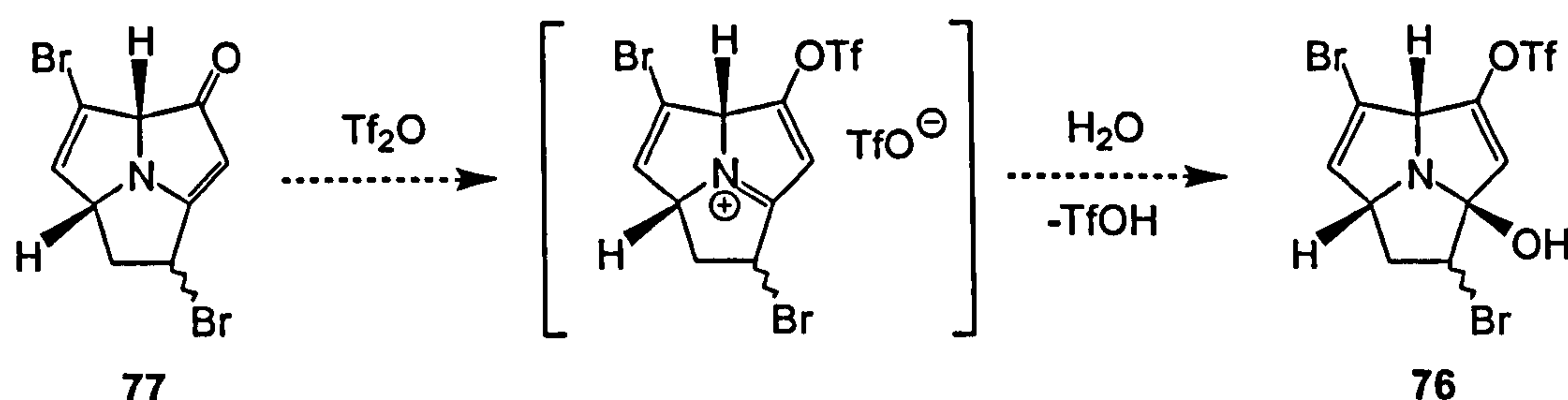
Another strategy relying upon enaminone type IV and type III reactivities was proposed and is presented in Scheme 34.



Scheme 34

Type IV reactivity of the enaminone moiety suggests that treatment of enaminone **55** with a base and then with NBS would introduce a bromine atom at C5 to give **77**. It could be possible to introduce the double bond in position C2-C3 using type III reactivity,<sup>38</sup> thus treating brominated enaminone **77** with trifluoromethanesulfonic anhydride should form the vinyltriflate **75** as shown Scheme 35. From this point, azatriquinacene could be synthesised by elimination of HBr and consequent reduction of the substituents with  $\text{Pd}^0/\text{Bu}_3\text{SnH}$  and/or  $\text{LiAlH}_4$ .<sup>39</sup>

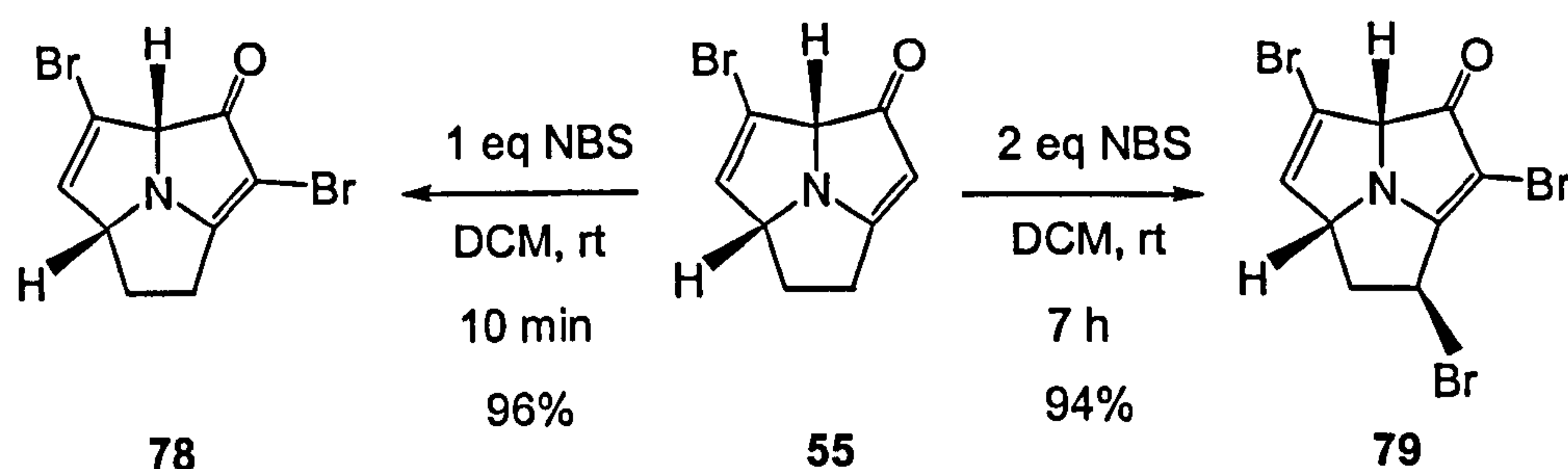




Scheme 35

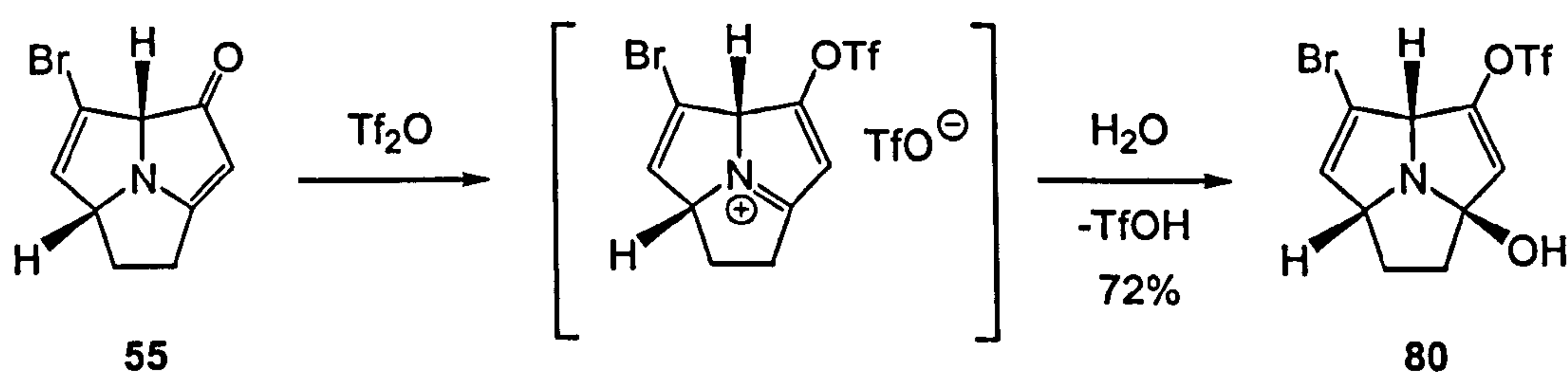
Two different bases, KHMDS and  $^n\text{BuLi}$ , were used to try to abstract the proton at C5 of **55**. NBS was then added to afford, in both cases, the  $\alpha$ -brominated product **78** instead of the desired  $\gamma$ -brominated product **77**. This can be explained by the fact that the base did not abstract the  $\gamma$ -proton and the  $\alpha$ -bromination was the result of type II reactivity of the enaminone. To test this theory, the enaminone **55** was treated with NBS in absence of a base and after 10 min the bromination in the  $\alpha$ -position took place to afford **78**. In the crystal structure of the enaminone (Figure 9, Section 2.3) is possible to see that the convex geometry of these compounds prevents carbon atoms C2, C3, C4 and C5 from adopting a coplanar disposition. Coplanarity is necessary for orbital overlap, responsible for the acidic characteristics of the  $\gamma$ -protons of enaminones.<sup>36</sup>

A selective bromination of an enaminone, in the  $\alpha$  or in the  $\alpha$  and  $\gamma$  positions with NBS, has been reported in the literature.<sup>40</sup> Based on this precedent, enaminone **55** was exposed to the bromination conditions and fortunately was observed that it underwent regioselective bromination using NBS. Exclusive  $\alpha$ -bromination was obtained using one equivalent of NBS to give **78** in 96% yield, whereas the  $\alpha,\gamma$ -dibrominated product **79** was formed in 94% yield when **55** was treated with two equivalents of NBS (Scheme 36). It is noteworthy that bromination at the C8-C9 double bond was not observed due to the lower reactivity of NBS compared with bromine.



Scheme 36

After this important result, which constitutes the first example of substitution of the bottom ring in enaminone **55**, a model reaction to introduce the double bond in position C2-C3 was attempted. Pleasingly, treatment of **55** with trifluoromethanesulfonic anhydride at  $-78^\circ\text{C}$  followed by hydrolysis of the resulting iminium salt afforded vinyltriflate **80** in 72% yield (Scheme 37).



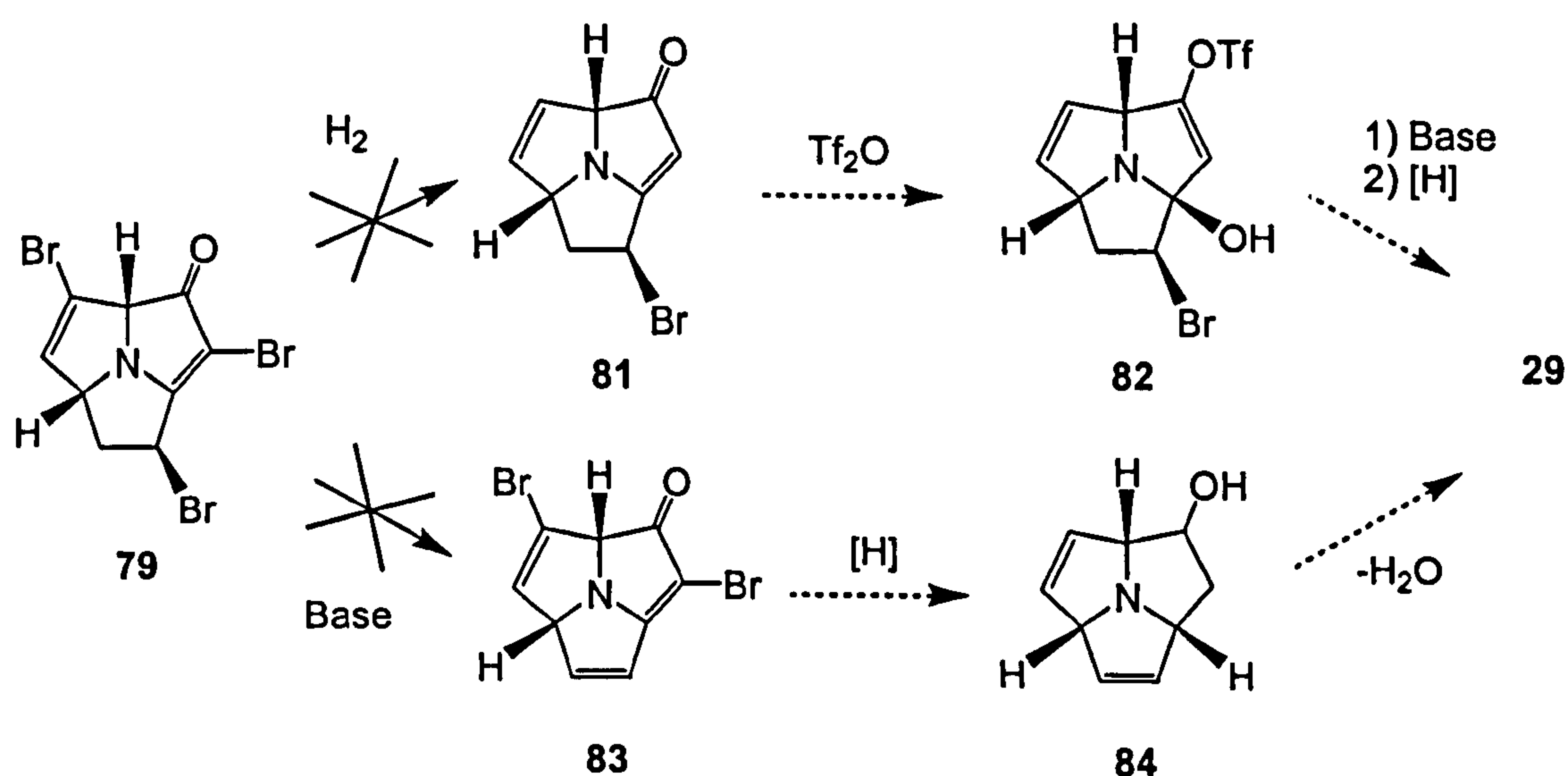
Scheme 37

However, when the reaction shown in Scheme 37 was attempted using **79**, it failed. This unsuccessful result was probably due to the presence of the  $\alpha$ -bromine atom which makes the enaminone poorly reactive through electronic and steric effects.<sup>41</sup>

With the aim of recovering the original reactivity of the system, the reduction of both vinyl bromides of **79** by hydrogenolysis<sup>42</sup> was attempted. Unfortunately the non-conjugated double bond was reduced under the reaction conditions and this fact was indicated by the absence in the  $^1\text{H-NMR}$  of the olefinic proton at C8.

It was then decided to eliminate HBr from the bottom ring of **79**. This was to be followed by reduction of the enaminone, then dehydration to give azatriquinacene **29**. The elimination reaction was attempted using DBU and KHMDS but unfortunately, decomposition occurred in both cases suggesting that **79** was unstable in the presence of strong bases.

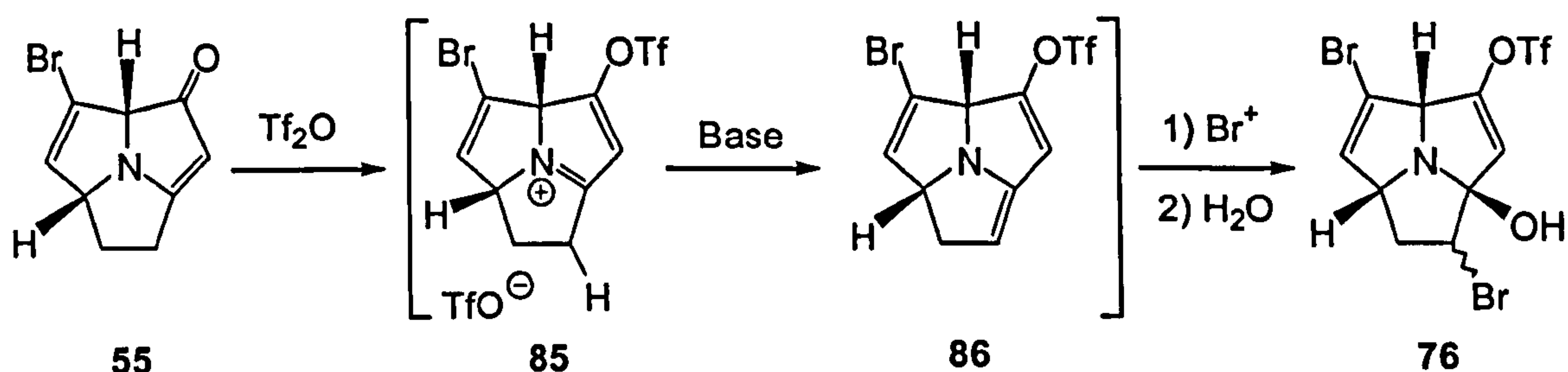
Both the reduction and elimination strategies are presented in Scheme 38.



Scheme 38

Bromination of the bottom ring of the iminium salt **85** was attempted. This would give a compound with the two top double bonds and the potential to form the third by elimination (Scheme 39). Unfortunately, treatment of the iminium salt **85** with a variety of bases ( $\text{NEt}_3$ , DBU and  $^t\text{BuOK}$ ) or a system of two bases ( $^t\text{BuNH}_2/\text{KHMDS}$ )<sup>43</sup> followed by NBS did not give any bromination of the 5-position. This unsuccessful result could be due to the fact that the presence of the double bonds at the 2- and 8-positions led to the product **86** being more strained than the parent enamine **26**, thus preventing deprotonation.



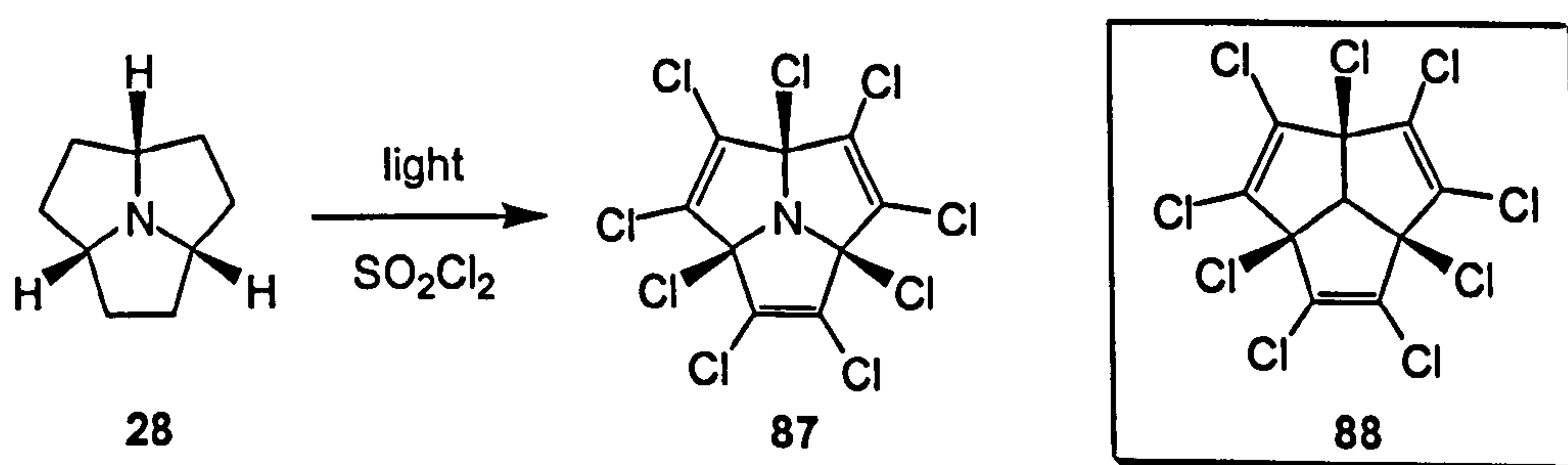


Scheme 39

### 2.4.3 Synthesis of azatriquinacene by reduction of 87

Following the problems and setbacks encountered with the substitution-elimination strategy and the difficulties in working with the  $\alpha,\gamma$ -dibromoenaminone **79**, new strategies were considered.

The nonachloroazatriquinacene **87**, prepared by Mascall and coworkers<sup>13</sup> from the saturated azatriquinane **28** by radical chlorination with sulfuryl chloride, constituted the first and unique example of the synthesis of an azatriquinacene structure (Scheme 40). However, all previous attempts to dechlorinate it by reduction in an analogous fashion to perchlorotriquinacene **88** failed.<sup>5</sup>

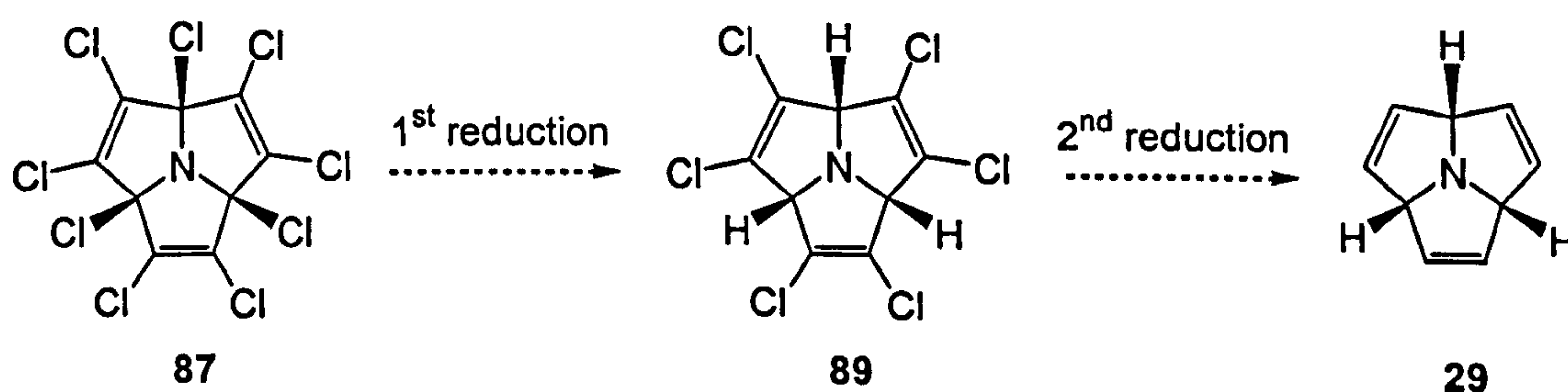


Scheme 40

In the final step of the synthesis of the diene **52** (Section 2.3), two vinyl bromides and the hydroxyl group of the hemiaminal were reduced in one step. This result suggested

that the reduction of the chlorine substituents in perchloroazatriquinacene **87** might be possible and should therefore be re-examined.

The reduction of a vinyl chloride is more difficult to achieve than that of a vinyl bromide. In the synthesis of triquinacene **3**, Hoffman used lithium in *tert*-butanol to reduce perchlorotriquinacene **88**.<sup>5</sup> In **87** the bridgehead chlorines are  $\alpha$  to a nitrogen and this fact makes these three chlorines easier to reduce than the bridgehead chlorines in **88**. In the case of the reduction of the hydroxyl group of the hemiaminal, the slow step is the formation of the iminium salt by expulsion of hydroxide by the electron lone pair of the nitrogen. This fact suggested that the reduction of the chlorines  $\alpha$  to nitrogen should be easier than the reduction of the hydroxyl group of the hemiaminal as chloride is a better leaving group than hydroxide. Thus, the strong conditions required to remove the vinyl chlorides and the mild conditions proposed to reduce the bridgehead chlorines suggested that the reduction of the nine chlorines of perchloroazatriquinacene **87** could be achieved in two steps (Scheme 41)..



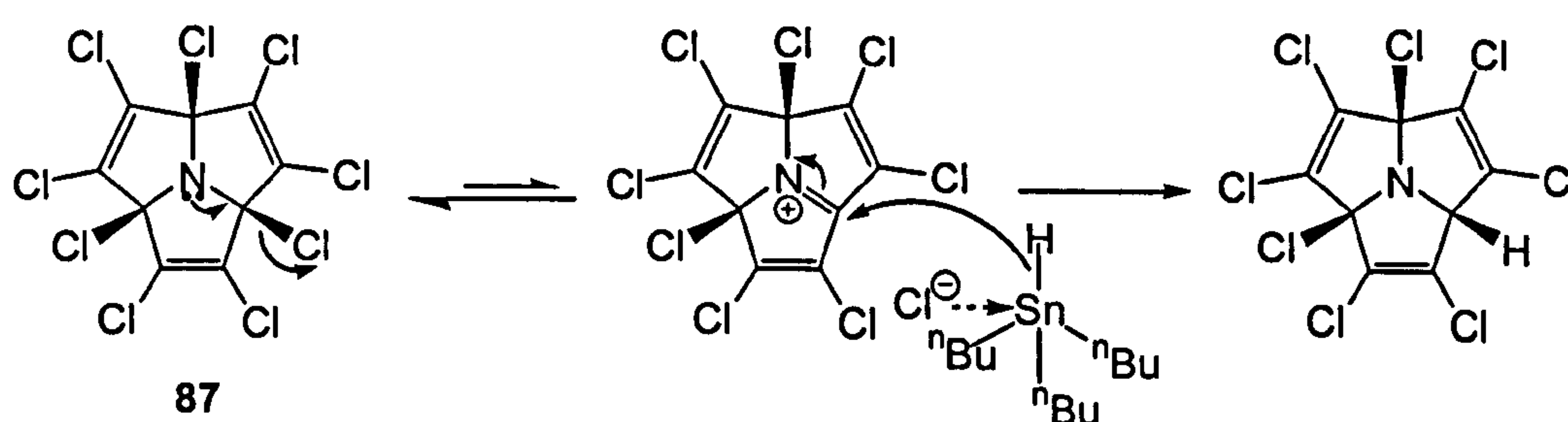
Scheme 41

Pleasingly, treating perchloroazatriquinacene **87** with tributyltin hydride at room temperature for 16 h<sup>44</sup> reduced the three chlorines  $\alpha$  to nitrogen to afford 2,3,5,6,8,9-hexachloroazatriquinacene **89** in 53% yield. This fact was supported by the presence



of a singlet at 4.75 ppm in the  $^1\text{H}$ -NMR spectrum which corresponded to the three protons  $\alpha$  to nitrogen.

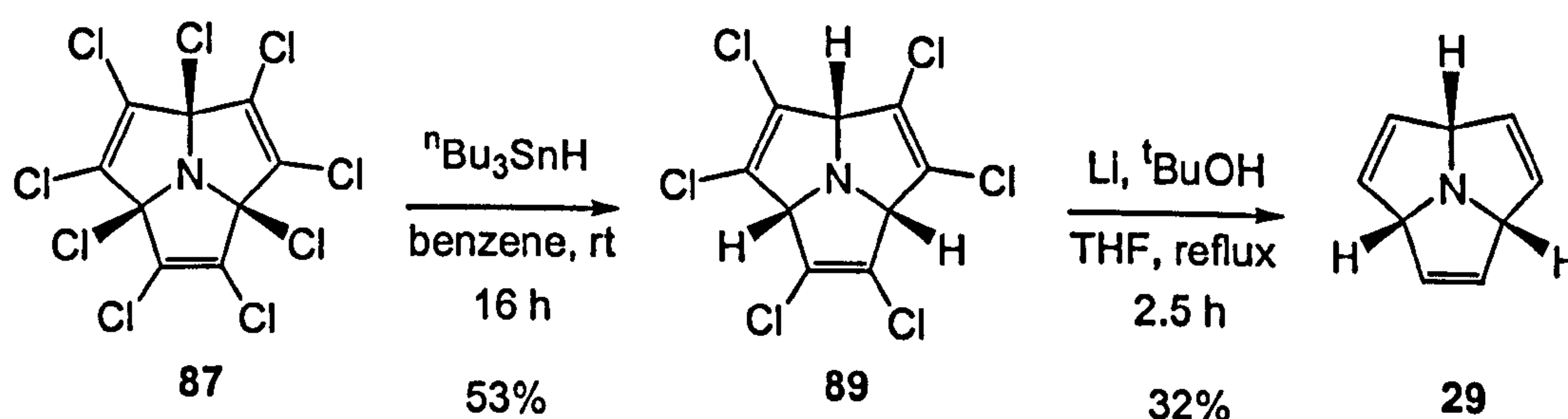
Due to the absence of a radical initiator and the temperature at which the reduction was carried out (room temperature), this reaction cannot be explained by a radical mechanism and must proceed *via* hydride transfer as shown in the Scheme 42. In this mechanism, chloride, a Lewis base, forms a pentacoordinated tin species that can act as a strong hydride donor.<sup>45</sup>



Scheme 42

With 89 in hand, the reduction of the remaining six chlorines was attempted using lithium and *tert*-butanol<sup>5</sup> and pleasingly, azatriquinacene 29 was obtained in 32% yield.<sup>27</sup> As in the case of the synthesis of diene 52 (Section 2.3), triene 29 was isolated as its trifluoroacetate salt as the free base is highly volatile.

The final steps in the synthesis of azatriquinacene 29 are presented in the Scheme 43.



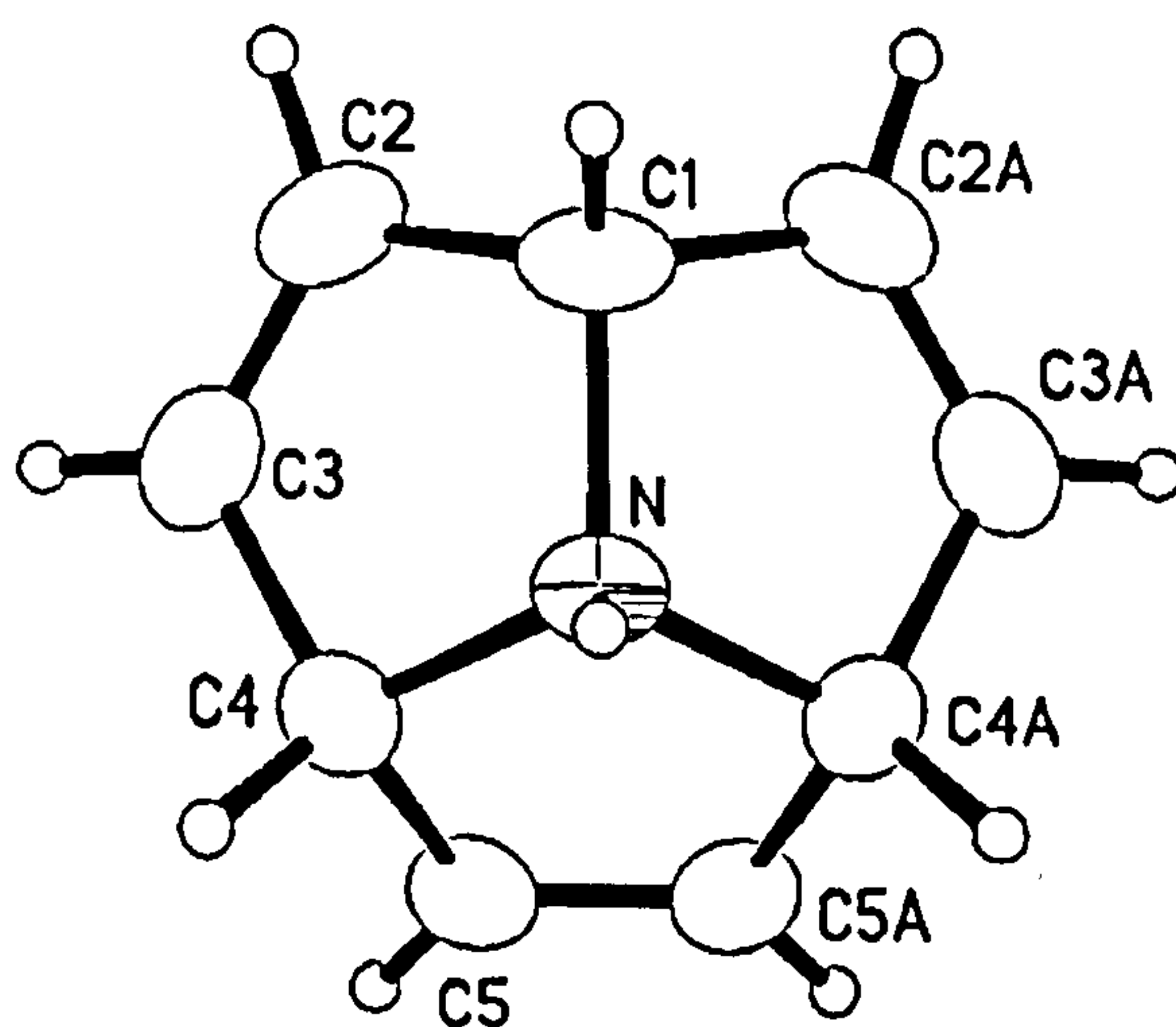
Scheme 43

The  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR data of its trifluoroacetate salt and the free base form are shown in Table 2.

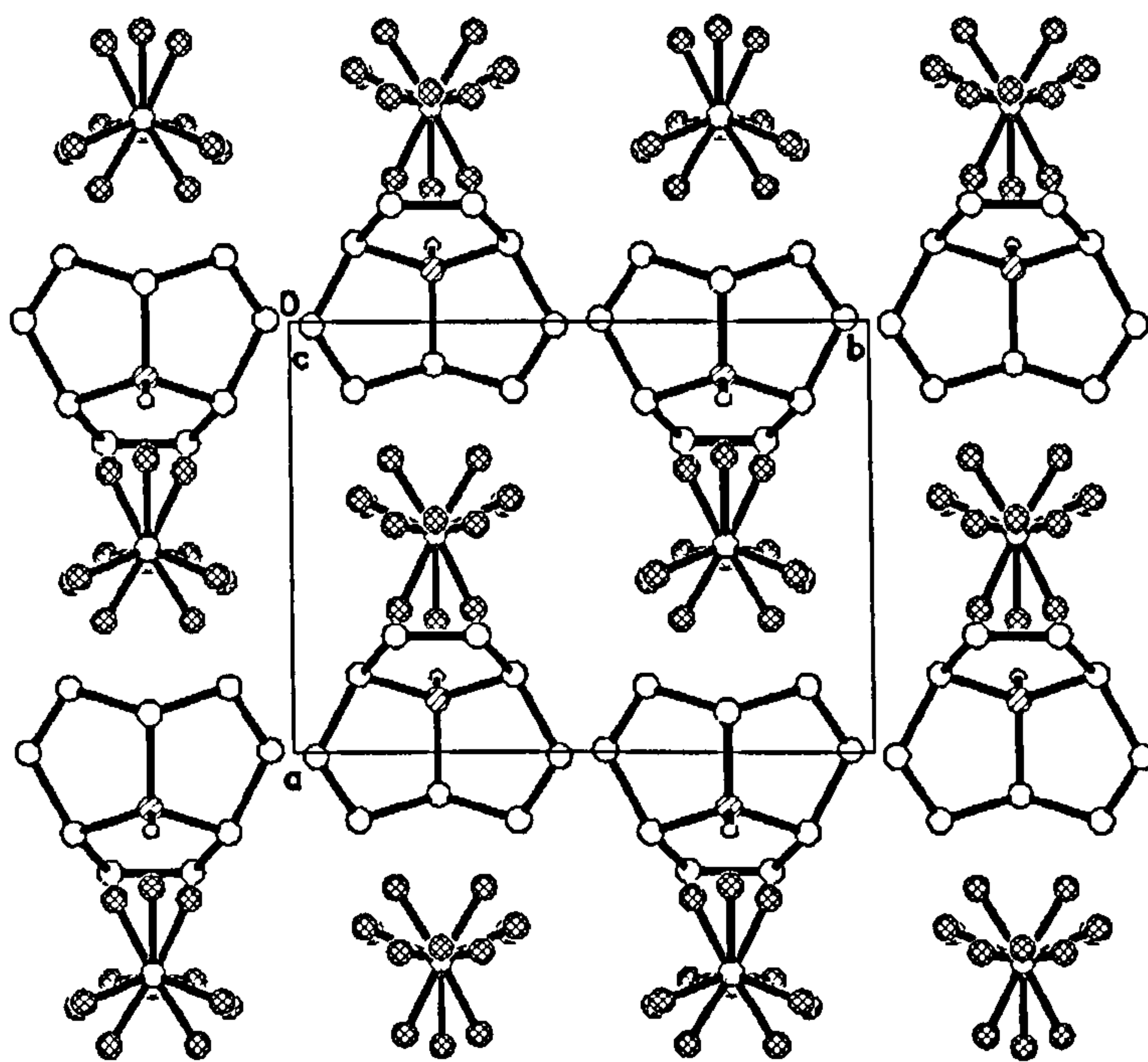
	$^1\text{H}$ -NMR( $\delta$ /ppm)		$^{13}\text{C}$ -NMR ( $\delta$ /ppm)	
	H2	H1	C2	C1
Azatriquinacene <b>29</b> ·HBF <sub>4</sub>	5.87 (s) <sup>a</sup>	5.69 (s) <sup>a</sup>	127.8 <sup>b</sup>	78.6 <sup>b</sup>
Azatriquinacene free base	5.79 (s) <sup>a</sup>	4.87 (s) <sup>a</sup>	130.3 <sup>c</sup>	78.6 <sup>c</sup>

**Table 2.** In all the cases the solvent used was CDCl<sub>3</sub>. <sup>a</sup>400 MHz, <sup>b</sup>125 MHz, <sup>c</sup>67.5 MHz.

The hydrochloride salt of this compound is very soluble in water whereas the trifluoroacetate salt is very soluble in organic solvents. The tetrafluoroborate salt is partially soluble in both, which makes it the most suitable salt for crystallisation studies. Crystals of the tetrafluoroborate salt of **29** grew as light yellow cubes by slow diffusion of ether into dichloromethane solution of **29**, and its X-ray crystal structure is presented in Figure 14. The tricycle and its counterion lie across a crystallographic mirror plane, which in the cation passes through C1 and N and bisects the C5-C5A double bond. An N-H...F hydrogen bond (N...F 2.77 Å and N-H...F 178°) links the cation and the anion, which pack in alternating corrugated layers (Figure 15).

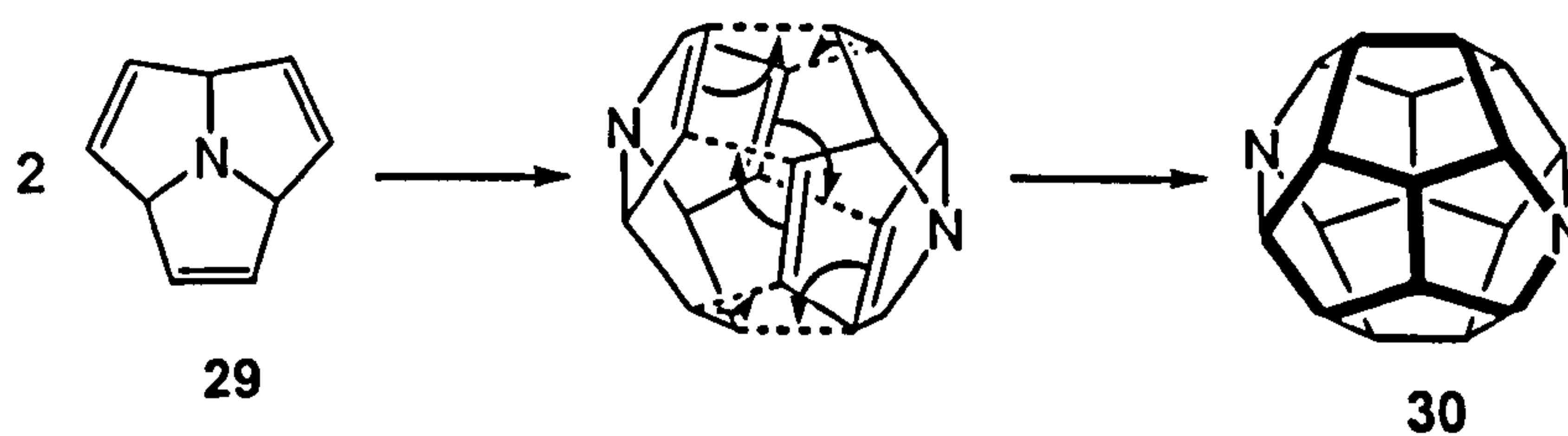


**Figure 14.** X-ray crystal structure of **29**·HBF<sub>4</sub>. The counterion is omitted for clarity.

Figure 15. Packing of 29·HBF<sub>4</sub>.

#### 2.4.4 Dimerisation attempts

As seen in the introduction, a great deal of effort has been focused on the dimerisation of triquinacene **3** to form dodecahedrane **1** *via* a  $[2+2+2+2+2+2]\pi$  cycloaddition reaction.<sup>1,4,5</sup> With azatriquinacene **29** in hand this strategy could be re-examined (Scheme 44).



Scheme 44

Figure 15 shows that, in the tetrafluoroborate salt of **29** there is no close approach between the *endo* faces of two molecules which makes topochemical dimerisation unlikely. However, it had been speculated that the aqueous solubility of **29** and its



perturbed electronics relative to **3** might prove advantageous in the photochemical  $[2+2+2+2+2+2]\pi$  dimerisation reaction which would give diazadodecahedrane **30** (Scheme 44). Thus, it was decided to do this study in solution and the conditions and outcomes of the preliminary attempts are summarised in Table 3.

Substrate	Irradiation Source	Solvent	Time	Photosensitiser	Salt	Metal
<b>29</b> ·CF <sub>3</sub> CO <sub>2</sub> H	300 W Sun lamp	H <sub>2</sub> O	48 h	No	Yes	No
<b>29</b> ·CF <sub>3</sub> CO <sub>2</sub> H	HV Mercury lamp	H <sub>2</sub> O	3.5 h	No	Yes	No
<b>29</b> ·CF <sub>3</sub> CO <sub>2</sub> H	HV Mercury lamp	Acetone	3.5 h	Yes	No	No
<b>29</b> ·CF <sub>3</sub> CO <sub>2</sub> H	HV Mercury lamp	H <sub>2</sub> O	3.5 h	Yes	No	No
<b>29</b> ·CF <sub>3</sub> CO <sub>2</sub> H	HV Mercury lamp	THF	144 h	No	No	No
<b>29</b> Free Base	HV Mercury lamp	H <sub>2</sub> O	144 h	Yes	No	Yes

**Table 3.** In all the cases acetone was used as the photosensitiser, LiCl as the inorganic salt and CuOTf·Benzene as the coordinating metal.

Starting material was recovered in all the entries except using the free base of **29** after a long period of irradiation in which decomposition was observed. Neither **29** nor its salt are more reactive than **3** in this capacity, and no evidence of diazadodecahedrane **30** has yet been found. In fact, compound **29** appears to be curiously inert, failing to react at all under conditions known to induce various photochemical transformations in **3**,<sup>46</sup> but efforts will continue.

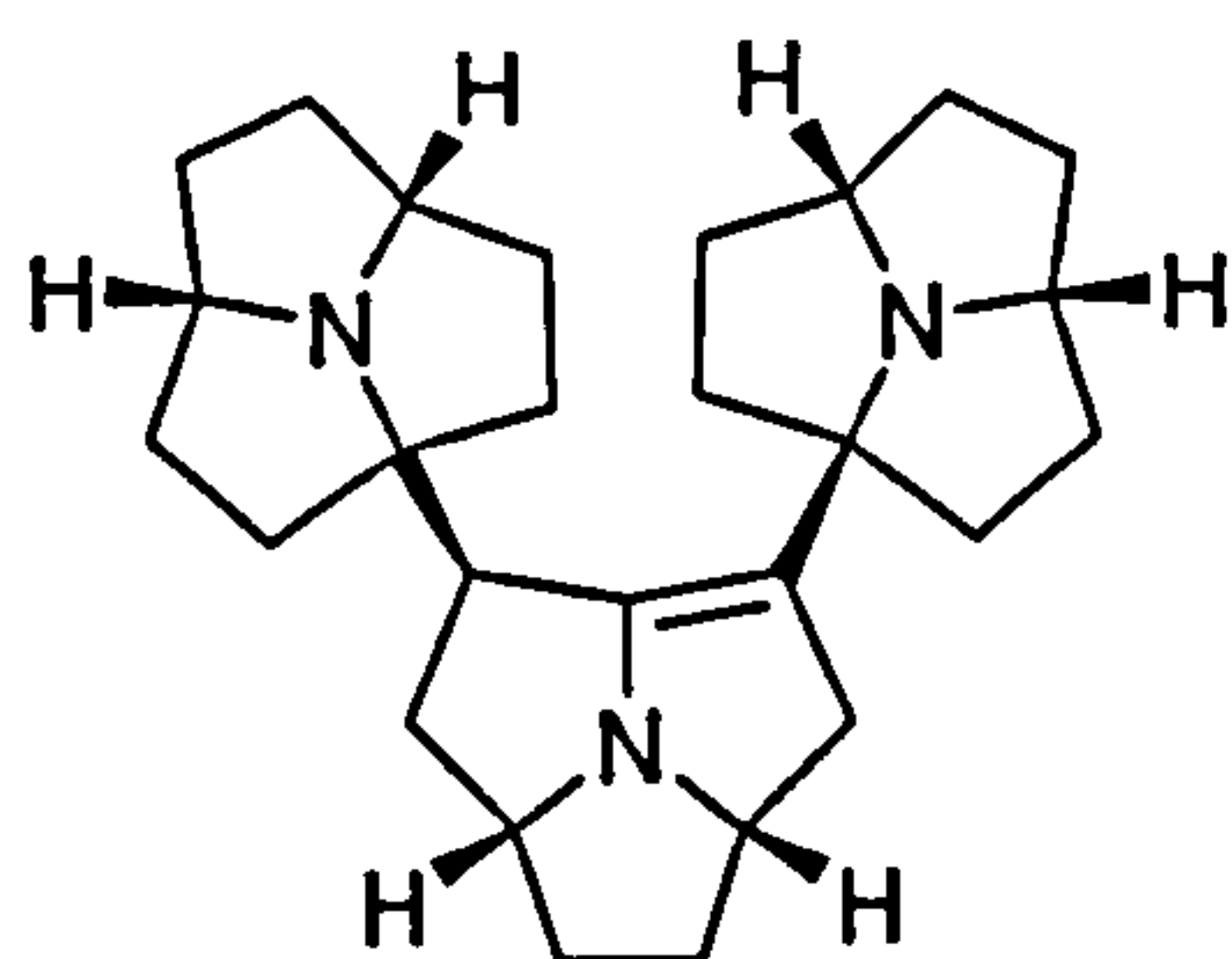
### 3. Experimental

*General Details.*- Melting points determinations were made on a Reichert Kofler micro hot-stage or in a Box 6402 Holliston MA 01746-6402 apparatus and are uncorrected. IR spectra were obtained using a Perkin-Elmer 1600 series FT-IR instrument as dilute solutions in spectroscopic grade chloroform. UV spectra were recorded as solutions in spectroscopic grade ethanol using a Philips PU 8700 spectrophotometer. Unless stated otherwise solutions in deuteriochloroform were used for the determination of NMR spectra. Shifts are expressed in ppm downfield from Me<sub>4</sub>Si, as internal standard. The <sup>1</sup>H and <sup>13</sup>C NMR spectra were obtained using a 270 MHz Jeol EX-270, 400 MHz Bruker AM400, 400 MHz Bruker AV400, or a 500 MHz Bruker DRX500 instrument. Multiplicities of signals are assigned using the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, m = multiplet, br = broad, app = apparent. Coupling constants (*J*) are given in Hertz. Assignments in the <sup>1</sup>H spectra were consistent with signal intensities, and in the <sup>13</sup>C spectra with the results of the DEPT pulse sequence. Signals corresponding to OH protons were not observed in the <sup>1</sup>H NMR spectra. Mass spectra were recorded on a MM-701CF instrument using electron impact ionisation at 70 eV, fast atom bombardment (FAB) or electrospray (ES) techniques. Microanalytical data were obtained on a Perkin-Elmer 24013 elemental analyser. Flash chromatography was performed using Merck silica gel 60. "Ether" refers to diethyl ether and "petrol" to the fraction bp 40-60 °C. All reactions were monitored by TLC using Merck silica gel 60 F254 precoated aluminum plates which were visualised with ultraviolet light and then with basic potassium permanganate solution. Organic extracts were dried over anhydrous magnesium sulfate prior to solvent removal using a Büchi rotary evaporator. Tetrahydrofuran was distilled from sodium benzophenone ketyl and



dichloromethane was distilled from calcium hydride before use in reactions. Other organic solvents and reagents were purified by the accepted literature procedures. Where necessary, reactions requiring anhydrous conditions were performed in a flame or oven dried apparatus under a nitrogen or argon atmosphere.

*rel*-(4*R*,7*S*,9*R*)-10-Azatricyclo[5.2.1.0<sup>1,10</sup>]-2,9-bis[1-(10*r*,13*S*,16*R*)-azatricyclo[5.2.1.0<sup>1,10</sup>]decane]dec-1-ene 43.

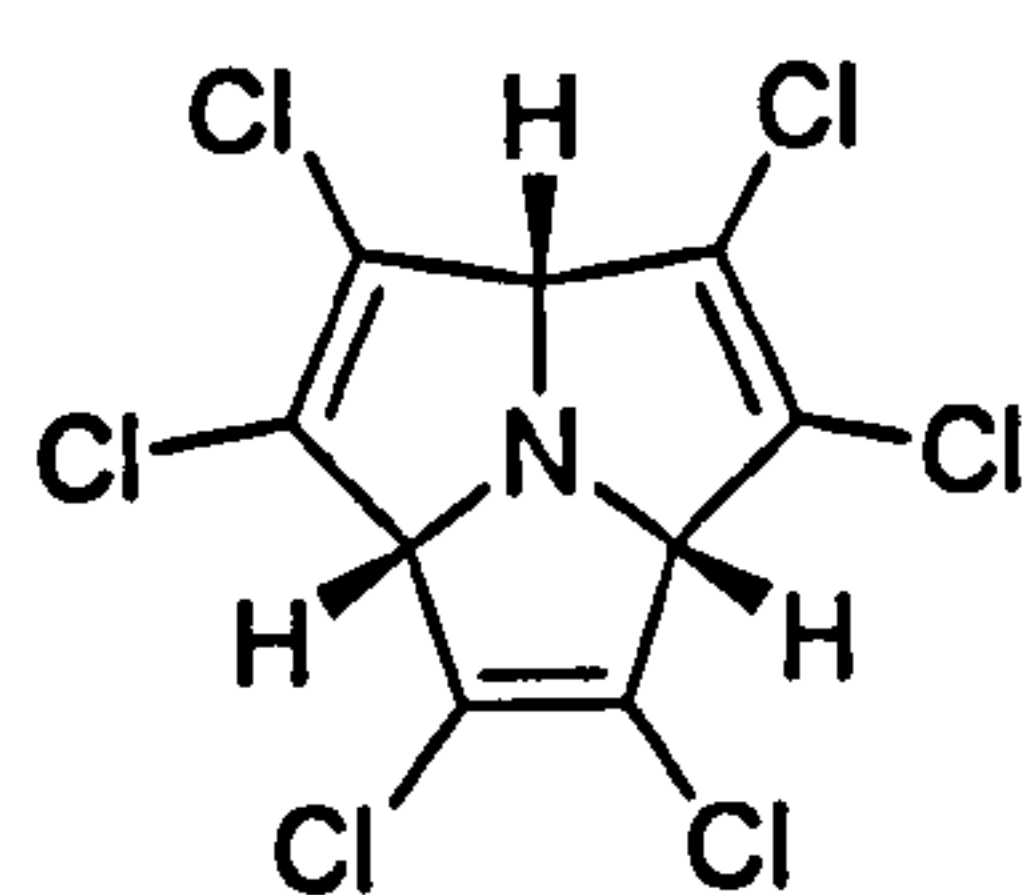


DCM (1 mL) was added to the freshly distilled enamine **26**<sup>13</sup> (0.781 g, 5.78 mmol) and the mixture was then heated at 140°C with stirring for 12 h. The reaction was cooled to RT and the resulting brown solid was purified by column chromatography (DCM:MeOH 10:1) to give the

monohydrochloride salt of **43** (0.254 g, 30%) as a pink crystalline solid, mp 127-130°C; (Found: (HRMS FAB)  $M^+ + H$  406.3223,  $C_{27}H_{39}N_3$  requires 406.3222);  $\nu_{\max}/\text{cm}^{-1}$  3696, 2935, 1678, 1602, 1459, 1348, 1068, 962, 892;  $\delta_{\text{H}}$ (400 MHz) 4.12 (1H, m), 4.04 (1H, m), 3.73 (1H, m), 3.58 (2H, m), 3.44 (1H, m), 2.99 (1H, dd,  $J$  15.0, 7.5), 2.87 (1H, m), 2.41 (1H, d,  $J$  15.1), 2.37-1.67 (25H, m), 1.62-1.50 (3H, m), 1.32-1.24 (2H, m);  $\delta_{\text{C}}$ (125 MHz) 147.6 (C), 112.9 (C), 81.8 (C), 76.1 (C), 67.7 (CH), 67.2 (CH), 66.8 (CH), 66.7 (CH), 62.3 (CH), 60.4 (CH), 43.1 (CH<sub>2</sub>), 42.6 (CH<sub>2</sub>), 36.9 (CH<sub>2</sub>), 36.8 (CH<sub>2</sub>), 36.3 (CH<sub>2</sub>), 32.8 (CH<sub>2</sub>), 32.2 (CH<sub>2</sub>), 31.8 (CH<sub>2</sub>), 31.0 (CH<sub>2</sub>), 30.4 (CH<sub>2</sub>), 30.2 (CH<sub>2</sub>), 30.0 (CH<sub>2</sub>), 29.9 (CH<sub>2</sub>), 29.8 (CH<sub>2</sub>), 29.7 (2 x CH<sub>2</sub>), 28.7 (CH<sub>2</sub>);  $m/z$  (FAB) 406 ( $M^+ + H$ , 53) 406 (53), 307 (26), 155 (32), 154 (100), 138 (36), 137 (67), 136 (86), 107 (26), 91 (29), 90 (24), 89 (20), 77 (22), 73 (68), 69 (23), 57 (32), 55 (29).

To obtain the free base of **43** a solution of KHMDS in toluene (0.5 M, 2.6 mL) was added dropwise to a stirred solution of the monohydrochloride salt (**43**·HCl) (0.191 g, 0.432 mmol) in THF (7 mL) at  $-78^{\circ}\text{C}$  under argon. The mixture was stirred 2 h at  $-78^{\circ}\text{C}$  and then 30 min at RT. The solvent was removed under argon and the residue extracted with dry ether (3 x 10 mL). The combined organic phase was filtered and the solvent removed under argon to give **43** (0.170 g, 97%) as a pale brown oil; (Found: (HRMS ES)  $\text{M}^+ + \text{H}$  406.3239,  $\text{C}_{27}\text{H}_{39}\text{N}_3$  requires 406.3222);  $\nu_{\text{max}}/\text{cm}^{-1}$  3652, 3155, 2947, 2866, 2253, 1817, 1794, 1651, 1458, 1382, 1348, 1320, 1299, 1094, 1064, 1022, 988, 947, 892, 644;  $\delta_{\text{H}}$ (400 MHz) 3.52-3.40 (4H, m), 3.32 (1H, m), 3.25 (1H, m), 3.05 (1H, d,  $J$  8.3), 2.80 (1H, dd,  $J$  14.4, 7.3), 2.36 (1H, m), 2.30-2.20 (2H, m), 2.01 (1H, m), 1.95-1.18 (26H, m), 0.85 (1H, m);  $\delta_{\text{C}}$ (100 MHz) 148.5 (C), 115.6 (C), 78.3 (C), 74.2 (C), 66.2 (CH), 66.0 (CH), 65.9 (CH), 65.3 (CH), 64.0 (CH), 60.4 (CH), 44.5 (CH<sub>2</sub>), 40.3 (CH<sub>2</sub>), 39.2 (CH<sub>2</sub>), 37.5 (CH<sub>2</sub>), 34.5 (CH<sub>2</sub>), 33.8 (CH<sub>2</sub>), 33.7 (CH<sub>2</sub>), 32.8 (CH<sub>2</sub>), 31.6 (CH<sub>2</sub>), 30.9 (CH<sub>2</sub>), 30.8 (CH<sub>2</sub>), 30.3 (CH<sub>2</sub>), 30.2 (2 x CH<sub>2</sub>), 30.0 (CH<sub>2</sub>), 29.9 (CH<sub>2</sub>), 28.6 (CH<sub>2</sub>).

**(1*r*,4*S*,7*R*)-2,3,5,6,8,9-Hexachloro-10-azatricyclo[5.2.1.0<sup>1,10</sup>]deca-2,5,8-triene **89**.**



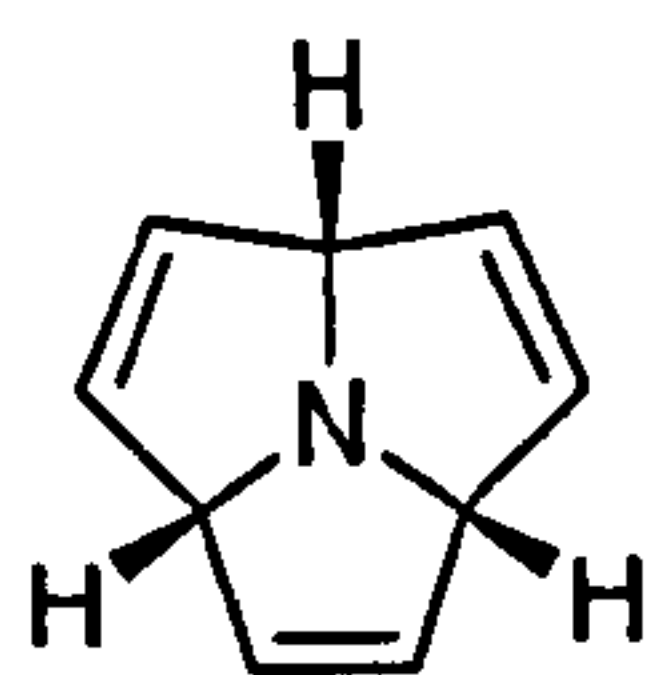
Tributyltin hydride (0.756 g, 2.60 mmol) was added dropwise to a stirred solution of **87** (0.265 g, 0.601 mmol) in benzene (20 mL) under nitrogen at RT. After 16 h the mixture was concentrated *in vacuo* and the residue purified by column chromatography (Petrol:AcOEt 10:1) to give

**89** (0.108 g, 53%) as a white solid, mp  $152-154^{\circ}\text{C}$ ; (Found: (HRMS EI)  $\text{M}^+$ , 334.8407,  $\text{C}_9\text{H}_3\text{NCl}_6$  requires 334.8397);  $\nu_{\text{max}}/\text{cm}^{-1}$  1636, 1073, 905;  $\delta_{\text{H}}$ (400 MHz) 4.75 (3H, s);  $\delta_{\text{C}}$ (100 MHz) 128.4 (C), 76.8 (CH);  $m/z$  (EI) 334 ( $\text{M}^+$ , 20) 341 (16), 340 (16), 339



(39), 338 (38), 337 (54), 336 (49), 335 (21), 334 (20), 304 (56), 302 (100), 300 (60), 267 (33), 265 (26), 232 (21), 230 (21).

**(1*r*,4*S*,7*R*)-10-Azatricyclo[5.2.1.0<sup>1,10</sup>]deca-2,5,8-triene 29.**



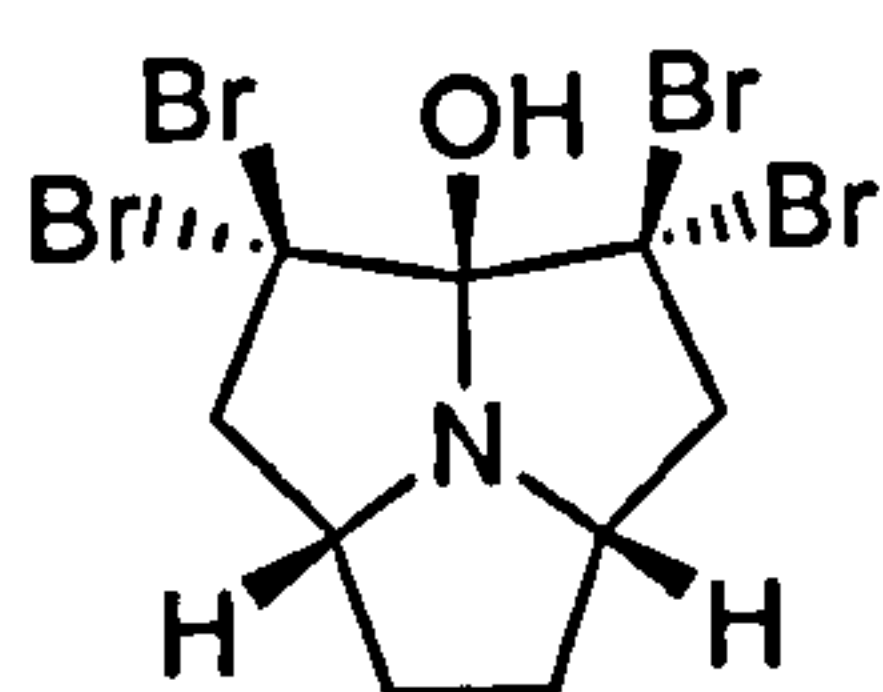
Lithium metal (0.234 g, 33.7 mmol) and *tert*-butanol (1.40 g, 18.9 mmol) were added to a stirred solution of 89 (0.338 g, 1.00 mmol) in THF (40 mL) at RT. A slow stream of nitrogen was passed over the mixture which was stirred for 20 min and then heated at reflux for 2.5 h. The mixture was allowed to cool to RT and poured into a mixture of ice / water / HCl (30 g / 20 mL / 5 mL) and stirred for 20 min. The mixture was washed twice with DCM and the aq layer basified with aq NaOH (2 M). The aq layer was extracted with DCM (3 x 40 mL) and trifluoroacetic acid (0.34 g, 3.0 mmol) was added to the organic phase which was then dried and the solvent was removed *in vacuo* to give the trifluoroacetate salt of 29 (79 mg, 0.31 mmol, 32%) as white crystals, mp 178-180°C; (Found: (HRMS EI)  $M^+$  131.0735,  $C_9H_9N$  requires 131.0735);  $\nu_{\max}/\text{cm}^{-1}$  2925, 1666, 1140, 973;  $\lambda_{\max}/\text{nm}$  202 ( $\epsilon$ , 4074), 253 (1585);  $\delta_{\text{H}}$ (400 MHz) 5.87 (6H, s), 5.69 (3H, s);  $\delta_{\text{C}}$ (100 MHz) 127.8 (CH), 78.6 (CH) (the signals corresponding to the trifluoroacetate were not observed);  $\delta_{\text{F}}$ (282 MHz) -76.1;  $m/z$  (EI) 131 ( $M^+$ , 100) 131 (100), 130 (76), 105 (55), 104 (26), 45 (32).

The crystalline tetrafluoroborate salt (29·HBF<sub>4</sub>) was prepared for X-ray analysis in essentially quantitative yield by dissolving 29·CF<sub>3</sub>CO<sub>2</sub>H in a 20-fold molar excess of saturated aq NaBF<sub>4</sub> followed by extraction with DCM.

To obtain the free base of 29, the trifluoroacetate salt (29·CF<sub>3</sub>CO<sub>2</sub>H) (0.102 g, 0.416 mmol) was introduced into a mixture of DCM (5 mL) and aq KOH (2 M, 1 mL). After vigorous agitation for 2 min, the layers were separated and the aq phase extracted with

DCM (2 x 5 mL). The combined organic phase was dried and the solvent cautiously removed *in vacuo* to give **29** (49 mg, 94%) as a volatile colourless solid; (Found: (HRMS EI)  $M^+$  131.0735,  $C_9H_9N$  requires 131.0735);  $\lambda_{\max}/\text{nm}$  205 ( $\epsilon$ , 9772);  $\delta_{\text{H}}$ (400 MHz) 5.79 (6H, s), 4.87 (3H, s);  $\delta_{\text{C}}$ (67.5 MHz) 130.3 (CH), 78.6 (CH);  $m/z$  (EI) 131 ( $M^+$ , 97) 131 (97), 130 (76), 105 (49), 69 (92), 55 (54).

**(1*r*,4*S*,7*R*)-2,2,9,9-Tetrabromo-10-azatricyclo[5.2.1.0<sup>1,10</sup>]decan-1-ol **50**.**

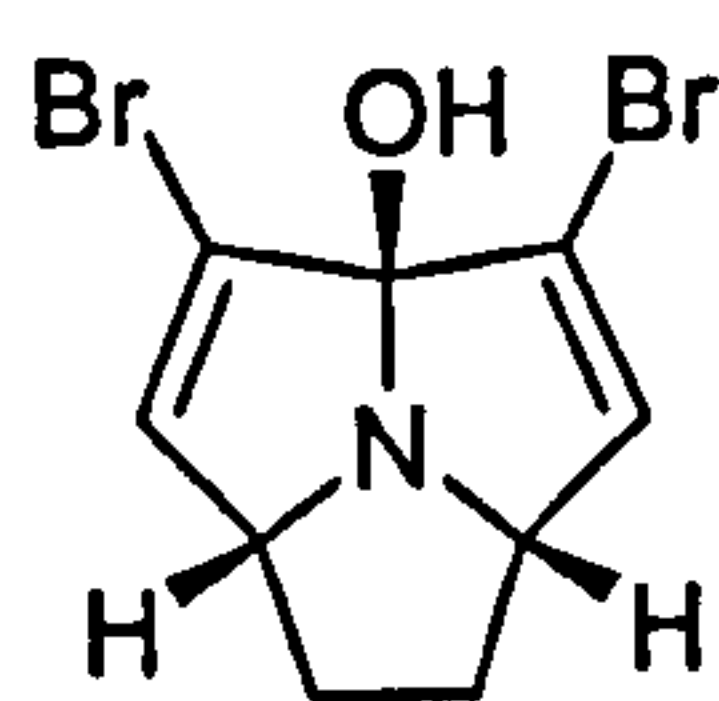


A solution of **26** (1.24 g, 9.17 mmol) in DCM (5 mL) was added dropwise to a stirred solution of bromine (7.40 g, 46.3 mmol) in DCM (30 mL) at  $-78^\circ\text{C}$  under nitrogen. After 5 min, a solution of triethylamine (4.66 g, 46.1 mmol) in DCM (12 mL) was added dropwise, and the mixture was stirred for an additional 5 min. The  $-78^\circ\text{C}$  bath was then replaced by an ice-acetone bath ( $-5^\circ\text{C}$ ) and stirring was continued for 10 min. Water (9 mL) was added and the mixture was stirred for a further 20 min. The layers were then separated and the aq phase extracted with DCM (3 x 60 mL). The combined organic phase was washed with aq sodium hydrogencarbonate (1 M, 80 mL) and water (3 x 60 mL) and dried. The solvent was removed *in vacuo* to leave a brown solid which was triturated with DCM (20 mL) to give **50** (0.843 g) as a white crystalline solid, mp  $132\text{--}134^\circ\text{C}$ . The DCM triturate could be purified by column chromatography (Petroleum ether:AcOEt 4:1) to give more **50** (0.748 g) as a yellow solid, total yield 37%; (Found: C, 23.0; H, 2.3; N, 2.9).  $C_9H_{11}NOBr_4$  requires C, 23.2; H, 2.4; N, 3.0); (Found (HRMS ES)  $M^+ + H$  465.7543  $C_9H_{12}NOBr_4$  requires 465.7652);  $\nu_{\max}/\text{cm}^{-1}$  3694, 3540, 2977, 2874, 1601, 1463, 1383, 1297, 1111, 976, 894, 613;  $\delta_{\text{H}}$ (400 MHz, DMSO- $d_6$ ) 3.71 (2H, m), 3.32–3.20 (4H, m), 2.20 (2H, m), 1.93 (2H, m);  $\delta_{\text{C}}$ (67.5 MHz, DMSO- $d_6$ )



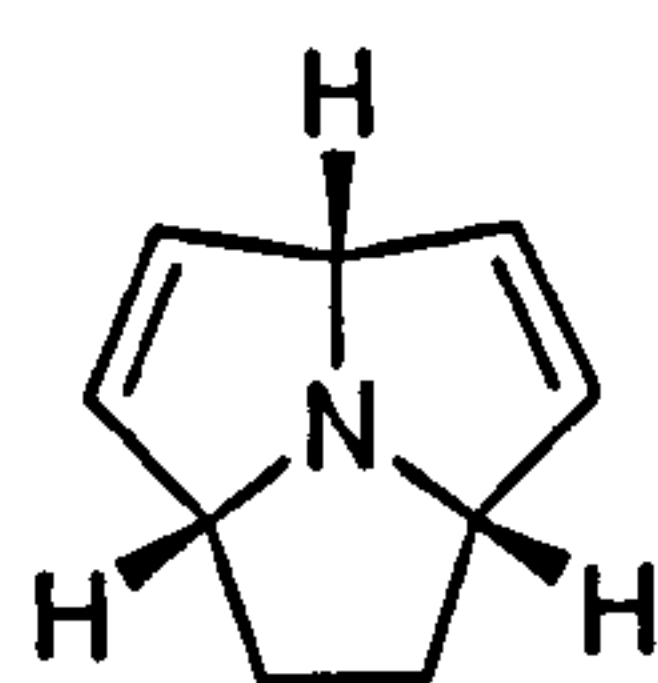
103.3 (C), 70.3 (C), 61.4 (CH), 53.8 (CH<sub>2</sub>), 29.1 (CH<sub>2</sub>); *m/z* (ES) 465 (M<sup>+</sup>+H, 18) 473 (1), 471 (40), 469 (100), 467 (96), 465 (18).

**(1*r*,4*S*,7*R*)-2,9-Dibromo-10-azatricyclo[5.2.1.0<sup>1,10</sup>]deca-2,8-dien-1-ol 51.**



A solution of KHMDS in toluene (0.5 M, 20 mL) was added dropwise to a stirred solution of **50** (1.17 g, 2.50 mmol) in THF (125 mL) at –78°C under nitrogen. The mixture was stirred 12 h during which it was allowed to warm to RT. Water (150 mL) and DCM (150 mL) were added and stirring was continued for 5 min. The layers were separated and the aq phase extracted with DCM (2 x 150 mL). The combined organic phase was dried and the solvent removed *in vacuo* to give **51** (0.649 g, 85%) as colourless crystals, mp 110-112°C; (Found: (HRMS ES) M<sup>+</sup>+H 305.9142, C<sub>9</sub>H<sub>10</sub>NOBr<sub>2</sub> requires 305.9129);  $\nu_{\text{max}}/\text{cm}^{-1}$  3571, 3153, 2967, 2898, 2795, 1626, 1457, 1338, 1307, 1295, 1150, 1129, 1105, 1081, 1070, 985, 950, 908;  $\delta_{\text{H}}$ (400 MHz) 6.09 (2H, s), 4.18 (2H, m), 2.09 (2H, m), 1.80 (2H, m);  $\delta_{\text{C}}$ (67.5 MHz) 134.9 (CH), 122.1 (C), 106.3 (C), 68.4 (CH), 31.8 (CH<sub>2</sub>); *m/z* (ES) 306 (M<sup>+</sup>+H, 29) 310 (25), 308 (100), 306 (30).

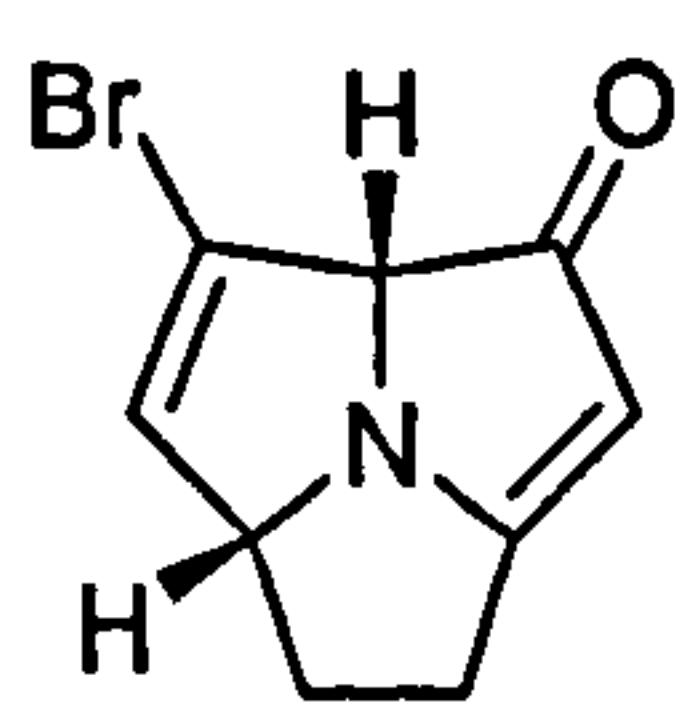
**(1*r*,4*S*,7*R*)-10-Azatricyclo[5.2.1.0<sup>1,10</sup>]deca-2,8-diene 52.**



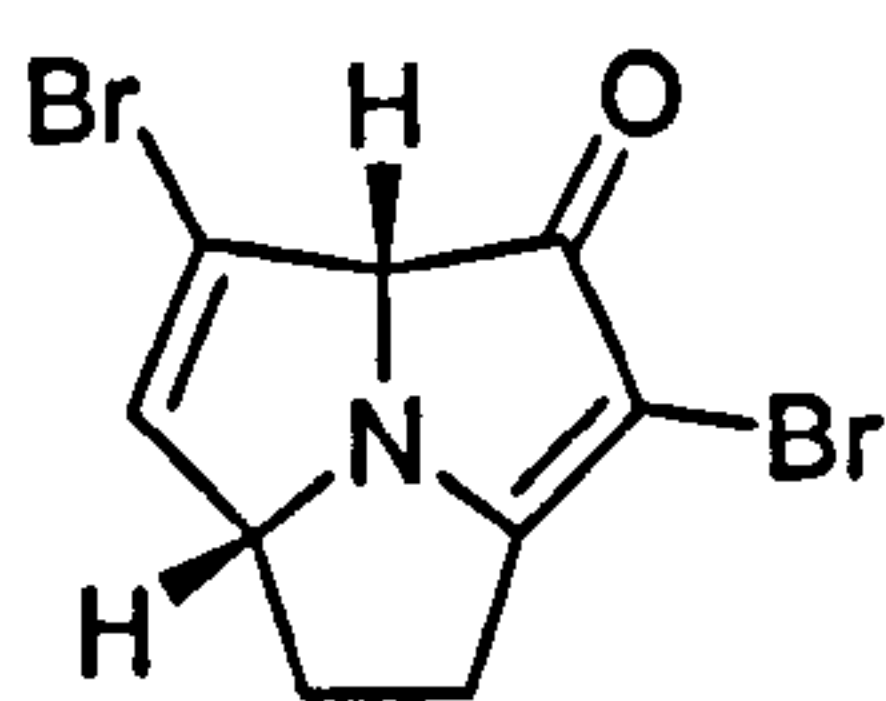
A solution of lithium aluminum hydride in THF (1.0 M, 14 mL) was added to a stirred solution of **51** (0.611 g, 2.00 mmol) in THF (40 mL) at RT under nitrogen. The mixture was then heated at reflux for 36 h, cooled to 0°C and water (2 mL) was cautiously added followed by aq NaOH (2 M, 10 mL). The resulting slurry was stirred at RT for 30 min and solid potassium carbonate (10 g) was added. The mixture was stirred for a further 1 h at RT and then filtered. The solid residue was washed with DCM and aq HCl (0.5 M, 150 mL) was added to



the combined filtrate. The layers were separated and the aq phase washed with DCM (150 mL). The aq layer was then basified with aq NaOH (2 M) and extracted with DCM (3 x 150 mL). Trifluoroacetic acid (0.68 g, 6.00 mmol) was added to the combined organic phase which was then dried. The solvent was removed *in vacuo* to give the trifluoroacetate salt of **52** (29 mg, 95%) as white crystals, mp 179-181°C; (Found: C, 53.4; H 5.0; N, 5.4.  $C_{11}H_{12}NO_2F_3$  requires C, 53.4; H, 4.9; N, 5.7 %); (Found: (HRMS EI)  $M^+$  133.0894,  $C_9H_{11}N$  requires 133.0892);  $\nu_{\max}/\text{cm}^{-1}$  2951, 1666, 1140, 1056, 838;  $\lambda_{\max}/\text{nm}$  207 ( $\epsilon$ , 1023), 260 (78);  $\delta_{\text{H}}$ (400 MHz) 5.82 (2H, dd,  $J$  6.3, 1.5), 5.69 (2H, dd,  $J$  6.3, 1.5), 5.46 (1H, s), 4.90 (2H, br s), 2.35 (2H, m), 1.90 (2H, m);  $\delta_{\text{C}}$ (100 MHz) 162.3 (q,  $^2J_{\text{CF}}$  36.3) (C), 128.0 (CH), 127.9 (CH), 116.4 (q,  $^1J_{\text{CF}}$  290.9) (C), 77.7 (CH), 64.8 (CH), 32.3 ( $\text{CH}_2$ );  $m/z$  (EI) 133 ( $M^+$ , 28) 133 (28), 105 (100), 104 (24), 45 (27). To obtain the free base **52**, the trifluoroacetate salt **52**· $\text{CF}_3\text{CO}_2\text{H}$  (0.152 g, 0.615 mmol) was introduced into a mixture of DCM (2 mL) and aq KOH (2M, 2 mL). After vigorous agitation for 2 min the layers were separated and the aq phase extracted with DCM (2 x 8 mL). The combined organic phase was dried and the solvent cautiously removed *in vacuo* to give **52** as a volatile colourless solid, (Found: (HRMS EI)  $M^+$  133.0896,  $C_9H_{11}N$  requires 133.0892);  $\nu_{\max}/\text{cm}^{-1}$  2937, 2868, 1667, 1453, 1351, 1117, 1080, 1065, 992, 964, 945;  $\lambda_{\max}/\text{nm}$  205 ( $\epsilon$ , 2818);  $\delta_{\text{H}}$ (400 MHz) 5.62 (2H, ddd,  $J$  5.9, 1.8, 1.8), 5.50 (2H, ddd,  $J$  5.9, 1.8, 1.8), 4.62 (1H, s), 4.00 (2H, m), 1.86 (2H, m), 1.45 (2H, m);  $\delta_{\text{C}}$ (100 MHz) 130.5 (CH), 130.0 (CH), 78.0 (CH), 71.2 (CH), 32.3 ( $\text{CH}_2$ );  $m/z$  (EI) 133 ( $M^+$ , 15) 133 (15), 105 (100).

***rel*-(1*R*,7*S*)-9-Bromo-10-azatricyclo[5.2.1.0<sup>1,10</sup>]deca-3,8-dien-2-one 55.**

A solution of DBU (2.70 mL, 2.75 g, 18.1 mmol) in THF (5 mL) was added dropwise over 15 min to a stirred solution of **50** (2.83 g, 6.04 mmol) in THF (50 mL) at  $-78^{\circ}\text{C}$  under nitrogen. Stirring was continued for 4.5 h during which the mixture was allowed to warm to RT. The solvent was removed *in vacuo* and the residue partitioned between DCM (50 mL) and water (35 mL). The mixture was shaken, the layers were separated and the aq phase was extracted with DCM (3 x 30 mL). The combined organic phase was washed with water (30 mL) and dried. The solvent was removed *in vacuo* to give a brown solid which was purified by column chromatography (AcOEt:Petroleum ether 1:1) to give **55** (312 mg, 23%) as orange crystals, mp  $100\text{--}103^{\circ}\text{C}$ ; (Found: (HRMS EI)  $M^{+}$  224.9793,  $\text{C}_9\text{H}_8\text{NOBr}$  requires 224.9789);  $\nu_{\text{max}}/\text{cm}^{-1}$  2925, 1687, 1584, 1368, 1045, 890;  $\delta_{\text{H}}$ (400 MHz) 5.87 (1H, br s), 5.24 (1H, br s), 4.53 (1H, m), 4.32 (1H, m), 2.91–2.72 (2H, m), 2.68–2.60 (1H, m), 2.02–1.93 (1H, m);  $\delta_{\text{C}}$ (67.5 MHz) 202.3 (C), 189.9 (C), 132.7 (CH), 119.1 (C), 101.1 (CH), 77.2 (CH), 65.6 (CH), 28.5 ( $\text{CH}_2$ ), 27.3 ( $\text{CH}_2$ );  $m/z$  (EI) 225 ( $M^{+}$ , 61) 227 (59), 225 (61), 199 (24), 197 (24), 118 (100), 117 (30), 51 (14).

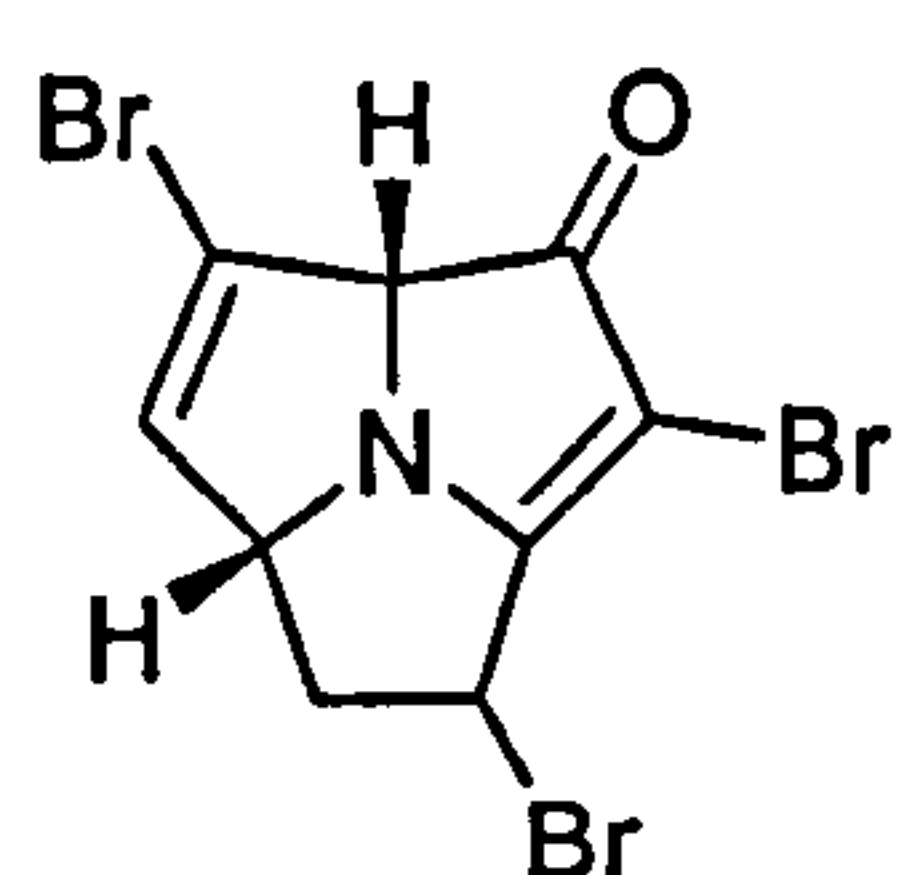
***rel*-(1*R*,7*S*)-3,9-Dibromo-10-azatricyclo[5.2.1.0<sup>1,10</sup>]deca-3,8-dien-2-one 78.**

*N*-Bromosuccinimide (12 mg, 0.067 mmol) was added portionwise to a stirred solution of **55** (14 mg, 0.062 mmol) in DCM (2 mL) at RT. The mixture was stirred for 10 min and DCM (10 mL) and 2 M NaOH (8 mL) were added. After vigorous agitation the layers were separated and the aq phase was extracted with DCM (2 x 10 mL). The combined organic phase was dried and the solvent removed *in vacuo* to give **78** (18 mg, 95%) as an oily yellow solid; (Found: (HRMS EI)  $M^{+}$  302.8884,  $\text{C}_9\text{H}_7\text{NOBr}_2$  requires 302.8894);  $\nu_{\text{max}}/\text{cm}^{-1}$



2925, 1702, 1588, 1360, 1064, 1043, 964, 882;  $\delta_{\text{H}}$ (400 MHz) 5.90 (1H, m), 4.62 (1H, m), 4.48 (1H, dd,  $J$  3.4, 2.2), 2.88 (2H, m), 2.69 (1H, m), 2.04 (1H, m);  $\delta_{\text{C}}$ (100 MHz) 194.4 (C), 187.0 (C), 133.2 (CH), 118.9 (C), 97.6 (C), 76.4 (CH), 66.6 (CH), 27.9 (CH<sub>2</sub>), 27.0 (CH<sub>2</sub>);  $m/z$  (EI) 303 ( $M^+$ , 20) 307 (19), 305 (51), 303 (20), 277 (12), 227 (23), 226 (43), 225 (25), 224 (51), 199 (12), 198 (11), 146 (11), 118 (45), 117 (100), 79 (27).

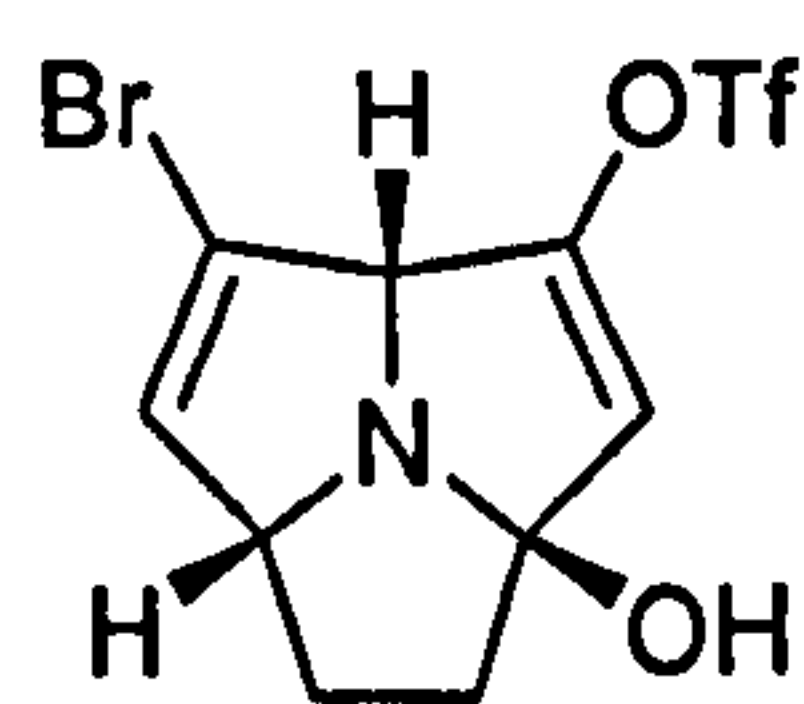
***rel*-(1*R*,5*R*,7*S*)-3,5,9-Tribromo-10-azatricyclo[5.2.1.0<sup>1,10</sup>]deca-3,8-dien-2-one 79.**



*N*-Bromosuccinimide (59 mg, 0.33 mmol) was added portionwise to a stirred solution of **55** (15 mg, 0.066 mmol) in DCM (2 mL) at RT.

The mixture was stirred for 7 h and DCM (10 mL) and 2 M NaOH (8 mL) were added. After vigorous agitation the layers were separated and the aq phase was extracted with DCM (2 x 10 mL). The combined organic phase was dried and the solvent removed *in vacuo* to give **79** (24 mg, 94%) as an oily orange solid; (Found: (HRMS ES)  $M^+ + H$  381.8044, C<sub>9</sub>H<sub>7</sub>NOBr<sub>3</sub> requires 381.8078);  $\nu_{\text{max}}/\text{cm}^{-1}$  2926, 2852, 1715, 1594, 1359, 1048;  $\delta_{\text{H}}$ (400 MHz) 5.91 (1H, dd,  $J$  2.2, 1.8), 4.98 (1H, dd,  $J$  7.3, 1.3), 4.82-4.77 (1H, m), 4.57 (1H, ddd,  $J$  3.1, 2.2, 0.6), 2.99 (1H, ddt,  $J$  15.3, 7.6, 1.0), 2.74 (1H, ddd,  $J$  15.3, 7.3, 5.2);  $\delta_{\text{C}}$ (67.5 MHz) 193.9 (C), 182.6 (C), 132.1 (CH), 118.7 (C), 92.8 (C), 75.5 (CH), 65.3 (CH), 40.8 (CH<sub>2</sub>), 36.4 (CH);  $m/z$  (ES) 382 ( $M^+ + H$ , 11) 388 (8), 386 (79), 384 (100), 382 (11).

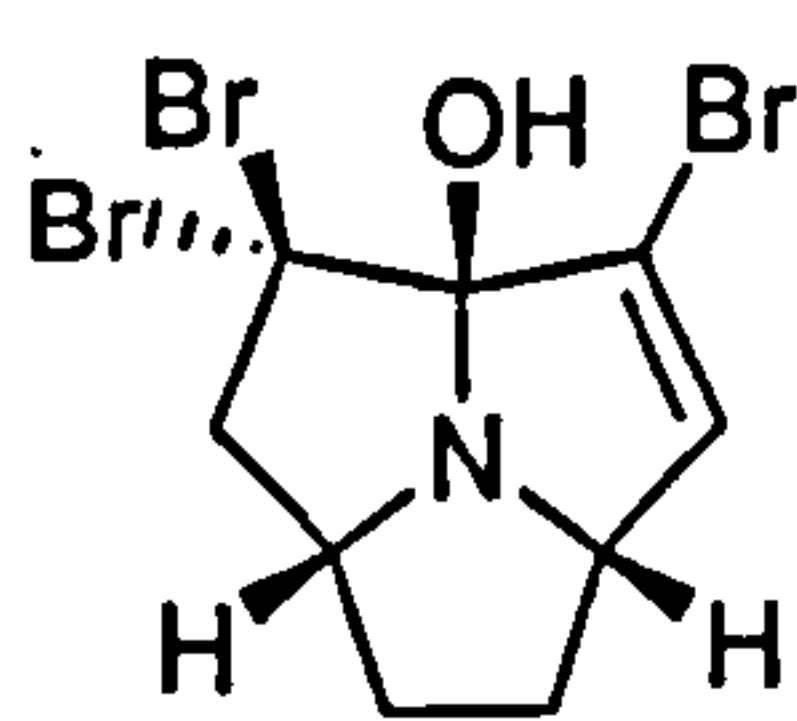
***rel*-(1*R*,4*R*,7*S*)-5-Bromo-3-trifluoromethanesulfonyloxy-10-azatricyclo[5.2.1.0<sup>1,10</sup>]deca-2,5-dien-1-ol 80.**



Triethylamine (30  $\mu$ L, 22 mg, 0.22 mmol) was added dropwise to a stirred solution of **55** (15 mg, 0.066 mmol) in DCM (2 mL) at  $-78^{\circ}\text{C}$  under nitrogen. A solution of trifluoromethanesulfonic anhydride (33

$\mu$ L, 55 mg, 0.20 mmol) in DCM (2 mL) was added dropwise and the mixture was stirred at  $-78^{\circ}\text{C}$  for 30 min. The reaction was allowed to warm to RT and DCM (10 mL) and water (8 mL) were added. Stirring was continued for an additional 5 min and the layers were separated. The organic phase was washed with aq sodium hydrogencarbonate (8 mL), dried and the volatiles removed *in vacuo* to give **80** (18 mg, 72%) as unstable white crystals; (Found: (HRMS ES)  $\text{M}^{\text{+}}\text{+H}$  375.9427,  $\text{C}_{10}\text{H}_{10}\text{NO}_4\text{BrF}_3\text{S}$  requires 375.9466);  $\nu_{\text{max}}/\text{cm}^{-1}$  3429, 2946, 1661, 1588, 1434, 1341, 1322, 1138, 1060, 998, 961, 867, 839, 638;  $\delta_{\text{H}}$ (400 MHz) 5.92 (1H, d,  $J$  1.4), 5.90 (1H, t,  $J$  1.8), 4.70 (1H, app quint,  $J$  1.7), 4.33 (1H, ddd,  $J$  5.1, 3.1, 1.7), 2.40 (1H, m), 2.15 (1H, ddd,  $J$  12.8, 6.7, 2.0), 1.97 (1H, m), 1.68 (1H, ddd,  $J$  12.5, 12.5, 7.3);  $\delta_{\text{C}}$ (100 MHz) 146.4 (C), 133.1 (CH), 118.5 (q,  $^1J_{\text{CF}}$  320.5) (C), 118.1 (CH), 116.9 (C), 103.1 (C), 75.2 (CH), 70.3 (CH), 36.7 ( $\text{CH}_2$ ), 30.1 ( $\text{CH}_2$ );  $m/z$  (ES) 378 ( $\text{M}^{\text{+}}\text{+H}$ , 100) 378 (100), 376 (62).

***rel*-(1*R*,4*S*,7*R*)-2,9,9-Tribromo-10-azatricyclo[5.2.1.0<sup>1,10</sup>]deca-2-en-1-ol 59.**

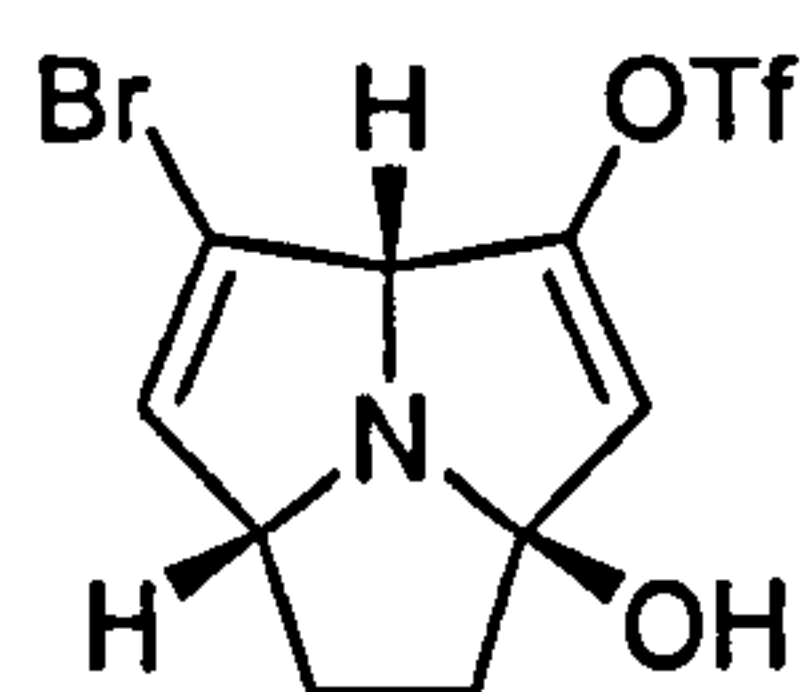


A solution of KHMDS in toluene (0.50 M, 1.63 mL, 0.82 mmol) was added dropwise to a stirred solution of **50** (101 mg, 0.215 mmol) in THF (10 mL) at  $-78^{\circ}\text{C}$  over 5 min. The mixture was stirred for an

additional 15 min and allowed to come to RT. DCM (20 mL) and water (10 mL) were immediately added, the mixture was stirred for 5 min and the layers were separated.

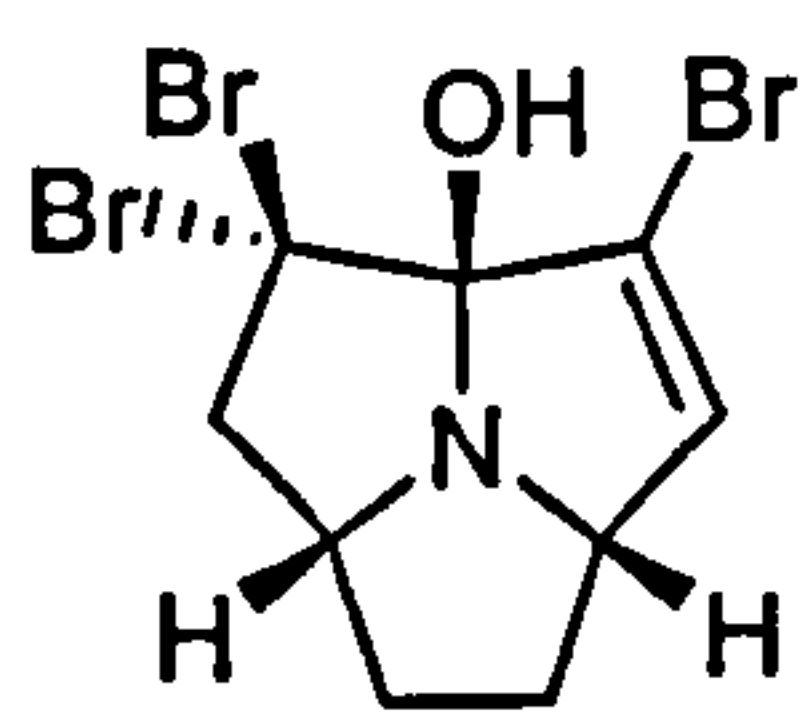


***rel*-(1*R*,4*R*,7*S*)-5-Bromo-3-trifluoromethanesulfonyloxy-10-azatricyclo[5.2.1.0<sup>1,10</sup>]deca-2,5-dien-1-ol 80.**



Triethylamine (30  $\mu$ L, 22 mg, 0.22 mmol) was added dropwise to a stirred solution of **55** (15 mg, 0.066 mmol) in DCM (2 mL) at  $-78^{\circ}\text{C}$  under nitrogen. A solution of trifluoromethanesulfonic anhydride (33  $\mu$ L, 55 mg, 0.20 mmol) in DCM (2 mL) was added dropwise and the mixture was stirred at  $-78^{\circ}\text{C}$  for 30 min. The reaction was allowed to warm to RT and DCM (10 mL) and water (8 mL) were added. Stirring was continued for an additional 5 min and the layers were separated. The organic phase was washed with aq sodium hydrogencarbonate (8 mL), dried and the volatiles removed *in vacuo* to give **80** (18 mg, 72%) as unstable white crystals; (Found: (HRMS ES)  $\text{M}^+\text{+H}$  375.9427,  $\text{C}_{10}\text{H}_{10}\text{NO}_4\text{BrF}_3\text{S}$  requires 375.9466);  $\nu_{\text{max}}/\text{cm}^{-1}$  3429, 2946, 1661, 1588, 1434, 1341, 1322, 1138, 1060, 998, 961, 867, 839, 638;  $\delta_{\text{H}}$ (400 MHz) 5.92 (1H, d,  $J$  1.4), 5.90 (1H, t,  $J$  1.8), 4.70 (1H, app quint,  $J$  1.7), 4.33 (1H, ddd,  $J$  5.1, 3.1, 1.7), 2.40 (1H, m), 2.15 (1H, ddd,  $J$  12.8, 6.7, 2.0), 1.97 (1H, m), 1.68 (1H, ddd,  $J$  12.5, 12.5, 7.3);  $\delta_{\text{C}}$ (100 MHz) 146.4 (C), 133.1 (CH), 118.5 (q,  $^1J_{\text{CF}}$  320.5) (C), 118.1 (CH), 116.9 (C), 103.1 (C), 75.2 (CH), 70.3 (CH), 36.7 ( $\text{CH}_2$ ), 30.1 ( $\text{CH}_2$ );  $m/z$  (ES) 378 ( $\text{M}^+\text{+H}$ , 100) 378 (100), 376 (62).

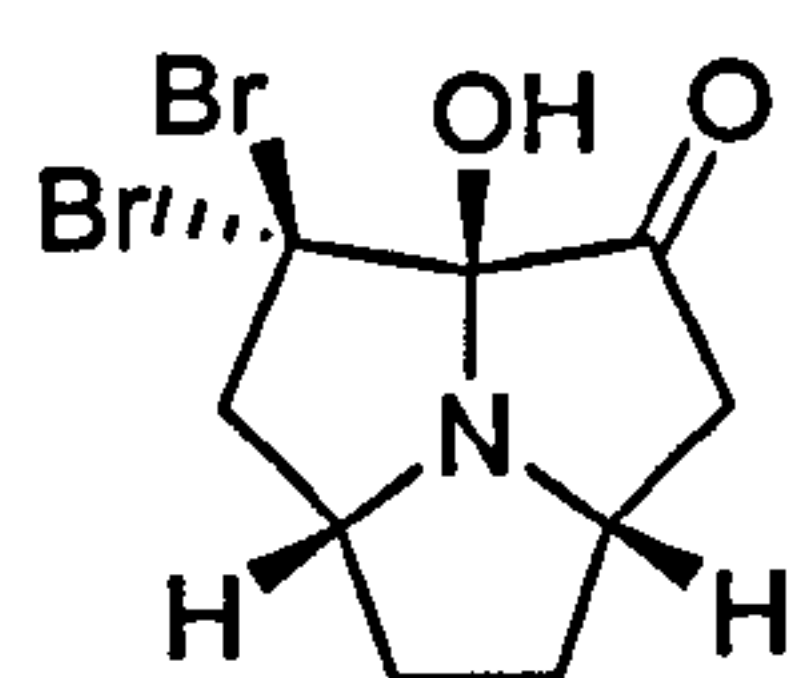
***rel*-(1*R*,4*S*,7*R*)-2,9,9-Tribromo-10-azatricyclo[5.2.1.0<sup>1,10</sup>]deca-2-en-1-ol 59.**



A solution of KHMDS in toluene (0.50 M, 1.63 mL, 0.82 mmol) was added dropwise to a stirred solution of **50** (101 mg, 0.215 mmol) in THF (10 mL) at  $-78^{\circ}\text{C}$  over 5 min. The mixture was stirred for an additional 15 min and allowed to come to RT. DCM (20 mL) and water (10 mL) were immediately added, the mixture was stirred for 5 min and the layers were separated.

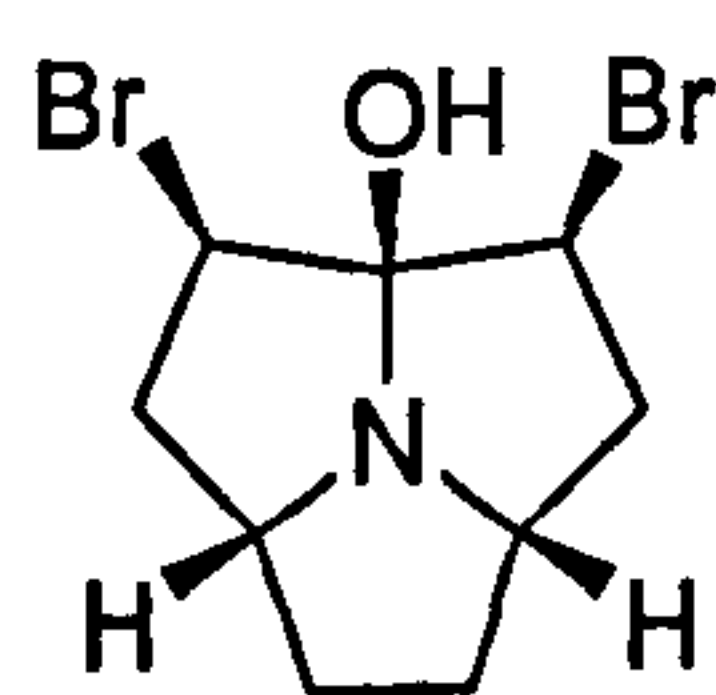
The aq phase was extracted with DCM (2 x 20 mL), the combined organic phase was dried and the solvent removed *in vacuo* to give **59** (69 mg, 83%) as orange crystals, mp 112-114°C; (Found: (HRMS ES)  $M^+ + H$  385.8380,  $C_9H_{11}NOBr_3$  requires 385.8391);  $\nu_{\max}/\text{cm}^{-1}$  3546, 2927, 1616, 1462, 1343, 1112, 1074, 949, 893, 642;  $\delta_H$ (400 MHz) 6.32 (1H, d,  $J$  2.0), 4.22 (1H, m), 3.92 (1H, m), 3.20 (1H, dd,  $J$  14.1, 5.9), 2.73 (1H, dd,  $J$  14.1, 7.5), 2.16-1.99 (3H, m), 1.88 (1H, m);  $\delta_C$ (100 MHz, DMSO- $d_6$ ) 138.7 (CH), 121.2 (C), 105.1 (C), 72.1 (C), 68.6 (CH), 60.9 (CH), 54.3 (CH<sub>2</sub>), 29.9 (CH<sub>2</sub>), 28.3 (CH<sub>2</sub>);  $m/z$  (ES) 386 ( $M^+ + H$ , 5) 392 (2), 390 (85), 388 (100), 386 (5).

***rel*-(1*R*,4*S*,7*R*)-9,9-Dibromo-1-hydroxy-10-azatricyclo[5.2.1.0<sup>1,10</sup>]decan-2-one **56**.**



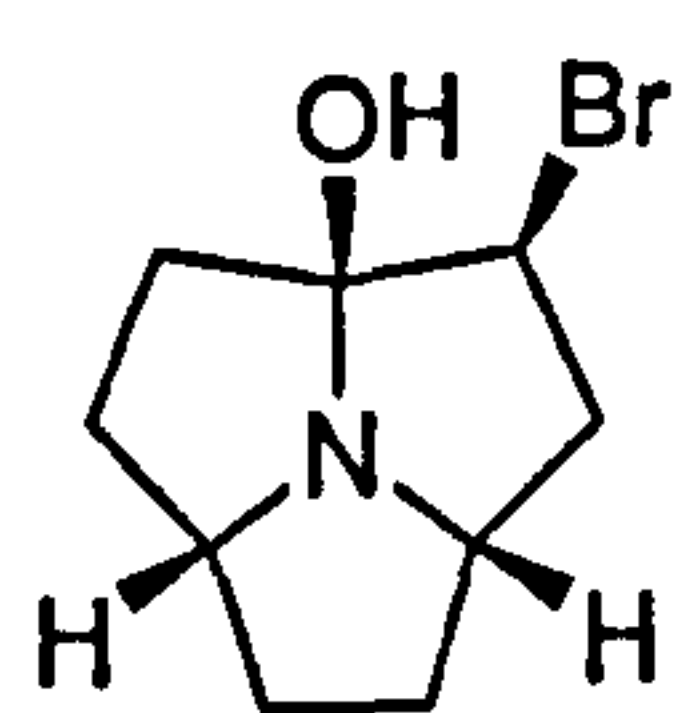
Compound **50** (48 mg, 0.10 mmol) was added to a stirred mixture of acetonitrile (30 mL) and sat aq sodium chloride (30 mL) and the resulting suspension was heated at reflux for 2 h. The reaction was cooled to RT and DCM (40 mL) and water (15 mL) were added. The mixture was stirred 5 min and the layers were separated. The aq phase was extracted with DCM (2 x 30 mL) and then the combined organic phase was dried and the solvent removed *in vacuo* to give a solid which was purified by column chromatography (AcOEt:Petroleum ether 3:1) to give **56** (10 mg, 30%) as colourless crystals, mp 97-99°C; (Found: (HRMS ES)  $M^+ + H$  323.9210,  $C_9H_{12}NO_2Br_2$  requires 323.9235);  $\nu_{\max}/\text{cm}^{-1}$  3696, 3533, 2946, 1760, 1698, 1601, 1463, 1354, 1146, 1125, 1098, 968, 894;  $\delta_H$ (400 MHz) 4.09 (1H, m), 3.87 (1H, m), 3.35 (1H, dd,  $J$  14.6, 6.6), 2.85 (1H, dd,  $J$  18.6, 7.3), 2.66 (1H, dd,  $J$  14.6, 6.7), 2.45 (1H, dd,  $J$  18.6, 7.4), 2.30-2.10 (2H, m), 2.01-1.92 (1H, m), 1.85 (1H, m);  $\delta_C$ (100 MHz) 209.6 (C), 97.4 (C), 68.1 (C), 62.7 (CH), 55.8 (CH), 55.6 (CH<sub>2</sub>), 43.6 (CH<sub>2</sub>), 32.5 (CH<sub>2</sub>), 29.0 (CH<sub>2</sub>);  $m/z$  (ES) 324 ( $M^+ + H$ , 30) 328 (25), 326 (100), 324 (30).



**(1*r*,2*R*,4*R*,7*S*,9*S*)-2,9-Dibromo-10-azatricyclo[5.2.1.0<sup>1,10</sup>]decan-1-ol 57.**

Diphenyl phosphite (2.37 mL, 2.90 g, 12.4 mmol) was added dropwise to a stirred solution of **50** (1.02 g, 2.19 mmol) in THF (10 mL) at RT.

The mixture was stirred 5 min and triethylamine (1.23 mL, 0.89 g, 8.82 mmol) was added dropwise. Stirring was continued for 30 min and the volatiles were removed *in vacuo* to give a residue which was partitioned between DCM (40 mL) and water (20 mL). The mixture was shaken and the layers were separated. The organic phase was washed with brine (60 mL) and water (2 x 50 mL), the solvent removed *in vacuo* and the residue was washed with ether (3 x 20 mL) to give **57** (249 mg, 37%) as a white solid, mp 138-140°C; (Found: (HRMS FAB)  $M^+ + H$  309.9420,  $C_9H_{14}NOBr_2$  requires 309.9442);  $\nu_{max}/cm^{-1}$  3632, 3548, 2958, 2872, 1732, 1462, 1266, 1106, 1068, 1016, 950;  $\delta_H$ (400 MHz) 4.23 (2H, t,  $J$  6.2), 3.86 (2H, app quint,  $J$  5.6), 2.52 (2H, dt,  $J$  13.5, 6.5), 2.15 (2H, dt,  $J$  13.5, 5.6), 1.95 (2H, m), 1.47 (2H, m);  $\delta_C$ (67.5 MHz) 100.0 (C), 61.9 (CH), 53.6 (CH), 40.4 (CH<sub>2</sub>), 29.8 (CH<sub>2</sub>);  $m/z$  (FAB) 310 ( $M^+$ , 6), 314 (6), 312 (9), 310 (6), 307 (26), 289 (14), 155 (30), 154 (100), 139 (14), 138 (35), 137 (66), 136 (72), 124 (12), 120 (12), 107 (23), 105 (11), 95 (10), 91 (15), 90 (14), 89 (18), 77 (19), 69 (14), 57 (19), 55 (17).

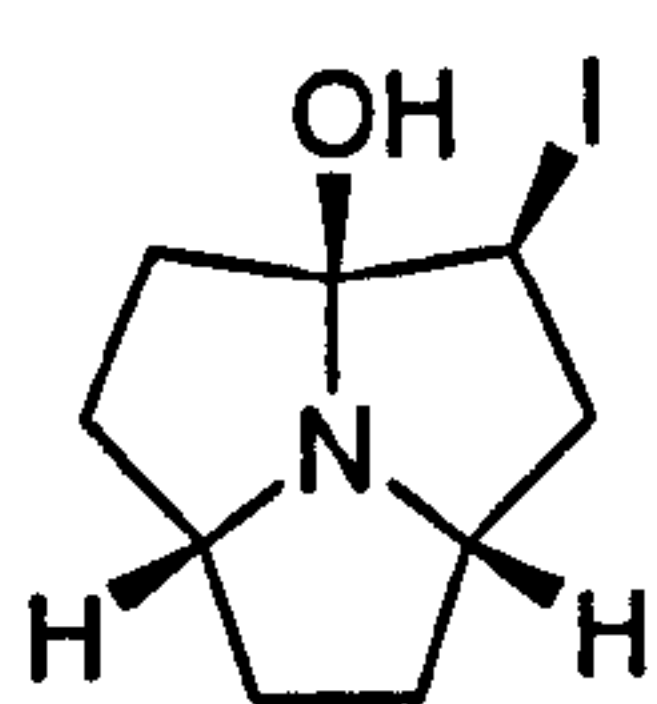
***rel*-(1*R*,2*R*,4*R*,7*S*)-2-Bromo-10-azatricyclo[5.2.1.0<sup>1,10</sup>]decan-1-ol 62.**

*N*-Bromosuccinimide (1.54 g, 8.65 mmol) was added portionwise to a stirred solution of enamine **26** (0.693 g, 5.13 mmol) in DCM (20 mL) at -78°C. When the addition was complete, the -78°C bath was replaced

by an ice bath and the mixture was stirred for 25 min. The reaction was then allowed to warm to RT and the mixture was stirred for a further 10 min. The solvent was removed *in vacuo* and the residue partitioned between DCM (50 mL) and 2 M NaOH

(30 mL). The mixture was shaken and the layers were separated. The aq phase was extracted with DCM (2 x 40 mL) and then, the combined organic phase was dried. The solvent was removed *in vacuo* to give a brown solid which was dissolved in a mixture of chloroform (50 mL) and conc aq HCl (10 mL) and the mixture was heated for 14 h at reflux with stirring. The reaction was cooled to RT and water (30 mL) and DCM (30 mL) were added. The layers were separated and the aq phase was extracted with DCM (2 x 30 mL). The combined organic phase was dried and the solvent removed *in vacuo* to give a solid which was purified by column chromatography (DCM:MeOH 10:1) to give **62** (0.131 g, 11%) as a white solid, mp 118-121°C; (Found: (HRMS ES)  $M^+ + H$  232.0354,  $C_9H_{15}NOBr$  requires 232.0337);  $\nu_{max}/cm^{-1}$  3564, 2943, 2870, 1463, 1355, 1293, 1140, 1106, 1078, 973;  $\delta_H$ (400 MHz) 4.33 (1H, t,  $J$  5.1), 3.87 (1H, m), 3.71 (1H, m), 2.40 (1H, m), 2.22-2.12 (1H, m), 2.07 (1H, ddd,  $J$  13.7, 7.1, 5.5), 2.00-1.72 (4H, m), 1.65-1.56 (1H, m), 1.53-1.43 (1H, m), 1.42-1.32 (1H, m);  $\delta_C$ (67.5 MHz) 101.9 (C), 64.9 (CH), 61.6 (CH), 58.7 (CH), 40.6 (CH<sub>2</sub>), 36.0 (CH<sub>2</sub>), 30.3 (CH<sub>2</sub>), 30.0 (CH<sub>2</sub>), 29.6 (CH<sub>2</sub>);  $m/z$  (ES) 232 ( $M^+ + H$ , 100) 234 (84), 232 (100).

***rel*-(1*R*,2*R*,4*R*,7*S*)-2-Iodo-10-azatricyclo[5.2.1.0<sup>1,10</sup>]decan-1-ol **63**.**



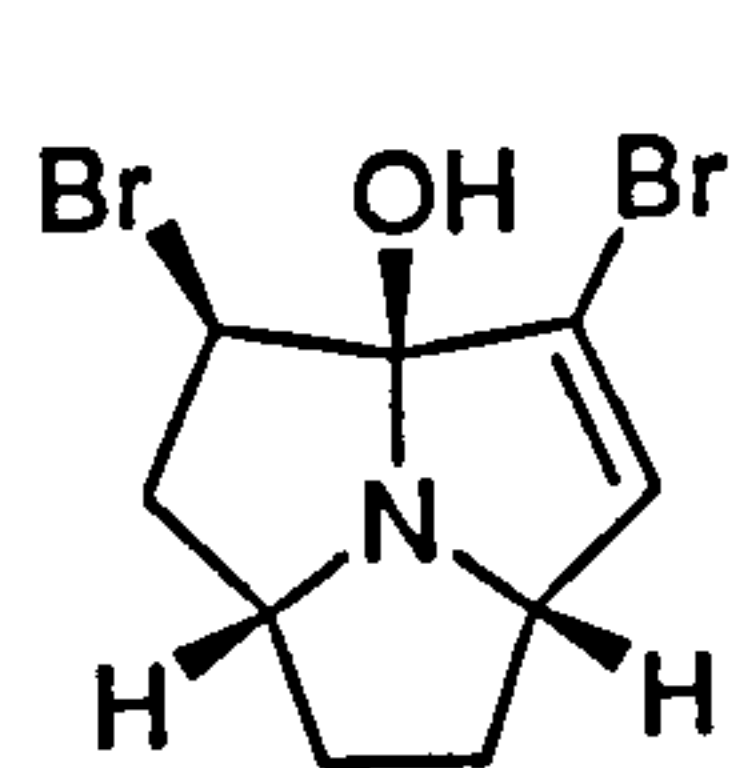
*N*-Iodosuccinimide (1.50 g, 6.67 mmol) was added portionwise to a stirred solution of enamine **26** (0.593 g, 4.35 mmol) in DCM (30 mL) at 0°C. The mixture was stirred at 0°C for 10 min and then at RT for 1 h.

Water (30 mL) and DCM (50 mL) were added and stirring was continued for a further 5 min. The layers were separated and the aq phase was extracted with DCM (2 x 40 mL). The combined organic phase was dried and the solvent removed *in vacuo* to give a brown solid which was dissolved in a mixture of chloroform (60 mL) and conc aq HCl (40 mL). The mixture was heated at reflux for 15 h the reaction was cooled to RT



and water (50 mL) and DCM (40 mL) were added. The mixture was shaken and the layers were separated. The aq phase was washed with DCM (2 x 40 mL), basified with aq NaOH (2 M) and extracted with DCM (3 x 40 mL). The combined organic phase was dried and the solvent removed *in vacuo* to give a brown oily solid which was purified by column chromatography (DCM:MeOH 10:1) to give **63** (79 mg, 6%) as an oily brown solid; (Found: (HRMS ES)  $M^+ + H$  280.0195,  $C_9H_{15}NOI$  requires 280.0198);  $\nu_{\max}/\text{cm}^{-1}$  3576, 2960, 2870, 1463, 1356, 1292, 1130, 1102, 1076, 1061, 970;  $\delta_H$ (400 MHz) 4.21 (1H, m), 3.73 (2H, m), 2.54 (1H, m), 2.15 (2H, m), 1.94 (2H, m), 1.85 (2H, m), 1.60-1.40 (3H, m);  $\delta_C$ (67.5 MHz) 101.9 (C), 64.6 (CH), 63.3 (CH), 42.0 (CH<sub>2</sub>), 35.7 (CH<sub>2</sub>), 35.0 (CH), 31.1 (CH<sub>2</sub>), 30.3 (CH<sub>2</sub>), 29.4 (CH<sub>2</sub>).

***rel*-(1*R*,4*R*,7*S*)-2,9-Dibromo-10-azatricyclo [5.2.1.0<sup>1,10</sup>]dec-2-en-1-ol **61**.**

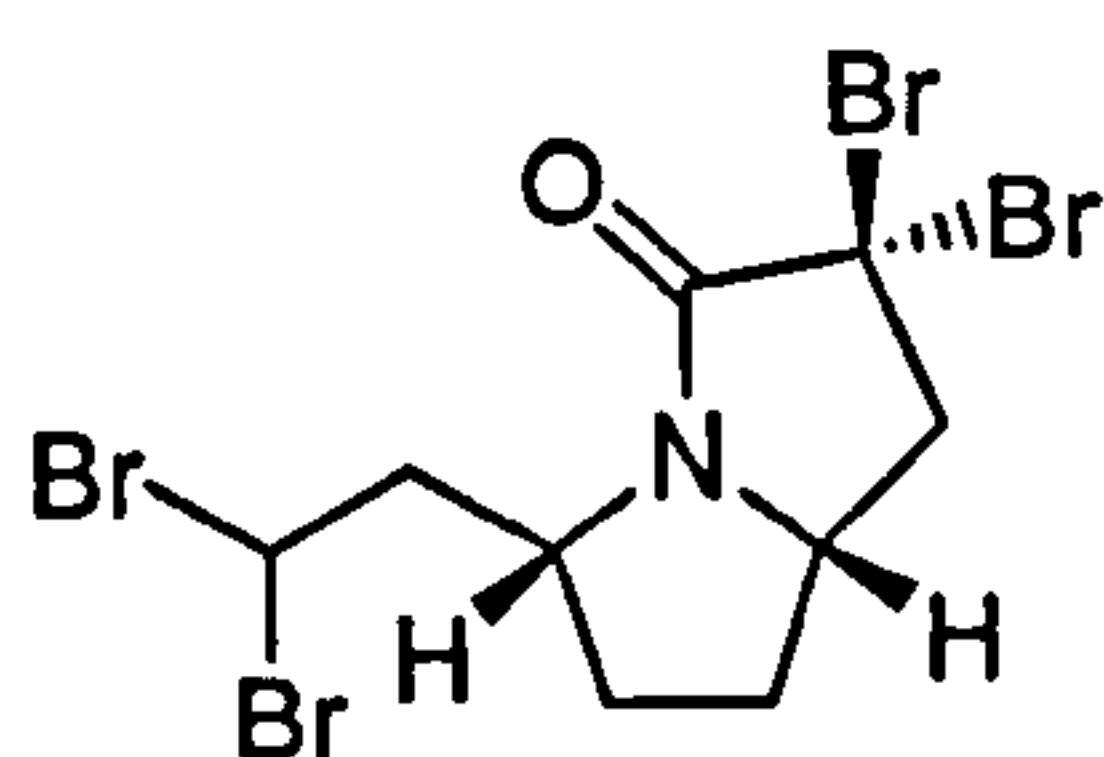


A solution of lithium triethylborohydride in THF (1.0 M, 4.7 mL, 4.7 mmol) was added to a stirred solution of **59** (295 mg, 0.761 mmol) in THF (11 mL) at RT under nitrogen. The mixture was then heated at

reflux for 48 h. The solvent was evaporated and the residue partitioned between water (10 mL) and conc aq HCl (8 mL). The mixture was stirred vigorously at RT for 15 min. The layers were separated and the aq phase was washed with DCM (10 mL). The aq layer was then made basic with 2 M NaOH and extracted with DCM (3 x 20 mL). Trifluoroacetic acid (3.4 mL, 5.0 g, 44 mmol) was added to the combined organic phase which was then dried and the solvent removed *in vacuo* to give the trifluoroacetate salt of **61** (83 mg, 26%) as colourless crystals, mp 185-187°C; (Found: (HRMS ES)  $M^+ + H$  307.9276,  $C_9H_{12}NOBr_2$  requires 307.9286);  $\nu_{\max}/\text{cm}^{-1}$  3505, 2942, 1624, 1458, 1355, 1101, 907;  $\delta_H$ (400 MHz) 6.07 (1H, d,  $J$  1.8), 4.54 (1H, t,  $J$  6.1), 4.28 (1H, ddd,  $J$  8.0, 4.6, 1.8), 3.92 (1H, app quint,  $J$  6.1), 2.47 (1H, dt,  $J$  13.6, 6.1),

2.20 (1H, dt,  $J$  13.6, 6.1), 2.11-1.92 (2H, m), 1.75 (1H, m), 1.51 (1H, m);  $\delta_{\text{C}}$ (67.5 MHz) 135.9 (CH), 120.5 (C), 103.6 (C), 68.7 (CH), 63.0 (CH), 56.2 (CH), 40.7 (CH<sub>2</sub>), 29.4 (CH<sub>2</sub>), 29.2 (CH<sub>2</sub>);  $m/z$  (ES) 308 ( $M^+ + H$ , 42) 312 (28), 310 (100), 308 (42).

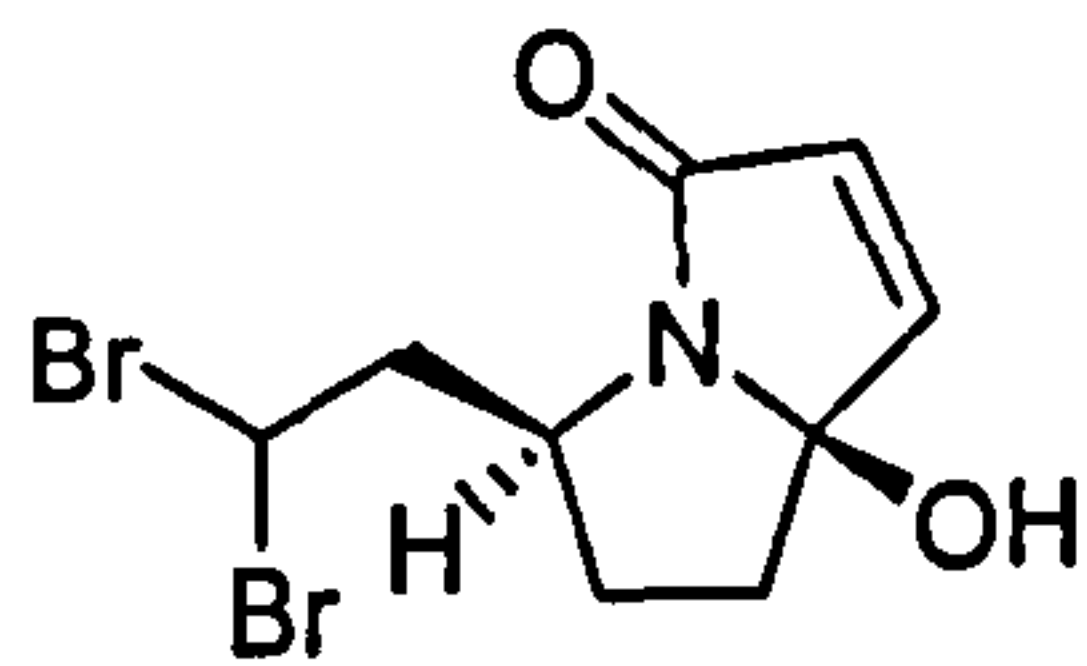
***rel*-(5*R*,8*S*)-2,2-Dibromo-5-(2,2-dibromoethyl)-pyrrolizidin-3-one 53.**



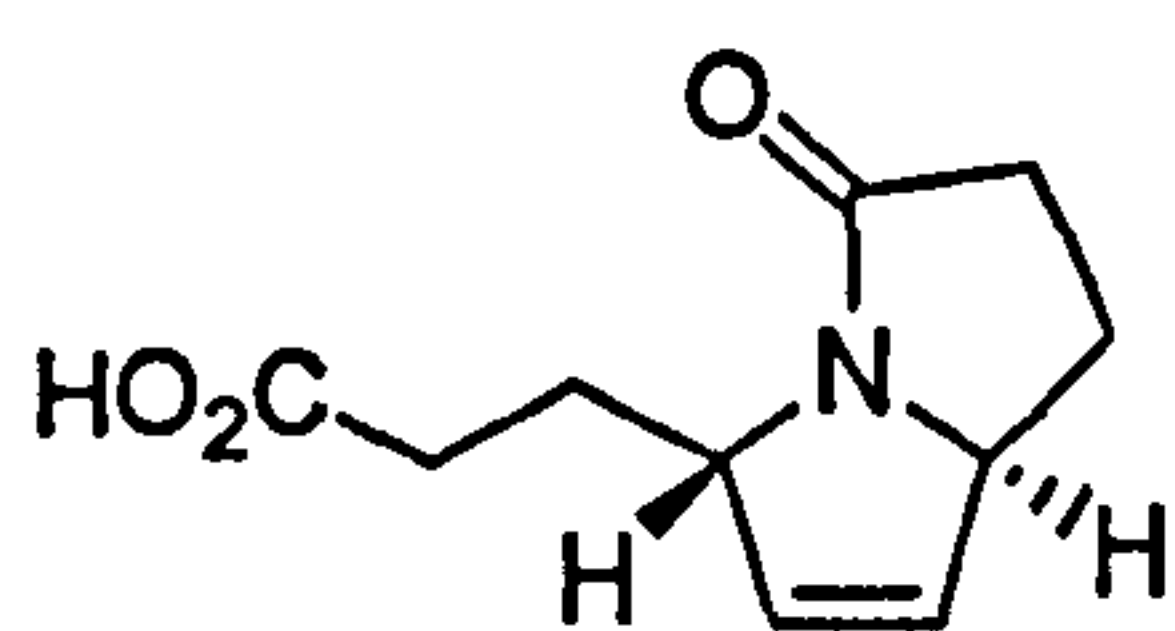
Solid potassium *tert*-butoxide (69 mg, 0.61 mmol) was added portionwise over 15 min to a stirred solution of **50** (101 mg, 0.215 mmol) in THF (5 mL) at 0°C under nitrogen. After 10 min

the solvent was removed *in vacuo* and the residue partitioned between water (10 mL) and DCM (15 mL). After vigorous agitation the layers were separated and the aq layer was extracted with DCM (2 x 10 mL). The combined organic phase was dried and the solvent removed *in vacuo* to give **53** (59 mg, 58%) as a pale yellow solid, mp 134-136°C; (Found: (HRMS EI)  $M^+$  464.7557, C<sub>9</sub>H<sub>11</sub>NOBr<sub>4</sub> requires 464.7574);  $\nu_{\text{max}}/\text{cm}^{-1}$  2957, 2931, 2875, 1715, 1405, 1352, 1300, 908;  $\delta_{\text{H}}$ (400 MHz) 6.10 (1H, dd,  $J$  7.1, 7.1), 3.99 (1H, m), 3.90 (1H, m), 3.38 (1H, dt,  $J$  14.7, 5.9), 3.29 (1H, dd,  $J$  13.5, 4.7), 2.69 (1H, dd,  $J$  13.4, 8.8), 2.49-2.34 (2H, m), 2.15-2.05 (2H, m), 1.58 (1H, m);  $\delta_{\text{C}}$ (67.5 MHz) 164.3 (C), 60.3 (CH), 60.0 (C), 54.1 (CH), 53.0 (CH<sub>2</sub>), 47.5 (CH<sub>2</sub>), 42.5 (CH), 33.6 (CH<sub>2</sub>), 28.0 (CH<sub>2</sub>);  $m/z$  (EI) 469 (4), 467 (3), 465 (0.3), 392 (7), 390 (19), 388 (19), 386 (7), 310 (4), 308 (7), 284 (18), 282 (35), 280 (18), 204 (93), 202 (100), 201 (10), 135 (10), 134 (13), 124 (9), 107 (6), 105 (5), 82 (31), 81 (11), 80 (30), 79 (9), 68 (17).



***rel*-(5*R*,8*S*)-5-(2,2-Dibromoethyl)-8-hydroxy-5,6,7,8-tetrahydropyrrolizin-3-one****54.**

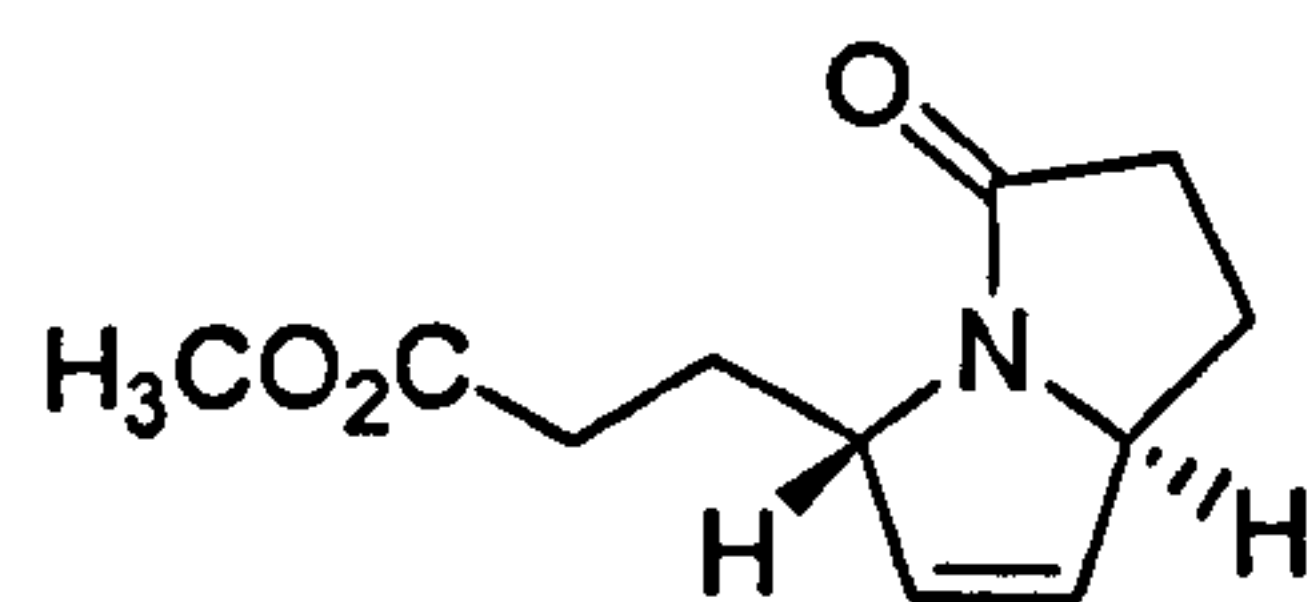
DBU (2.80 mL, 2.85 g, 18.7 mmol) was added dropwise over 5 min to a stirred solution of **50** (1.73 g, 3.69 mmol) in THF (150 mL) at RT under nitrogen. After 20 min the solvent was removed *in vacuo* and the residue partitioned between water (40 mL) and DCM (60 mL). After vigorous agitation the layers were separated and the aq layer was extracted with DCM (2 x 50 mL). The combined organic phase was dried and the solvent was removed *in vacuo* to give a brown oil which was purified by column chromatography (AcOEt:Petroleum ether 5:1) to give **54** (49 mg, 4%) as a colourless crystalline solid, mp 124-126°C; (Found: (HRMS ES)  $M^+ + Na$  345.9023,  $C_9H_{11}NO_2Br_2Na$  requires 345.9054);  $\nu_{max}/cm^{-1}$  3579, 3400, 2947, 2876, 1711, 1593, 1458, 1366, 1347, 1296, 1148, 1296, 1148, 1100, 1028, 1001, 908;  $\delta_H$ (400 MHz) 7.10 (1H, d,  $J$  5.7), 5.96 (1H, d,  $J$  5.7), 5.87 (1H, dd,  $J$  9.4, 3.8), 3.87 (1H, m), 2.82 (1H, ddd,  $J$  14.7, 8.8, 3.8), 2.72 (1H, ddd,  $J$  14.7, 9.5, 3.7), 2.54 (1H, m), 2.34 (1H, m), 2.17 (1H, dd,  $J$  12.7, 6.7), 1.60 (1H, ddd,  $J$  12.6, 12.6, 7.9);  $\delta_C$ (100 MHz) 174.4 (C), 150.2 (CH), 126.4 (CH), 98.7 (C), 54.3 (CH), 53.5 (CH<sub>2</sub>), 43.0 (CH), 34.3 (CH<sub>2</sub>), 34.2 (CH<sub>2</sub>);  $m/z$  (ES) 346 ( $M^+ + Na$ , 28) 350 (20), 348 (100), 346 (28).

**3-(*rel*-(5*R*,8*S*)-5-Oxo-5,6,7,8-tetrahydro-3H-pyrrolizin-3-yl)-propionic acid **71**.**

Pyrrole **23** (0.269 g, 1.12 mmol) was added portionwise to a stirred suspension of zinc dust (1.11 g, 17.0 mmol) in HCl (20%, 5 mL) at 0°C over a period of 15 min. Conc aq HCl (2 mL) was added and the mixture was stirred at 0°C for 30 min. The reaction was warmed to RT and stirring was continued for a further 3 h. The mixture was filtered

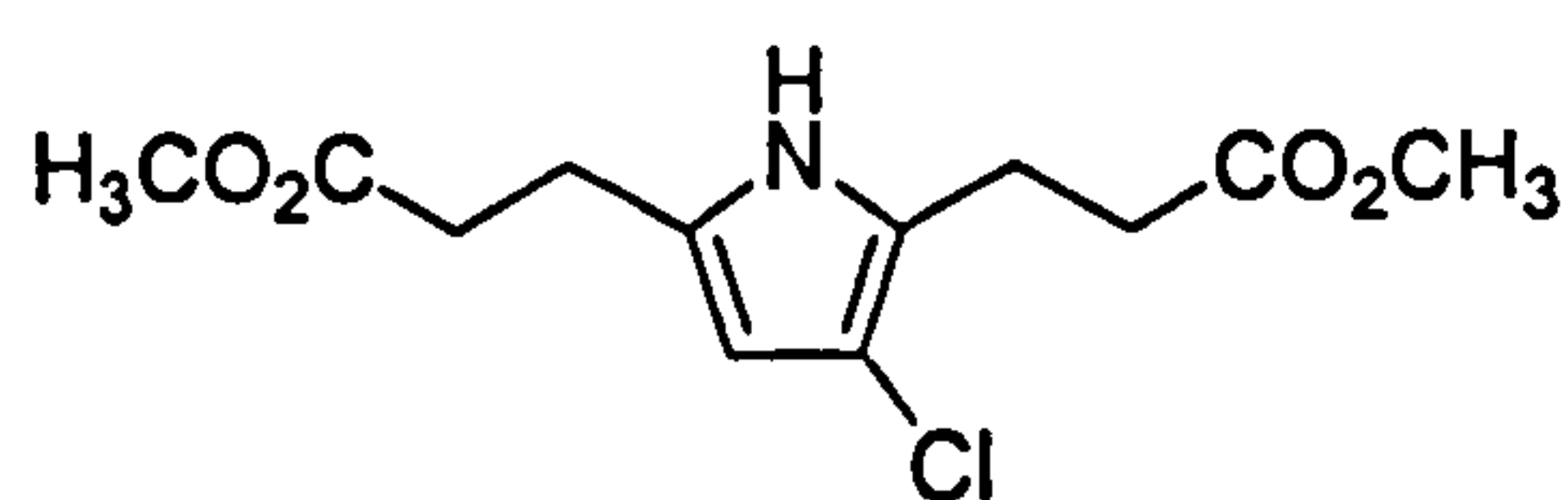
and the filtrate basified with aq NaOH (2 M), followed by acidification with dil aq HCl. The mixture was extracted with DCM (3 x 80 mL) and then the combined organic phase was dried and the solvent removed *in vacuo* to give **71** (39 mg, 17%) as an oily yellow solid; (Found: (HRMS EI)  $M^+$  195.0902,  $C_{10}H_{13}NO_3$  requires 195.0895);  $\nu_{\max}/\text{cm}^{-1}$  2928, 1738, 1658, 1382, 1095, 671;  $\delta_{\text{H}}$ (400 MHz) 5.90 (1H, d,  $J$  6.1), 5.80 (1H, d,  $J$  6.1), 4.69 (1H, m), 4.60 (1H, m), 2.74 (1H, m), 2.48-2.31 (4H, m), 1.94 (1H, m), 1.80 (1H, m), 1.61 (1H, m);  $\delta_{\text{C}}$ (67.5 MHz) 178.9 (C), 176.7 (C), 131.7 (CH), 130.6 (CH), 67.1 (CH), 60.9 (CH), 34.0 ( $\text{CH}_2$ ), 31.3 ( $\text{CH}_2$ ), 29.0 (2 x  $\text{CH}_2$ );  $m/z$  (EI) 195 ( $M^+$ , 6) 195 (6), 139 (22), 134 (24), 122 (60), 121 (9), 80 (10), 55 (100).

**3-(*rel*-(5*R*,8*S*)-5-Oxo-5,6,7,8-tetrahydro-3H-pyrrolizin-3-yl)-methylpropionate **72**.**



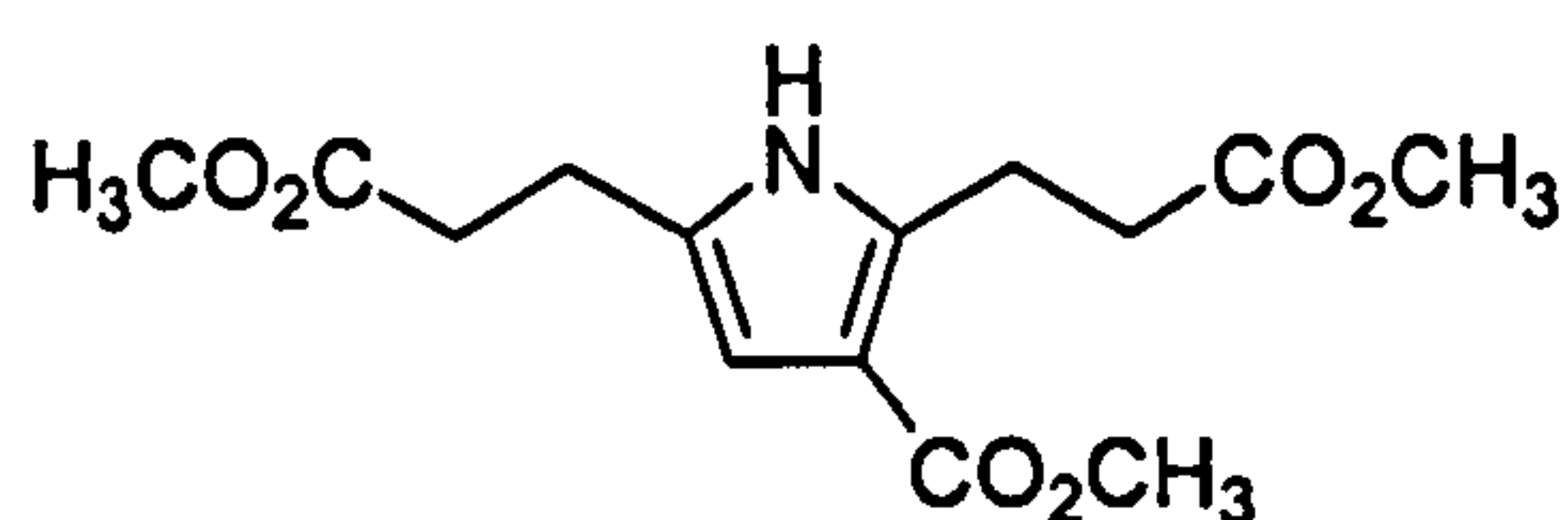
Two drops of conc sulfuric acid were added to a stirred solution of **71** (59 mg, 0.31 mmol) in MeOH at RT. The solution was stirred for 2 h and the solvent was removed *in vacuo* to give an orange oil which was dissolved in DCM (10 mL) and treated with aq KOH (2 M, 8 mL). The layers were separated and the aq phase extracted with DCM (2 x 10 mL). The combined organic phase was dried and the solvent removed *in vacuo* to give **72** (48 mg, 82%) as an oily yellow solid; (Found: (HRMS EI)  $M^+$  209.1051,  $C_{11}H_{15}NO_3$  requires 209.1052);  $\nu_{\max}/\text{cm}^{-1}$  2952, 2874, 1732, 1683, 1388, 1346, 1149, 1015;  $\delta_{\text{H}}$ (400 MHz) 5.87 (1H, d,  $J$  6.0), 5.78 (1H, d,  $J$  6.0), 4.63 (2H, m), 3.62 (3H, s), 2.69 (1H, m), 2.45-2.25 (4H, m), 1.95 (1H, m), 1.76 (1H, m), 1.62 (1H, m);  $\delta_{\text{C}}$ (67.5 MHz) 178.4 (C), 173.9 (C), 131.8 (CH), 130.6 (CH), 67.0 (CH), 61.2 (CH), 51.6 ( $\text{CH}_3$ ), 34.2 ( $\text{CH}_2$ ), 30.8 ( $\text{CH}_2$ ), 29.7 ( $\text{CH}_2$ ), 29.7 ( $\text{CH}_2$ );  $m/z$  (EI) 209 ( $M^+$ , 19) 209 (19), 178 (19), 153 (55), 134 (64), 122 (100), 83 (27), 80 (32), 55 (68).



**Dimethyl 1*H*-pyrrole-3-chloro-2,5-dipropanoate 66.**

*N*-Chlorosuccinimide (0.388 g, 2.88 mmol) was added to a stirred solution of dimethyl 1*H*-pyrrole-2,5-dipropanoate **23** (0.621 g, 2.61

mmol) in chloroform (6 mL) at 0°C under nitrogen. The solution was allowed to warm to RT and stirred for 1 h, then heated at reflux for 2 h. DCM (5 mL) was added and the mixture was washed with water (3 x 3 mL). The layers were separated and the aq phase extracted with DCM (3 x 3 mL). The combined organic phase was dried and the solvent removed *in vacuo* to give **66** (0.669 g, 94%) as an orange solid, mp 62-64°C; (Found: (ES)  $M^+ + H$  274.0821,  $C_{12}H_{17}ClNO_4$  requires 274.0846);  $\nu_{\max}/\text{cm}^{-1}$  3412, 2954, 1732, 1589, 1455, 1366, 1310, 1155, 1071, 986;  $\delta_H$ (400 MHz) 8.72 (1H, br s), 5.77 (1H, d,  $J$  3.0), 3.73 (3H, s), 3.72 (3H, s), 2.87 (2H, t,  $J$  6.9), 2.82 (2H, t,  $J$  6.9), 2.61 (2H, t,  $J$  6.9), 2.60 (2H, t,  $J$  6.9);  $\delta_C$ (67.5 MHz) 174.2 (C), 173.8 (C), 128.9 (C), 125.0 (C), 108.4 (C), 105.3 (CH), 51.7 (2 x  $CH_3$ ), 33.9 ( $CH_2$ ), 33.4 ( $CH_2$ ), 22.7 ( $CH_2$ ), 19.9 ( $CH_2$ );  $m/z$  (ES) 274 ( $M^+ + H$ , 100) 276 (18), 274 (100) .

**Dimethyl 1*H*-pyrrole-3-methoxycarbonyl-2,5-dipropanoate 67.**

Methyl chloroformate (0.12 mL, 1.6 mmol) and aluminium trichloride (19 mg, 0.15 mmol) were added to a stirred solution of dimethyl 1*H*-

pyrrole-2,5-dipropanoate **23** (0.153 g, 0.640 mmol) in chloroform (3 mL) under nitrogen and the mixture was heated at reflux for 12 h. DCM (2 mL) was added and the solution was washed with dil aq sodium hydrogencarbonate (2 mL). The layers were separated and the aq phase extracted with DCM (3 x 2 mL). The combined organic phase was dried and the solvent removed *in vacuo* to give **67** (0.169 g, 92%)

as a pale yellow solid, mp 82-84°C; (Found: C, 56.3; H, 6.4; N, 4.5; C<sub>14</sub>H<sub>19</sub>NO<sub>6</sub> requires C, 56.6; H, 6.4; N, 4.7%); (Found: (HRMS EI) M<sup>+</sup> 297.1208, C<sub>14</sub>H<sub>19</sub>NO<sub>6</sub> requires 297.1212);  $\nu_{\max}/\text{cm}^{-1}$  3416, 2953, 1729, 1697, 1458, 1365, 1093;  $\delta_{\text{H}}$ (400 MHz) 9.12 (1H, br s), 6.19 (1H, d, *J* 3.0), 3.79 (3H, s), 3.72 (3H, s), 3.67 (3H, s), 3.20 (2H, t, *J* 7.0), 2.82 (2H, t, *J* 6.9), 2.66 (2H, t, *J* 6.9), 2.59 (2H, t, *J* 6.9);  $\delta_{\text{C}}$ (67.5 MHz) 174.8 (C), 174.4 (C), 166.2 (C), 137.7 (C), 129.9 (C), 111.3 (C), 107.4 (CH), 52.3 (CH<sub>3</sub>), 52.2 (CH<sub>3</sub>), 51.1 (CH<sub>3</sub>), 34.3 (CH<sub>2</sub>), 33.9 (CH<sub>2</sub>), 22.7 (CH<sub>2</sub>), 22.2 (CH<sub>2</sub>); *m/z* (EI) 297 (M<sup>+</sup>, 3) 297 (3), 237 (3), 224 (8), 192 (9), 164 (5), 132 (6), 88 (7), 86 (39), 84 (66), 83 (4), 49 (100), 47 (16).

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## **Part II**

# **SYNTHESIS OF BACKBONE MODIFIED DNA USING TRANSITION METAL CATALYSIS**

**Supervised by Dr Christopher J. Hayes**

## Abstract

- The synthesis of a DNA building block containing a linker with an amine has been accomplished and its capacity to accommodate interesting molecules in an internal position of an oligonucleotide was proved by the attachment of the amino acid arginine.
- The coupling of the above dinucleotide with *bis*(disopropylamino)cyanoethyl phosphite has been achieved with the aim of making this material suitable for solid support synthesis as used in automated DNA synthesisers.
- A new methodology for the synthesis of *H*-phosphonates has been developed based upon three nucleophilic substitutions onto phosphorus trichloride in one pot.
- We have developed an easy and efficient way to carry out the palladium catalysed cross-coupling reactions using sealed, thick walled reaction vials.
- A new method for the synthesis of alkynylphosphonates from 1,1-dibromo-1-alkenes has been developed and the synthesis of a new alkyne-containing thymidine dimer has been achieved.
- Vinylphosphonate-linked dinucleotides have been prepared using an olefin cross-metathesis reaction as a key step.



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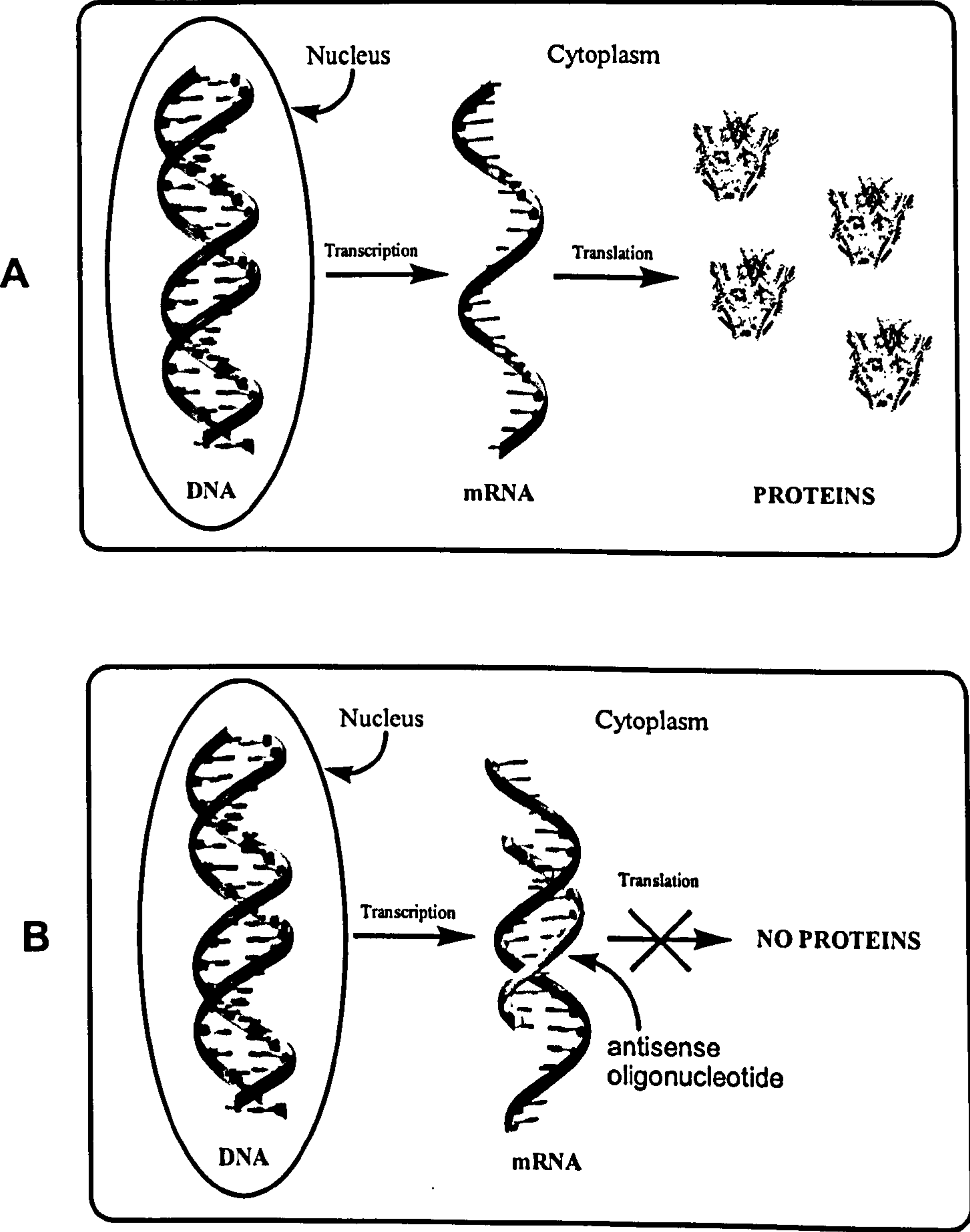
## 1. Introduction

Even today, despite great advances in technology and knowledge, new drugs are usually not discovered by rational drug design, even though this would be highly desirable for the medically oriented chemist. On average, it is still necessary to synthesise and test about 10,000 new compounds in order to discover a new active substance worth developing. In many cases the active substance is directed against proteins such as enzymes, receptors, or ion channels, the structure and mode of action of which are usually very complicated and often not completely understood. On the other hand, therapeutic intervention at the level of the nucleic acid appears to offer a number of advantages.

On transcription, every gene gives rise to a relatively small number of copies of messenger ribonucleic acid (mRNA), which are translated into a large number of protein molecules. For this reason inhibition of gene expression ought to be more efficient than inhibition of the resulting protein product. There is already one drug on the market whose activity is based on direct interaction with deoxyribonucleic acid (DNA).

The main goal of this strategy is an efficient gene-specific approach to chemotherapy.<sup>1</sup> The disease targets can be broadly defined as any diseases that involve the production of harmful proteins. This category includes bacterial, viral and fungal diseases, as well as certain types of cancer. The basis of this approach is the “antisense method” for controlling gene expression (protein synthesis)<sup>2</sup> and this is illustrated in Figure 1. The antisense method is a naturally occurring<sup>3</sup> gene regulation mechanism that relies upon the ability of messenger RNA, which is a single-stranded nucleic acid, to be recognised in a sequence-specific manner by complementary nucleic acid molecules. This recognition occurs by the familiar and highly reliable Watson-Crick base pairing

that is responsible for the specificity of the genetic code. The inhibitory effect of antisense oligonucleotides was first observed in 1978 by Zamecnik and Stephenson, who used a 13-mer oligonucleotide to inhibit the growth of Rous sarcoma virus in cell culture.<sup>4</sup>



**Figure 1.** (A) Normal process of synthesis of proteins. (B) Inhibition of synthesis of proteins by antisense oligonucleotides.



In the natural form of this gene regulation method, an antisense RNA molecule is synthesised inside the cell by transcription. This high molecular weight nucleic acid binds to its target mRNA and prevents the message from being translated into proteins in the ribosomes. Observed in both prokaryotes<sup>5</sup> and eukaryotes,<sup>6</sup> antisense regulation can also be introduced artificially *via* transfection with antisense genes. These processes have no “drug delivery problem” because the antisense reagent is produced inside the cell.

The chemical version of the antisense technique employs antisense sequences that are synthesised outside the cell, such as on a DNA synthesiser. For several reasons, including enhanced cellular uptake and lower cost, synthetic antisense molecules are much lower in molecular weight (typically 17-20 nucleotides long) than the full-length gene transcripts employed in the natural antisense method.<sup>7</sup> Since, statistically, the base sequence of a 17-mer oligonucleotide occurs just once in the sequence of the human genome, extremely selective intervention ought to be possible with antisense oligonucleotides of this length.

The mechanism of action of the short, synthetic antisense sequences does not necessarily parallel the natural antisense mechanism, which involves sequestering the target mRNA by binding it to a complementary RNA strand. Instead of merely binding to specific mRNA sequences, DNA drugs act in concert with the cellular enzyme Ribonuclease H (RNase H) to destroy the target mRNA.<sup>2</sup> RNase H is known to digest the RNA strand of DNA-RNA duplexes, and it provides a catalytic pathway for antisense DNA drugs to operate.

Given the apparent specificity and catalytic nature of the chemical antisense approach, it may be surprising that only one antisense drug is in active use. Several clinical trials are now in progress,<sup>8</sup> but a number of difficulties remain to be overcome. Examples of

such difficulties include: (1) drug delivery across cell membranes,<sup>9</sup> (2) drug delivery to the correct intracellular regions,<sup>10</sup> (3) *in vivo* stability of DNA drugs to nucleases enzymes and other biochemical degradation agents, and (4) cost of the reagents.

Many advances have been made in the chemistry of DNA analogues<sup>11</sup> that retain Watson-Crick base-pairing ability, while offering improved delivery and stability properties.<sup>12</sup> However, a problem with using such analogues is that the enzyme RNase H is highly specific about which nucleic acids it will accept as templates for RNA digestion,<sup>13</sup> it is a DNA-dependent RNA hydrolysis catalyst. Thus, RNA does not promote RNase H-mediated RNA cleavage, nor do most DNA analogues. Therefore, the dilemma arises that chemical modifications designed to enhance the stability and uptake of DNA drugs may actually block the catalytic pathway.

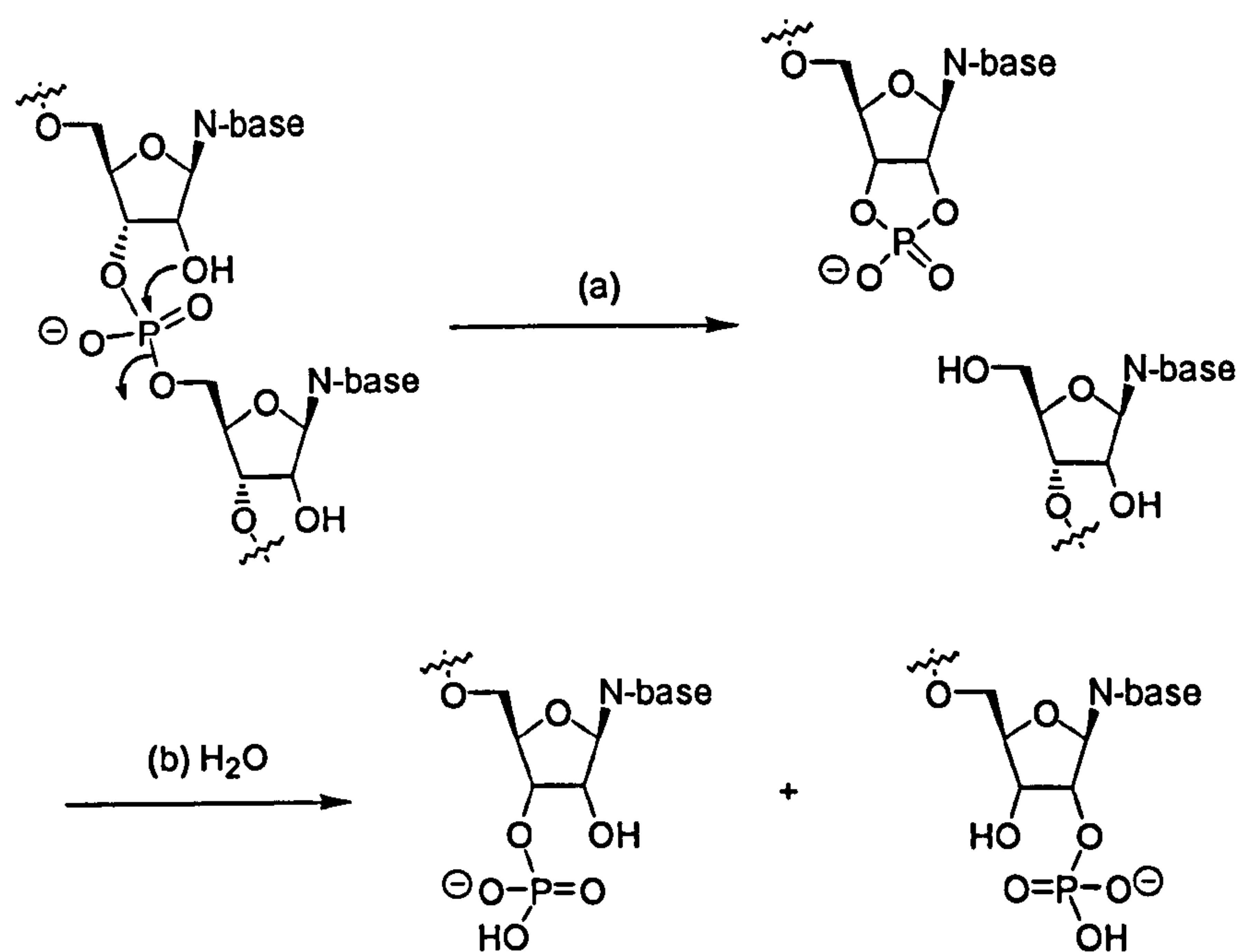
## 1.1 Ribozyme Mimics

Up until a few years ago it was generally believed that only proteins catalyse chemical reactions *in vivo*. However, it has been discovered recently that ribozymes are catalytic nucleic acids that often, as part of a larger mRNA structure, catalyse the self-splicing of the primary transcript.<sup>14</sup>

Functional mimics of ribozymes and ribonucleases are defined as “synthetic molecules that cleave RNA in a sequence-directed manner, using biomimetic chemical reactions such as transesterification and hydrolysis”. For simplicity, the term “ribozyme mimics” will be used to represent these compounds. The transesterification and hydrolysis reactions are grouped together as “nucleophilic cleavage reactions” for several reasons (Scheme 1). These reactions are closely related by the nucleophilic attack on phosphorus (V) that each employs. The mechanistic connection between these reactions was unified by Perreault and Anslyn.<sup>15</sup> For transesterification, the



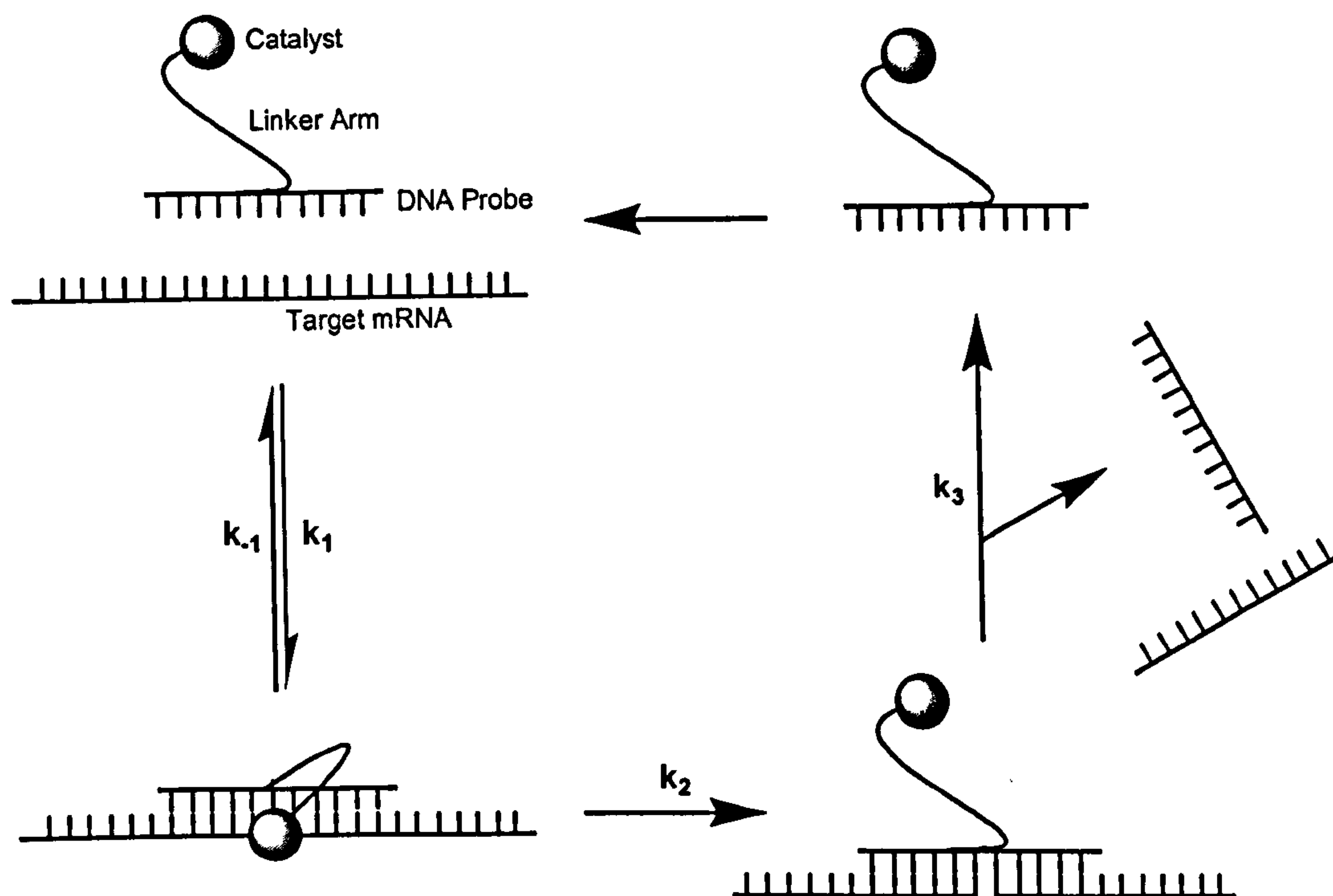
nucleophile is an alcohol or alkoxide, while for hydrolysis, the nucleophile is almost always water or hydroxide.<sup>16</sup> Furthermore, most inorganic reagents that catalyse RNA transesterification go on to hydrolyse the resulting 2',3'-cyclic monophosphate to a mixture of 2'- and 3'-phosphate monoesters, as indicated in Scheme 1b.



**Scheme 1.** Nucleophilic cleavage of RNA: (a) Transesterification with concomitant cleavage of the phosphodiester backbone. (b) Hydrolysis of the 2',3'-cyclic monophosphate to a mixture of 2'- and 3'-monophosphates.

Ribozyme mimics can be constructed by covalent incorporation of active RNA cleavage catalysts into DNA oligonucleotides or their analogues. The specificity of these reagents is consequently derived from the Watson-Crick hydrogen bonding of the DNA strand to its complementary RNA sequence (Scheme 2), and it exhibits the full specificity of the genetic code. The chemoselectivity of the mimics arises from the relative ease of nucleophilic cleavage of RNA compared with DNA.<sup>17</sup> This, in turn, is derived from the facile intramolecular nature of the nucleophilic attack that typically drives RNA cleavage (Scheme 1). DNA lacks the 2'-OH functionality and has proved

almost completely inert to hydrolysis or transesterification by small molecule catalysts, with a few notable exceptions.<sup>18</sup>



**Scheme 2.** Potential catalytic cycle of a ribozyme mimic. The first step involves recognition and binding of the target mRNA ( $k_1$ ). In the second step, the RNA target is cleaved in a site-specific manner ( $k_2$ ). Finally, the product fragments are released ( $k_3$ ), and the mimic is ready for another cycle.

A balance is needed between the on-rate for nucleic acid binding (Scheme 2,  $k_1$ ) and the rate of chemical cleavage (Scheme 2,  $k_2$ ). Too high a rate of chemical cleavage will result in nonspecific RNA degradation. For catalytic turnover to be achieved with ribozyme mimics, the off-rate for release of cleaved RNA fragments by the DNA strand (Scheme 2,  $k_3$ ) is also of fundamental importance.

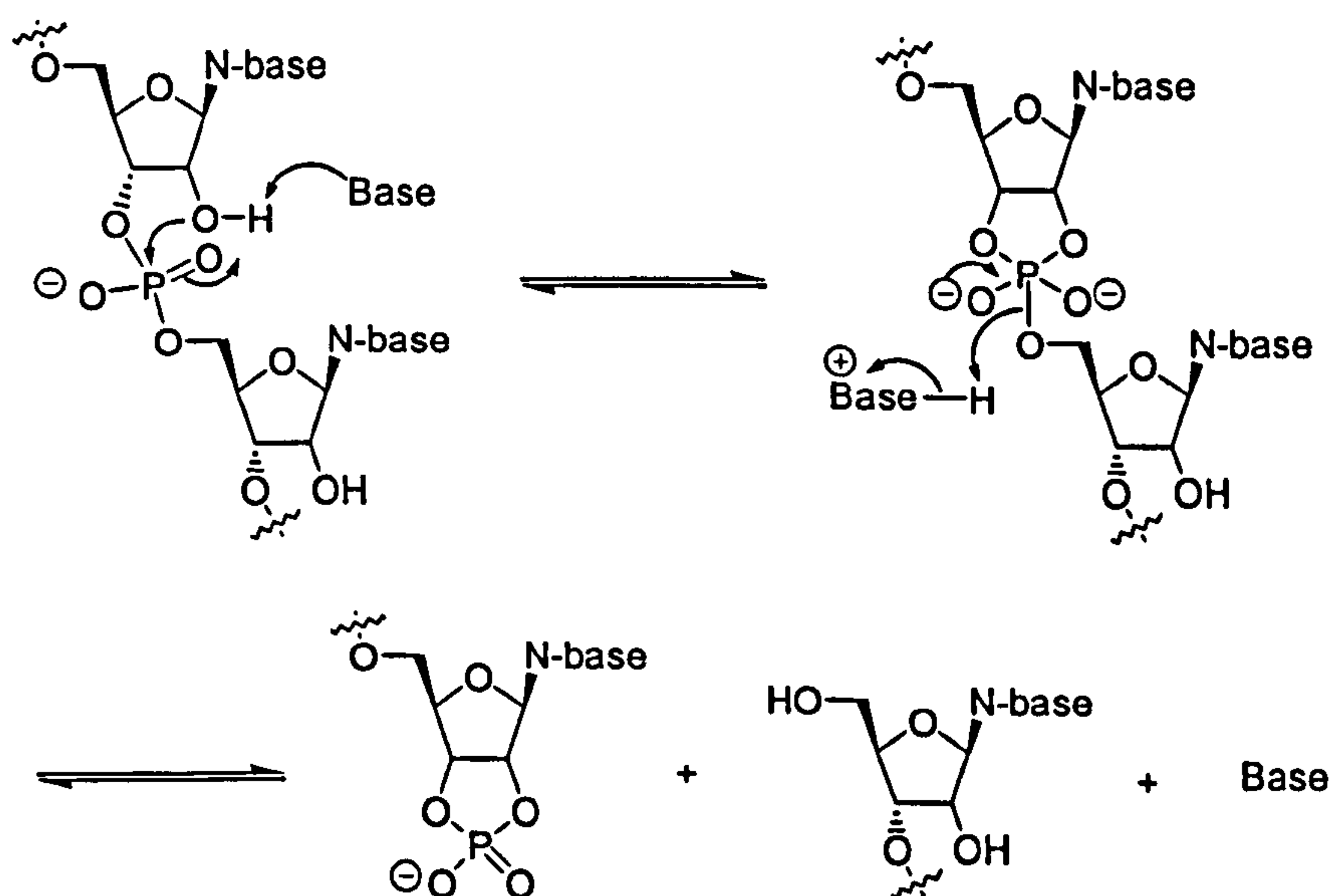
## 1.2 Catalysts and their Mechanisms of RNA Cleavage

One remarkable feature of RNA transesterification and hydrolysis is that these reactions are catalysed by a large variety of species that span almost the entire Periodic



Table. Protons, hydroxide, amines and other nitrogen derivatives, Mg(II), Ca(II), Fe(III), Ni(II), Cu(II), Zn(II), Pb(II), trivalent lanthanides,  $\text{UO}_2^{2+19}$  and Th salts<sup>20</sup> are just some of the species known to cleave RNA through nucleophilic paths. Of course, enzymes and ribozymes must be added to the list. Most of these individual catalyst types have been reviewed recently.<sup>21</sup>

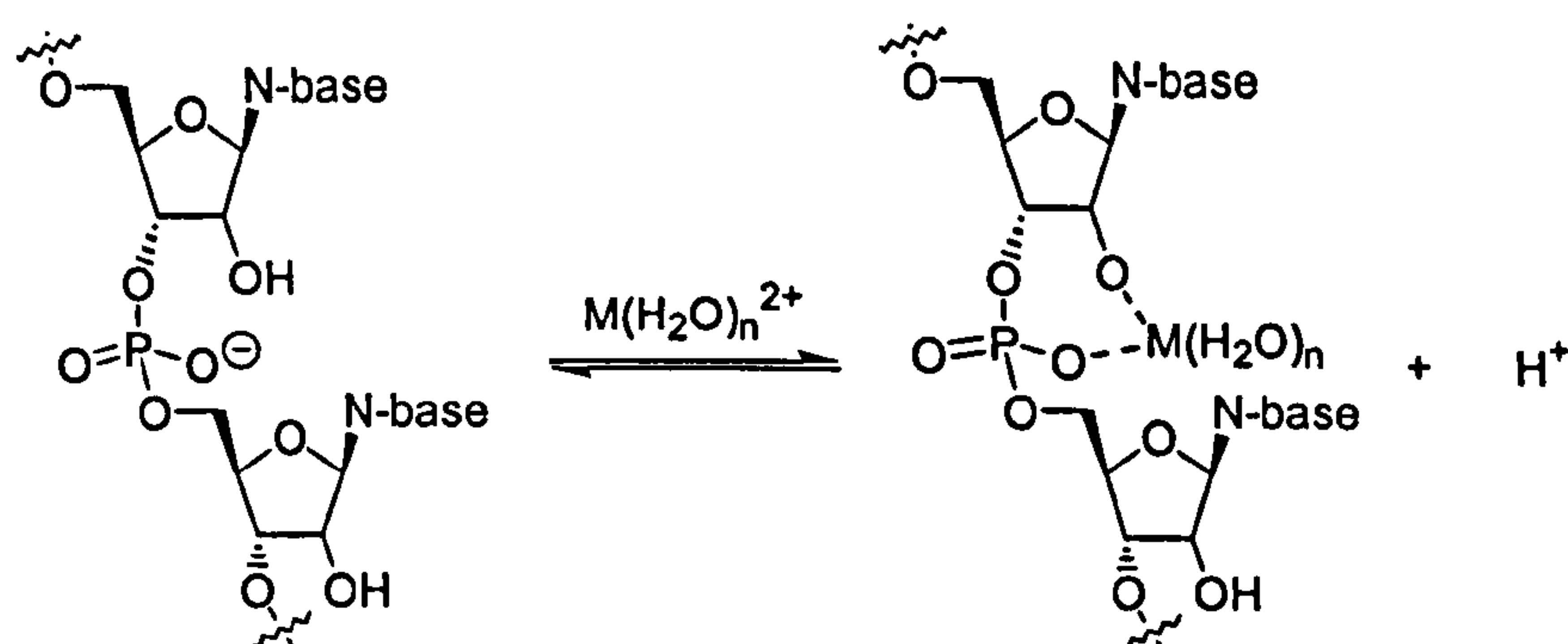
Certain features are generally applicable to RNA transesterification. However, for any given combination of a particular catalyst and a particular RNA substrate, the order of events, the order of the reaction, the simultaneous or stepwise nature of the processes, and importance of each individual step can vary dramatically.



Scheme 3

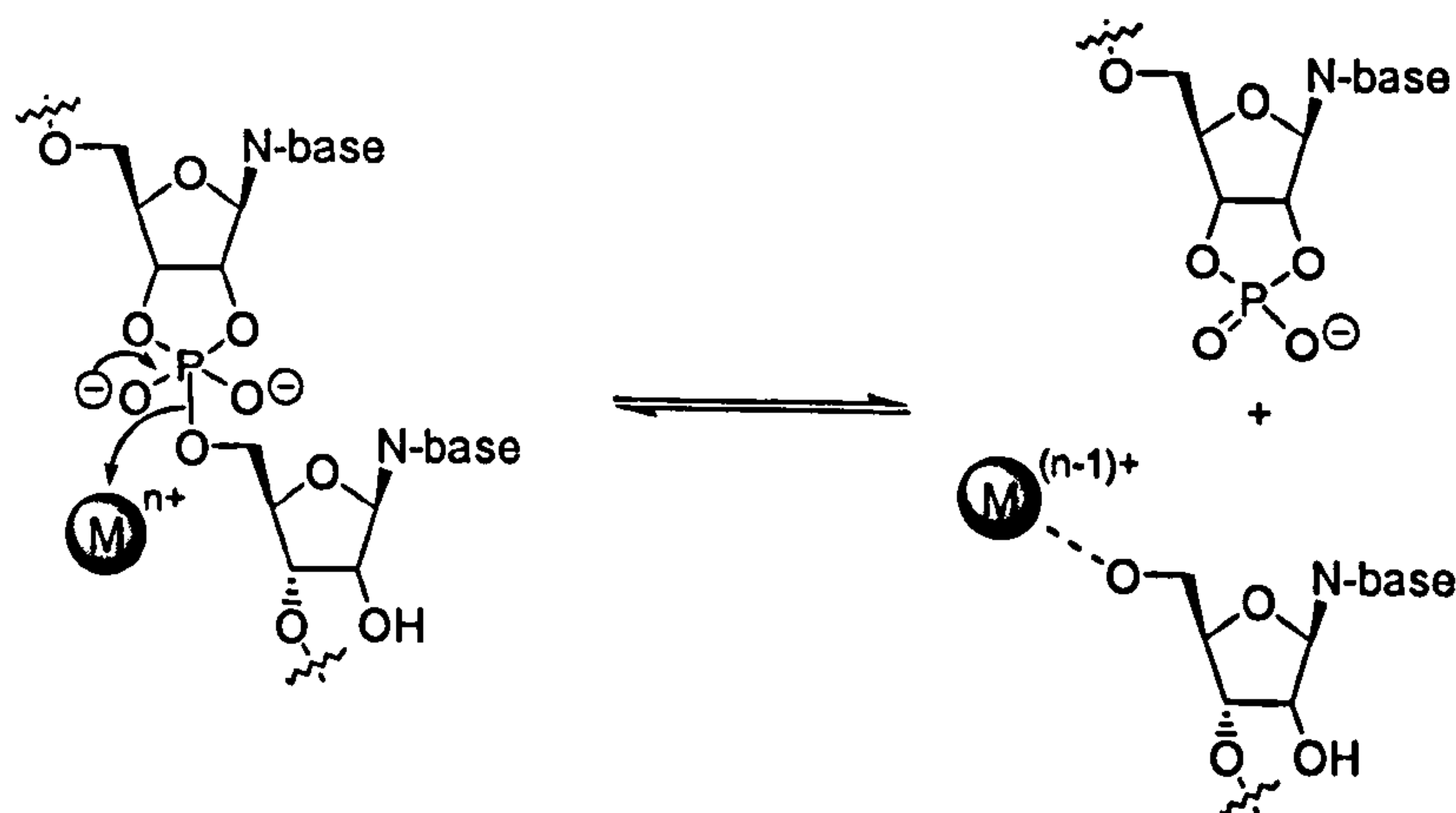
Transesterification of RNA normally requires participation of the 2'-OH as a nucleophile<sup>22</sup> (except for some ribozymes, which employ other nucleophilic hydroxyls). During the reaction, the 2'-OH must be deprotonated, and the leaving 5'-alkoxy group must be protonated. Both steps can be accomplished by one round of base catalysts, for example by imidazole or a metal hydroxide (Scheme 3).

It is possible that deprotonation of the nucleophile may occur by prior coordination of a metal ion to the 2'-OH, since this would lower the relevant  $pK_a$ .<sup>23</sup> The effective nucleophile would then be a metal alkoxide (Scheme 4).



Scheme 4

It is also possible that protonation of the leaving group is of secondary importance to stabilisation of the departing alkoxide by a metal ion (Scheme 5).



Scheme 5

In general terms, the nucleophile derived from the 2'-OH must attack a tetrahedral phosphodiester, generating either a phosphorane intermediate or a shorter-lived activated complex. Nucleophilic attack on an anionic phosphodiester is notoriously unfavourable from an electrostatic perspective.<sup>17</sup> Therefore, some means of preventing

a large negative charge from building up in the activated complex is typically necessary. Protons or Lewis acids may provide this charge stabilisation.

The number of metal ions involved in metal-mediated RNA cleavage is a matter of some interest and dispute, whether the reaction is performed by enzymes, ribozymes or small metal complexes. To design the most effective ribozyme mimic, it is of vital importance to know how many metal ions must be delivered to the substrate.

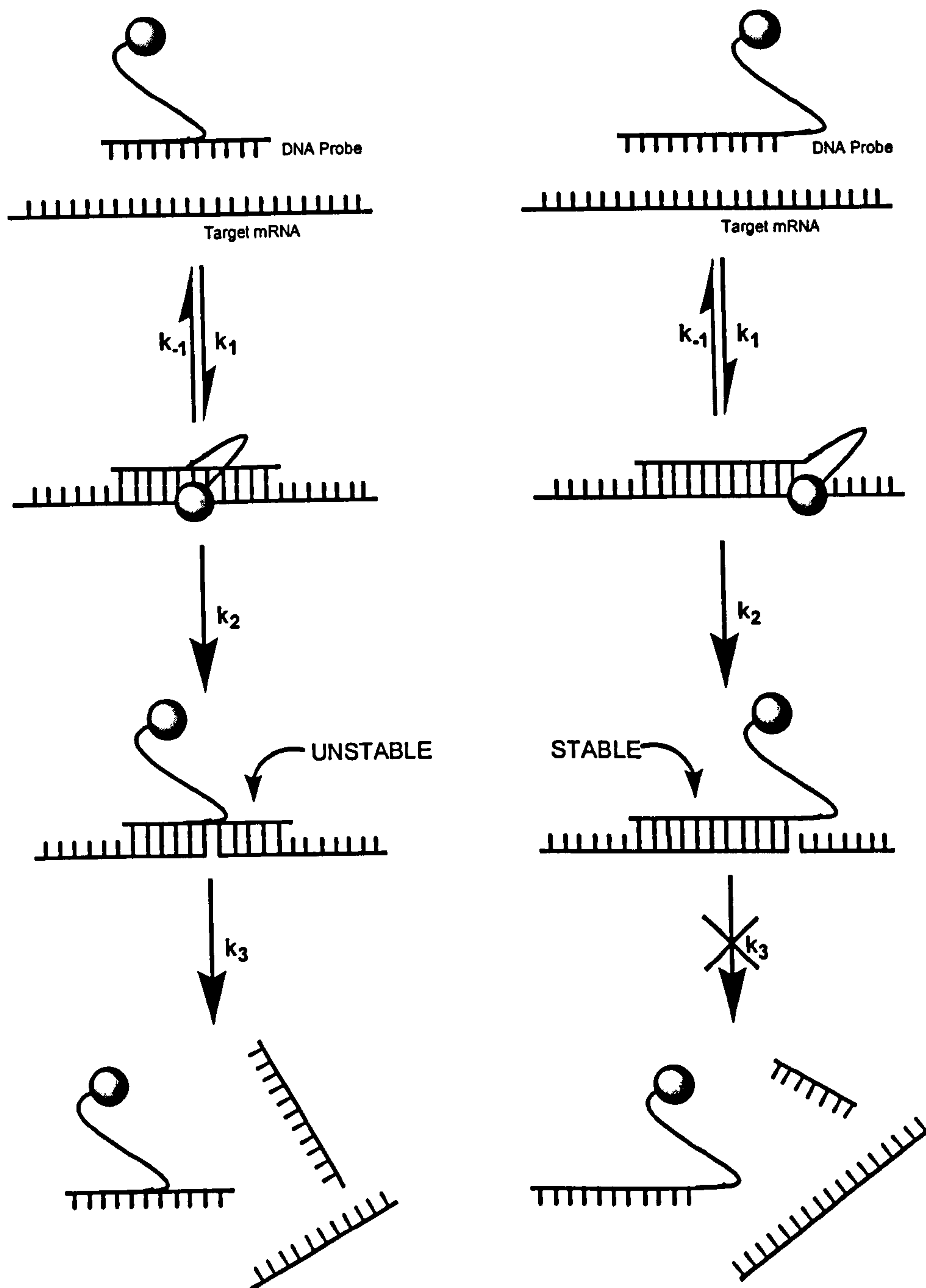
## 1.3 Sequence-Specific Nucleophilic RNA Cleavage

### 1.3.1 Key Design Features.

It is by now generally understood that certain design features are critical to the development of effective ribozymes mimics. One of the most heavily emphasised requirements is that of “cleavage within the DNA-RNA duplex region”.<sup>24</sup> This design element is *essential for catalytic turnover* because the DNA-RNA binding constant is strongly length-dependent. Cleavage within the duplex greatly reduces the binding constant between the ribozyme mimic and its RNA target, which allows the product fragments to be released. This is illustrated in Scheme 6, along with the alternative design which places the cleavage agent at the end of the duplex region. Reagents for external cleavage are usually simpler to prepare than reagents for internal cleavage. However, external cleavage results in complete product inhibition, because the DNA-RNA binding constant is not decreased by the cleavage event, so the “catalyst” is entombed in an RNA strand.

The length of the linker arm between the DNA probe and the pendant RNA cleavage catalyst is of major importance. Molecular modelling is a useful method for estimating the optimum linker arm length for delivering the catalyst to the RNA target. Early designs have used flexible linkers sacrificing precision to achieve activity.





Scheme 6. Cleavage of RNA within the duplex region versus cleavage outside the duplex region.

### 1.3.2 Preparation and Behavior of Sequence-Specific RNA Cleavage Agents

#### 1. Ribozyme Mimics Based on Organic Catalysts

Synthetic ribonucleases have been developed by the covalent attachment of organic RNA transesterification catalysts to deoxyoligonucleotides. This work started with the ideas reported by Cohen<sup>25</sup> and with subsequent papers by the Bashkin group on the

synthesis of DNA building blocks containing organic catalysts.<sup>26</sup> Komiyama and coworkers were the first to report successful sequence-specific RNA cleavage using this method.<sup>27</sup> Their mimic consisted of a urethane-linked ethylenediamine at the 5'-end of a 19-mer DNA probe complementary to A44-A62 of tRNA<sup>Phe</sup> (Figure 2). It was prepared by the treatment of a solid-supported DNA 19-mer with 1,1'-carbonyldiimidazole, followed by diethylenetriamine. This artificial enzyme site specifically cleaved about 10% of the tRNA after 4 h at 50°C. Cleavage occurred just outside the duplex region adjacent to the ethylenediamine residue. Reactions consisted of 0.1 mM probe, 1  $\mu$ M tRNA and 1 mM EDTA at pH 8.0. The EDTA was added to the reaction mixture to sequester any metal ions and rule out the participation of metal ions in the cleavage reaction. Site-specific cleavage of a linear 30-mer oligoribonucleotide was also achieved using the same construct.<sup>28</sup> The extent of cleavage was 10% after 4 h at pH 8.0 and 50°C. The concentrations of conjugate and RNA were 100 and 1.0  $\mu$ M, respectively.

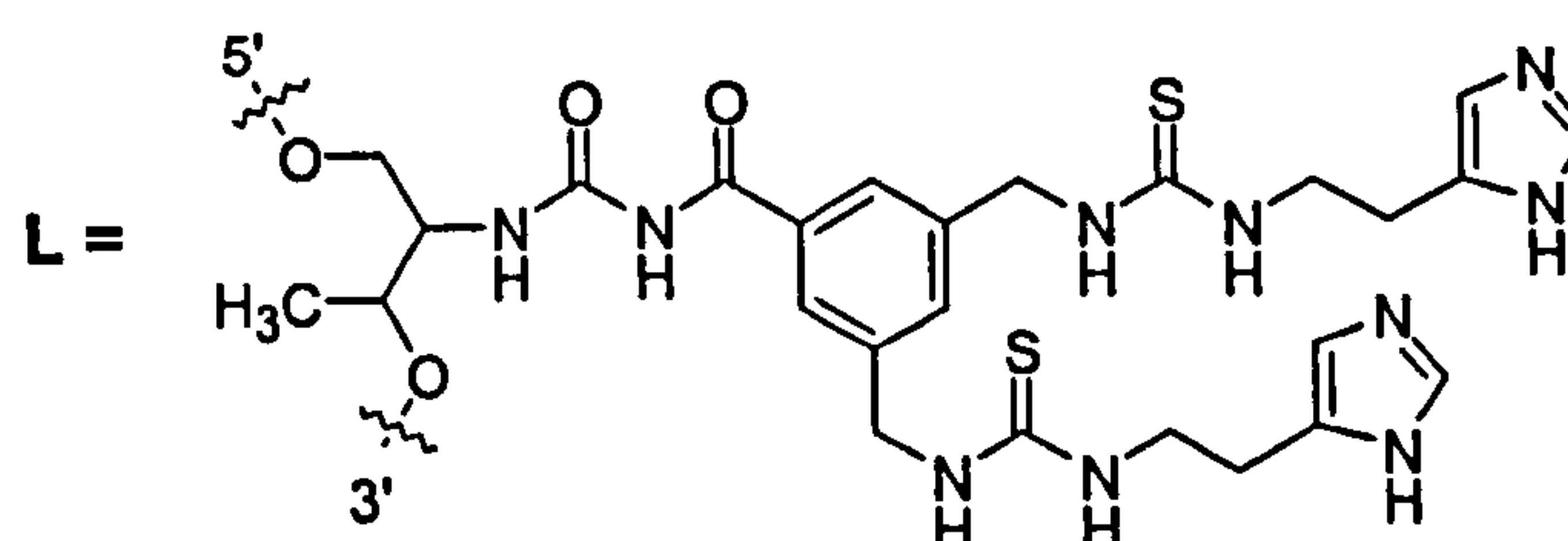


Figure 2. An ethylenediamine-DNA hybrid.

Reynolds *et al.* made an imidazole-based synthetic ribonuclease.<sup>29</sup> Recognising that single-stranded RNA is more susceptible to nucleophilic cleavage than duplexed RNA (and also the importance of cleavage within the duplex region for release of the cleaving agent), they engineered into their probe a nonnucleotide-linker L with attached imidazole groups in place of one of the complementary bases (Figure 3).

RNA 3' - AUCGAAGGAAUCGAGGACGUAC - 5'

MPO 5' - TAGCTTCCTTLCGCTCCTG - 3'



**Figure 3.** Structure of the antisense methylphosphonate oligonucleotide (MPO) cleaving agent and its RNA target.

Using this technique, the authors hoped to introduce added flexibility into the opposite RNA strand, thereby allowing it to adopt a conformation more favourable for transesterification. Two of their probes yielded site-specific cleavage of a 22-mer RNA strand within the duplex region. Less than 10% cleavage was observed after 5 days at 25°C and pH 7.2, with a final probe concentration of 75  $\mu$ M. EDTA (1.0 mM) was also added to each reaction to scavenge metals, thus ruling out the participation of metal contaminants in the reaction.

Quite recently, Vlassov *et al.*<sup>30</sup> obtained remarkable site-specific cleavage for an organic-based synthetic ribonuclease. They made deoxyoligonucleotide derivatives conjugated with moieties containing two histamine residues that mimic the catalytic active site of RNase A. Deoxyoligonucleotides with the modifications at either the 3'- or 5'-end, separately or together, site specifically cleaved tRNA. Maximum cleavage (60%) of the target was attained after 8 h at 37°C (pH 7.0). Two probes were used and the longer of them with the modification at the 5'-end gave the most efficient cleavage (Figure 4). The cleavage pattern corresponded to regions on the RNA just opposite the histamine groups, outside the duplex region. An additional cleavage site was found away from the expected targeted region. Apparently this site is close, in a three-



dimensional sense, to the targeted cleavage region after folding. To show that the cleavage was not due to metals binding to the histamines, all solutions contained 1 mM EDTA.

5' - TCCAGGACACAAGCTAG - R

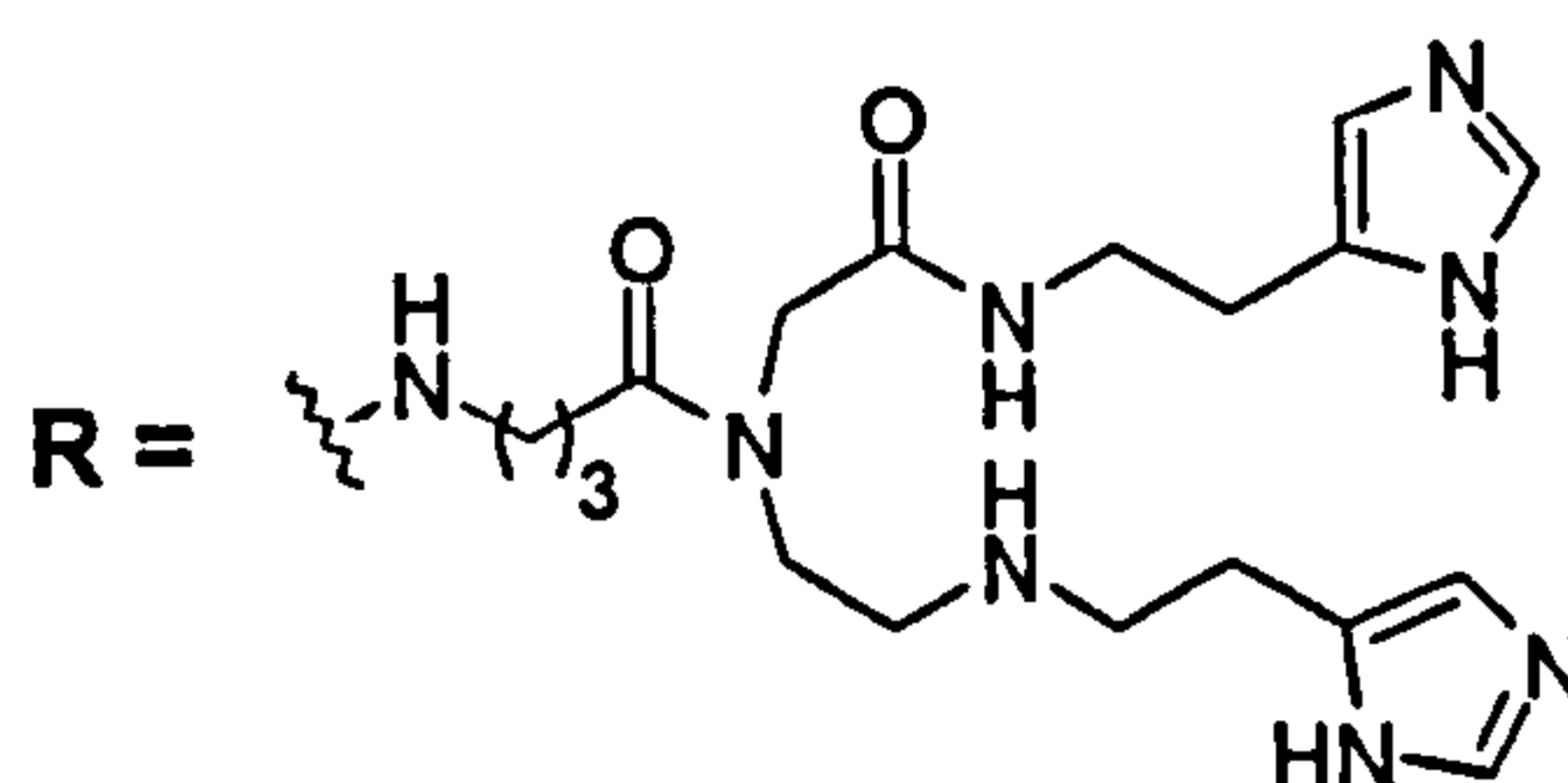


Figure 4. Oligonucleotide conjugate containing a diamidazole construct attached at the 3'-end.

## 2. Hybrid Enzymes

The first reported examples of artificial site-specific cleaving agents for RNA were created by the covalent attachment of nuclease enzymes to deoxyoligonucleotides. Through Watson-Crick base pairing interactions, the deoxyoligonucleotides delivered the relatively nonspecific enzymes to defined sites on the target RNA molecules. Zuckermann and Schultz<sup>31</sup> attached a mutant Staphylococcal nuclease (Lys-116 to Cys-116) to the 3'-end of a deoxyoligonucleotide (14-mer) *via* a disulfide linkage (Figure 5). Treatment of a 59-mer single stranded RNA target with this construct (in the presence of  $\text{Ca}^{2+}$ ) resulted in cleavage over a 3- to 5-nucleotide region directly adjacent to the hybridisation site. Unfortunately, the reagent's specificity decreased with increasing reaction times, possibly as a result of self-cleavage of the DNA-enzyme construct. Staphylococcal nucleases hydrolyse the phosphodiester bonds of both single stranded RNA and DNA, which means that it can also destroy the DNA probe to which it is attached.

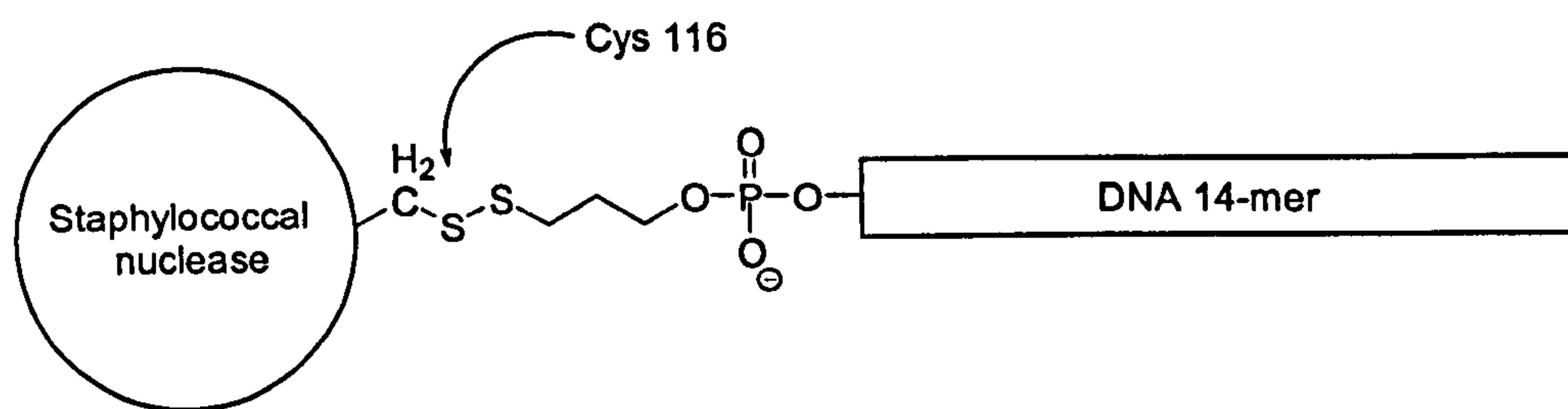


Figure 5

As a possible solution to this problem, the same group coupled RNase S (a subtilisin digested version of RNase A) to a deoxyoligonucleotide (14-mer) to create a sequence-specific ribonuclease.<sup>32</sup> In contrast to the hybrid staphylococcal nuclease, this hybrid was incapable of self-cleavage because RNase S is specific for RNA only.

As was expected, the oligonucleotide delivered the enzymatic activity to a specific site on its 62-mer RNA target. Cleavage occurred at one pyrimidine-purine site adjacent to the site of hybridisation. Unfortunately, cleavage efficiency and specificity decreased at elevated temperatures ( $>37^{\circ}\text{C}$ ), and the authors attributed this to the probable dissociation of the RNase S adduct into its two protein fragment components.

Kanaya and colleagues<sup>33</sup> later created a hybrid enzyme by covalently linking *E. coli* RNase H to the 5'-terminus of a 9-mer deoxyoligonucleotide (Figure 6). This was accomplished using site-directed mutagenesis to substitute a cysteine residue for Glu<sup>135</sup> in a mutant form of the enzyme. The free cysteine residue allowed for coupling of the enzyme to a maleimide group, which was attached at the 5'-end of the 9-mer deoxyoligonucleotide *via* a flexible tether. The target for this conjugate was a synthetic 9-mer single-stranded RNA, and sequence-specific cleavage was achieved between the fifth and sixth residues of the target. An important property of this particular hybrid is that catalytic turnover was achieved. This property can better be explained by the fact that the cleavage event must occur within the duplex region (since RNase H only cleaves RNA in DNA-RNA duplexes), and this must lower the



binding affinity enough to allow for the dissociation of the cleavage products from the hybrid and free it for another cycle. Subsequent melting temperature studies performed on the DNA 9-mer and the RNA cleavage products indicated that a dramatic decrease in the stability of the DNA-RNA duplex would occur after cleavage by RNase H. The same group later reported the preparation of a series of the same hybrid in which the length of the linker arm was varied (18, 24 and 27 Å). The hybrid with the 27 Å linker arm cleaved a 22-mer RNA target at almost exclusively one position.

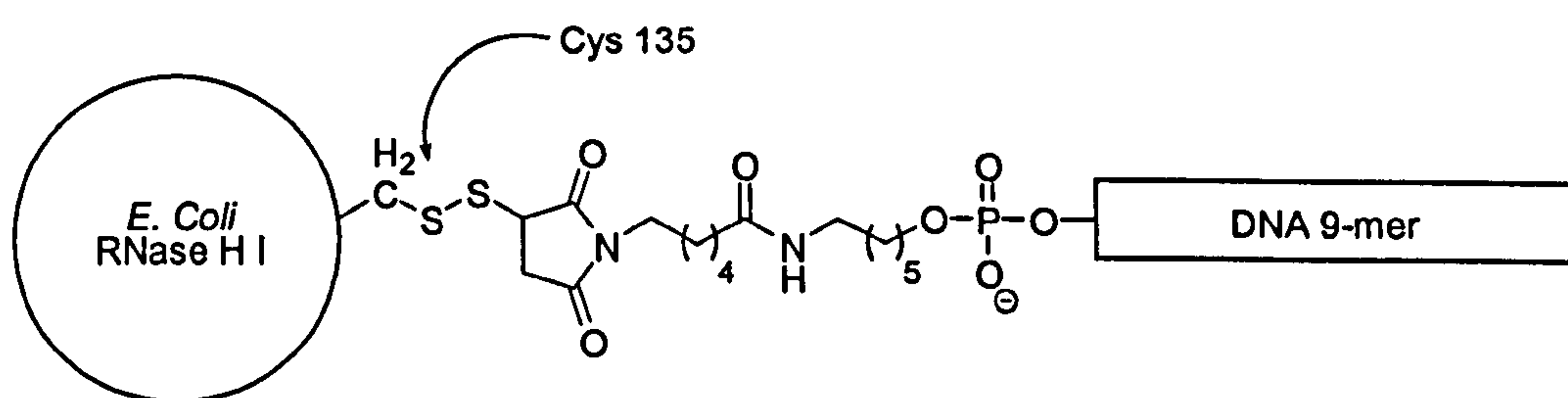


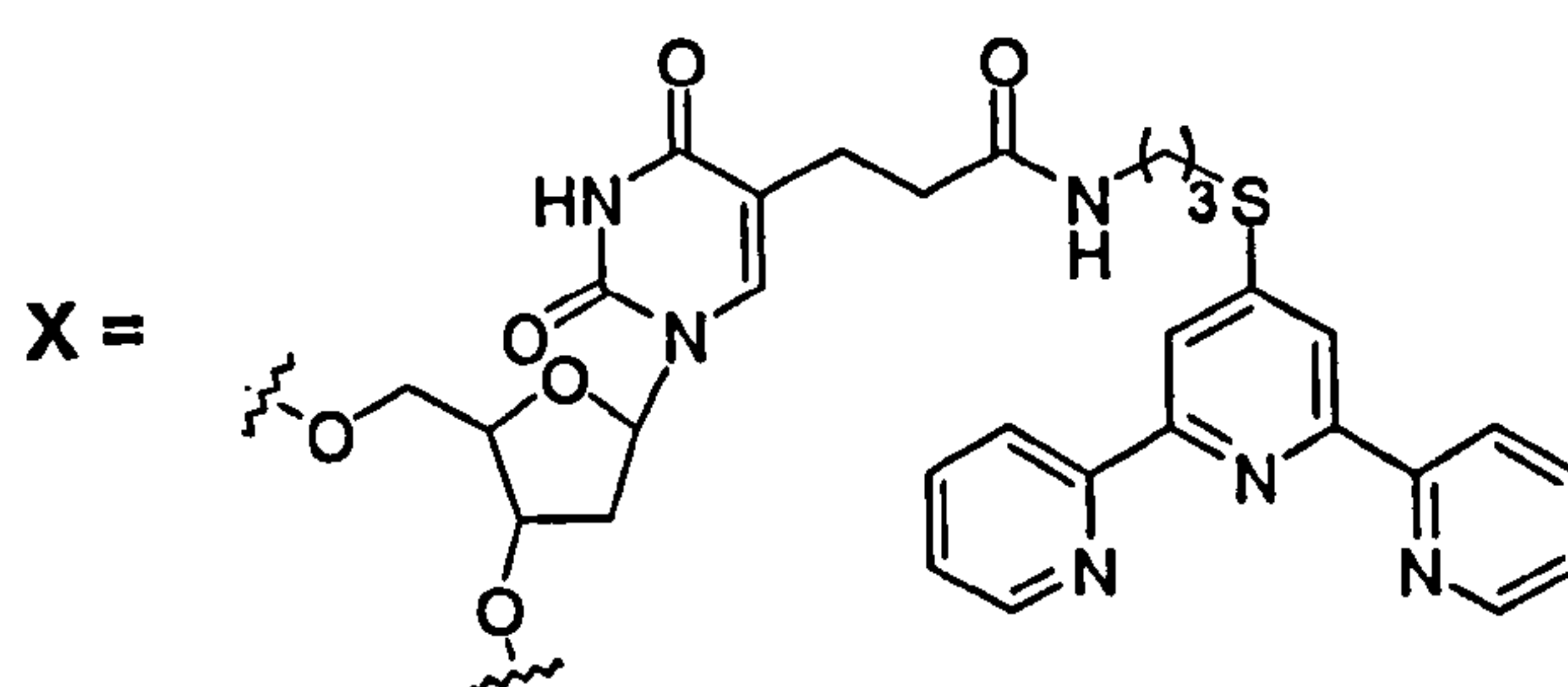
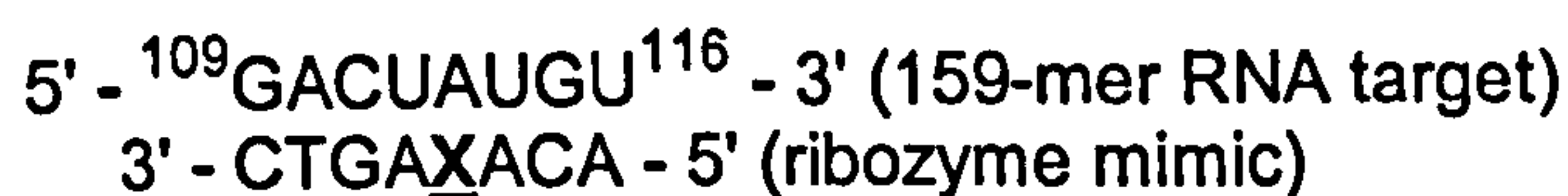
Figure 6

### 3. Ribozyme Mimics Based on Metal Complexes

While hybrid enzymes exhibit highly specific and efficient cleavage of their RNA targets, their large molecular weights and difficulty of preparation probably preclude their use in practical applications. Recently, several groups have covalently attached metal complexes to oligonucleotides to form ribozymes mimics. In 1994, the first example of a *wholly synthetic*, functional mimic of a ribozyme was reported.<sup>24</sup> This mimic consisted of a 17-mer DNA oligonucleotide with a covalently attached terpyridine ligand at C-5 of an internal uracil residue (Figure 7). The mimic was synthesised (*via* solid-phase DNA chemistry) using a modified DNA building block. The target was a 159-mer RNA sequence derived from the *gag*-mRNA of HIV, and sequence-specific cleavage was observed at physiological pH (7.5) in the presence of  $\text{CuCl}_2$ . The cleavage was located at two positions within the duplex region opposite

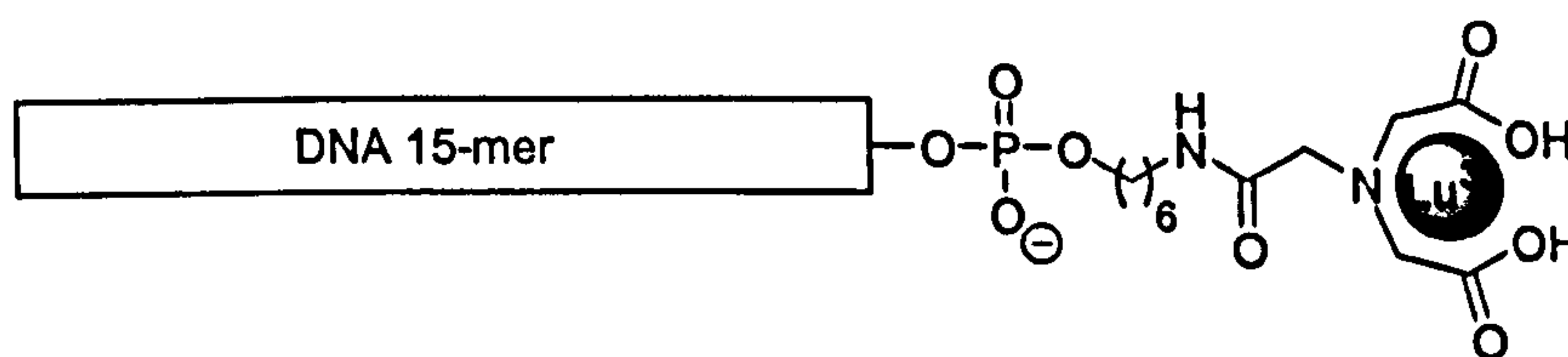


the modified base. The efficiency of cleavage was 11% at 37°C and 18-25% at 45°C over a period of 72 h, with a probe concentration of 5  $\mu\text{M}$  and a RNA target concentration of  $10^{-3}$   $\mu\text{M}$ . These results were pivotal in that they proved the concept that ribozyme mimics can be constructed by covalently linking RNA transesterification catalysts to DNA. Thus, the complex catalytic region of a natural ribozyme was replaced with a small molecule catalyst.



**Figure 7.** The first wholly synthetic, functional mimic of a ribozyme.

Shortly thereafter, other examples of sequence-specific RNA cleavage agents based on metal complexes were reported. Matsumura *et al.*<sup>34</sup> prepared a 15-mer deoxyoligonucleotide which was functionalised at the 5'-end with a lanthanide-complexing aminodiacetate residue (Figure 8).



**Figure 8.** A DNA 15-mer functionalised at the 5'-end with an aminodiacetate residue.

This conjugate was synthesised using a modification in which a DNA 15-mer with an amino group at its 5'-end was reacted with the 4-nitrophenyl ester of the metal-

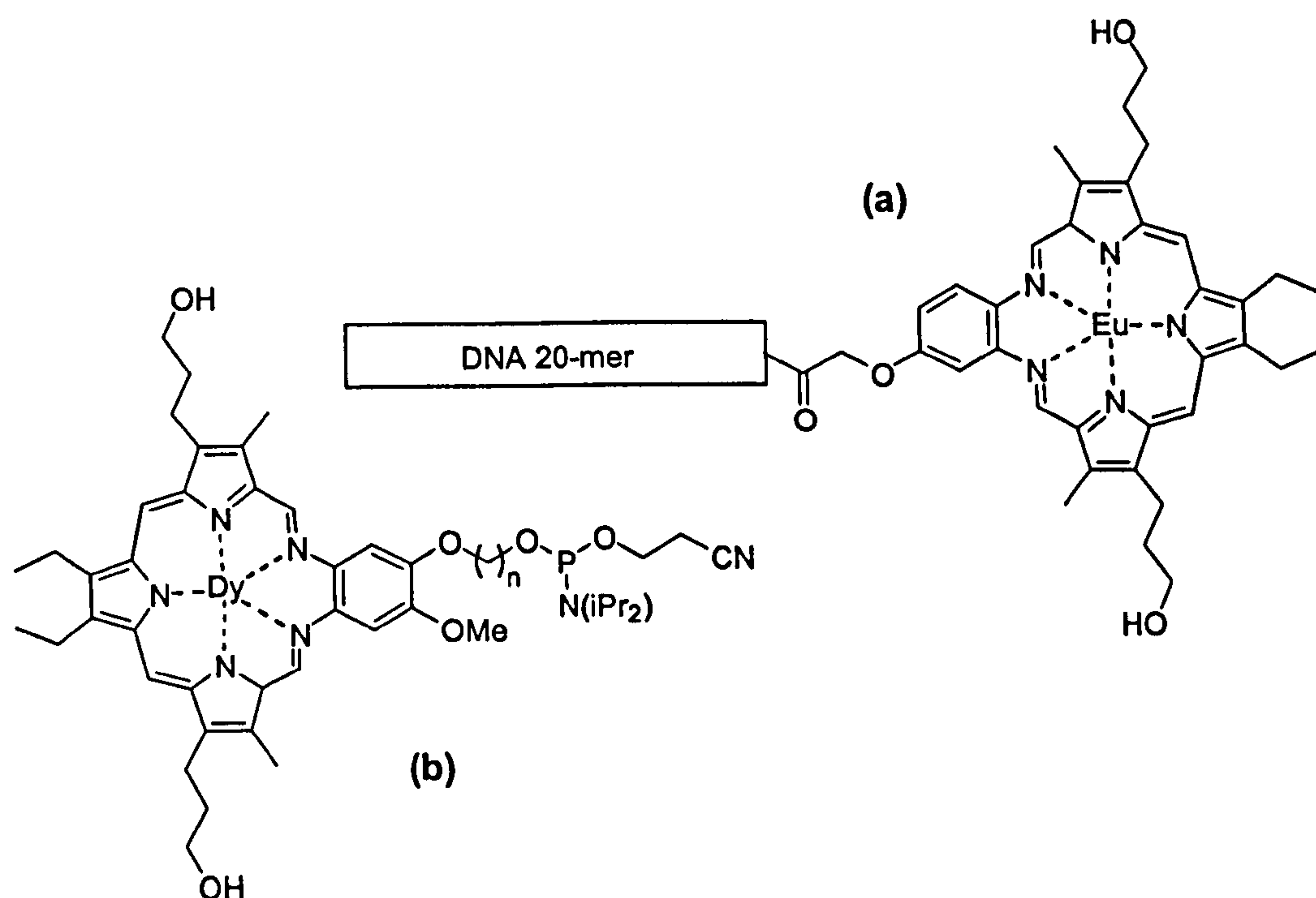
complexing moiety. In the presence of various lanthanide ions [i.e., Lu(III), Th(III) and Eu(III)], the modified oligo cleaved a synthetic 39-mer RNA target outside the duplex region, opposite the metal complex. The efficiency of cleavage was 7.3% after 4 h and 17% after 8 h. Reactions were performed at 37°C and pH 8, and the concentrations of probe and RNA target were 10 and 0.3  $\mu$ M, respectively.

Magda *et al.*<sup>35</sup> synthesised ribozyme mimics by attaching Eu(III), chelated by a monoanionic, pentadentate texaphyrin ligand (EuTx), to 20-mer DNA probes. Their strategy involved the synthesis of DNA oligonucleotides containing alkylamine groups either at a C-5 of an internal thymine residue or at a 5'-terminal phosphate. This was followed by treatment of the deoxyoligonucleotides with the europium(III)-texaphyrin carboxylic acid to effect amide coupling to the alkylamine groups (Figure 9a). The conjugate with the texaphyrin complex attached at the 5'-end of the DNA strand site specifically cleaved a chemically synthesised 30-mer RNA target near the expected location. Approximately 30% cleavage was observed after 24 h at 37°C and pH 7.5 in a reaction containing 2.5 nM probe and 1 nM RNA target. In contrast, no cleavage was observed with the internally modified deoxyoligonucleotides. A major contribution of this work was the use of the stable preformed Eu(III) complex. Whereas other approaches required the addition of free metal ion cofactors for the cleavage event to take place, this method allowed the cleavage reaction to occur independently of such cofactors. This may prove to be extremely important when considering the use of ribozyme mimics for *in vivo* applications in the presence of competing protein ligands and other bioavailable metals.

More recently, Magda reported a variation of this approach in which the synthesis of similar cleavage agents was accomplished using a dysprosium (III) texaphyrin phosphoramidite as an auxiliary reagent on a commercial DNA synthesiser (Figure



9b).<sup>36</sup> The objective was to avoid the need for solution-phase conjugation of the preformed metal complex and postsynthetic addition of a metal cation to a DNA-bound ligand. The complex was attached at the 5'-end of a 20-mer deoxyoligonucleotide during the course of automated DNA synthesis, and sequence-specific cleavage of a complementary 36-mer RNA target was achieved. A series of structural variants of this conjugate was constructed, and one mimic cleaved approximately 80% of the RNA target after 6 h at 37°C and pH 7.5. The concentrations of conjugate and RNA target were 50 nM and 2.0 nM, respectively.

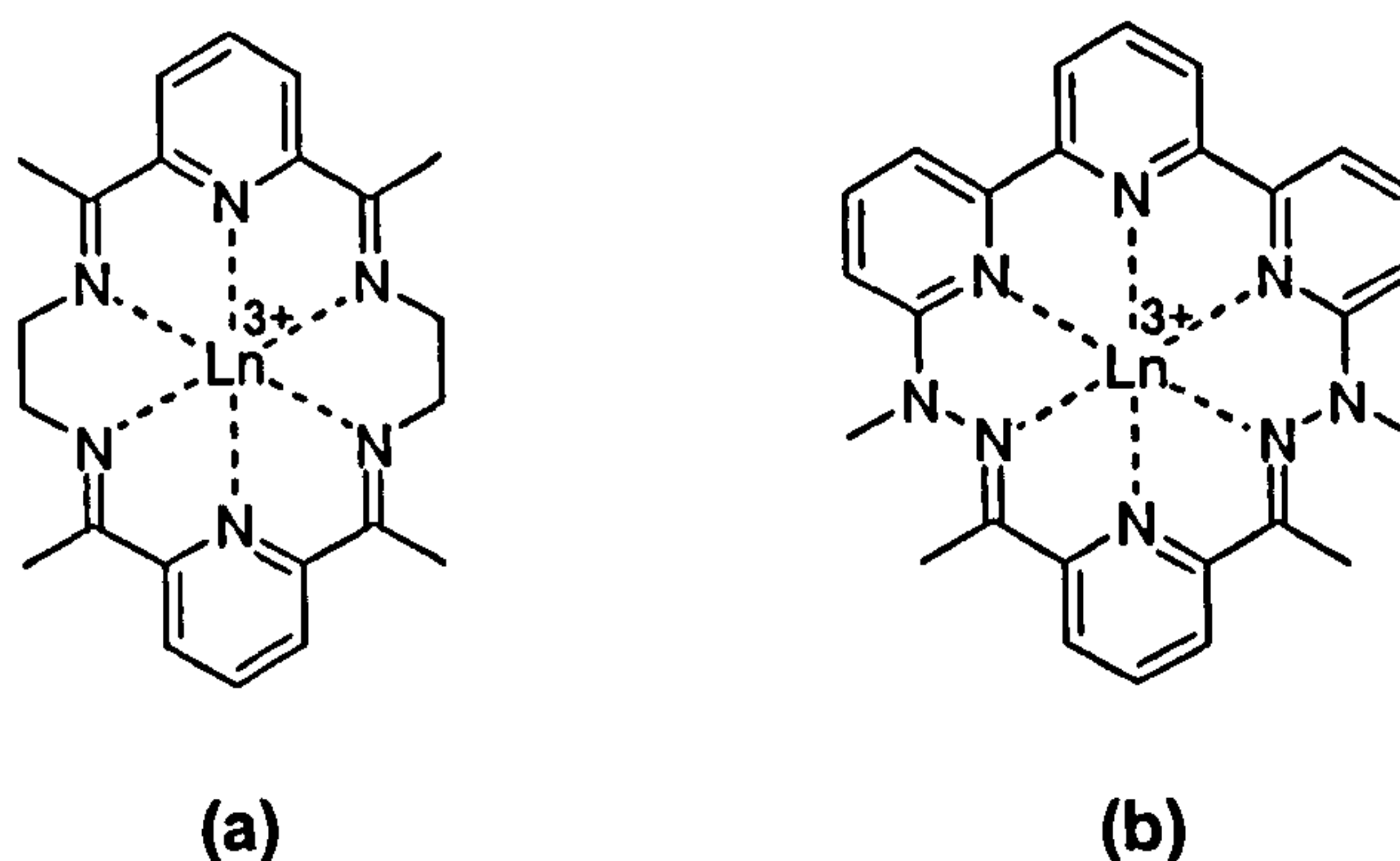


**Figure 9.** (a) Eu(III) texaphyrin conjugate used for sequence-specific RNA cleavage and (b) Dy(III) texaphyrin phosphoramidite used as a reagent for the automated synthesis of ribozyme mimics.

Much success has been achieved by Hall *et al.*<sup>37</sup> with the terpyridine-derived, lanthanide macrocyclic complexes linked covalently to deoxyoligonucleotides. Previous work by Morrow *et al.* revealed that related, hexadentate Schiff base macrocyclic lanthanide complexes (Figure 10a) were efficient RNA transesterification



catalysts.<sup>1</sup> Unfortunately, these complexes were susceptible to hydrolytic decomposition in aqueous solution, and this prompted the synthesis of the terpyridine derived macrocyclic complexes with hydrazone-type linkages in place of the imine linkages for added stability (Figure 10b).



**Figure 10.** (a) Hexadentate Schiff base macrocyclic lanthanide complex. (b) Hexadentate terpyridine macrocyclic lanthanide complex.

Europium (III) complexes of this type were linked to a 5'-hexylamino-derivatised deoxyoligonucleotide (20-mer) by using postsynthetic strategies, and the resulting conjugates (Figure 11) were tested for their ability to effect the specific cleavage of a synthetic 29-mer RNA target. Analysis revealed near-quantitative cleavage (88%) of the target RNA strand at almost exclusively one site outside of the duplex region after incubation at 37°C and pH 7.4 for 16 h. Reactions contained an RNA target concentration of 10-50 nM and a probe concentration of 400 nM.

Lönnberg and co-workers<sup>38</sup> developed a sequence-specific RNA cleavage agent by tethering a histamine group to the 3'-terminus of a 10-mer deoxyoligonucleotide (Figure 12). This was accomplished by attaching an ester function to the deoxyoligonucleotide during chain assembly.

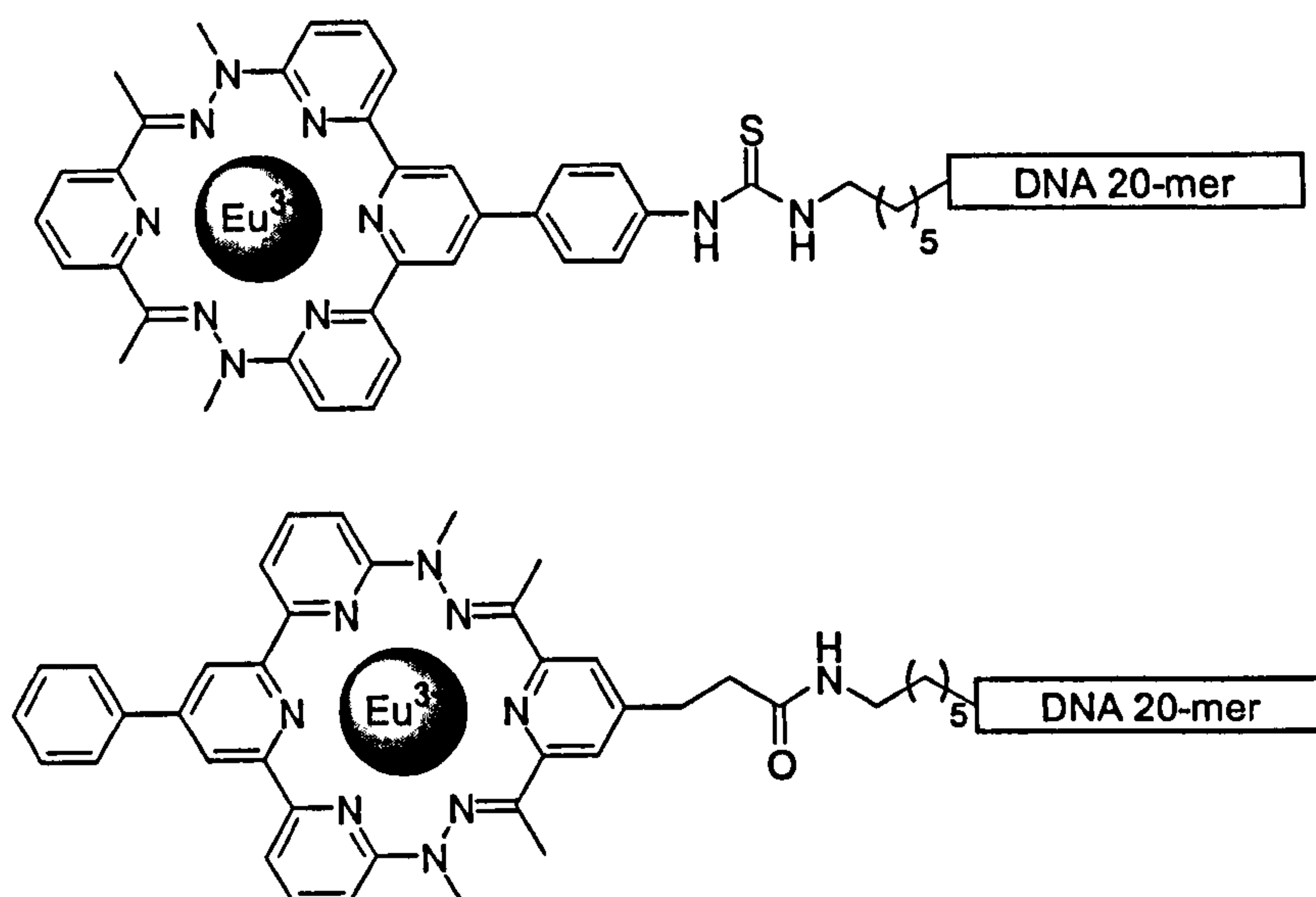


Figure 11

Treatment of the deoxyoligonucleotide shown in Figure 12 with the appropriate primary amine afforded the desired conjugate. Site-specific cleavage of a synthetic 16-mer RNA target was observed only in the presence of zinc (II) ion. The extent of cleavage was determined to be 2-5% after 19 h at room temperature in a reaction containing 2.5  $\mu\text{M}$  deoxyoligonucleotide conjugate, 50 mM  $\text{Zn}^{2+}$  and 0.5  $\mu\text{M}$  RNA target at pH 7.0. Cleavage was achieved with only the 3'-end modified probe and not with probes containing the histamine at the 5'-end or with 1'-modified deoxyoligonucleotides containing the histamine at an internal duplex position.

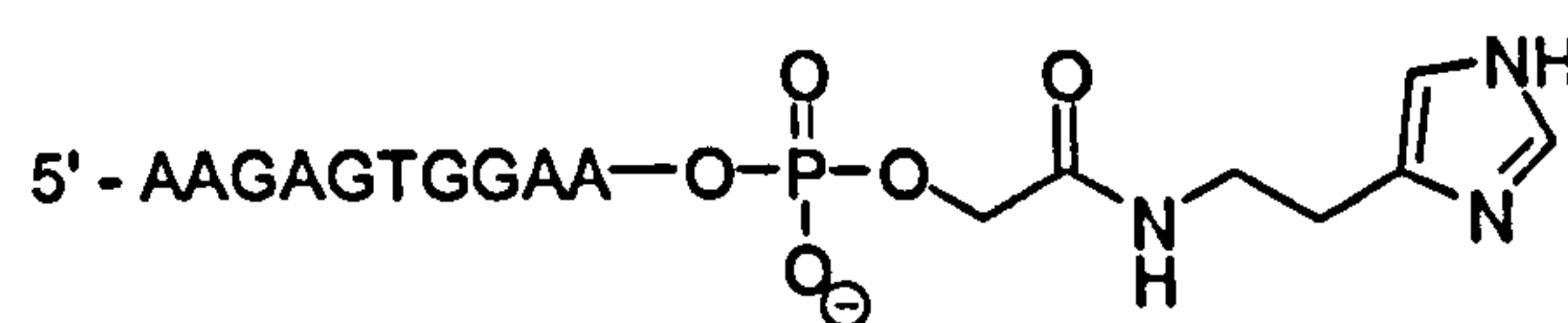
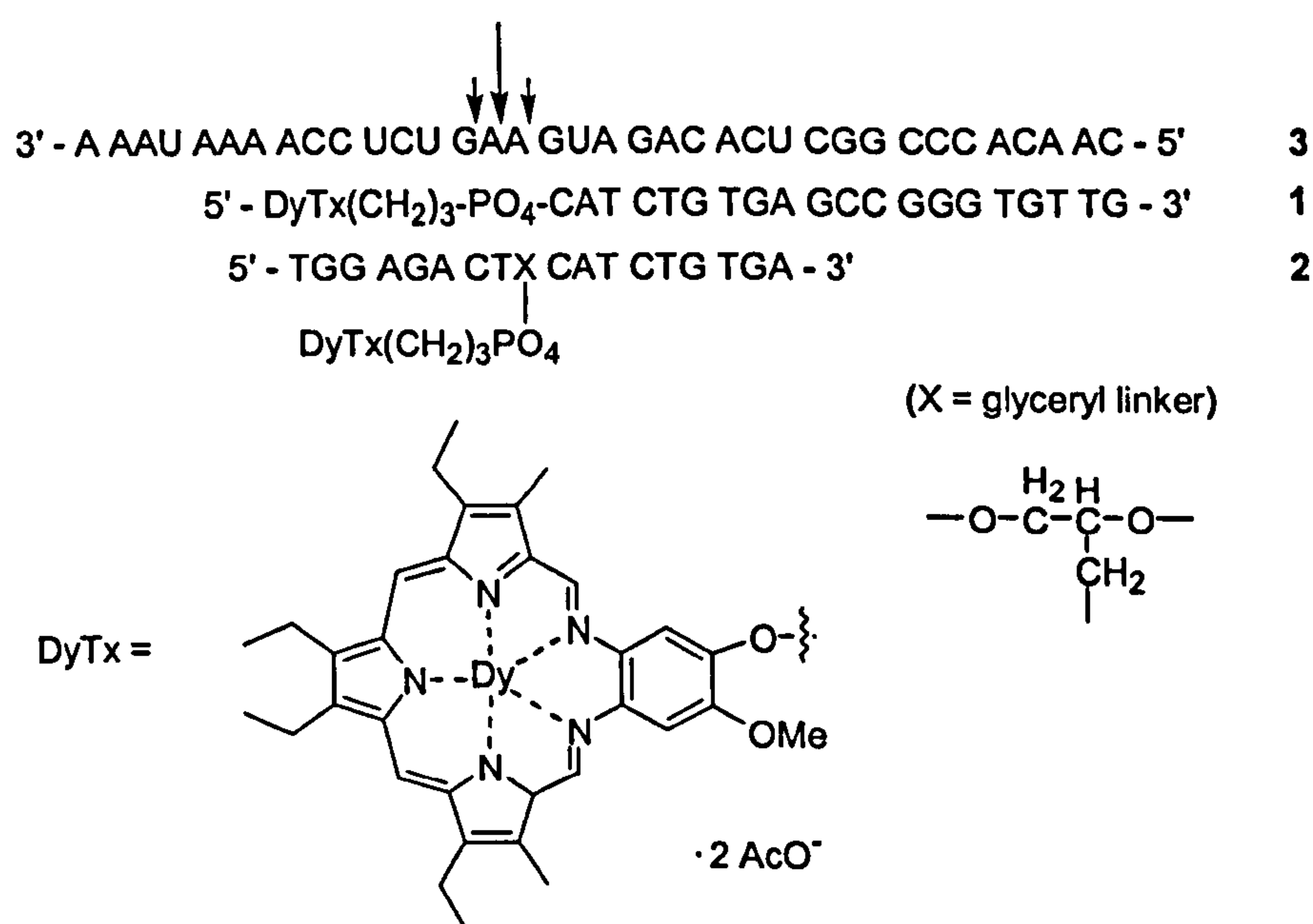


Figure 12. A ribozyme mimic with a histamine at the 5'-end of the DNA probe.

In 1997, Magda *et al.*<sup>39</sup> reported an important result in this area. They prepared a probe which contained a dysprosium (III) texaphyrin metal complex attached at an internal position within the DNA oligomer (Figure 13). Addition of a 10-fold excess of RNA

(500 nM RNA) this conjugate cleaved 67% of the total RNA. This level of cleaved RNA, 335 nM after 24 h corresponded to a value that is 6.7 times the concentration of the DyTx-DNA conjugate **2** present in the reaction medium. The above data supported the contention that the DyTx-DNA conjugate **2** was able to exhibit catalytic turnover. A similar conjugate where the DyTx complex was attached to the 5'-end (**1**) cleaved only 5% of the total RNA under the same conditions. This critical difference between the activity of the two conjugates confirmed the hypothesis explained previously in Scheme 6.



**Figure 13.** Structure and sequence of DyTx-DNA conjugates **1** and **2** analysed in this study. Also shown is the sequence of complementary 5'-<sup>32</sup>P-radiolabeled RNA target **3**. The larger arrow indicates the major site of cleavage produced upon incubation of this RNA target with conjugate **1** or **2** at 37°C.

Figure 14 shows the molecular structure of natural DNA and the structure of the conjugate **2** (Figure 13). In spite of its successful activity, it is important to mention that in **2** the structure of DNA is highly modified. It contains five extra atoms and two extra negative charges in comparison with the natural structure of DNA. Also, they are prepared as diastereomeric mixtures due to the chirality at C(\*).



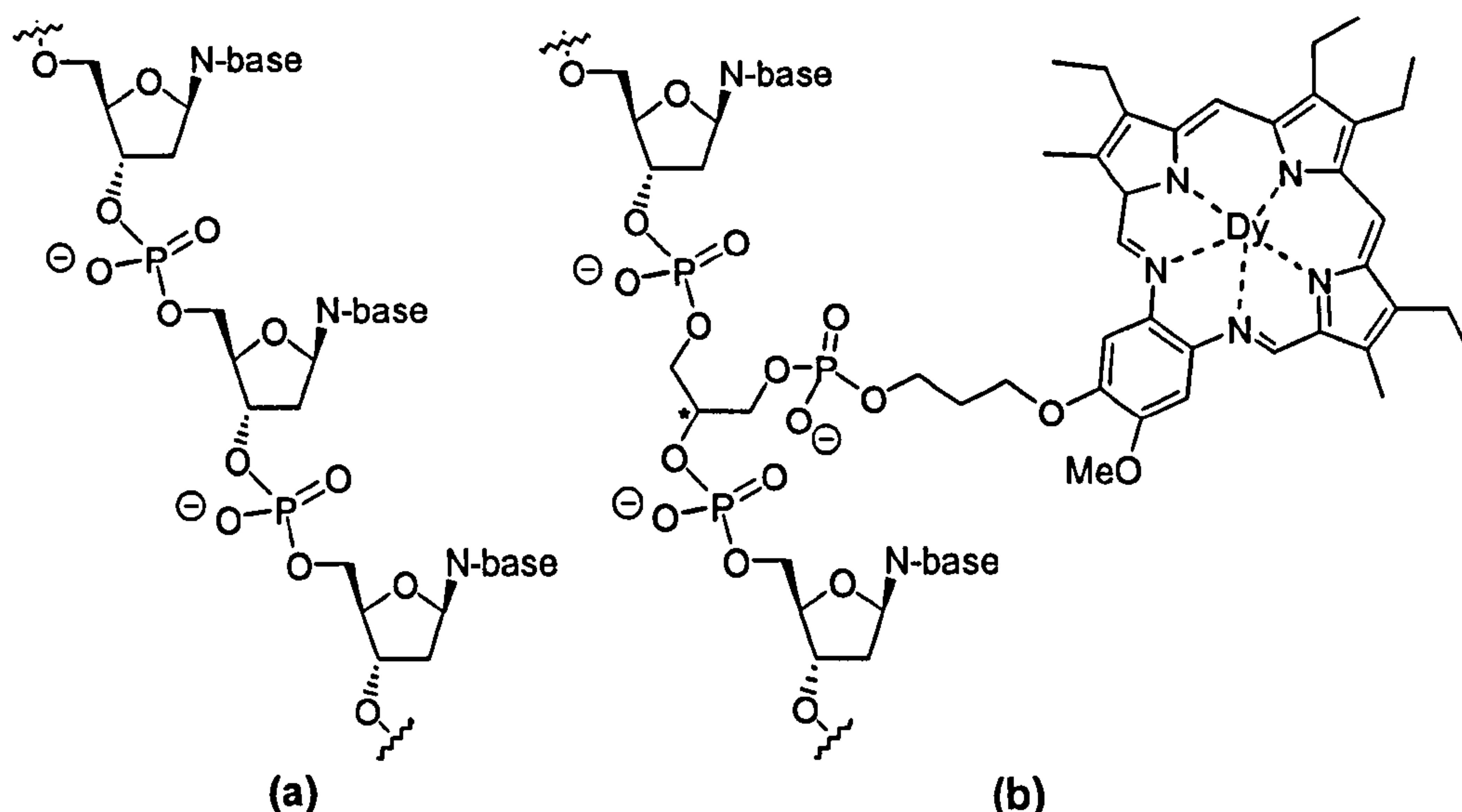


Figure 14. (a) Molecular structure of natural DNA. (b) Molecular structure of conjugate 2 (Figure 13).

An important modification in the structure of the nucleic acids affects the Watson-Crick base pairing and consequently the binding constant between the probe and its RNA target. These highly modified drugs could also present problems of specificity.

It may be possible to improve the catalytic activity of these probes if diastereomerically pure conjugates with a more similar molecular structure to that present in natural DNA were synthesised.

Additionally, there are other interesting molecules that could be attached to nucleic acids, other than RNA cleavage catalysts. These include molecular transporters<sup>40</sup> which can facilitate the cellular uptake of drugs, and fluorescent molecules which are important for a number of studies such as organ distribution.

The objective of the work described in the following chapters of this thesis is to synthesise backbone modified nucleic acids using transition metal catalysis, ie. palladium catalysed cross-coupling or olefin cross-metathesis reactions, to allow the attachment of “interesting” molecules at internal positions of nucleic acids.

## 2. Results and Discussion

As seen in the introduction, it is important to synthesise backbone modified nucleic acids that can facilitate conjugation with other molecules in an internal position of an oligonucleotide. With this aim, we propose the synthesis of the following building blocks:

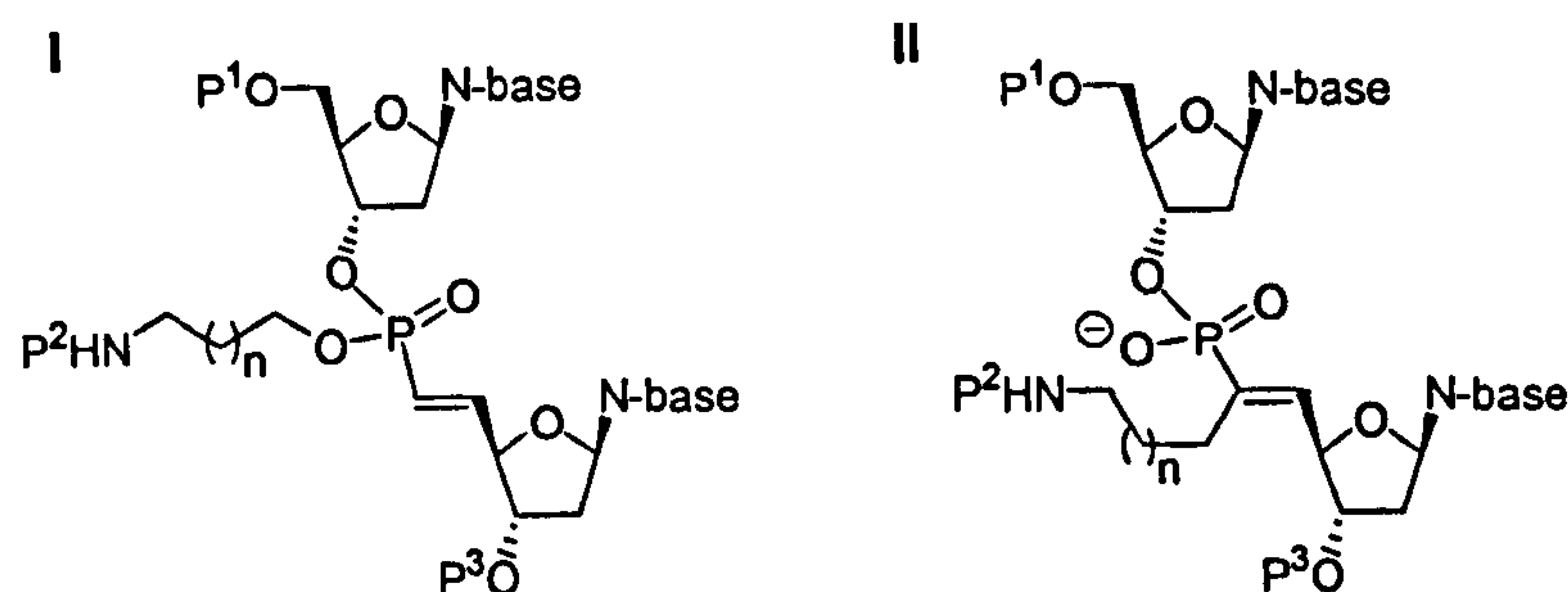


Figure 15. (I) Non-ionic building block (chiral at P). (II) Ionic building block (non-chiral at P).

Both molecules contain a vinylphosphonate moiety which makes them stable to hydrolysis by nucleases and their backbones have the same number of atoms as in natural DNA. The value of the P-C=C bond angle  $\alpha$  is  $120^\circ$  ( $sp^2$  hybridised) and the torsion angle  $\beta$  is locked at  $180^\circ$  which are very similar to the angles  $\alpha$  and  $\beta$  in natural DNA (Figure 16).

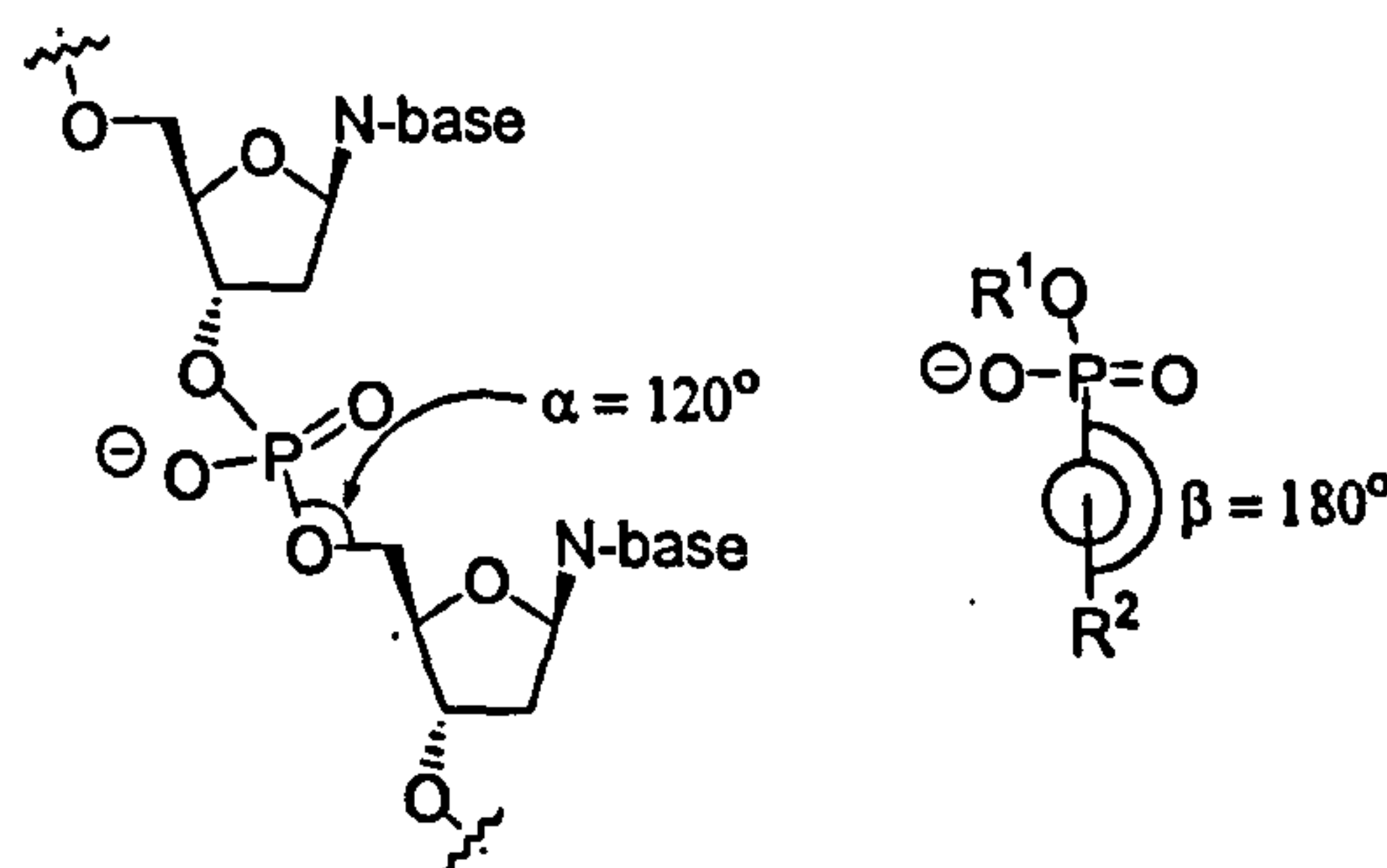


Figure 16. 5'-O-bridge bond angle  $\alpha$  and torsion angle  $\beta$  (Newman projection) in natural DNA.

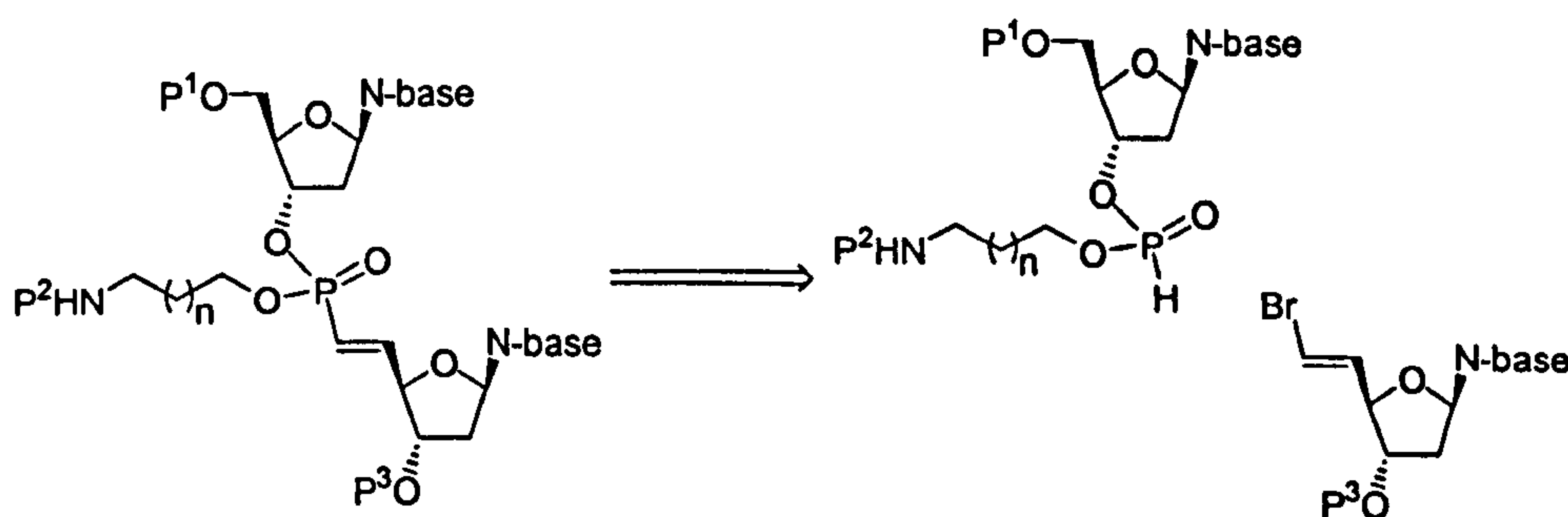
Both compounds contain hydrocarbon linkers with pendant amines with the aim of attaching other molecules to these structures (for example *via* peptide coupling).

In the building block I, the linker and the amine are attached to the oxygen of the phosphonate to give a neutral compound which is chiral at phosphorus, whereas in building block II the linker and the amine are attached to a carbon atom to give an anionic compound which is not chiral at phosphorus.

Both compounds present interesting synthetic challenges, and our approach to each will be described below.

## 2.1 Synthesis of the Building Block I

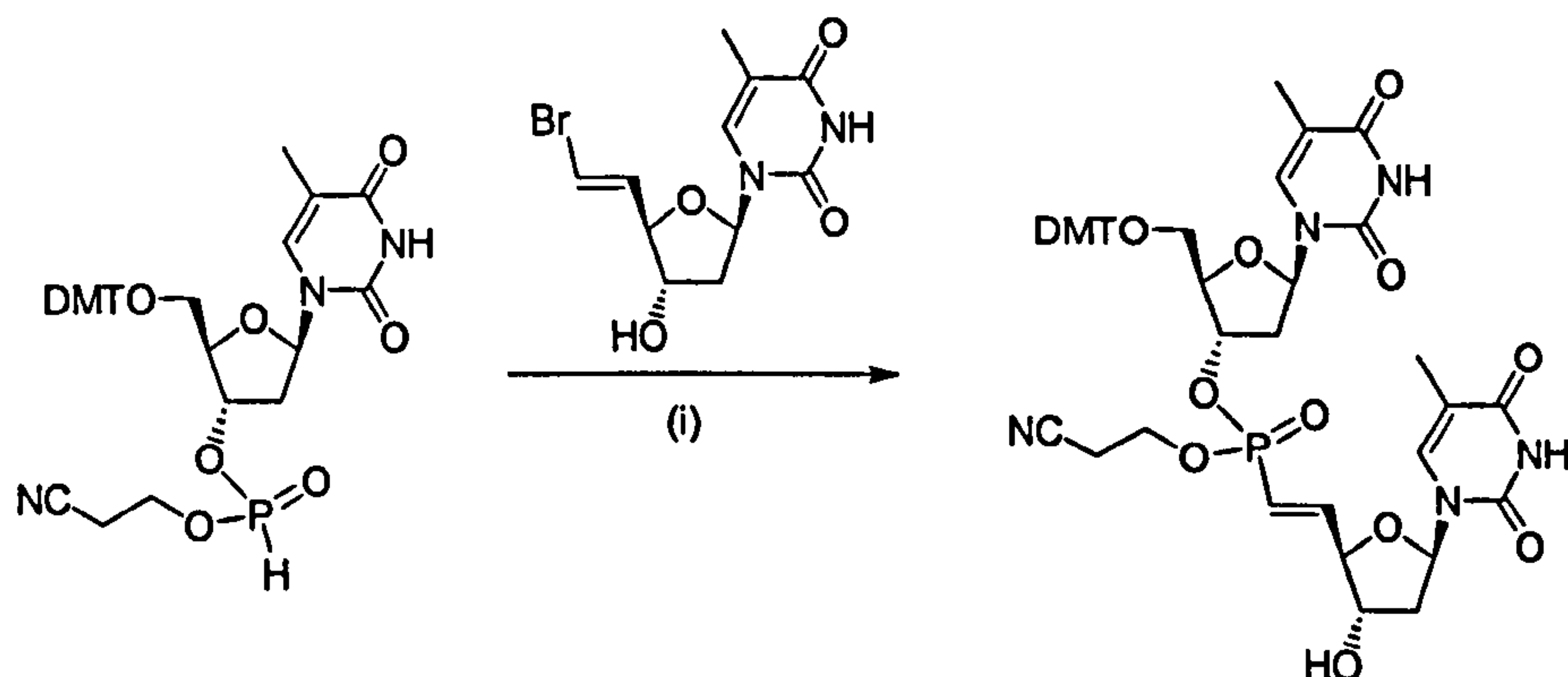
As seen previously, building block I contains the amine linker derived attached to the oxygen of the phosphonate to give a neutral compound which is chiral at phosphorus. The following scheme illustrates the retrosynthetic analysis proposed for building block I:



Scheme 7. Retrosynthetic analysis proposed for building block I.

In principle, this building block can be prepared using methodology developed previously in our research group, in which a vinylphosphonate linked dinucleotide is synthesised *via* a palladium catalysed cross-coupling reaction between a vinyl bromide and an *H*-phosphonate.<sup>41</sup> These reactions proceed in good to excellent yield, and are stereospecific with retention of configuration at phosphorus (Scheme 8).

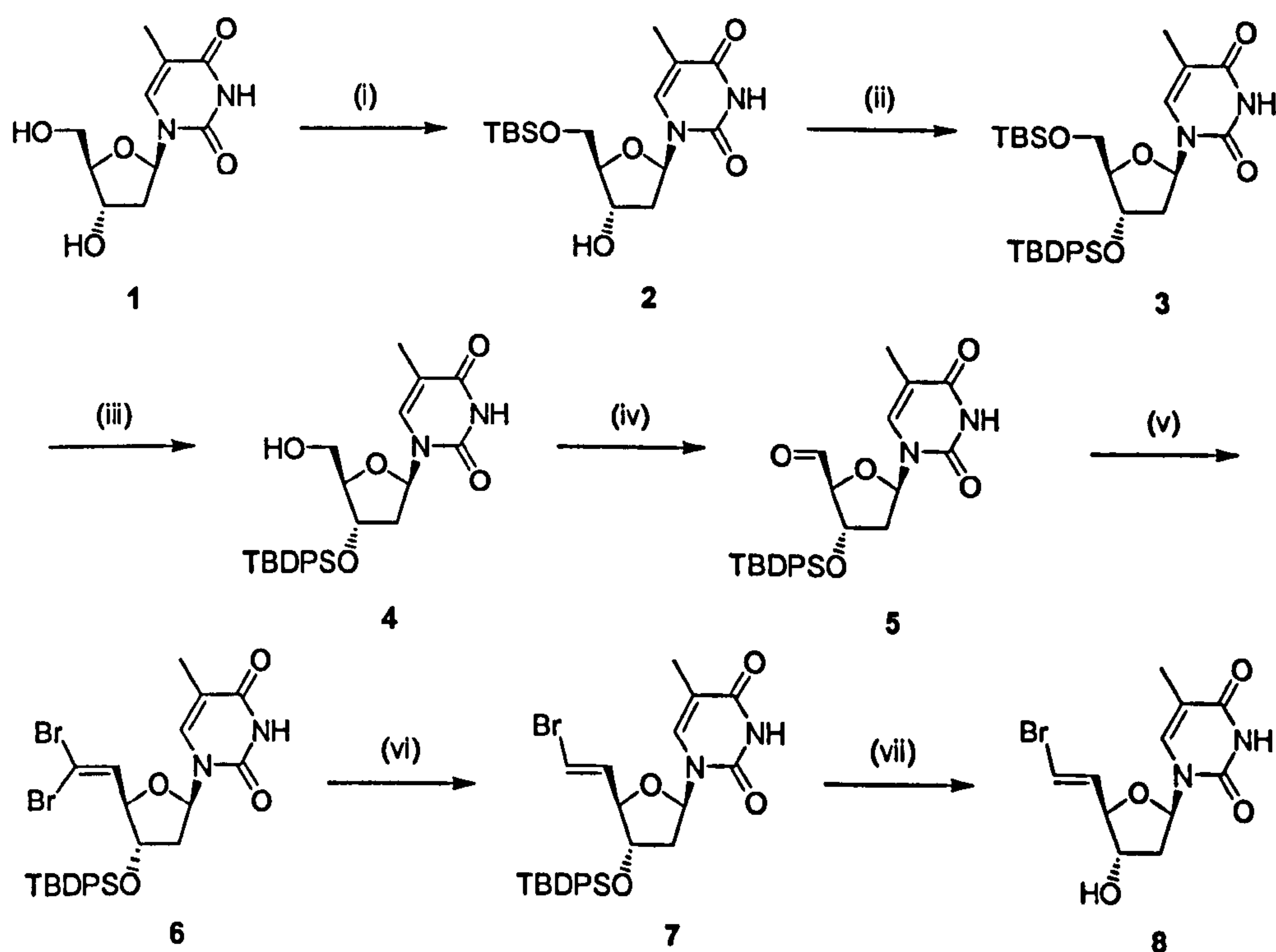




**Scheme 8.** Palladium catalysed cross-coupling reaction between a vinylbromide and an *H*-phosphonate.

(i)  $\text{Pd}(\text{OAc})_2$ , dppf, propylene oxide, THF, 14 h, 60%.

The vinylbromides **7** and **8** were prepared using our previously described methodology<sup>41</sup> (Scheme 9).



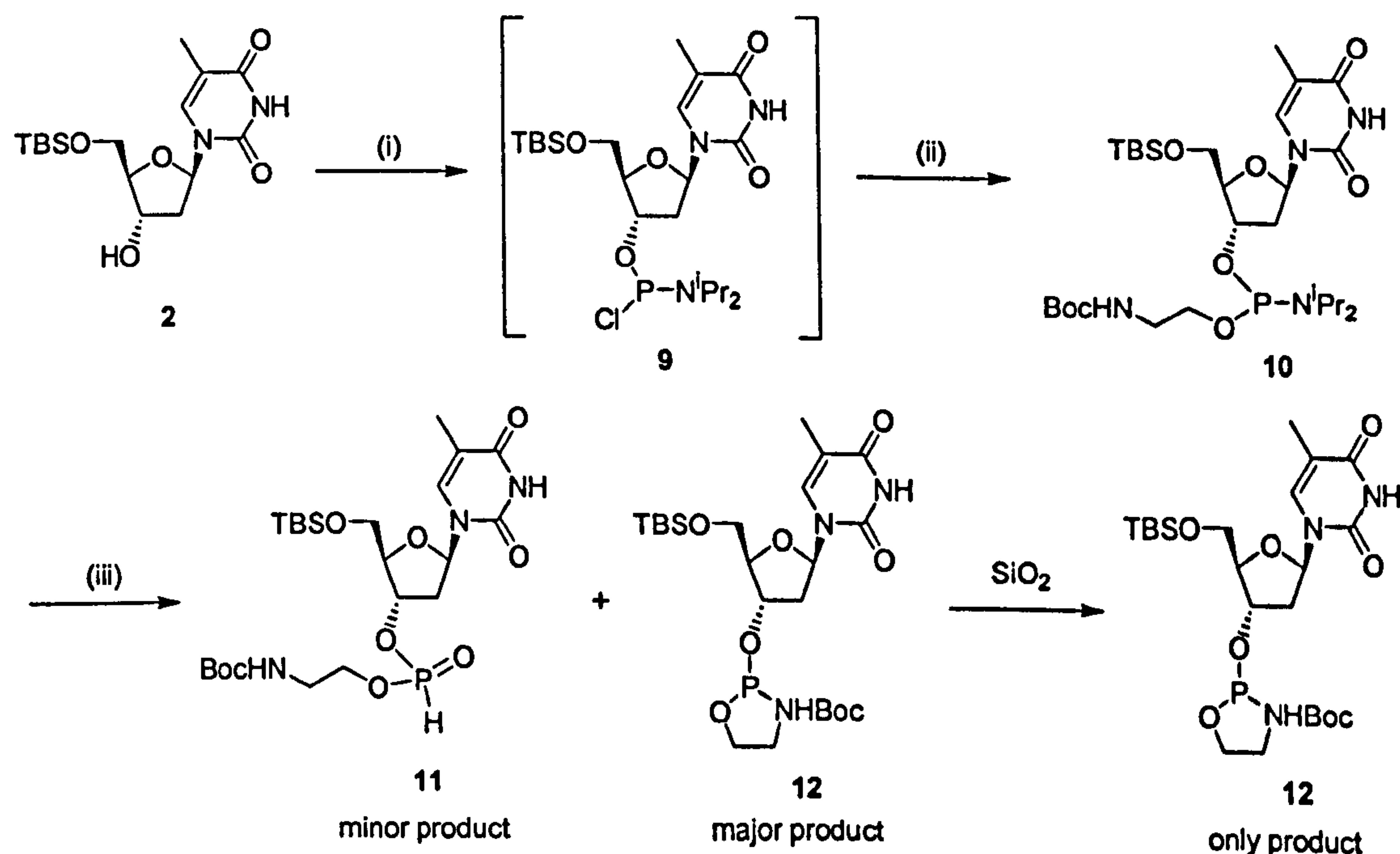
**Scheme 9.** Synthesis of the vinylbromides **7** and **8**. (i) TBS-Cl (1.3 eq), imidazole (1.5 eq), DMF, 0°C to RT, 14 h, 98%; (ii) TBDPS-Cl (1.3 eq), imidazole (1.5 eq), DMF, 0°C to RT, 14 h, 98%; (iii) aq HF (12 eq),  $\text{CH}_3\text{CN}$ , RT, 1.5 h, 90%; (iv) Dess-Martin periodinane (1.2 eq), DCM, RT, 1 h, 83%; (v)  $\text{CBr}_4$  (2 eq),  $\text{Ph}_3\text{P}$  (4 eq), DCM, 0°C to RT, 2 h, 60%; (vi)  $(\text{CH}_3\text{O})_2\text{P}(\text{O})\text{H}$  (4 eq),  $\text{NEt}_3$  (4 eq), DMF, RT, 14 h, 47%; (vii) TBAF (2 eq), THF, RT, 1 h, 84%.

This synthesis starts with selective protection of the secondary alcohol of thymidine 1 to give alcohol 4, which after oxidation and a Wittig reaction affords dibromide 6. Vinylbromide 7 is obtained by reduction of 6 and can be converted into vinylbromide 8 by treatment with TBAF.

Vinylbromide 7 was prepared with the aim of synthesising (3'-O-TBDPS)-dinucleotides, whereas vinylbromide 8 was used for the synthesis of 3'-OH dinucleotides.

### 2.1.1 Synthesis of the *H*-Phosphonate Coupling Partners

As shown in Scheme 10, it was necessary to prepare *H*-phosphonates with a linker and an amine attached to the oxygen of the phosphonate moiety. The first approach attempted was the synthesis of *H*-phosphonates containing a two methylene linker and a Boc-protected amine using known methodology.<sup>42</sup>



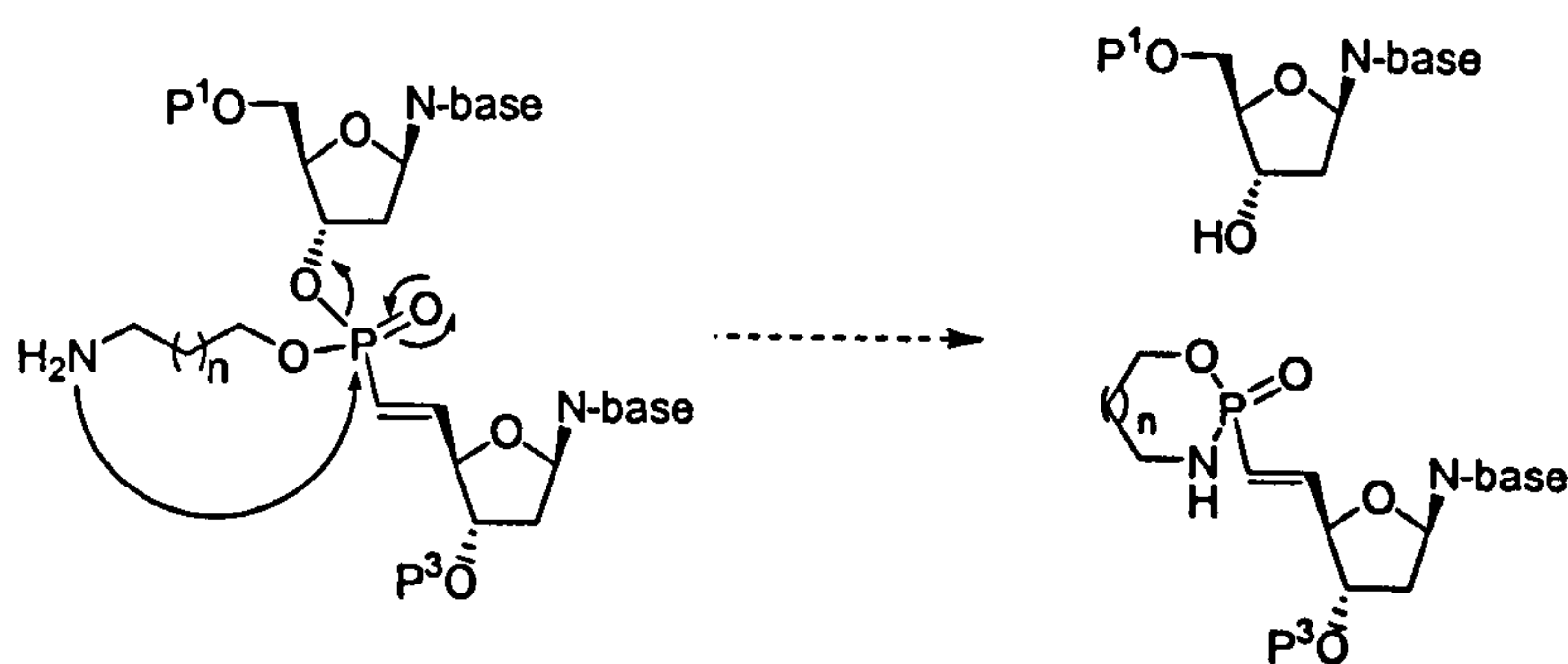
**Scheme 10.** (i) Diisopropylphosphoramidous chloride (1.5 eq), NEt<sub>3</sub> (20 eq), DCM, -10°C, 30 min; (ii) N(Boc)-ethanolamine (2 eq), DCM, -10°C, 20 min and then RT, 1 h, 26% over two steps; (iii) 1*H*-tetrazole (2 eq), DCM, RT, 15 min, H<sub>2</sub>O, RT, 1 h, 53%.

Unfortunately, due to the high electrophilic character of the phosphorus, the cyclic product **12** was formed as the major product in the last step (Scheme 10). The mixture of **11** and **12** was chromatographed but only **12** was obtained, suggesting that **11** was unstable and maybe decomposed during the purification process on SiO<sub>2</sub>.

Another drawback of this strategy was the low yield of the first two steps due to the moisture sensitive characteristics of the trivalent phosphorus species **9**.

The structure of cyclic product **12** was confirmed by <sup>1</sup>H-NMR and MS. The formation of this product suggested that it is necessary to diprotect the amine to completely eliminate its nucleophilic character and avoid any possibility of cyclisation.

The presence of **12** also questioned the stability of the proposed building blocks (Figure 15) due to the possibility of intramolecular nucleophilic cleavage of the dinucleotide after deprotection of the amine (Scheme 11).

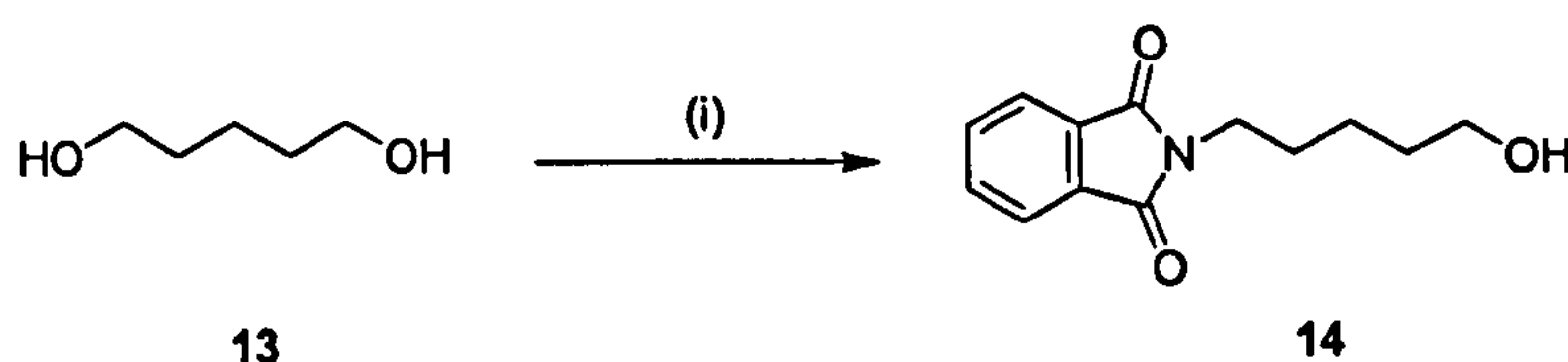


Scheme 11. Possible intramolecular cleavage of the dinucleotide.

It was therefore decided to attempt the synthesis of *H*-phosphonates with an amine protected as a phthalimide and a linker with five methylene groups. The diprotection of the amine as a phthalimide completely eliminates the nucleophilic character of the nitrogen and a linker of five methylene groups would reduce the tendency for cyclisation as this would lead to the unfavourable formation of an eight membered ring.

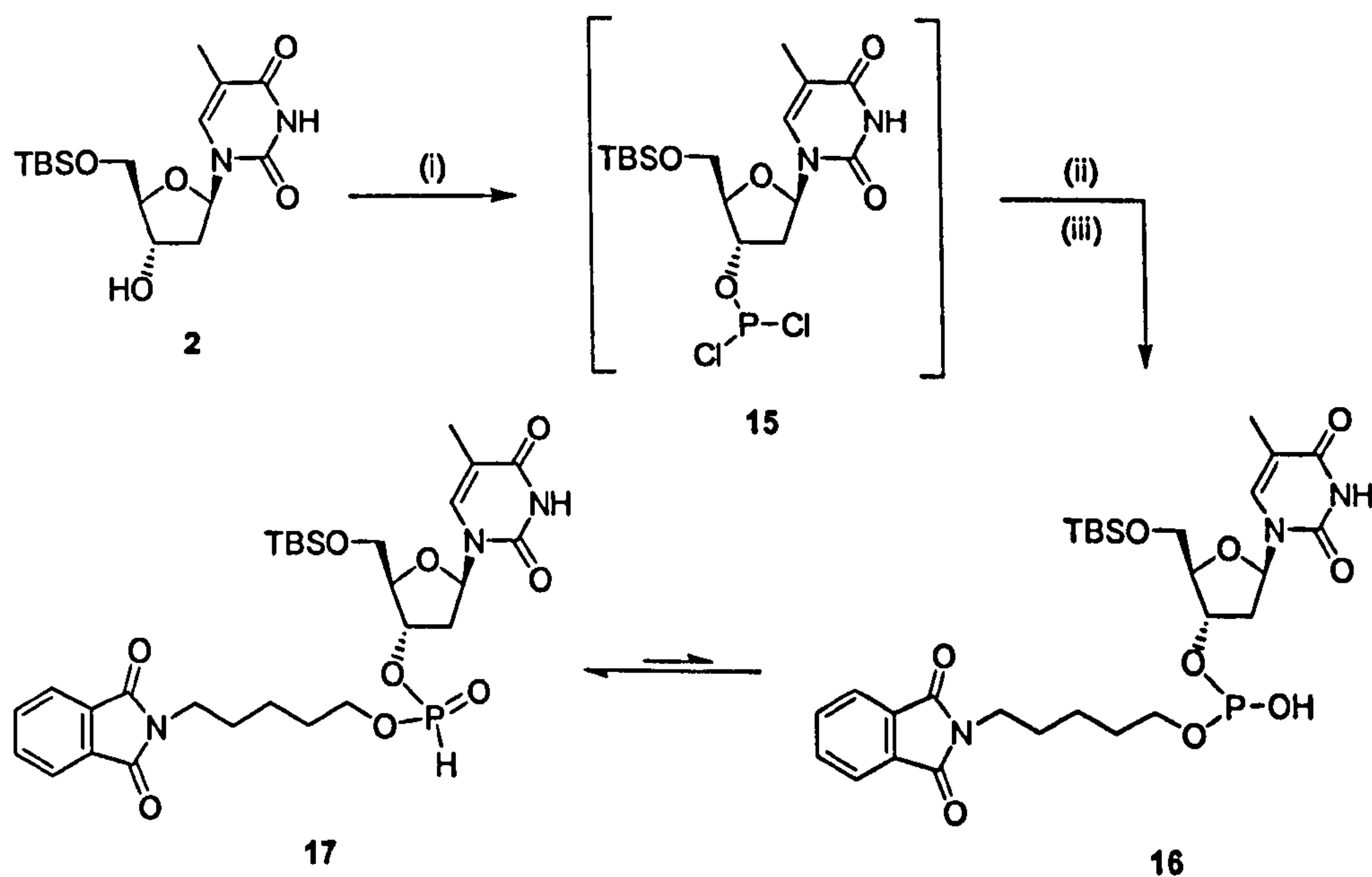


Firstly, alcohol **14** was prepared using Mitsunobu conditions (Scheme 12).<sup>43</sup>



Scheme 12. (i) DIAD (1.2 eq),  $\text{Ph}_3\text{P}$  (1.2 eq), phthalimide (1.2 eq), THF,  $0^\circ\text{C}$  to RT, 15 h, 51%.

The desired *H*-phosphonate **17** was prepared in a method analogous to Scheme 10 but again the yields were very low. It was necessary to develop new methodology for the synthesis of this *H*-phosphonate, and after several attempts, the desired *H*-phosphonate coupling partner was synthesised in 76% overall yield using the strategy illustrated in Scheme 13.



Scheme 13. New methodology developed for the synthesis of the *H*-phosphonate coupling partners **17**.

(i)  $\text{PCl}_3$  (4.5 eq), pyridine (5 eq), THF,  $-78^\circ\text{C}$  to reflux, 3.3 h; (ii) Alcohol **14** (1 eq), pyridine (5 eq), THF, RT to reflux, 1 h; (iii)  $\text{H}_2\text{O}$ , RT, 5 min, 76% over 3 steps, 1:1 mixture of diastereomers separable by column chromatography.

This strategy consists of three nucleophilic substitutions onto phosphorus trichloride, first by the 3'-OH of (5'-O-TBS)-thymidine, second by alcohol 14 and the third by water. After tautomerisation of 16, the desired *H*-phosphonate 17 was obtained as a 1:1 mixture of diastereomers at phosphorus which were readily separated by column chromatography.

At the moment, we do not know the stereochemistry at phosphorus in the *H*-phosphonates 17 and with this aim, crystallisation experiments<sup>44</sup> and NOE studies have been performed without success. Therefore, we are going to name 17(**fast**) the least polar diastereomer and 17(**slow**) the most polar diastereomer (Figure 17).\*

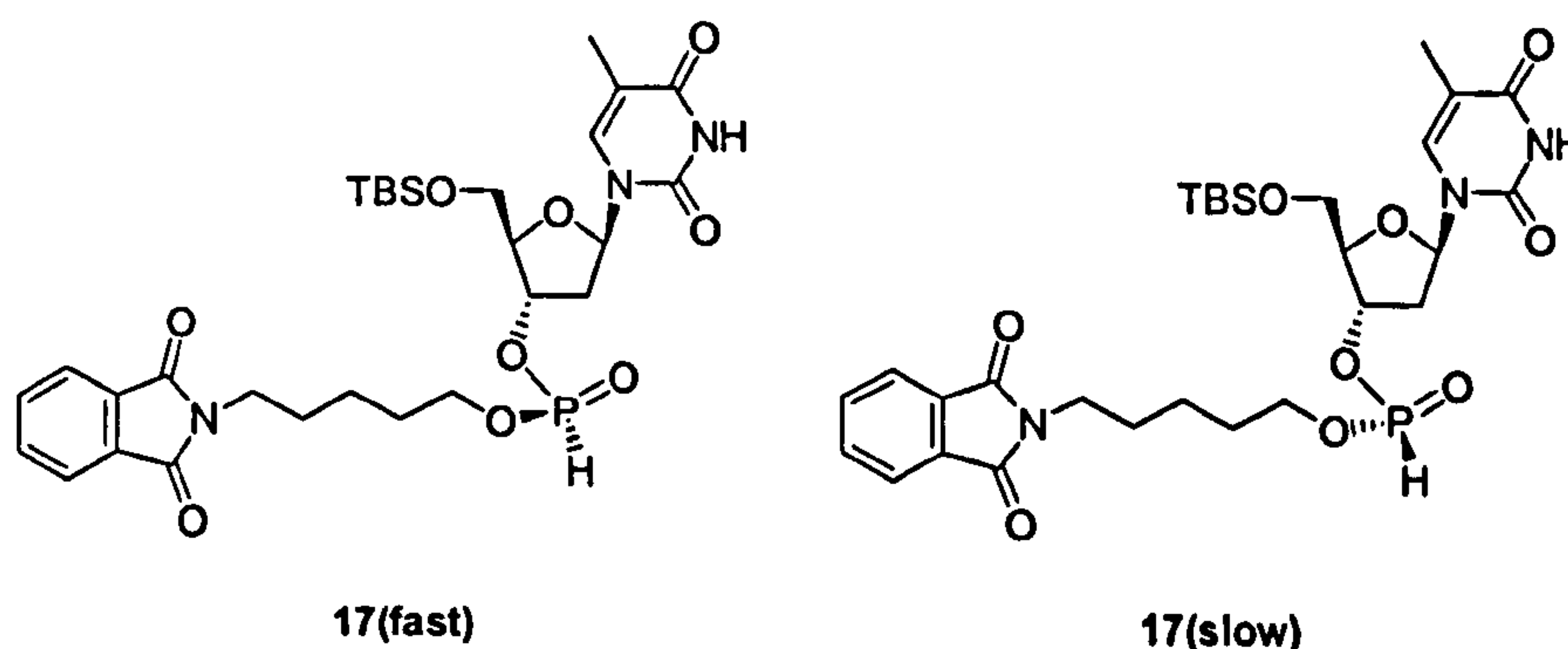
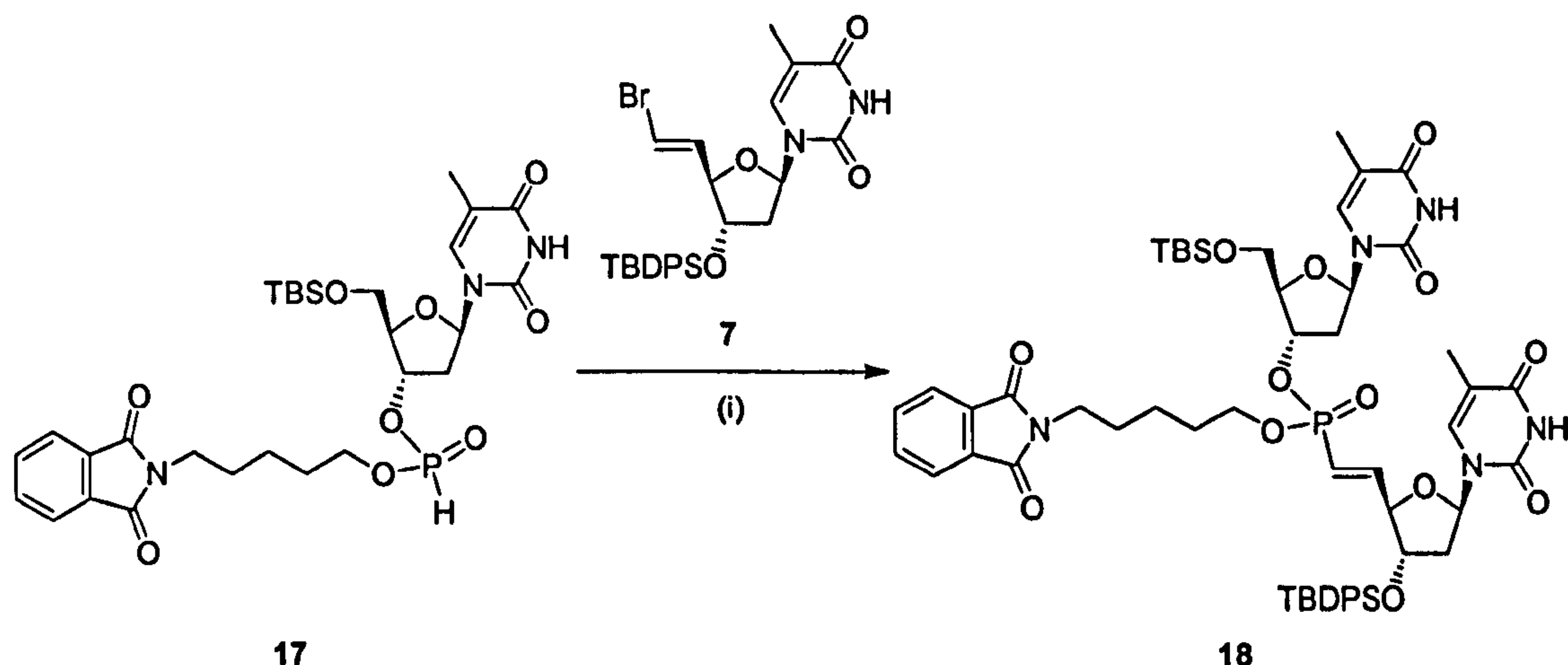


Figure 17

### 2.1.2 Palladium Catalysed Cross-Coupling Attempts

With the desired *H*-phosphonates in hand, the palladium catalysed cross-coupling reaction with vinylbromide 7 was attempted using *H*-phosphonate 17 as a 1:1 mixture of diastereomers [17(**fast**) and 17(**slow**)]. Pleasingly, the desired dinucleotide 18 was obtained in 60% yield as a 1:1 mixture of diastereomers showing that both *H*-phosphonates are reactive under the palladium catalysed cross-coupling conditions (Scheme 14).

\* An arbitrary stereochemistry at phosphorus is shown.



**Scheme 14.** Palladium catalysed cross-coupling reaction using (3'-O-TBDPS)-vinylbromide **7** (1.0 eq) and *H*-phosphonate **17** (1.2 eq) as a 1:1 mixture of diastereomers. (i) Pd(OAc)<sub>2</sub> (0.2 eq), dppf (0.4 eq), propylene oxide (5 eq), THF, 70°C, 15 h, 60%, 1:1 mixture of diastereomers.

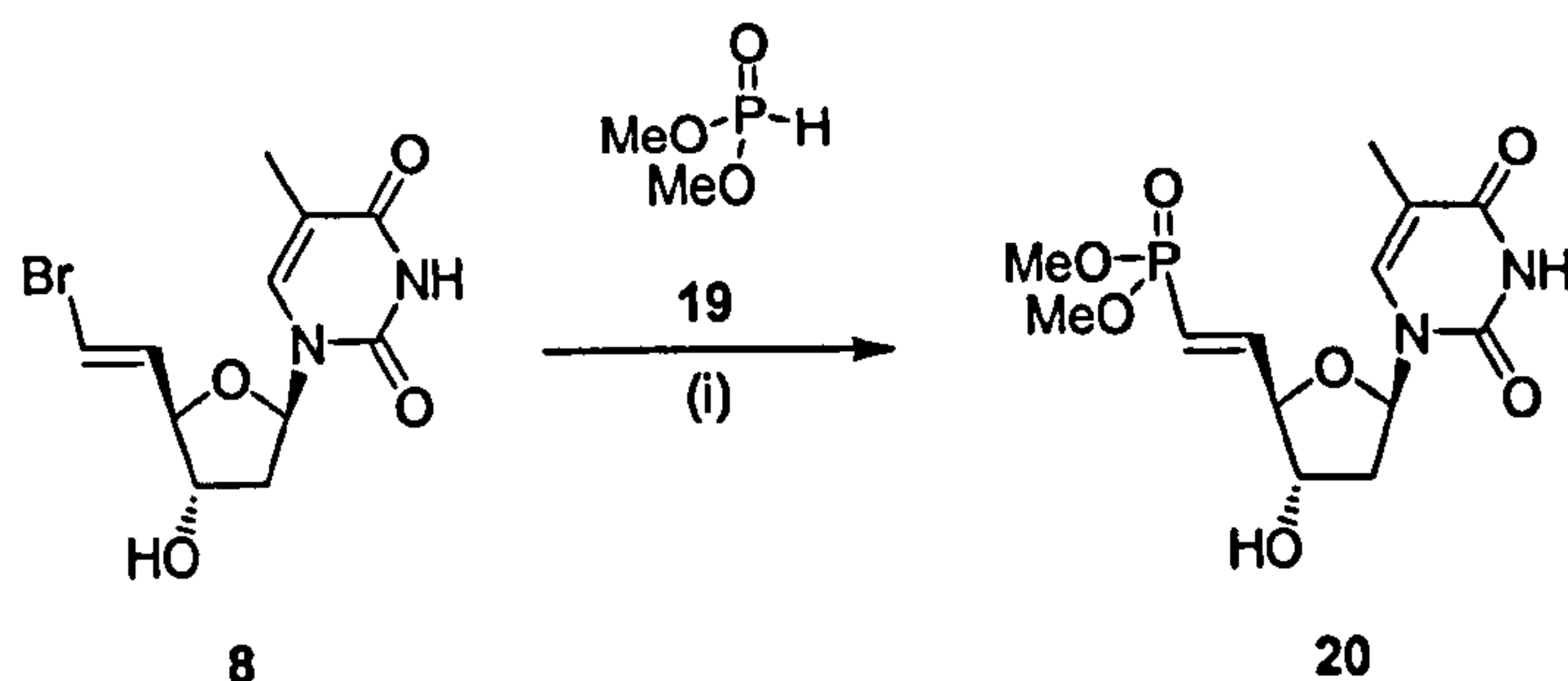
Eventually, these dinucleotides need to be incorporated into an oligomer using a DNA synthesiser. For this purpose, it is necessary to use vinylbromide **8** to prepare dimers with a free 3'-OH group, which can be further elaborated into a 3'-phosphoramidite.

Unfortunately, when the palladium cross-coupling reaction was attempted using vinylbromide **8**, the desired dinucleotide **21** was formed in very low yield. This reaction was performed several times and it was observed that better yields were obtained when smaller flasks were used. Due to the volatile character of propylene oxide (bp 34°C) and the temperature at which the palladium catalysed cross-coupling reaction takes place (70°C) the reaction proceeded until no more propylene oxide was present in the reaction mixture. Thus, the possibility of performing these reactions in sealed, thick walled reaction vials instead of the conventional flask fitted with a condenser was investigated.

A model reaction using vinylbromide **8** and dimethyl phosphite **19** as the *H*-phosphonate was studied using different ligands "L" (dppf, TFP, Ph<sub>3</sub>P and Ph<sub>3</sub>As) and different HBr scavengers "B" (propylene oxide, epichlorohydrin, NEt<sub>3</sub> and K<sub>2</sub>CO<sub>3</sub>) in

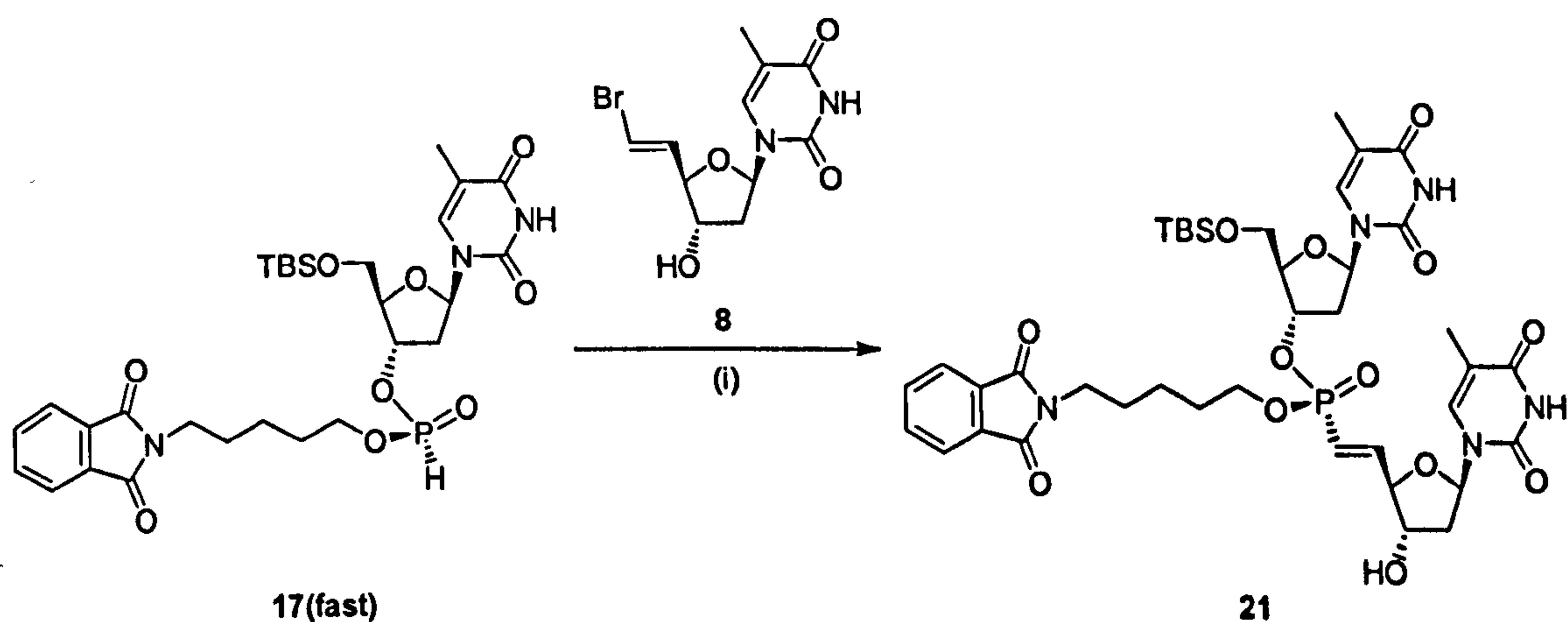


sealed, thick walled reaction vials (Scheme 15). It was found that propylene oxide was the best “base” for this transformation and  $\text{Ph}_3\text{P}$  the best ligand, giving the desired coupling product **20** in 70% yield.



**Scheme 15.** Model reaction using vinylbromide **8** (1.0 eq) and dimethylphosphite **19** (2.0 eq). (i)  $\text{Pd}(\text{OAc})_2$  (0.1 eq), “L” (0.2 eq), “B” (2 eq except with propylene oxide: 20 eq), THF, 70°C, 15 h.

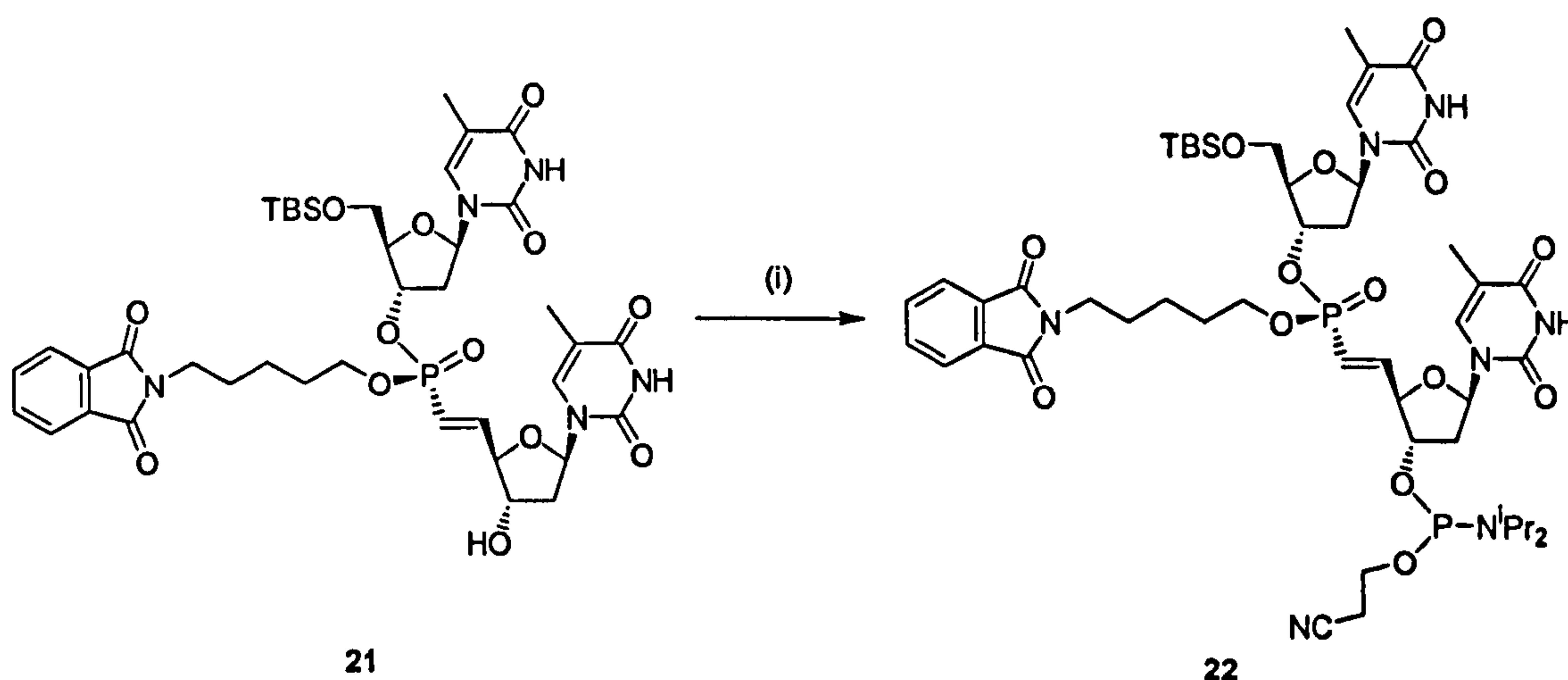
Pleasingly, when the diastereomerically pure *H*-phosphonate **17(fast)** was used instead of dimethyl phosphite the desired dinucleotide **21** with a free 3'-OH was obtained in 80% yield (Scheme 16). The reaction with *H*-phosphonate **17(slow)** has not yet been attempted but we anticipate that it should also work, and this will be completed in due course.



**Scheme 16.** Palladium catalysed cross-coupling reaction using vinylbromide **8** with the free 3'-OH (1.0 eq) and *H*-phosphonate **17(fast)** (1.25 eq). (i)  $\text{Pd}(\text{OAc})_2$  (0.1 eq),  $\text{Ph}_3\text{P}$  (0.4 eq), propylene oxide (20 eq), THF, 70°C, 15 h, 80%.

The above study not only provided us with good conditions to perform the desired reaction with the free 3'-OH, but also revealed that the use of sealed, thick walled reaction vials in these palladium catalysed cross-coupling reactions generally improves the yield and constitutes a very easy method to carry out these transformations.

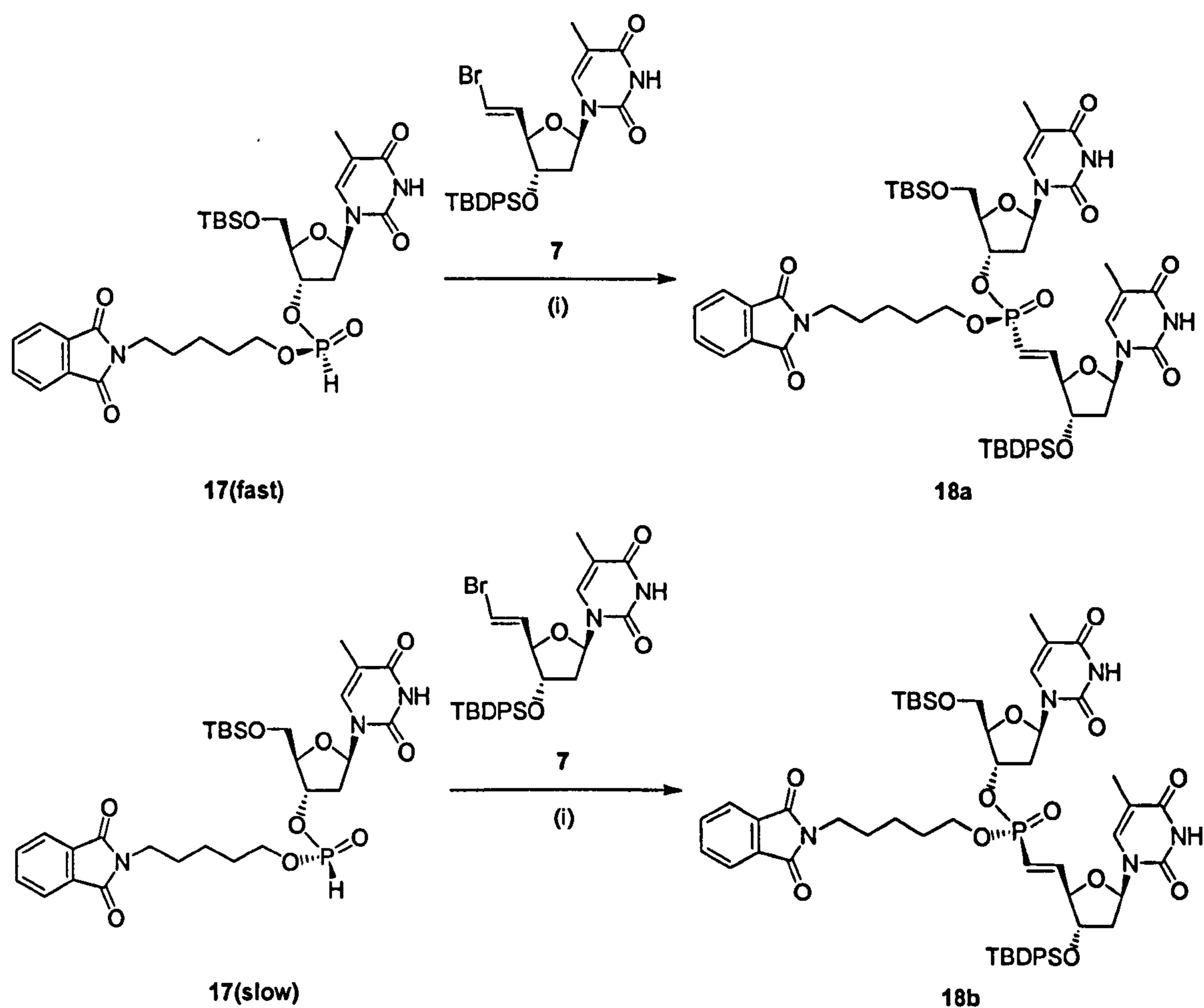
With the aim of making dinucleotide **21** suitable for solid support synthesis used in DNA synthesisers, the coupling of **21** with bis(diisopropylamino)cyanoethylphosphite<sup>42</sup> was attempted, and pleasingly the desired dinucleotide **22** was obtained in 80% yield (Scheme 17).



**Scheme 17.** Coupling of dinucleotide **21** with *bis*(diisopropylamino)cyanoethylphosphite. (i) *Bis*(diisopropylamino)cyanoethylphosphite (1.5 eq), 1*H*-tetrazole (1.0 eq), DCM, RT, 1 h, 80%, 1:1 mixture of diastereomers at the trivalent phosphorous.

### 2.1.3 Amine Deprotection and Arginine Attachment

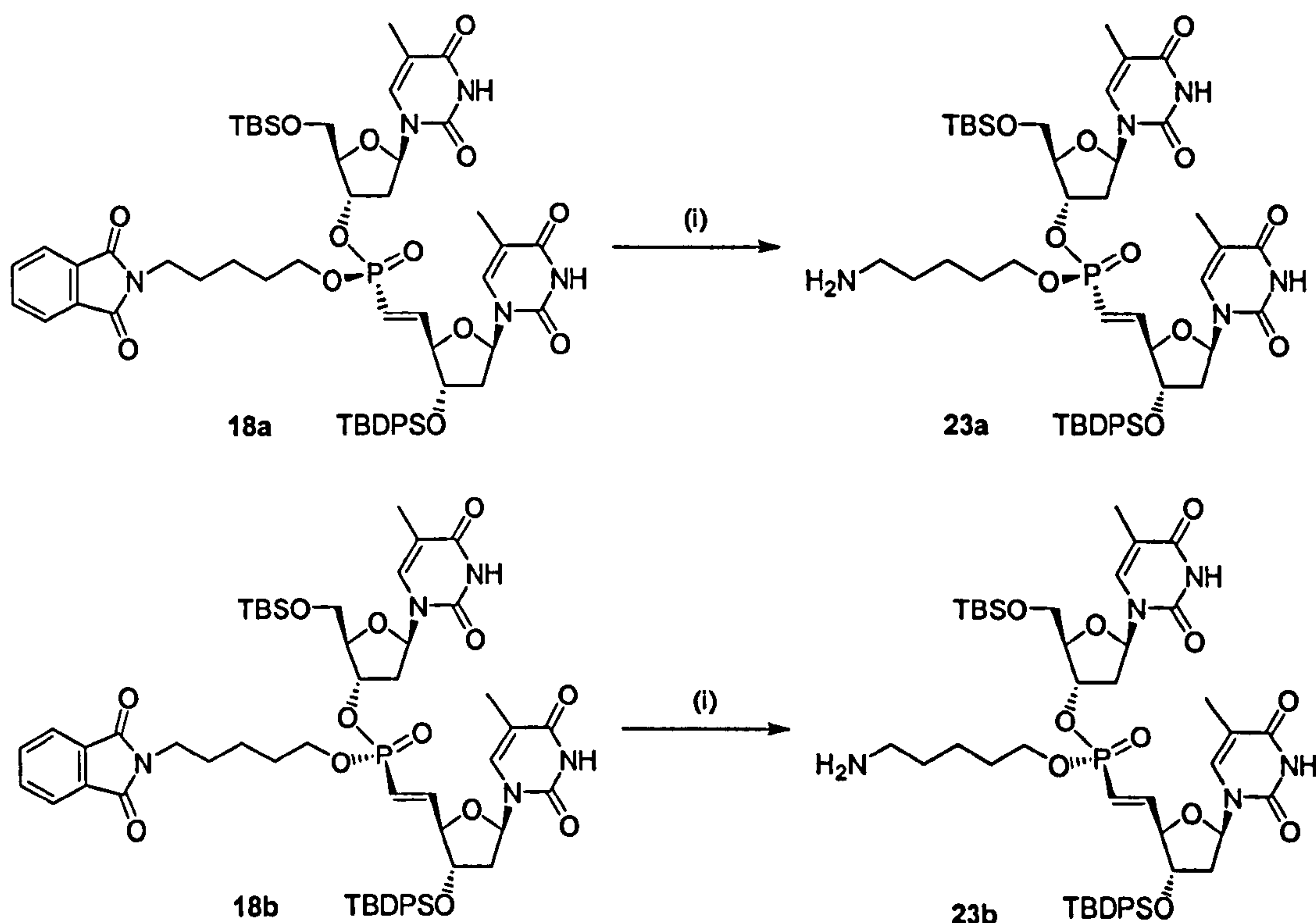
Fortunately, the palladium catalysed cross-coupling reactions of the diastereomerically pure *H*-phosphonates **17**(fast) and **17**(slow) with vinylbromide **8** in sealed, thick walled reaction vials gave the desired diastereomerically pure dinucleotides **18a** and **18b** in 50% and 67% yields, respectively (Scheme 18).



**Scheme 18.** Palladium catalysed cross-coupling reactions using vinylbromide **7** (1.0 eq) and diastereomerically pure *H*-phosphonate **17(fast)** (1.01 eq) and **17(slow)** (1.08 eq). (i) Pd(OAc)<sub>2</sub> (0.1 eq), Ph<sub>3</sub>P (0.4 eq), propylene oxide (20 eq), THF, 70°C, 15 h, 50% and 67% respectively.

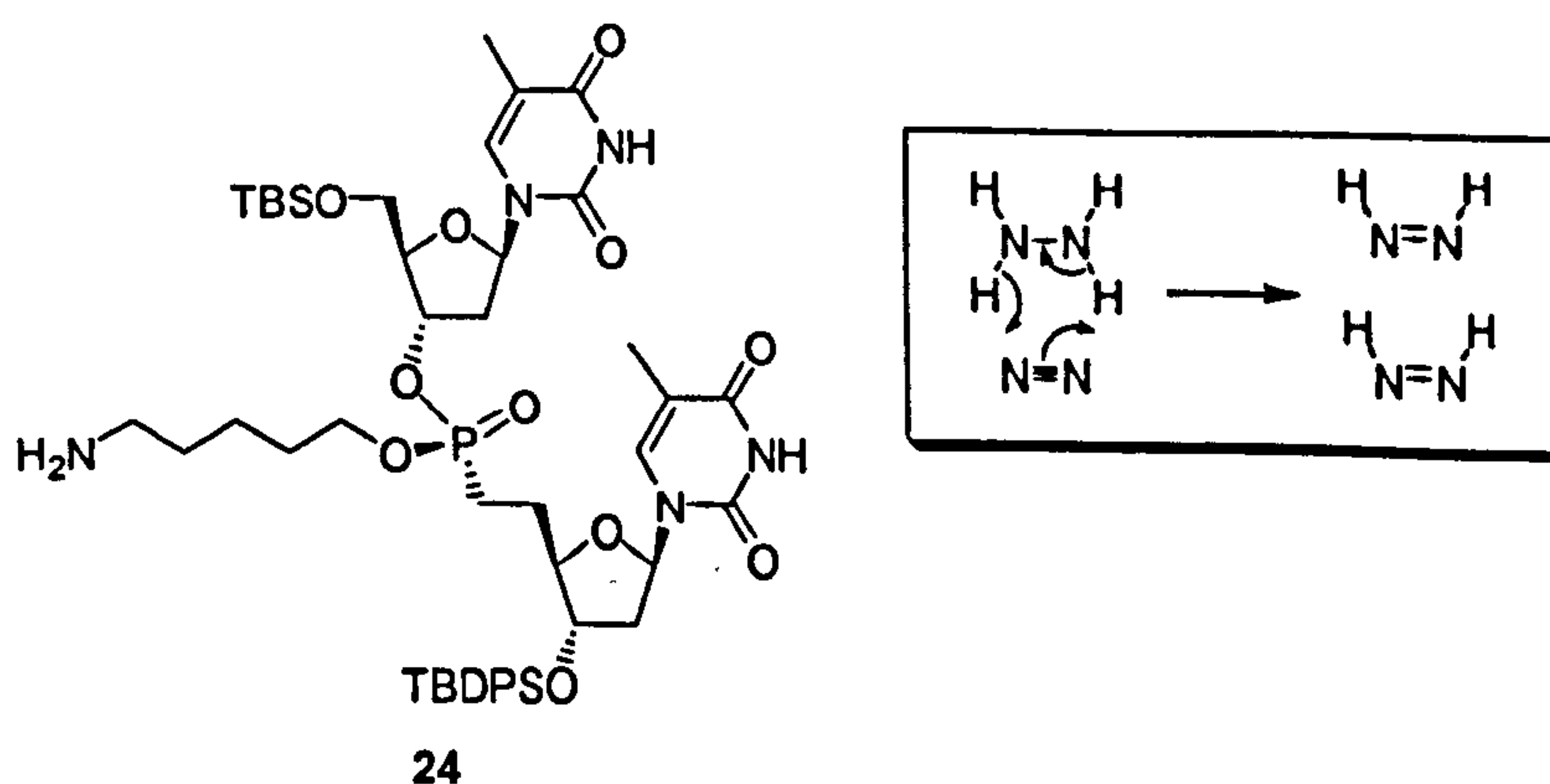
With the dinucleotides **18a** and **18b** in hand, we needed to prove that it was possible to conjugate these with other molecules *via* the amine moiety. For this purpose, we first needed to selectively deprotect the amine of both diastereomers. These deprotections were accomplished on the 3'-O-TBDPS protected dinucleotides **18a** and **18b** with hydrazine monohydrate in methanol using literature procedures<sup>43</sup> to give the free amines **23a** and **23b** (Scheme 19).





Scheme 19. Selective deprotection of the amine. (i)  $\text{H}_2\text{N}-\text{NH}_2 \cdot \text{H}_2\text{O}$  (30 eq), MeOH, RT, 2 h.

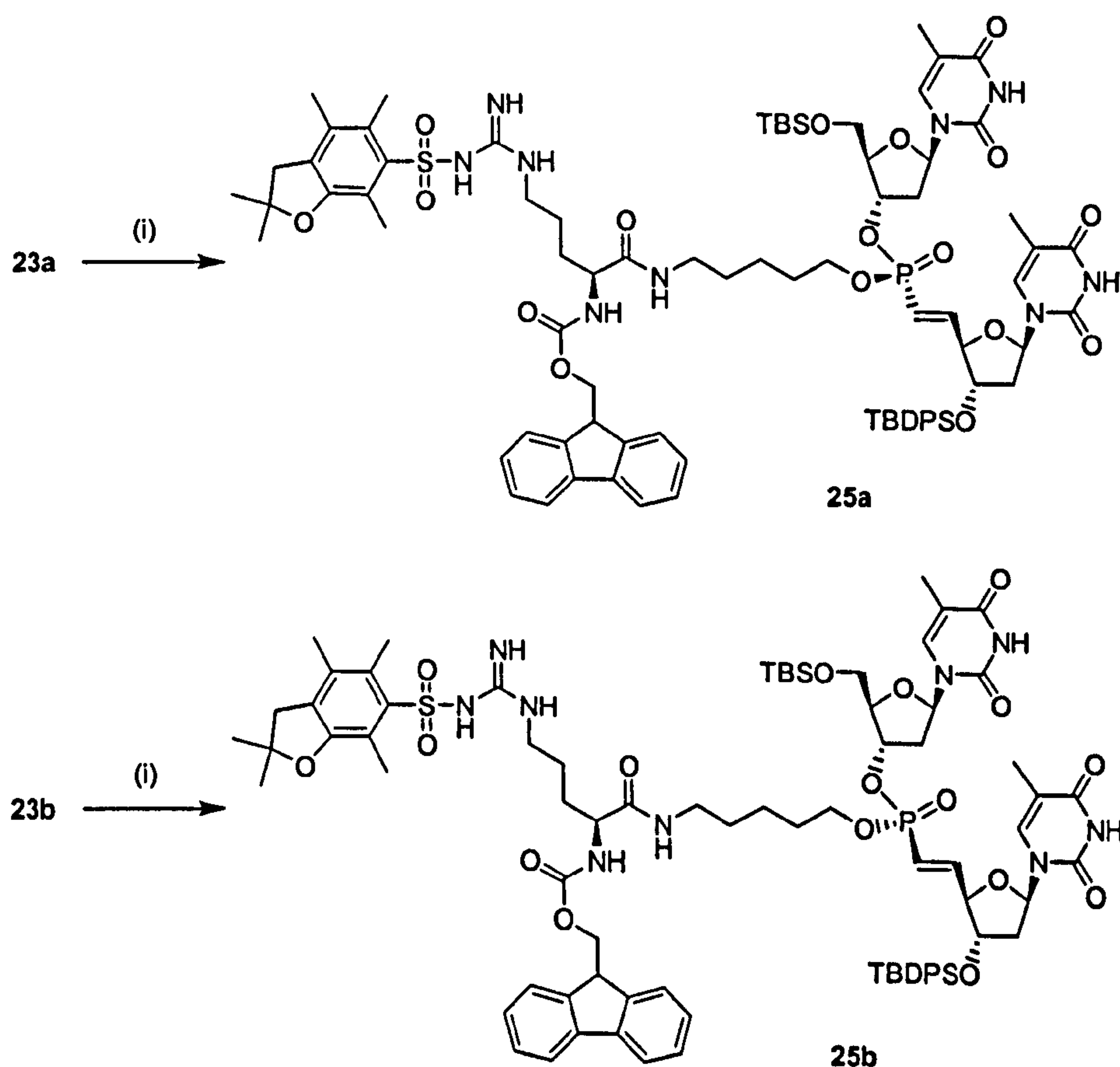
Interestingly, it was observed that longer periods of reaction (15 h) caused the reduction of the double bond of the vinylphosphonate moiety to give **24**, whose structure was confirmed by  $^1\text{H}$ -NMR and MS (Scheme 20). It is known that double bonds are reduced under mild conditions with hydrazine in the presence of nitrogen due to the formation of the reducing agent diimide.<sup>45</sup>



Scheme 20. Reduced product **24** after 15 h reaction and mechanism for the formation of diimide.

The reduction of the double bond was not a problem because the deprotection of the amines could be followed easily by electrospray MS which showed that this reaction finished after 2 h.

After the successful deprotection of the amines, the next step was to attempt the conjugation of a molecule to these amines. As mentioned in the introduction, molecular transporters are molecules that enable or enhance cellular uptake. Some of these molecules are polypeptides (peptoid molecular transporters), and one that presents excellent activity is polyarginine.<sup>40</sup>



**Scheme 21.** Peptide coupling of 23a and 23b with diprotected arginine. (i) Fmoc-Arg(Pbf)-OH (1.1 eq), EDC (1.1 eq), BtOH (1.1 eq), NMM (1.5 eq), DCM, 0°C to RT, 15 h, 49% and 67% respectively from 18a and 18b.

Due to the molecular transporter characteristics of polyarginine and the complex structure of arginine, this amino acid was chosen as a good candidate to attempt this reaction. Pleasingly, treatment of the crude amines **23a** and **23b** with diprotected arginine under peptide coupling conditions, gave the coupled products **25a** and **25b** in 49% and 67% overall yield over two steps (amine deprotection and peptide coupling) (Scheme 21).

These results successfully prove that it is possible to attach complex molecules to this type of modified dinucleotide. The introduction of these dimers into an oligonucleotide sequence using a DNA synthesiser remains to be accomplished.



## 2.2 Approaches to the Synthesis of Building Block II

After the successful preparation of building block I, the synthesis of building block II, in which the linker and the amine are attached to a backbone carbon atom (Figure 18), was examined.

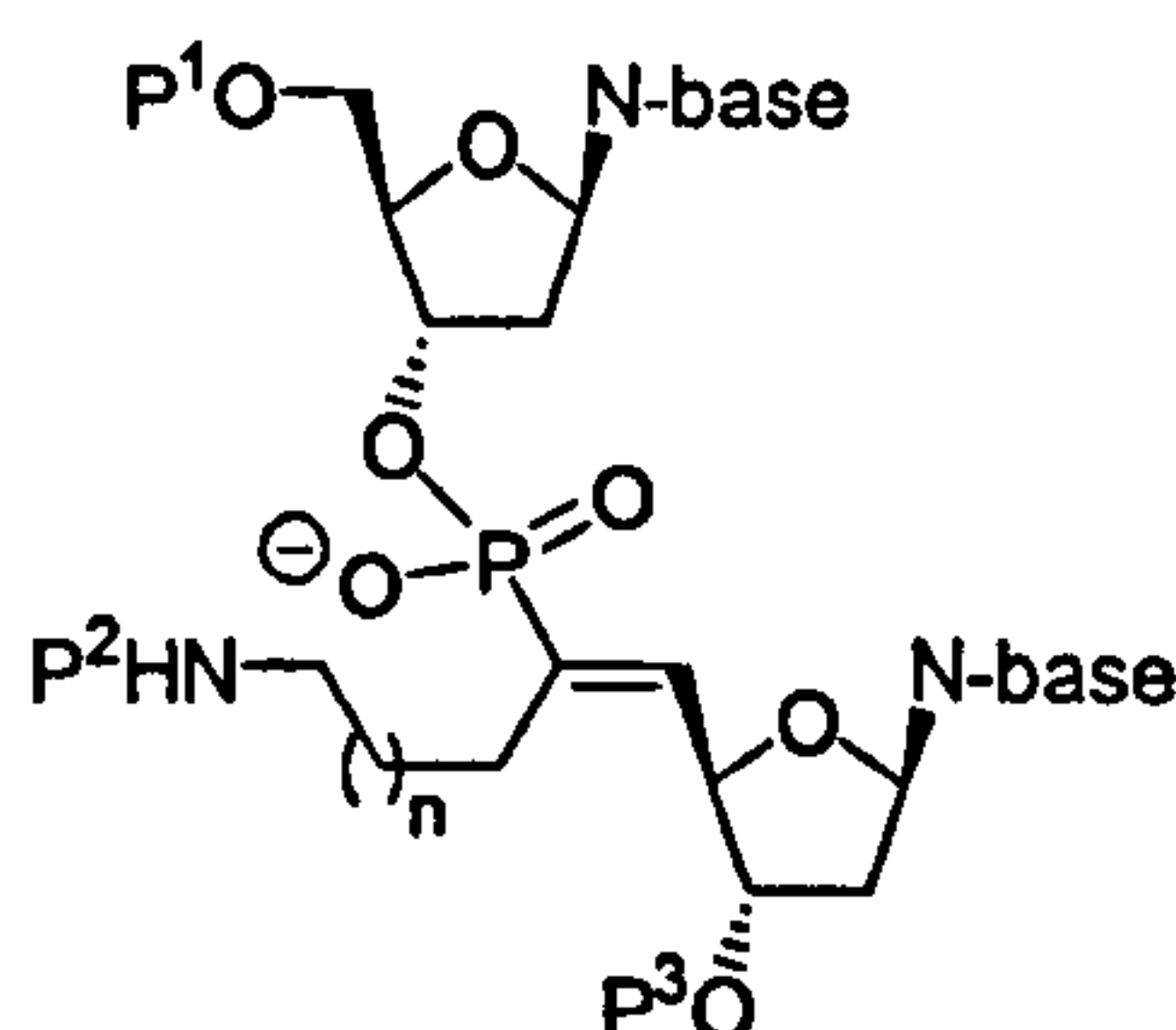
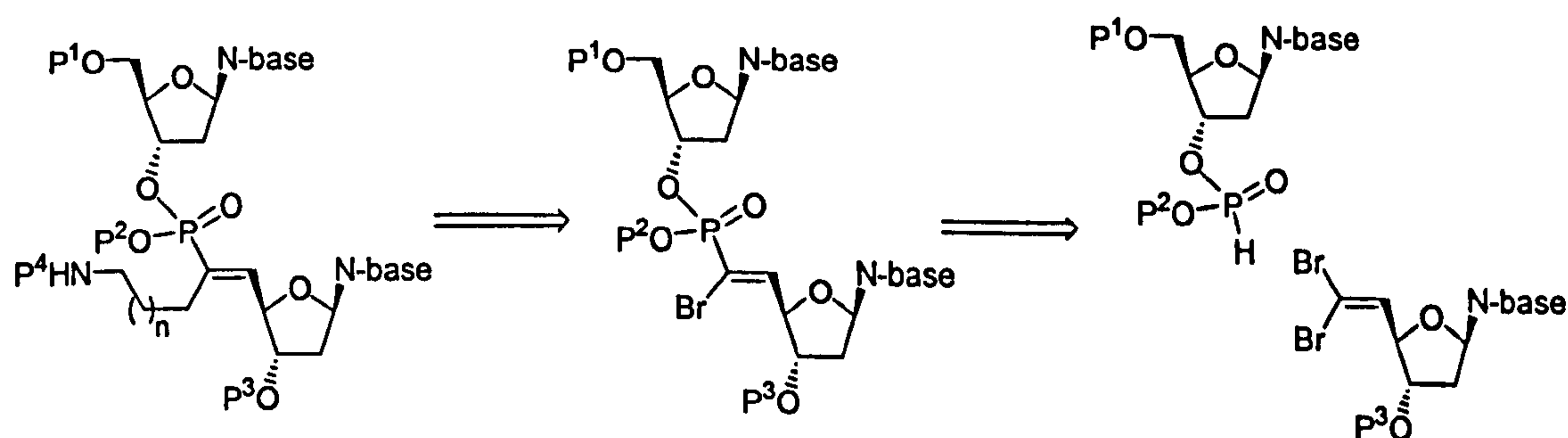


Figure 18. Structure of the building block II.

### 2.2.1 Palladium Catalysed Cross-Coupling Approach

#### 2.2.1.1 Synthesis of Alkynylphosphonates

Scheme 22 illustrates the proposed retrosynthetic analysis for the synthesis of building block II.

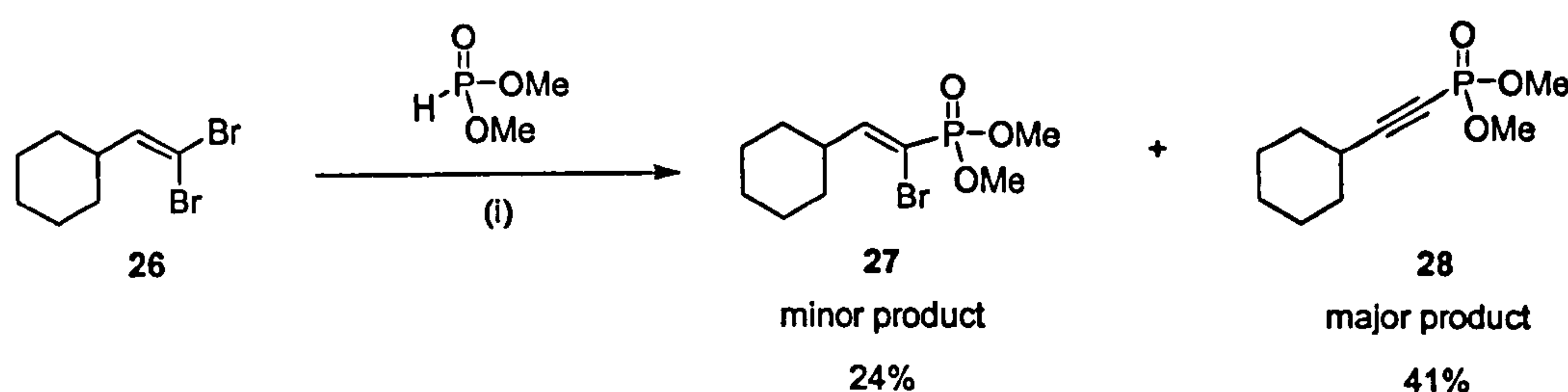


Scheme 22. Retrosynthetic analysis proposed for building block II.

Building block II could potentially be synthesised from a vinylphosphonate dinucleotide which contains a vinylbromide that may allow the introduction of the linker and the amine *via* a Suzuki<sup>46</sup> or Stille<sup>47</sup> coupling. The vinylbromide dinucleotide could in turn be prepared *via* a palladium catalysed cross-coupling reaction similar to

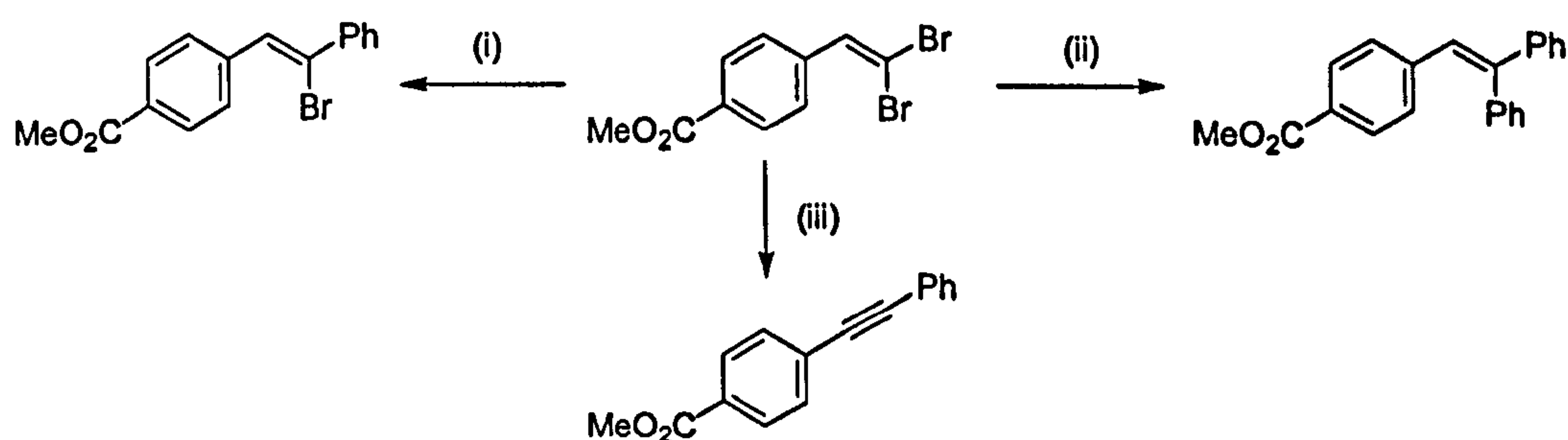
the reaction seen for the preparation of building block I, the difference being that instead of a mono-vinylbromide a 1,1-dibromoalkene is used.<sup>48</sup>

The first reaction attempted was a model reaction between dibromoalkene **26**\*\* and dimethyl phosphite, and surprisingly the major product isolated was the alkynylphosphonate **28** (Scheme 23).



Scheme 23. (i) Pd(OAc)<sub>2</sub> (0.2 eq), dppf (0.4 eq), propylene oxide (3 eq), dimethyl phosphite (2 eq), THF, 70°C, 15 h, 24% yield of **27** and 41% yield of **28**.

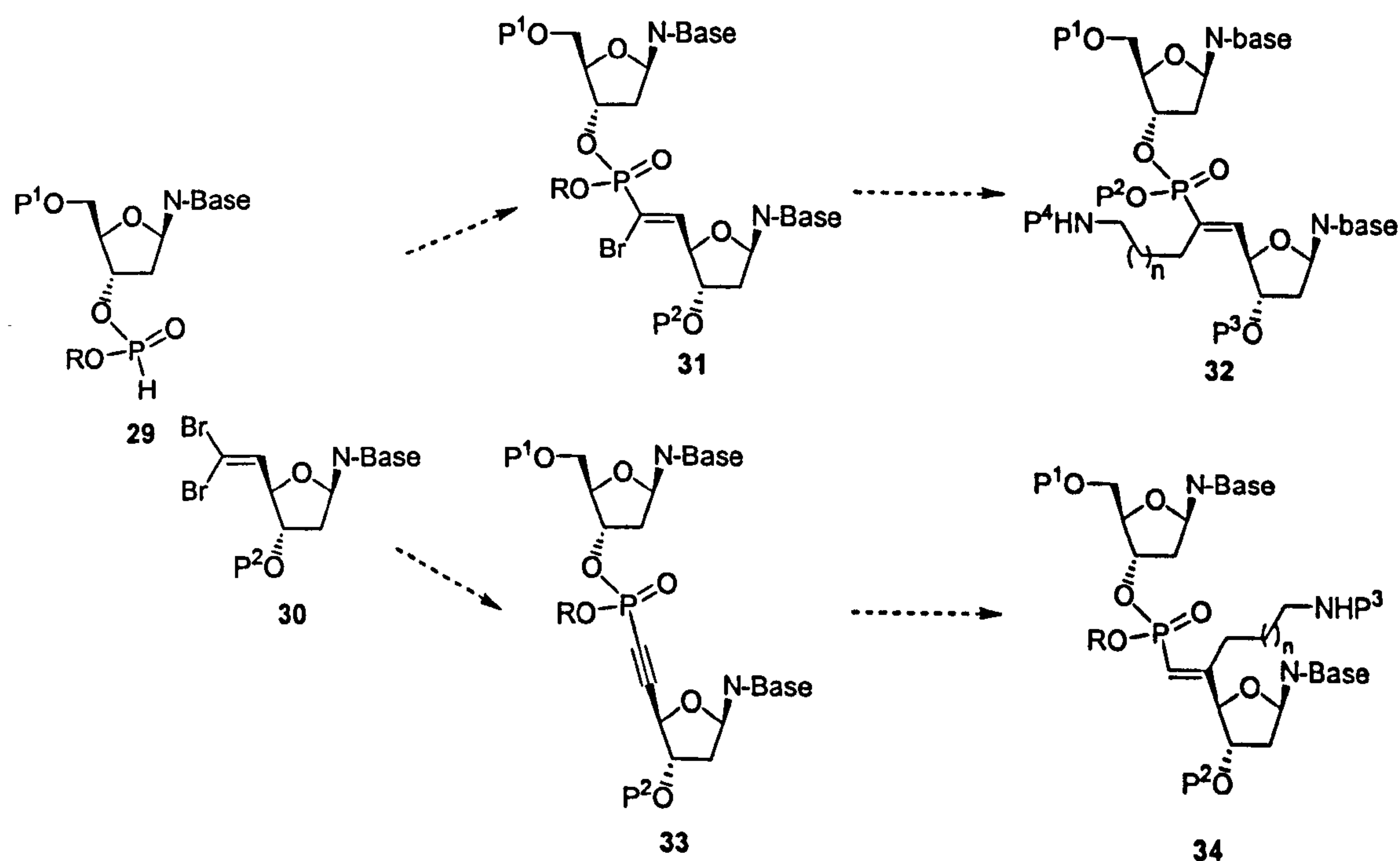
A literature search revealed an article in which Shen *et al.*<sup>49</sup> synthesised selectively mono- and di-coupled products or acetylenes by Stille coupling using 1,1-dibromoalkenes and vinylstannanes (Scheme 24). They found that the polarity of the solvent was crucial for the selective formation of the different type of compounds.



Scheme 24. (i) PhSnMe<sub>3</sub> (1.05 eq), Pd<sub>2</sub>(dba)<sub>3</sub> (0.025 eq), TFP (0.15 eq), PhMe, 100°C, 20 h, 92%. (ii) PhSnMe<sub>3</sub> (2.2 eq), Pd<sub>2</sub>(dba)<sub>3</sub> (0.025 eq), TFP (0.15 eq), PhMe, 105°C, 48 h, 100%. (iii) PhSnMe<sub>3</sub> (1.05 eq), Pd<sub>2</sub>(dba)<sub>3</sub> (0.025 eq), TFP (0.15 eq), DIPEA (1.5 eq), DMF, 80°C, 10 h, 91%.

\*\* 1,1-dibromoalkenes were prepared from the corresponding aldehydes using the Corey-Fuchs procedure.

Both types of transformations are very important for the present work. Using the nucleotide substrates **29** and **30**, a monocoupling reaction would form **31**, a possible precursor to synthesise building block II (**32**) (Scheme 22), and the acetylene formation is important for a number of reasons. Firstly, an alkynylphosphonate modified dinucleotide **33** has not yet been prepared and constitutes an interesting backbone modification in nucleic acid chemistry. Secondly, vinylphosphonates are not good Michael acceptors, whereas Michael additions using alkynylphosphonates have been performed.<sup>50</sup> This could provide us with other interesting building block (**34**) in which the linker and the amine are attached to the other  $sp^2$  carbon atom (Scheme 25).

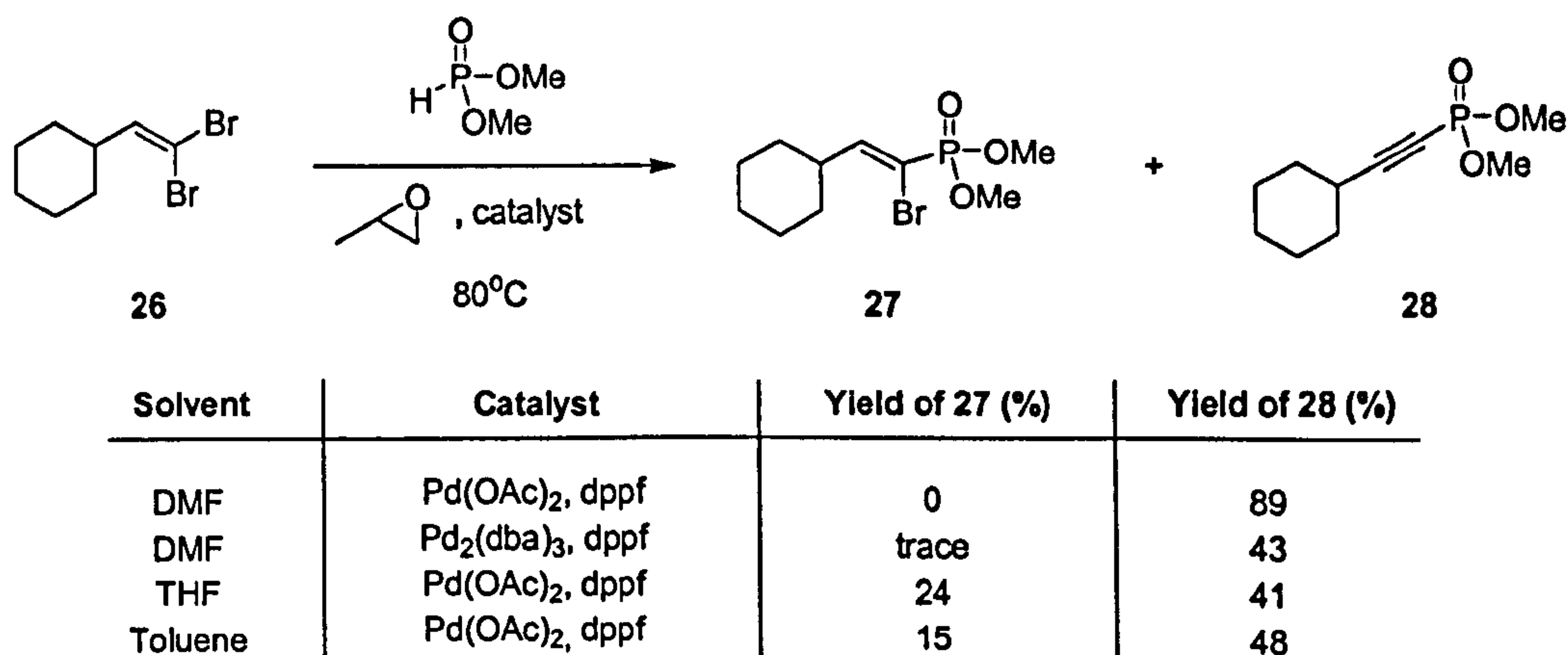


**Scheme 25.** Possible products that could be obtained *via* the palladium catalysed cross-coupling reaction using a 1,1-dibromoalkene and the possible formation of building block II (**32**) and building block **34**.

Due to its potential, the reaction shown in Scheme 23 was examined using different conditions, and pleasingly the use of palladium acetate, dppf, and DMF at 80°C



selectively formed alkynylphosphonate **28** in good yield. The results of this study are shown in Table 1.



**Table 1.** Different conditions used for the selective synthesis of the alkynylphosphonate **28**. Pd(OAc)<sub>2</sub> or Pd<sub>2</sub>(dba)<sub>3</sub> (0.2 eq), dppf (0.4 eq), propylene oxide (3.0 eq), dimethyl phosphite (2.0 eq), 80°C, 15 h.

Table 1 shows that there is a solvent effect on the yield and product ratio and that DMF was the best solvent to use for the selective formation of the alkynylphosphonate. Using these conditions, the transformation was successfully accomplished with a number of substrates and the results are summarised in Table 2.<sup>51</sup>

#### 2.2.1.1.1 Results using dppf as the Ligand

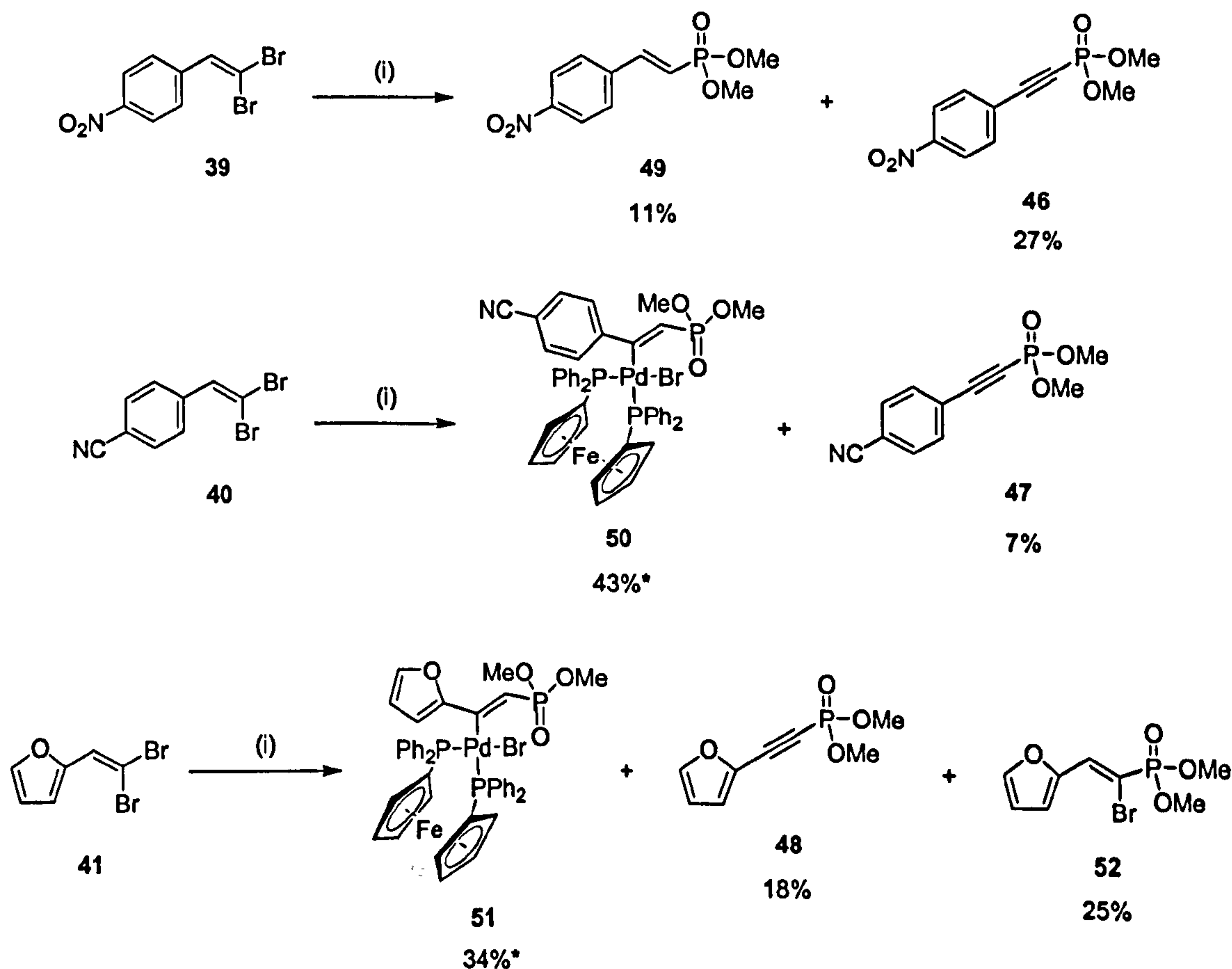
Using dppf with alkyl-substituted dibromoalkenes (Table 2, entries 1 and 2), the alkynylphosphonates **28** and **42** were obtained in good yield (89% and 66%, respectively). Aryl dibromides with *para* electron donor groups **36** and **37** (Table 2, entries 3 and 4) and phenyl-substituted dibromide **38** (Table 2, entry 5) also gave the corresponding alkynylphosphonates **43**, **44** and **45** in good yield (73%, 68% and 63%, respectively).

Entry	R	Dibromo	L	Yield (%)	Product
1		26	dppf TFP	89 33	28
2		35	dppf TFP	66 27	42
3		36	dppf	73	43
4		37	dppf	68	44
5		38	dppf	63	45
6		39	dppf TFP	27 31	46
7		40	dppf TFP	7 29	47
8		41	dppf TFP	18 60	48

**Table 2.** Synthesis of alkynylphosphonates. Pd(OAc)<sub>2</sub> (0.2 eq), ligand (L): dppf (0.4 eq) or TFP (0.8 eq), propylene oxide (3.0 eq), dimethyl phosphite (2.0 eq), DMF, 80°C, 15 h.

In all of the above cases the alkynylphosphonate was the only product isolated after the reaction. However, when the palladium catalysed cross-coupling reaction was attempted with dppf using electron deficient aryl (Table 2, entries 6 and 7) or furyl-substituted dibromides (Table 2, entry 8), lower yields of the corresponding alkynylphosphonates were obtained (27%, 7% and 18%) and other products were formed. In the case of the *p*-nitrophenyl dibromide 39 (Table 2, entry 6) vinylphosphonate 49 was formed in 11% yield. Using a *p*-cyanophenyl substituted dibromide (Table 2, entry 7) only a small amount of acetylene 47 was produced, along with a major product which had a very complicated <sup>1</sup>H- and <sup>13</sup>C-NMR. Fortunately,

crystals were grown and its structure was determined by X-ray crystallography (Figure 19a). When this reaction was attempted using a furan substituent (Table 2, entry 8) three products were obtained. The monocoupling product **52** was the major product formed in this reaction, and its structure and stereochemistry were confirmed by X-ray crystallography (Figure 20). The other two products were the desired acetylene **48** and a small amount of the palladium complex **51** whose structure was again elucidated by X-ray crystallography (Figure 19b) and was found to be analogous to the structure of the palladium complex **50** (Figure 19a). The results of entries 6, 7 and 8 (Table 2) using dppf as the ligand are summarised in Scheme 26.



Scheme 26. Different products obtained in the palladium catalysed cross-coupling reactions Table 2, entries 6, 7 and 8 using dppf. (i) Pd(OAc)<sub>2</sub> (0.2 eq), dppf (0.4 eq), propylene oxide (3.0 eq), dimethyl phosphite (2.0 eq), DMF, 80°C, 15 h.\* Yield based in the amount of Pd(OAc)<sub>2</sub>.



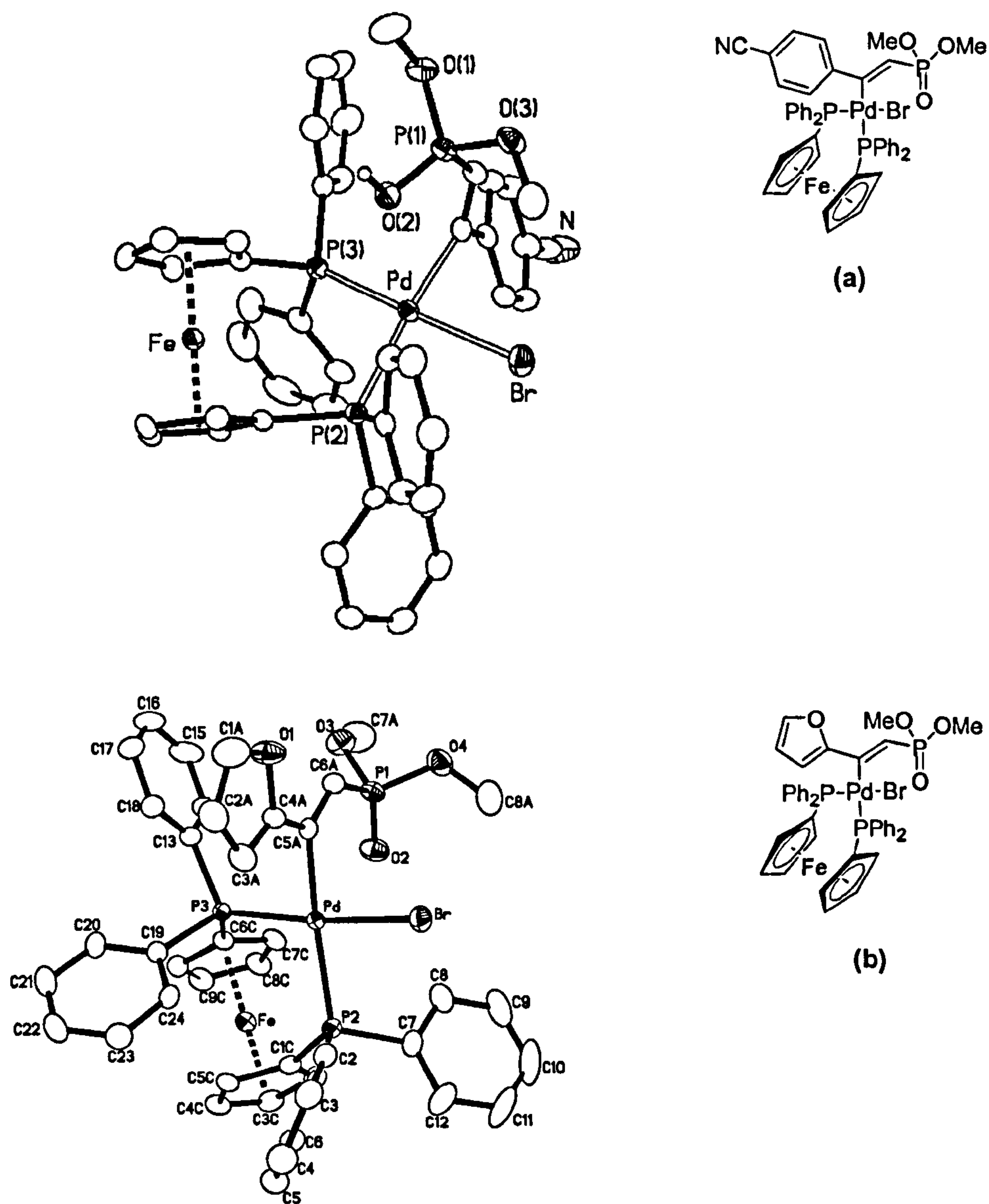


Figure 19. X-ray crystal structures of the palladium complexes 50 (a) and 51 (b).

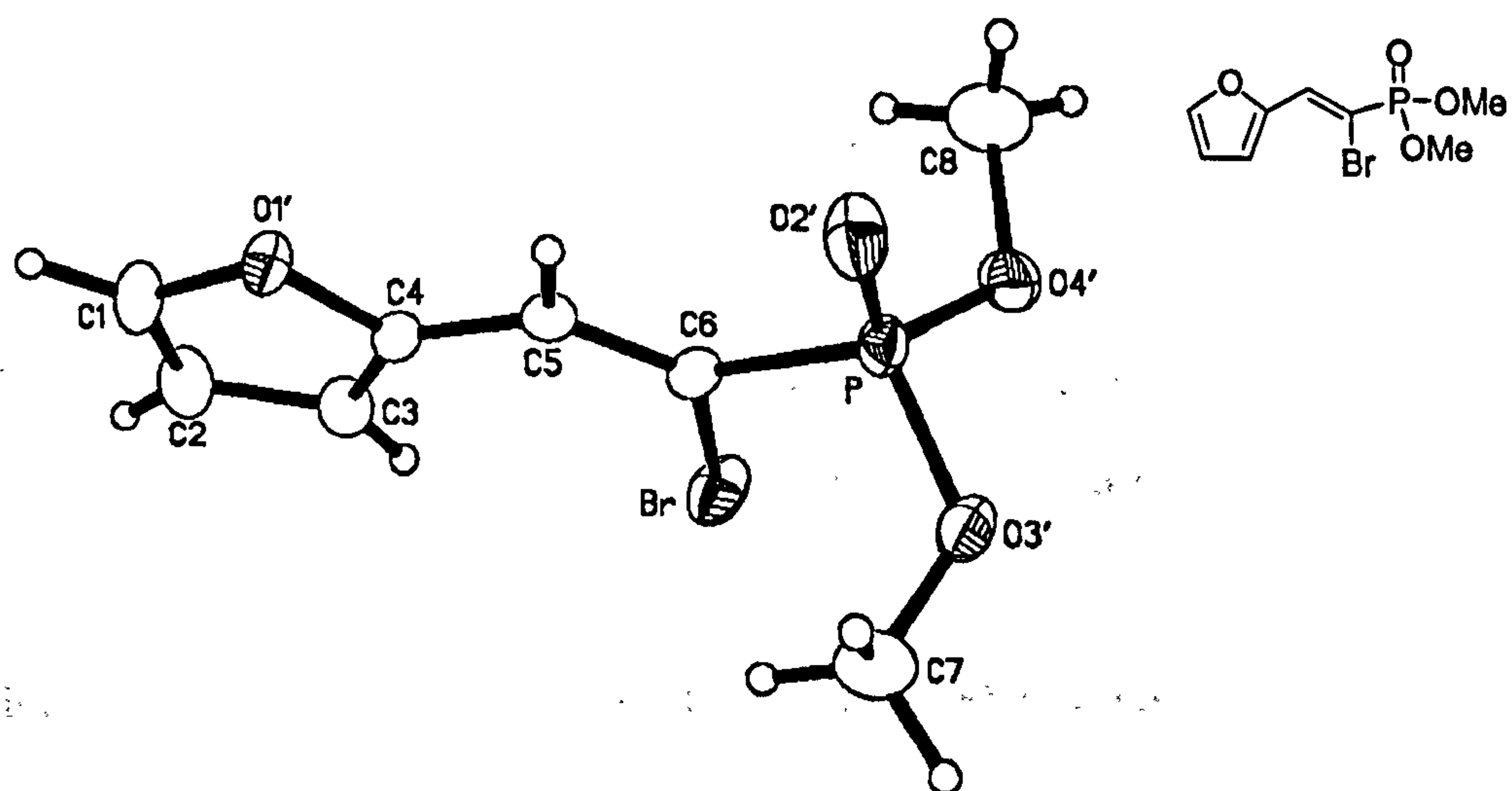
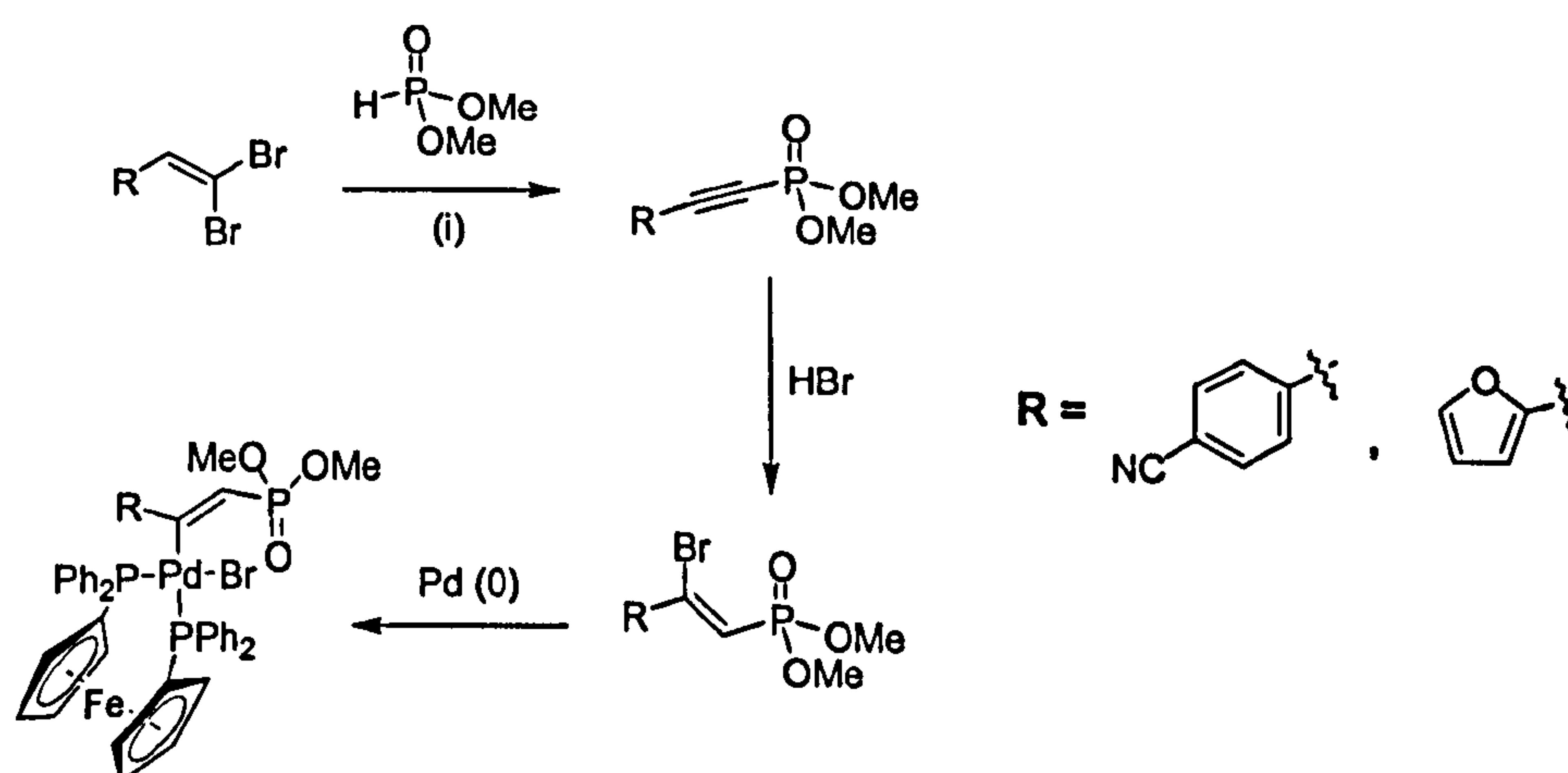


Figure 20. X-ray crystal structure of the monocoupling product 52.

The palladium complexes shown in Figure 19 have been formed using the *p*-cyanophenyl and the furan substituted dibromides **40** and **41** (Table 2, entries 7 and 8), however using *p*-nitrophenyl dibromide **39** (Table 2, entry 6) vinylphosphonate **49** was obtained, which could come from the protonation of a similar palladium species. This could mean, that the formation of a palladium complex could have taken place in the three cases where the palladium catalysed cross-coupling reaction using dppf afforded low yields of the corresponding alkynylphosphonates.

The most interesting feature observed in the palladium complexes **50** and **51** (Figure 19) is that the palladium atom is attached to the  $\beta$ -carbon instead to the  $\alpha$ -carbon. Our explanation for the formation of these two complexes is that in both cases the desired alkynylphosphonates were formed in the reaction, however they reacted further. They could react *via* an addition of HBr followed by a palladium oxidative insertion into the C-Br bond (Scheme 27).



**Scheme 27.** Possible explanation for the formation of the palladium complexes **50** and **51**. (i)  $\text{Pd}(\text{OAc})_2$  (0.2 eq), dppf (0.4 eq), propylene oxide (3.0 eq), dimethyl phosphite (2.0 eq), DMF,  $80^\circ\text{C}$ , 15 h.

Interestingly, the palladium complexes **50** and **51** are stable enough to survive column chromatography and they did not react further with another molecule of dimethyl

phosphite, in spite of the addition of two equivalents of this reagent. This fact could be explained by an impossible access to the Pd metal due to steric hindrance. One face of the square-planar palladium structure is blocked by the phosphonate group and the other by the aromatic groups of dppf (See X-rays crystal structures in Figure 19).

#### 2.2.1.1.2 Results using TFP as the Ligand

In the article mentioned previously by Shen *et al.*,<sup>49</sup> TFP was generally used as the ligand in the Stille coupling reactions with 1,1-dibromo compounds. Thus, it was decided to examine the palladium catalysed cross-coupling reaction using this ligand.

Interestingly, using TFP better yields were obtained and cleaner reactions took place using electron deficient aryl (Table 2, entries 6 and 7) or furyl-substituted dibromides (Table 2, entry 8). Using the aryl dibromides 39 and 40 (Table 2, entries 6 and 7) compounds 46 and 47 were obtained in 31% and 29% yield respectively, and in the case of the furyl-substituted dibromide 41 (Table 2, entry 8) alkynylphosphonate 48 was formed in 60% yield.

After these results, the palladium catalysed cross-coupling reaction using TFP was examined with 1,1-dibromoalkenes which gave a good yield in the alkynylphosphonate formation with dppf. Dibromo compounds 26 and 35 were exposed to the reaction conditions using TFP affording the corresponding alkynylphosphonates 28 and 42 in lower yields (33% and 27%, respectively).

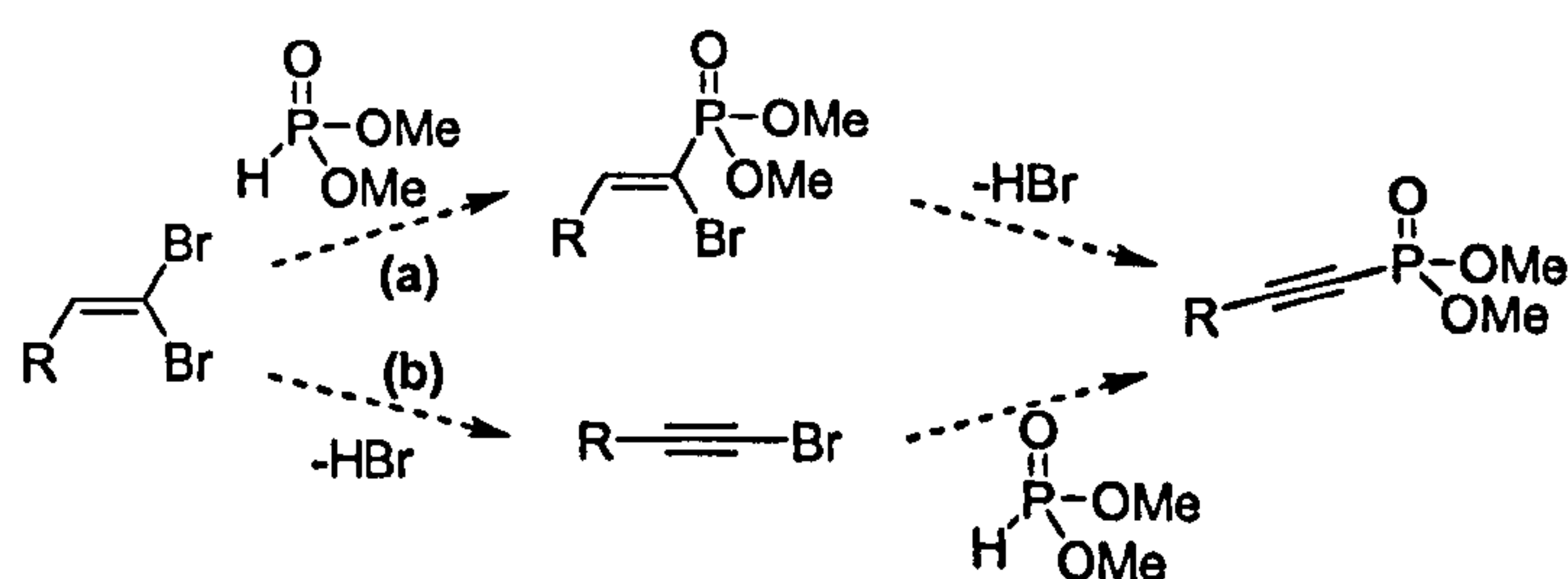
The use of TFP as the ligand has improved the yield and the ratio of the desired alkynylphosphonates in the cases where using dppf the reactions did not work well. However, with substrates that afforded good yields of the alkynylphosphonate with dppf, the use of TFP gave lower yields of the products suggesting that there is a



correlation between the substrate and the ligand and that there is not a general ligand for this transformation.

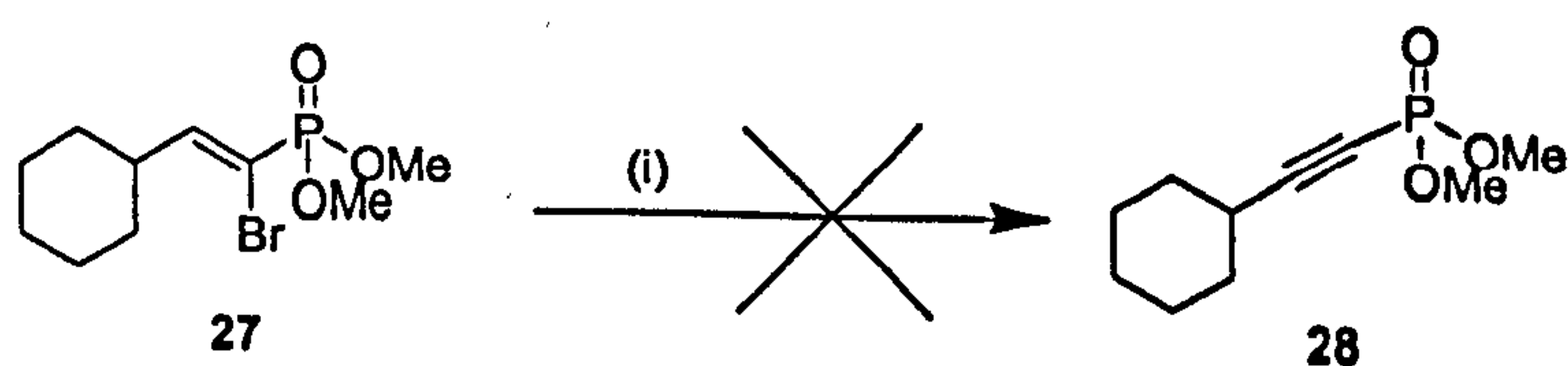
### 2.2.1.2 Mechanistic Studies

At this point, the mechanism of acetylene formation was investigated. It is relatively easy to propose a mechanism in which the alkynyl phosphonate is formed from the monocoupled product *via* an elimination of HBr, with or without promotion by palladium (Scheme 28a). It also could be possible that the elimination of HBr is the first step in the reaction and the resulting alkynyl bromide undergoes coupling with the *H*-phosphonate to give the acetylene (Scheme 28b).



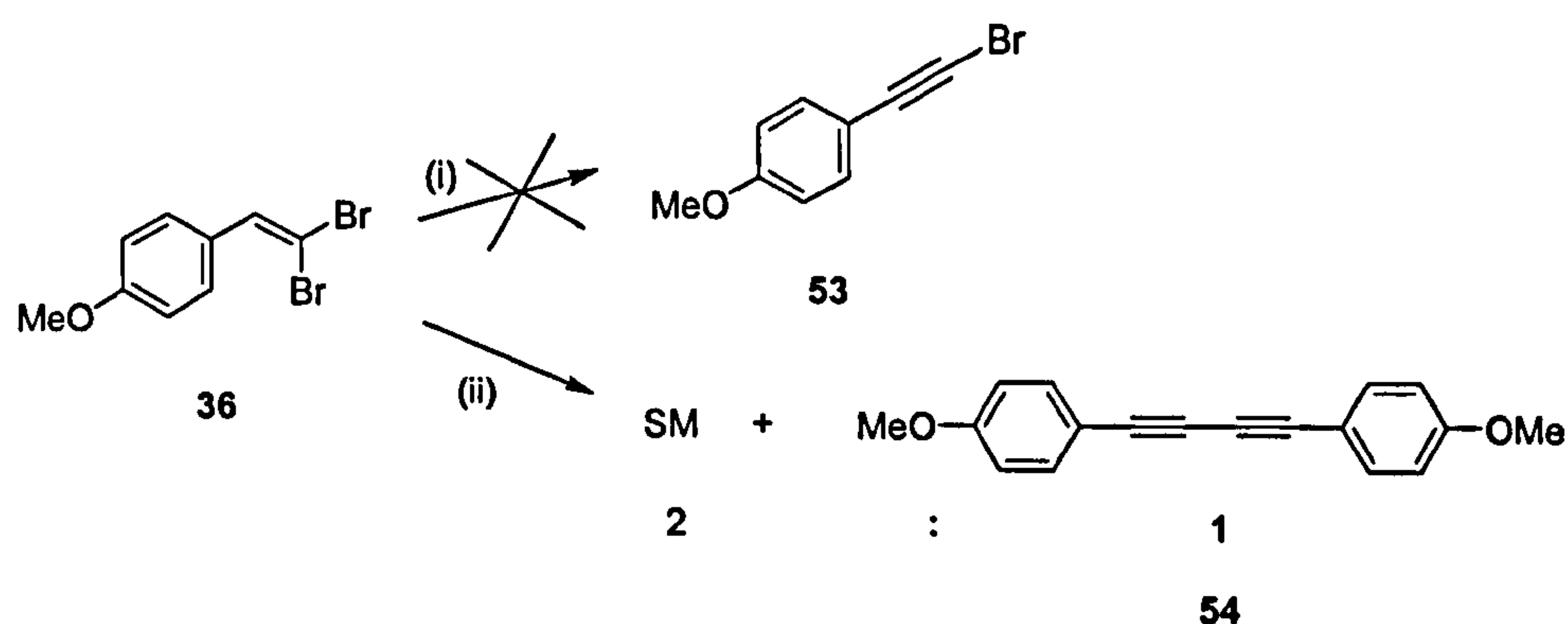
Scheme 28. Possible mechanisms for the formation of the alkynylphosphonates.

The following reactions were performed with the aim of elucidating the correct mechanism. Monocoupled product **27** was exposed to the palladium coupling conditions to try to prove mechanism (a) and only starting material was obtained suggesting that the monocoupled products are not the precursors of the alkynylphosphonates (Scheme 29).



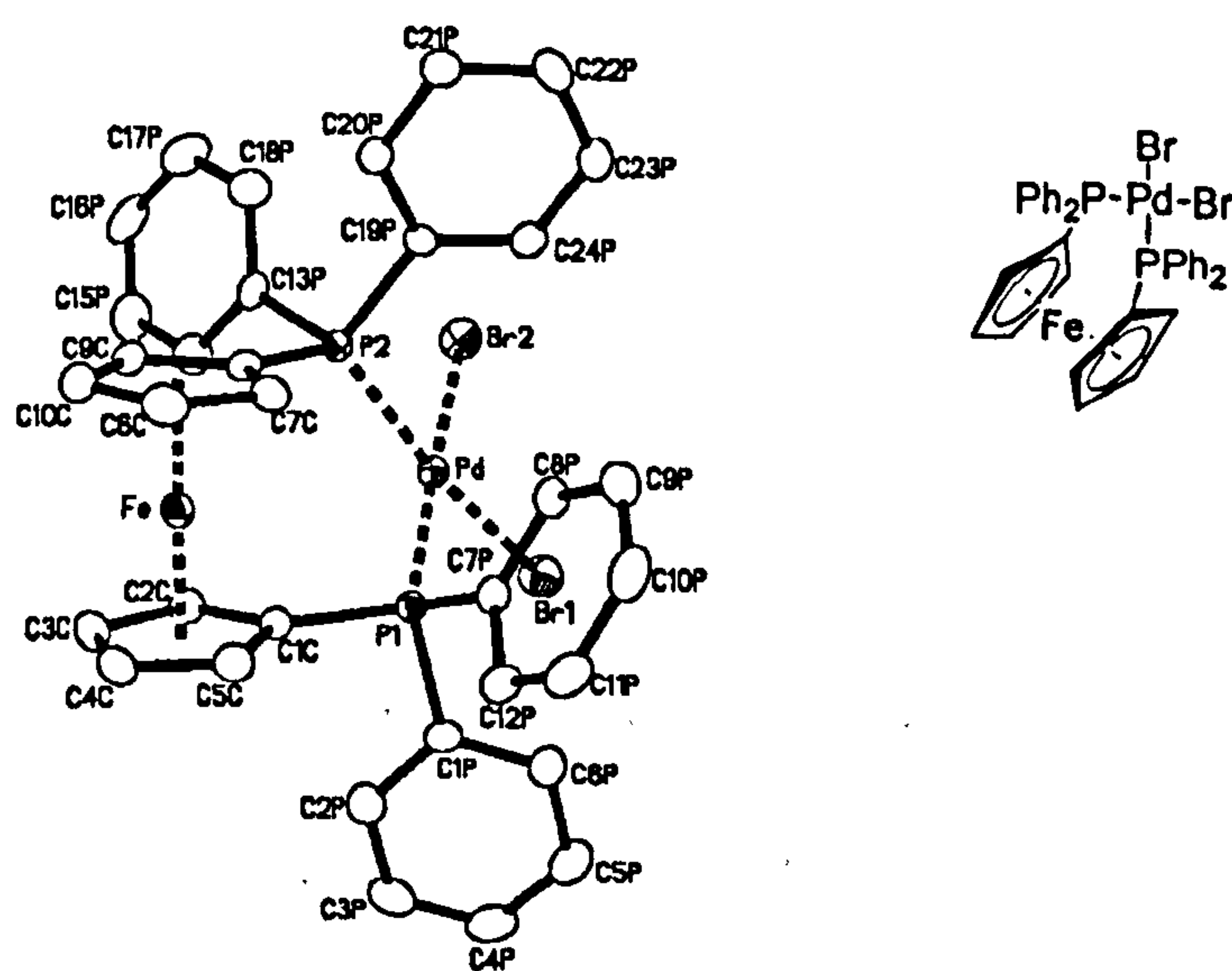
Scheme 29. Attempt of the synthesis of alkynylphosphonate **26** from the monocoupled product **25**. (i) Pd(OAc)<sub>2</sub> (0.2 eq), dppf (0.4 eq), propylene oxide (3.0 eq), DMF, 80°C, 15 h.

With the aim of proving mechanism (b) the reactions shown in Scheme 30 were attempted. The elimination of HBr was attempted in the absence of the catalytic system to see if propylene oxide alone was responsible for the elimination of HBr. The reaction was also attempted in the presence of the catalyst  $[\text{Pd}(\text{OAc})_2 / \text{dppf}]$  to see if the elimination of HBr is a palladium catalysed process.



**Scheme 30.** Attempts to eliminate HBr from 1,1-dibromoalkene 36. (i) Propylene oxide (3.0 eq), DMF, 80°C, 15 h. (ii)  $\text{Pd}(\text{OAc})_2$  (0.2 eq), dppf (0.4 eq), propylene oxide (3.0 eq), DMF, 80°C, 15 h.

When the reaction was performed without the catalyst, only starting material was recovered suggesting that the elimination of HBr is not possible when only propylene oxide is present in the reaction, however the formation of the compound 54 does not eliminate the possibility of a catalytic elimination.

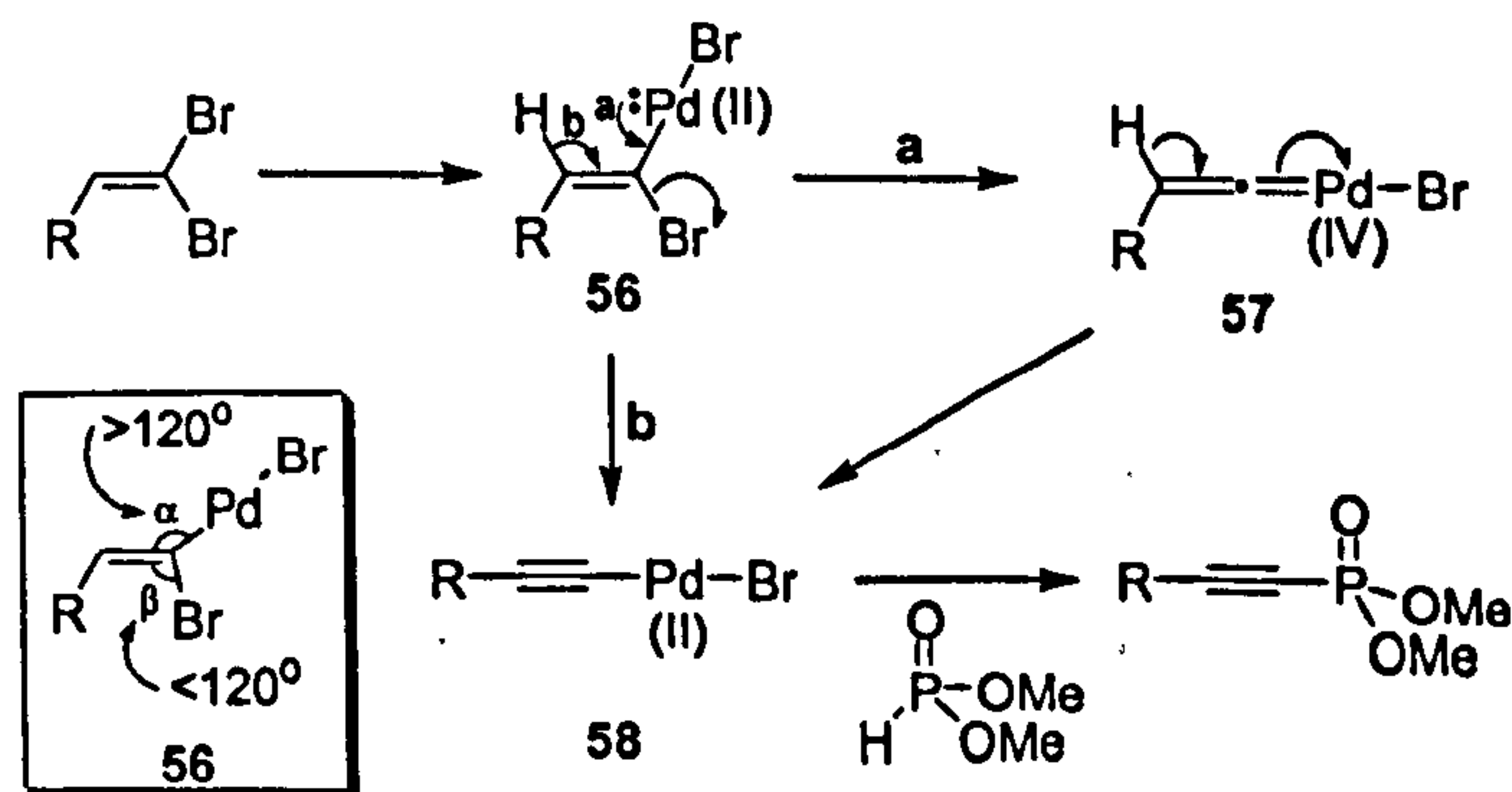


**Figure 21.** X-ray crystal structure of the palladium complex 55.

In all of the reactions to give alkynylphosphonates the palladium complex **55** was detected, the structure of which was confirmed by X-ray crystallography (Figure 21). This complex could be the end of the catalytic system in all of these transformations, but unfortunately it does not give us any information about the mechanism.

The mechanism of the alkynylphosphonate formation is unclear and we propose the two mechanisms (a and b) shown in the Scheme 31. Firstly, the Pd(0) is inserted into the *trans* C-Br bond of the 1,1-dibromoalkene and the resulting Pd(II) species **56** undergoes elimination of HBr *via* the mechanism a or the mechanism b to give the palladium-alkyne complex **58** which is coupled with the *H*-phosphonate to form the alkynylphosphonate.

In the mechanism a HBr is eliminated in two steps, a bromide is first eliminated to give the palladium (IV) carbenoid species **57** which undergoes the rearrangement shown in the scheme to give the palladium-alkyne complex **58**. In the mechanism b, complex **58** is directly formed from the Pd(II) species **56**. Based in literature precedents,<sup>52</sup>  $\alpha$ -halopalladium complexes such as **56**, have unusual values for the  $\alpha$  and  $\beta$  angles (Scheme 31). The elimination of HBr in these structures could be facilitated by the fact that the value for the  $\alpha$  angle is usually higher than  $120^\circ$  and the value for the  $\beta$  angle is usually lower than  $120^\circ$ .



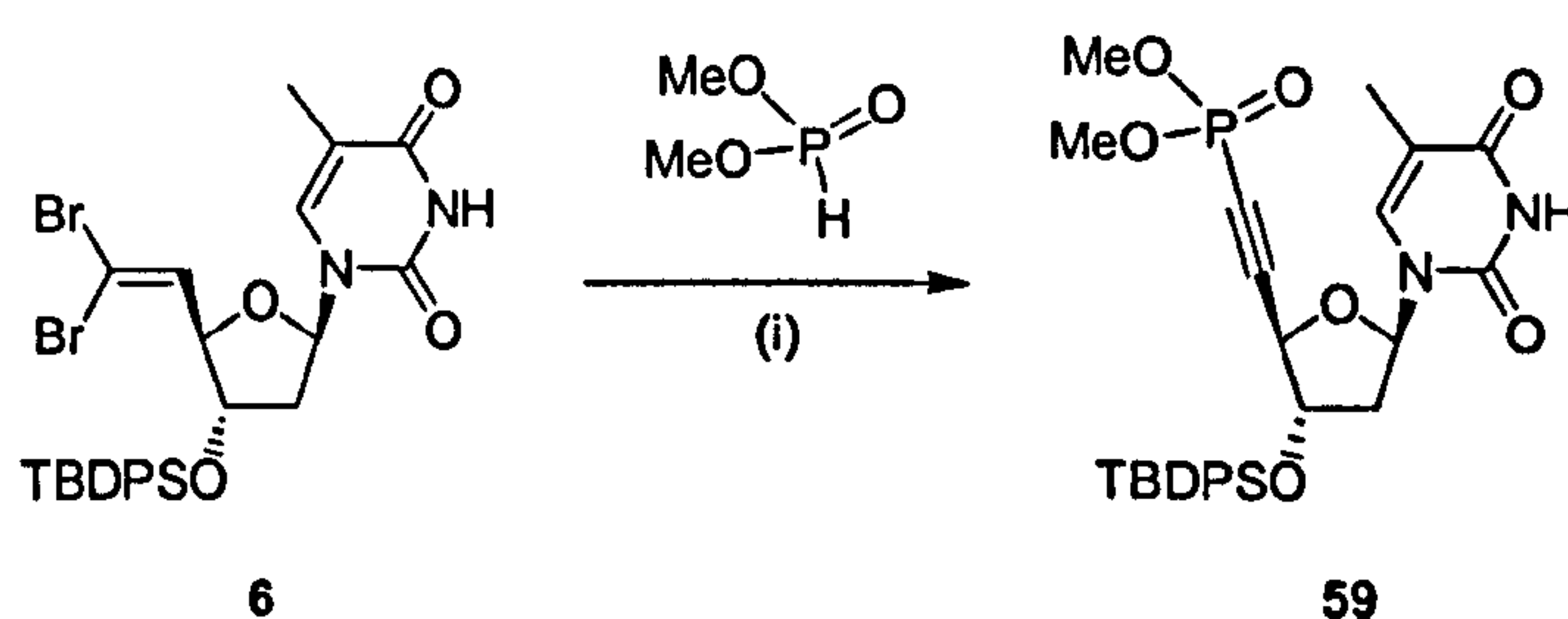
Scheme 31. Mechanisms proposed for the formation of the alkynylphosphonates.



### 2.2.1.3 Synthesis of an Alkynylphosphonate Dinucleotide

With the successful conditions for the synthesis of alkynylphosphonates in hand, the synthesis of a dinucleotide containing this modification was examined.

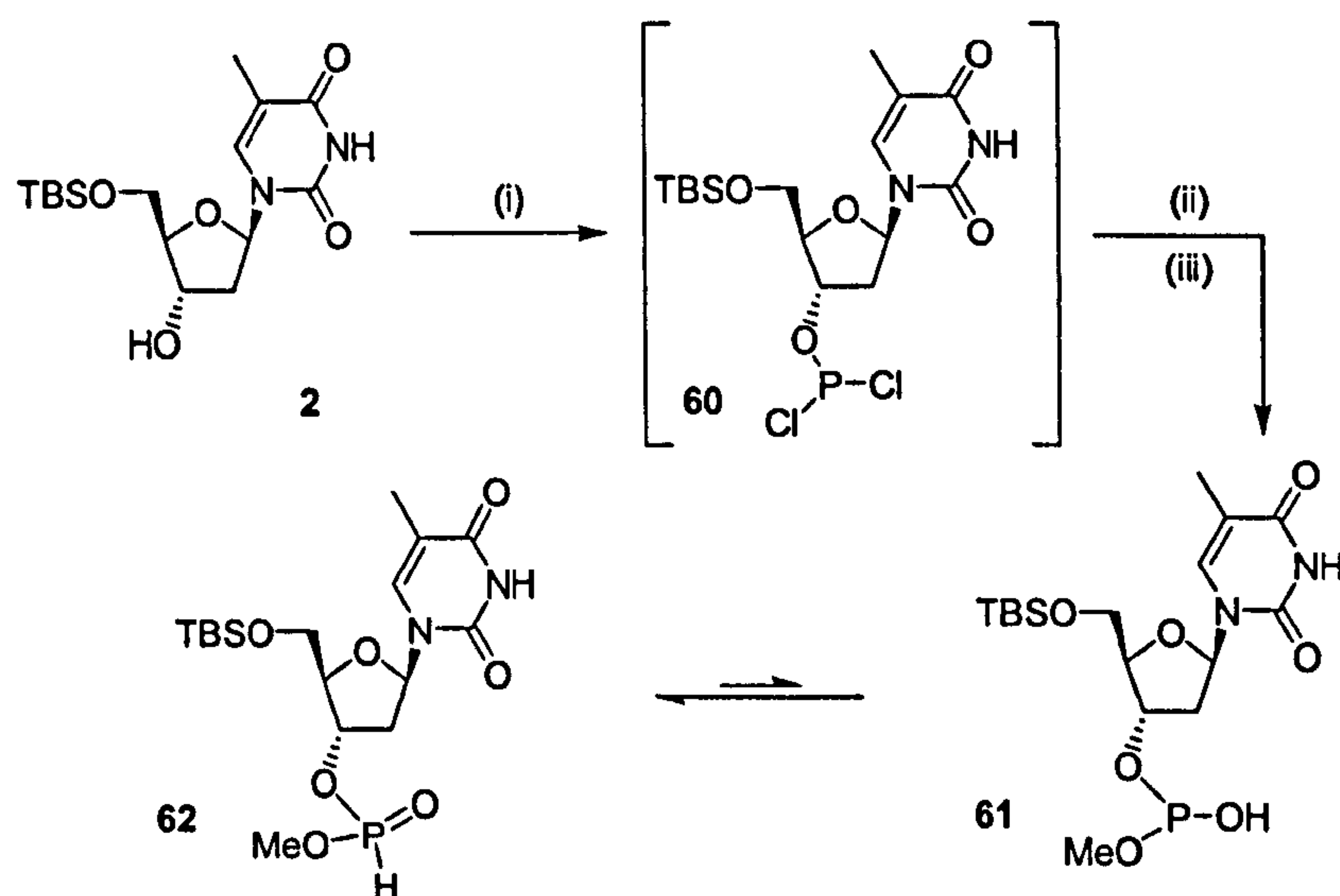
First, a model reaction using 1,1-dibromonucleotide **6** with dimethylphosphite was attempted to see if the conditions were tolerant to the functionality present in nucleotides (Scheme 32).



**Scheme 32.** Model reaction using 1,1-dibromonucleotide **6** and dimethylphosphite. (i) Pd(OAc)<sub>2</sub> (0.2 eq), dppf (0.4 eq), propylene oxide (3.0 eq), DMF, 80°C, 15 h, 63%.

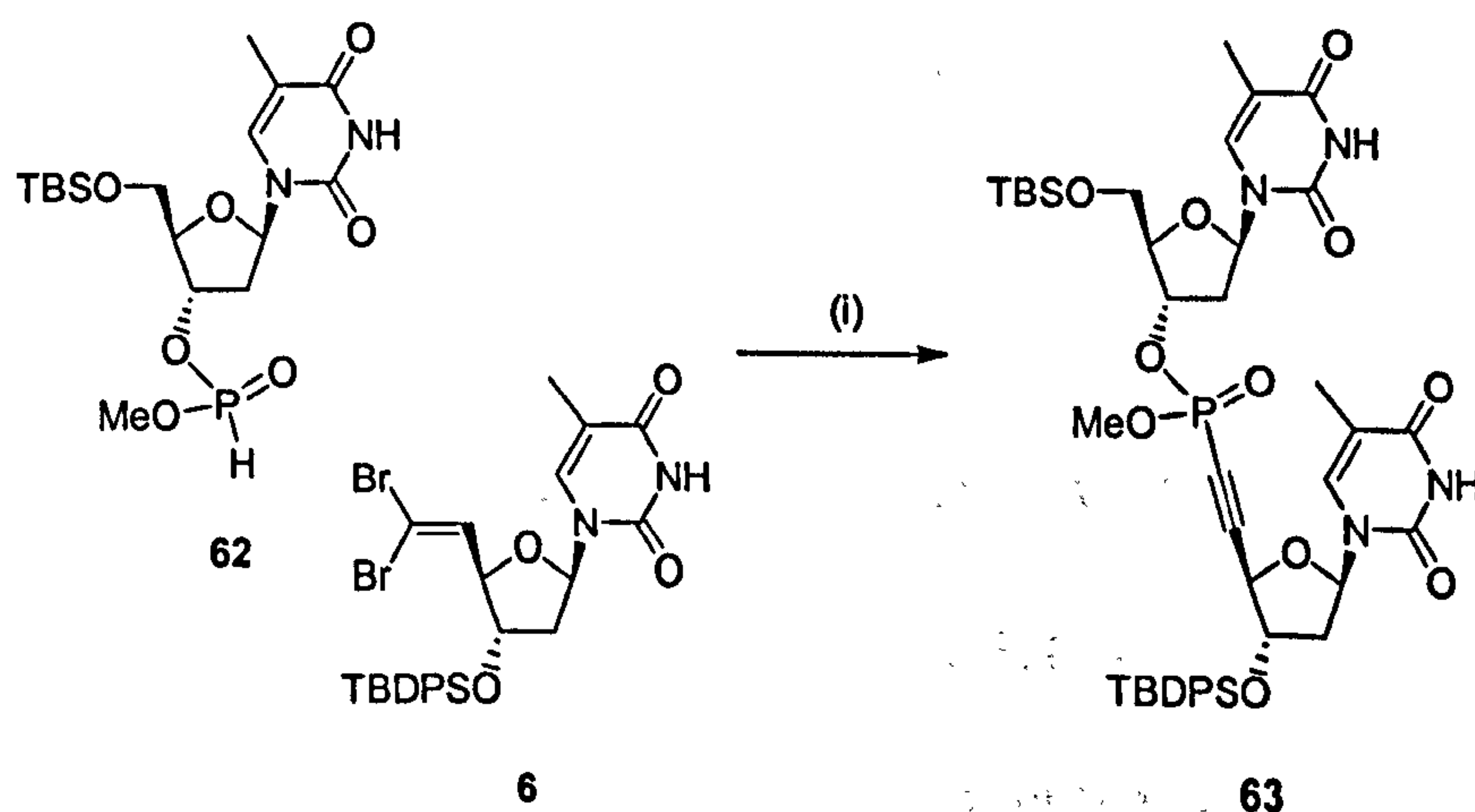
Pleasingly, when dibromo **6** was exposed to the palladium catalysed cross-coupling reaction conditions, the desired alkynylphosphonate **59** was formed in good yield (63%).

Having shown that the thymidine-derived dibromide **6** successfully coupled with dimethyl phosphite, the next step was to attempt this reaction with the more functionalised *H*-phosphonate **62**. For this purpose, it was necessary first to synthesise *H*-phosphonate **62**, and this compound was prepared using the methodology developed for the synthesis of *H*-phosphonate **17** (Scheme 13). Instead of alcohol **14**, methanol was used to give the desired methyl *H*-phosphonate **62** as a 1:1 mixture of diastereomers in 60% overall yield. These reactions are illustrated in the following Scheme.



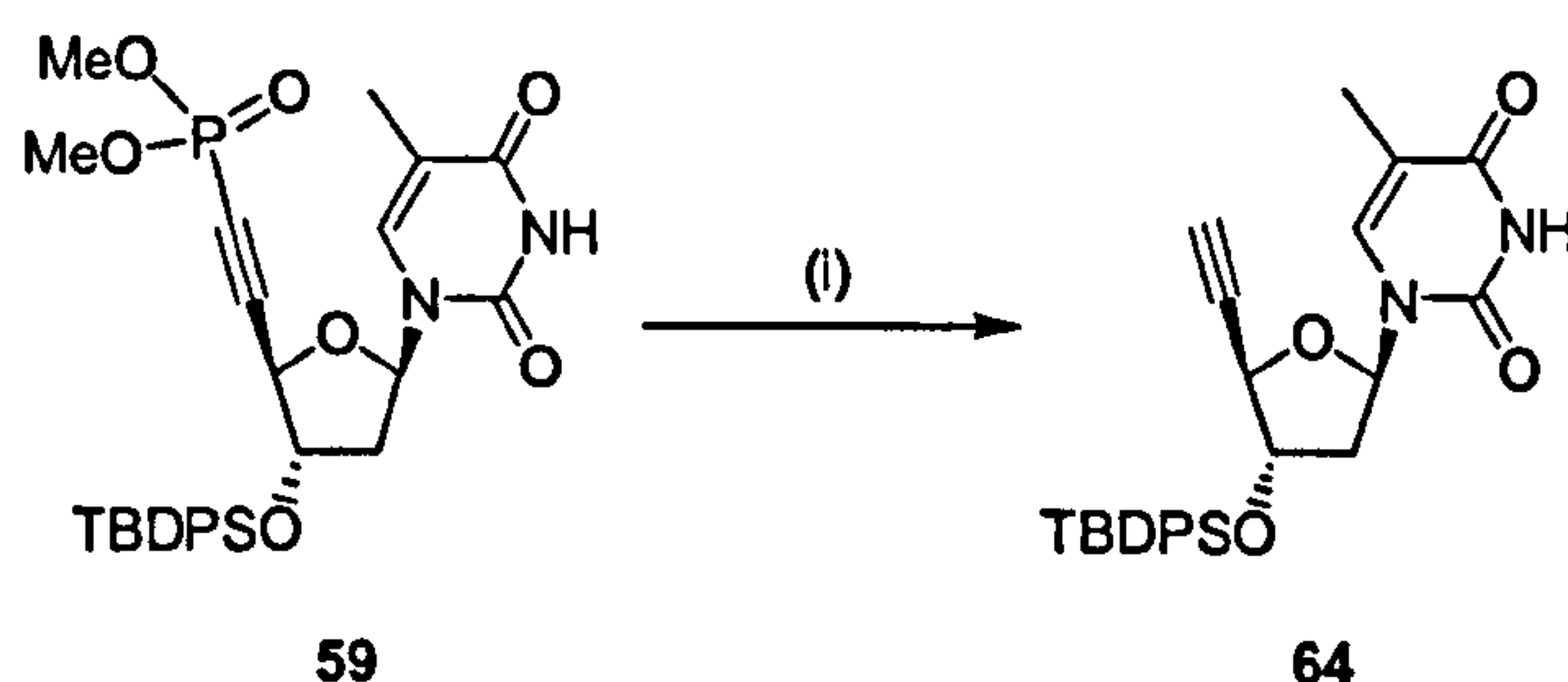
**Scheme 33.** (i)  $\text{PCl}_3$  (4.5 eq), pyridine (5 eq), THF,  $-78^\circ\text{C}$  to reflux, 3.3 h; (ii) MeOH (1 eq), pyridine (5 eq), THF, RT to reflux, 1 h; (iii)  $\text{H}_2\text{O}$ , RT, 5 min, 60% over 3 steps, 1:1 mixture of diastereomers at P.

With methyl *H*-phosphonate **62** in hand, the synthesis of a modified nucleotide dimer was attempted, and pleasingly the desired alkyne-containing thymidine dimer **63** was formed in 51% yield (+12% of recovered **6**) as a 1:1 mixture of diastereomers which were readily separated by column chromatography (Scheme 34).<sup>51</sup> Considering the complexity of this product, we believe that this result is particularly impressive and it clearly demonstrates the potential of this methodology to allow access to highly functionalised targets.



**Scheme 34.** (i)  $\text{Pd}(\text{OAc})_2$  (0.2 eq), dppf (0.4 eq), propylene oxide (3.0 eq), **62** (1.4 eq), DMF,  $80^\circ\text{C}$ , 15 h, 51% (+12% of recovered **6**), 1:1 mixture of diastereomers separable by column chromatography.

As alkynylphosphonates are known Michael acceptors,<sup>50</sup> model reactions using **59** to investigate the possibility of introducing nucleophiles into this system were attempted. Heating alkynylphosphonate **59** in the presence of amyl amine, unfortunately afforded decomposition and treatment of **59** with the organocopper compound derived from <sup>n</sup>BuLi and CuI gave the terminal alkyne **64** (Scheme 35).



**Scheme 35.** Model reaction of Michael addition using alkynylphosphonate **59**. (i) CuI (3.4 eq), <sup>n</sup>BuLi (6.0 eq), THF, -78°C, 15 min, alkynylphosphonate **59** (1.0 eq), THF, -78°C to RT, 1.5 h, 38%.

The formation of terminal alkyne **64** could be explained by the fact that the organocopper species was not formed in the reaction and **64** was the result of nucleophile attack of <sup>n</sup>BuLi onto the phosphorus atom.

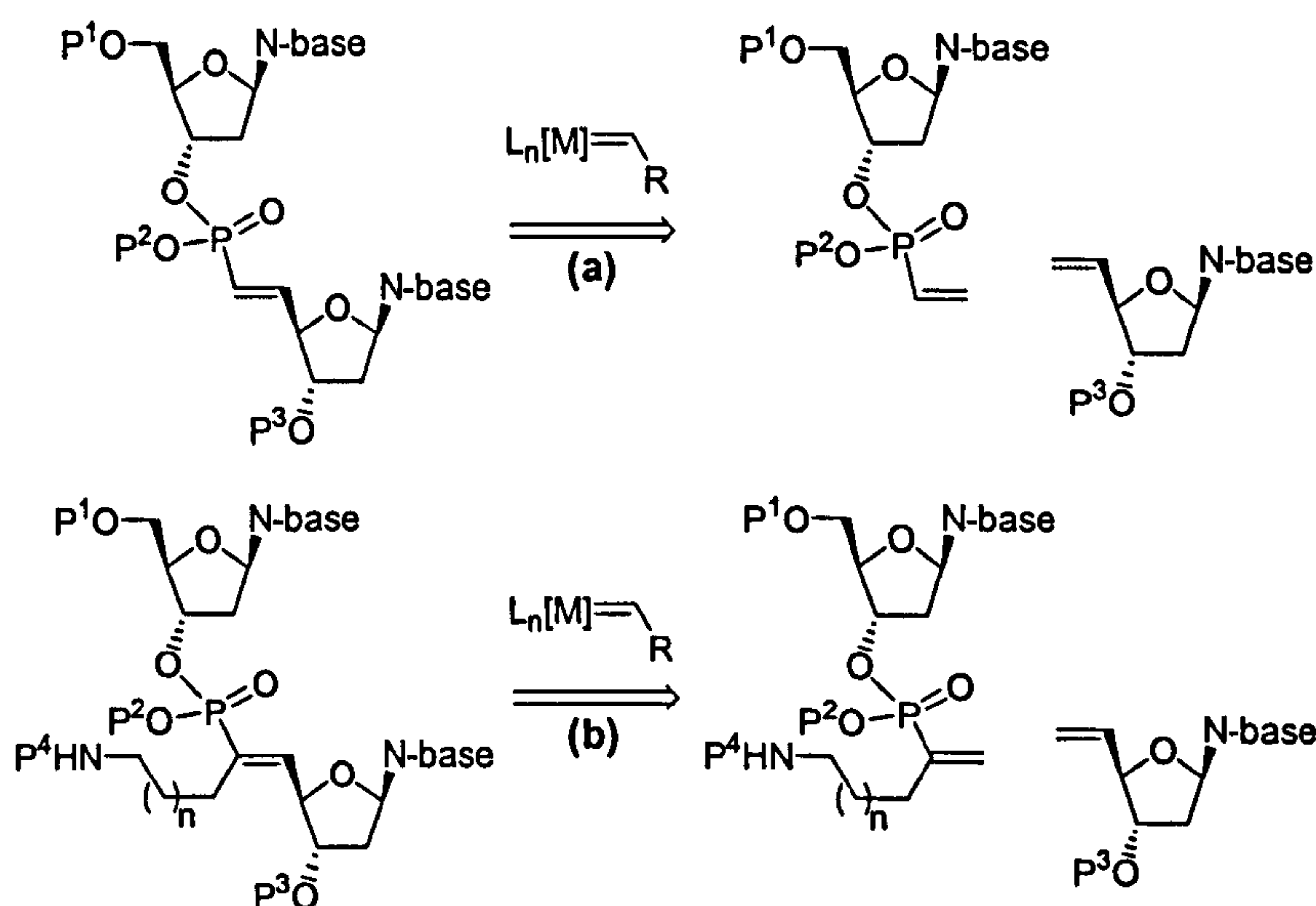
Following the problems encountered in the Michael addition experiments, a new strategy based in an olefin cross-metathesis reaction, to synthesise a building block with a linker and an amine attached to an sp<sup>2</sup> carbon atom, was considered and it will be discussed below.

### 2.2.2 Olefin Cross-Metathesis Approach. A New Method for the Synthesis of Vinylphosphonate-linked Nucleic Acids

As seen previously, the use of 1,1-dibromoalkene **6** in the palladium catalysed cross-coupling reaction with *H*-phosphonate **62** afforded alkynylphosphonate **63** instead of the desired monocoupled product necessary for the synthesis of building block II.



At this point, we wondered if it would be possible to access the vinylphosphonate internucleotide linkage using an olefin cross-metathesis reaction. This reaction was investigated with the aims of having a novel approach to these backbone modified nucleic acids (Scheme 36a) and to re-examine the synthesis of building block II (Scheme 36b).



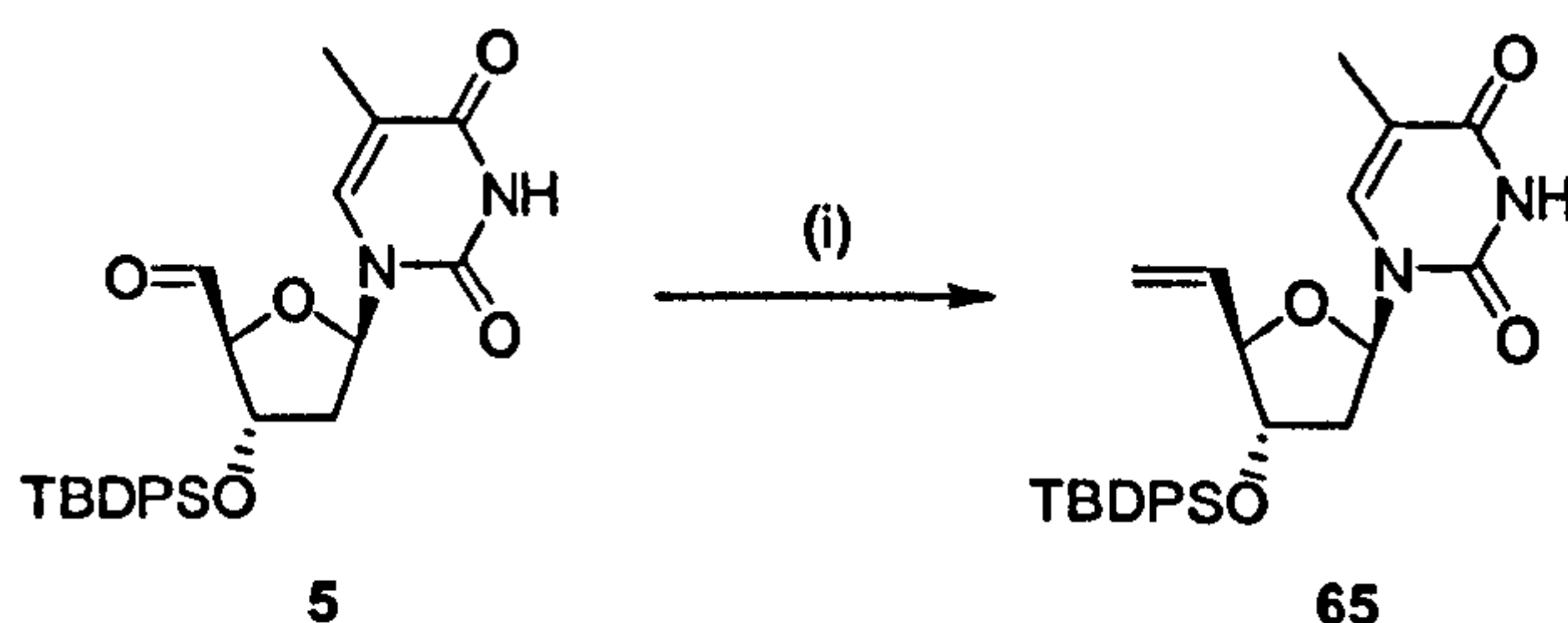
**Scheme 36.** (a) Retrosynthetic analysis proposed for the synthesis of vinylphosphonate-linked nucleic acids *via* an olefin cross-metathesis reaction. (b) Retrosynthetic analysis proposed for the synthesis of building block II.

Olefin cross-metathesis reactions usually proceed with good yields and good *E/Z* ratios.<sup>53</sup> Grubbs *et al.* have recently published a method for the synthesis of vinylphosphonates *via* an olefin cross-metathesis reaction.<sup>54</sup> A vinylphosphonate-linked dinucleotide might be synthesised by a cross-metathesis reaction between a vinylphosphonate and a terminal alkene. For the synthesis of building block II it is necessary to use 1,1-disubstituted olefins, and because olefin metathesis processes are more favourable when less substituted olefins are used due to steric effects, the

synthesis of a vinylphosphonate dinucleotide using terminal olefin containing nucleotides (Scheme 36a) was investigated first.

### 2.2.2.1 Synthesis of Vinylphosphonate-linked Nucleic Acids by Olefin Cross-Metathesis

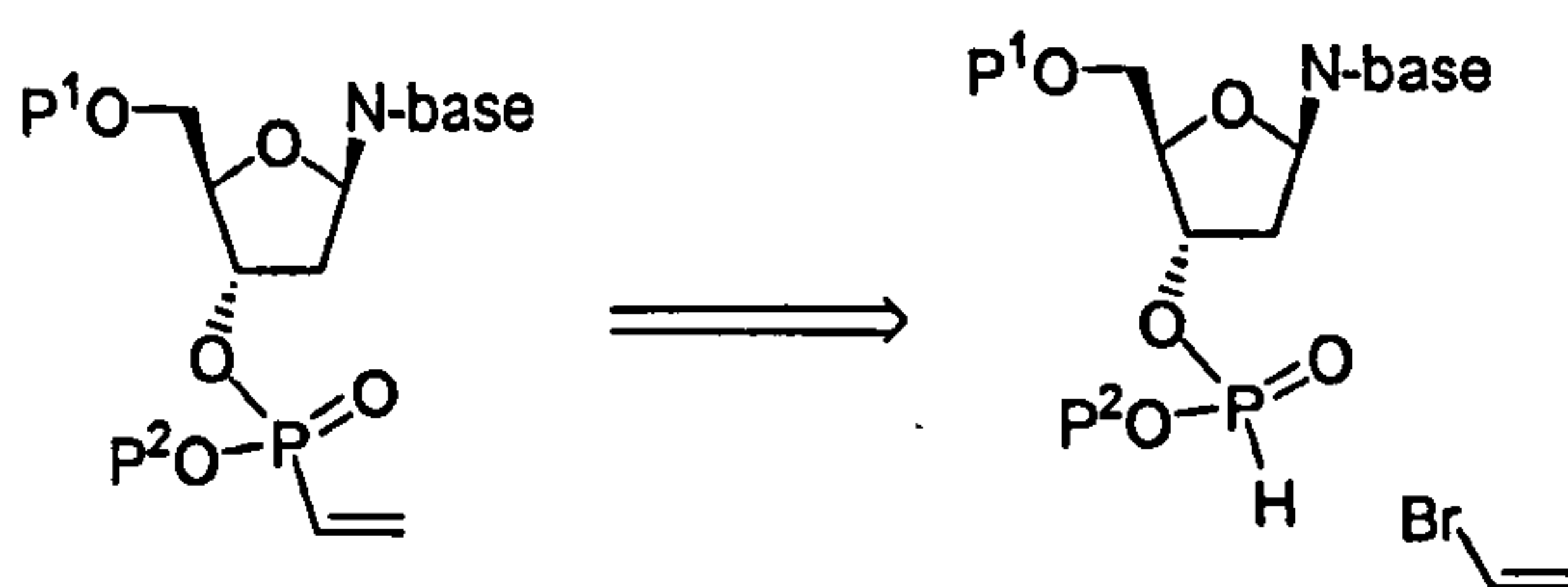
To attempt this reaction it was necessary to prepare both coupling partners. The synthesis of terminal alkene **65** was accomplished according to literature procedures<sup>55</sup> based on a Wittig reaction from the corresponding known aldehyde **5** (Scheme 37).



**Scheme 37.** Synthesis of terminal alkene **65**. (i) Methyltriphenylphosphonium bromide (4 eq), <sup>n</sup>BuLi (4 eq), THF, 0°C to RT, 18 h, 24%.

The low yield obtained in this reaction was attributed to the presence of two acidic protons in compound **5**, the proton of the thymine and the proton  $\alpha$  to the aldehyde function, which can be abstracted by the non-stabilised ylide formed from <sup>n</sup>BuLi and methyltriphenylphosphonium bromide. Other bases, such as KHMDS and <sup>t</sup>BuOK were used in an attempt to improve the yield, but without success. Other reagents used for the methylenation of aldehydes and ketones when acidic protons are present, such as Tebbe<sup>56</sup> and Nysted,<sup>57</sup> were also attempted. A better yield (37%) was obtained in the case of the Tebbe reagent experiment and when the Nysted reagent was used, the reaction did not go to completion however, in both cases problems in purification of the resulting alkene **65** were found.

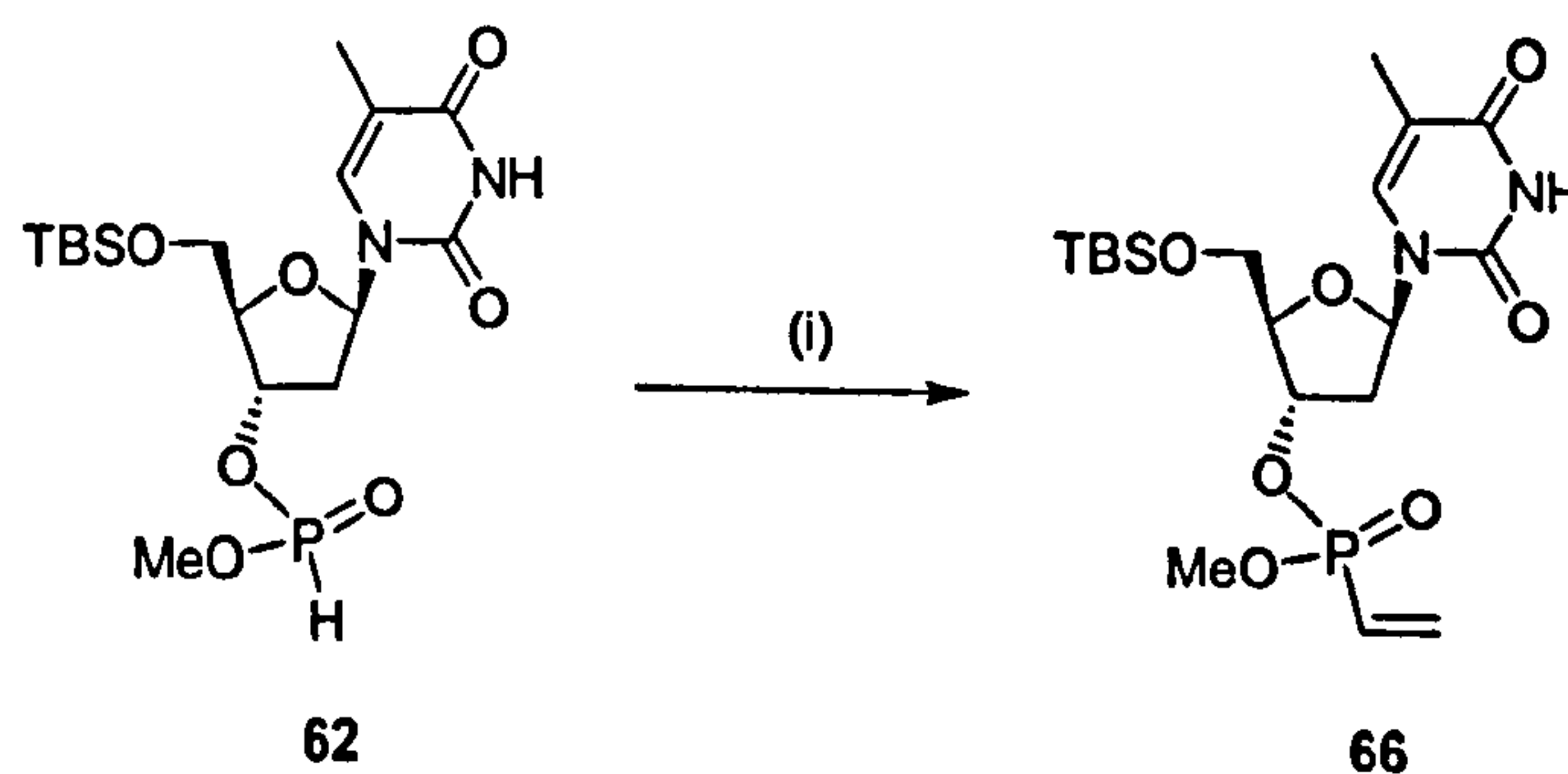
The synthesis of terminal vinylphosphonates was examined next. These coupling partners may be prepared using a similar palladium cross-coupling reaction as seen previously (Section 2.1) for the formation of the P-C bond between an *H*-phosphonate and vinylbromide (Scheme 38).



Scheme 38. Retrosynthetic analysis proposed for the vinylphosphonate coupling partners.

*H*-phosphonate **62** was prepared as a 1:1 mixture of diastereomers at phosphorus as seen in the previous section and vinylbromide is a commercial available compound as a 1.0 M solution in THF.

This reaction was initially attempted using a conventional round bottomed flask fitted with a condenser, but only starting material was recovered due to the high volatility of vinylbromide (bp 16°C). Pleasingly, when this reaction was performed in a sealed, thick walled reaction vial and using an excess of vinylbromide the desired vinylphosphonate **66** was obtained in 64% yield as a 1:1 mixture of diastereomers at phosphorus (Scheme 39).



Scheme 39. (i) Pd(OAc)<sub>2</sub> (0.1 eq), Ph<sub>3</sub>P (0.2 eq), vinylbromide (4.1 eq), propylene oxide (17 eq), THF, 70°C, 15 h, 64%, 1:1 mixture of diastereomers at phosphorus.



With the two desired coupling partners in hand, the olefin cross-metathesis reaction was examined. Among several catalysts available for olefin cross-metathesis reactions, the ruthenium based Grubbs catalysts **67** and **68**<sup>58</sup> (Figure 22) were chosen for this study due to their excellent functional group tolerance, their reasonable moisture and air stability and because they are commercial available compounds.

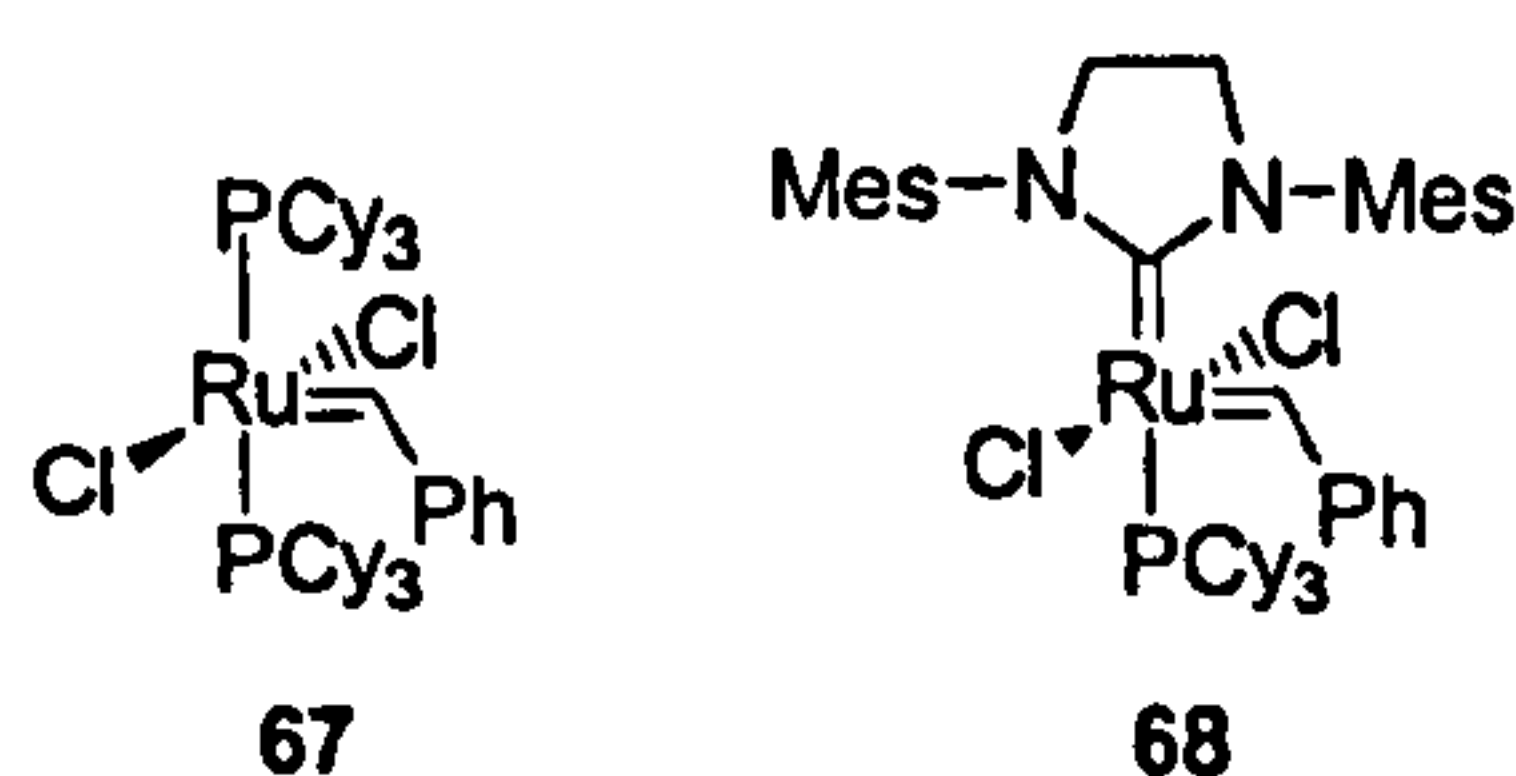


Figure 22. Grubbs catalysts chosen to attempt the olefin cross-metathesis reaction.

Initially, the olefin cross-metathesis reaction of **65** and **66** was attempted with the first generation Grubbs catalyst **67**. In this case, the desired product **69** was not detected by <sup>1</sup>H-NMR of the crude reaction mixture, which consisted only of unreacted starting materials. This lack of reactivity could be due to the formation of relatively stable chelate structures (Figure 23).<sup>59</sup>

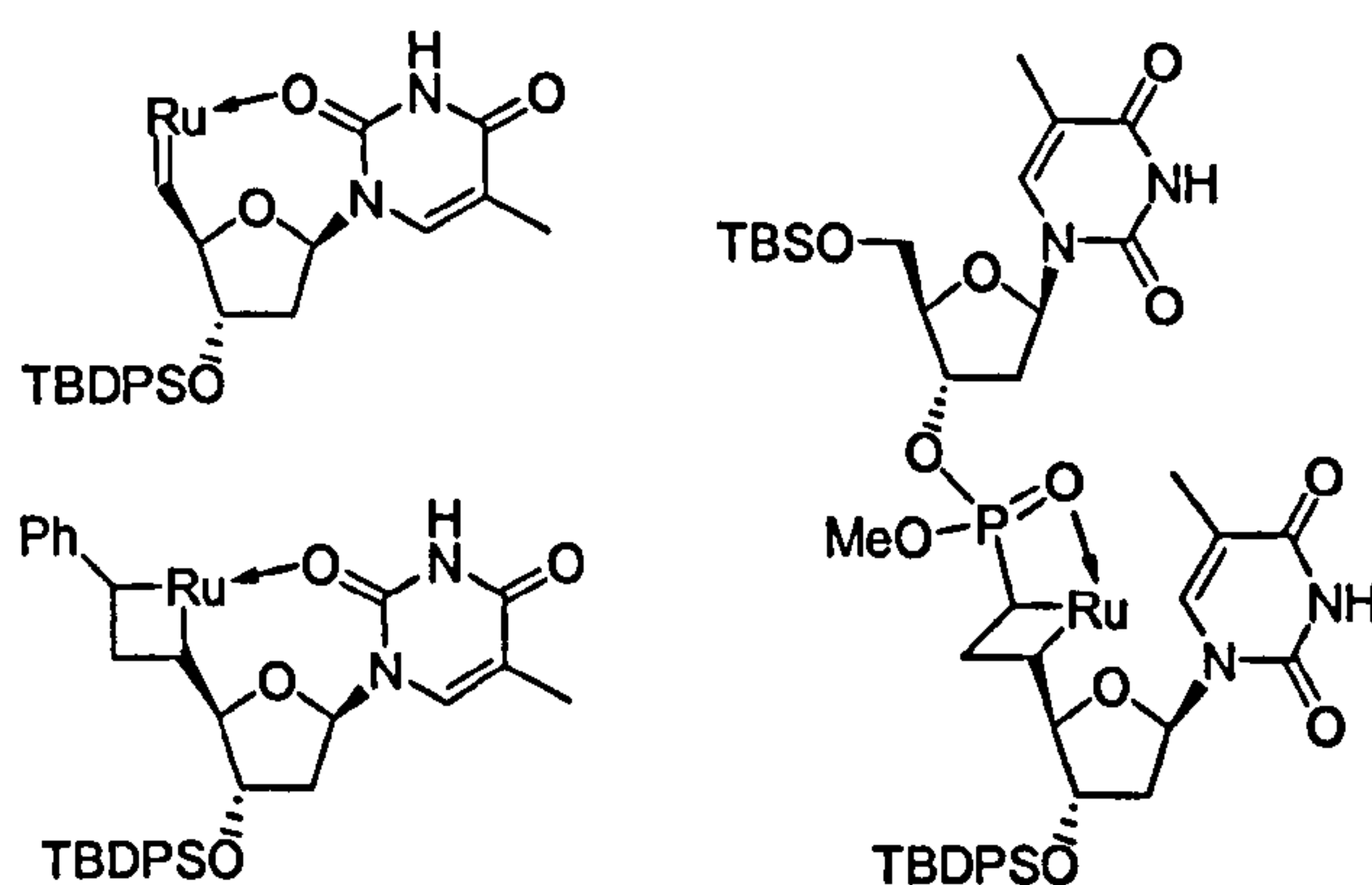


Figure 23. Possible stable chelate structures formed using catalyst **67**.

Catalyst **68** contains a 1,3-dimesityl-4,5-dihydroimidazol-2-ylidene group which makes it a more electron rich catalyst compared with **67**. It has been proposed that the more electron rich ruthenium centre is less prone to chelate formation. Another

advantage of having a more electron rich ruthenium centre is that formation of the “productive” metallo-cyclobutane shown in Figure 24 should be relatively more facile, based upon electronic grounds, resulting in better catalyst turnover and increased yields of the desired cross-metathesis product.

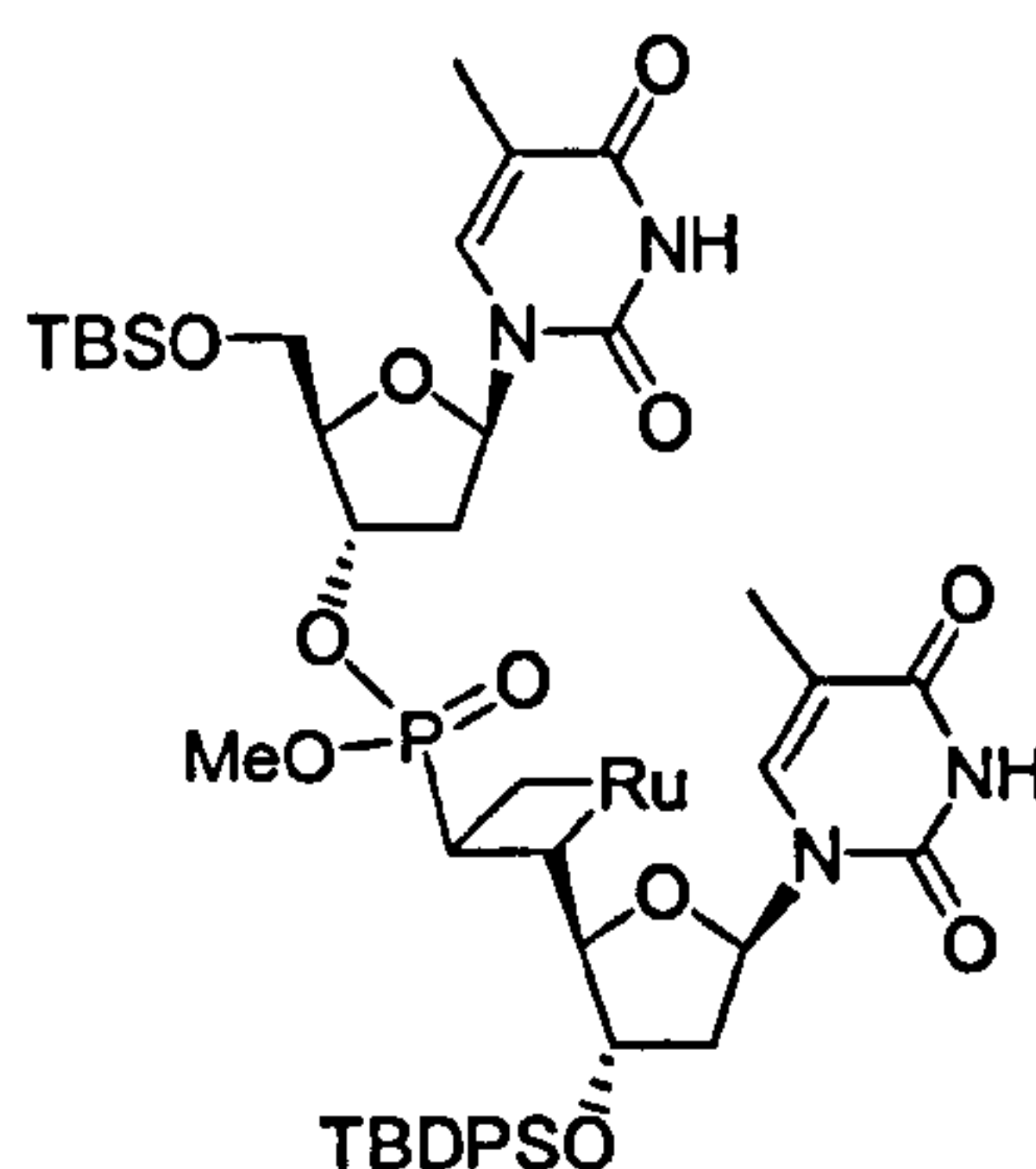
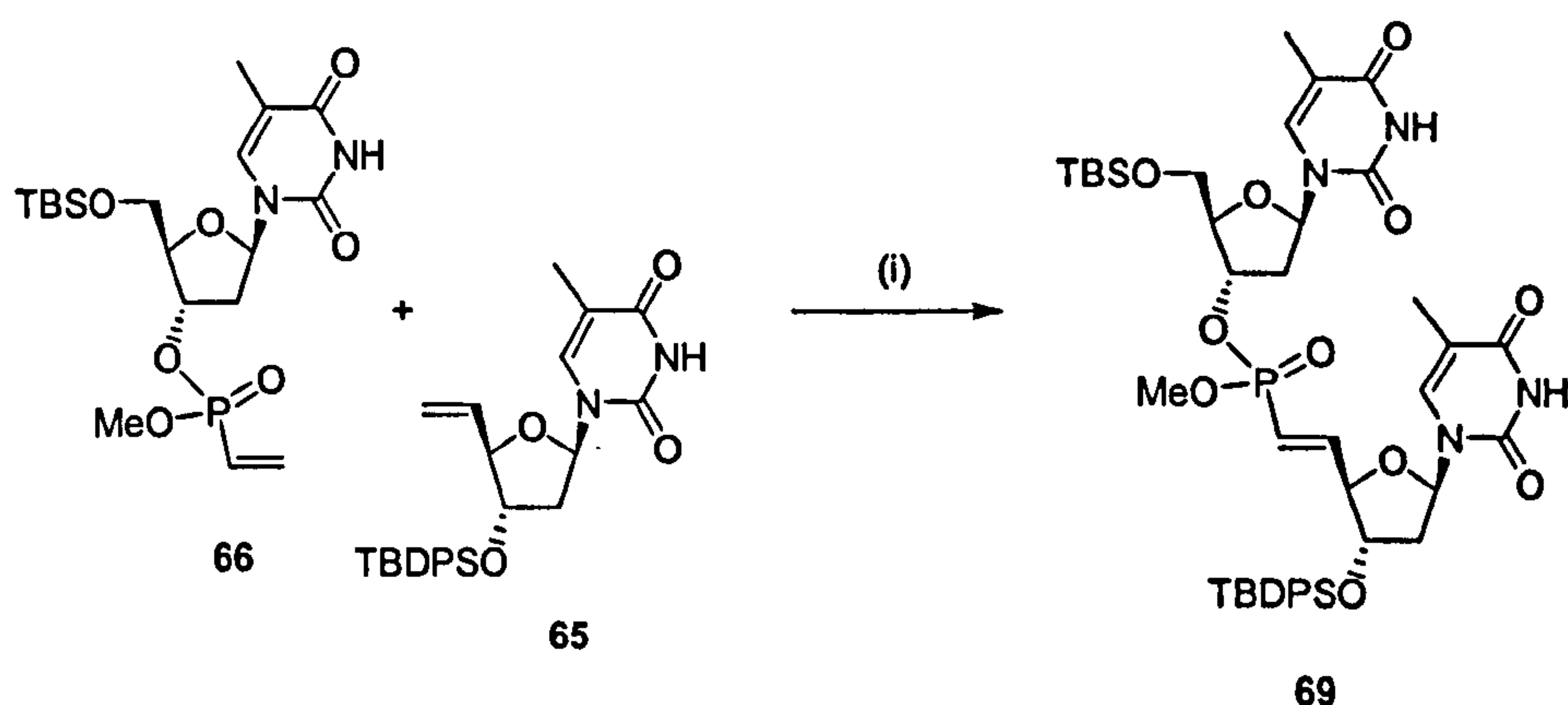


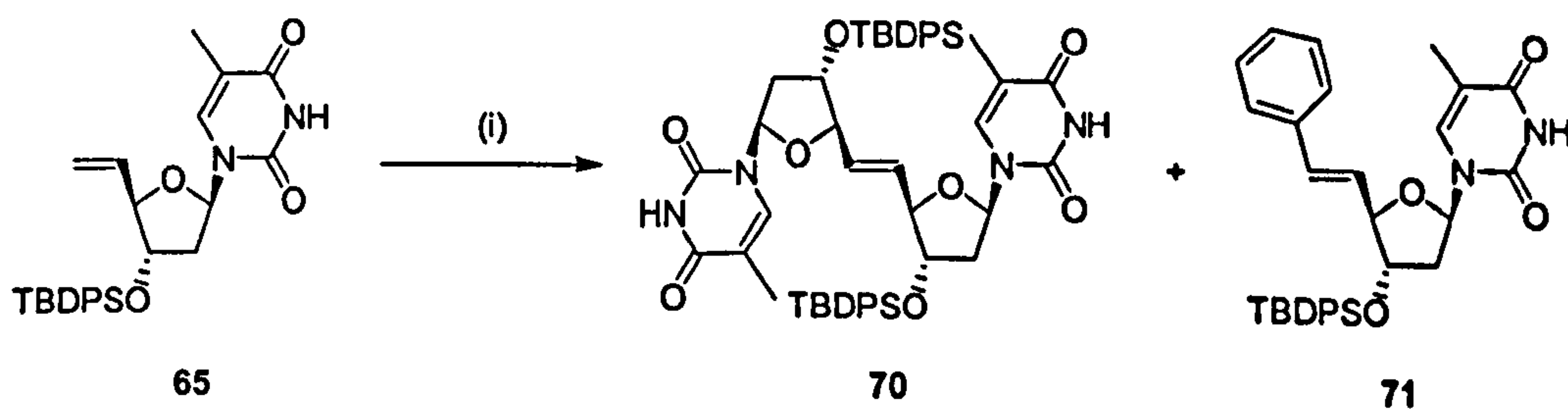
Figure 24. “Productive” metallo-cyclobutane.

Pleasingly, when the olefin cross-metathesis was attempted using the second generation Grubbs catalyst 68, the desired dinucleotide 69 was obtained as a 1:1 mixture of diastereomers at phosphorus in 58% yield (70% based on recovered 65) (Scheme 40).<sup>60</sup> This product was identical in all respects to material that was prepared previously in our research group using a palladium (0) catalysed P-C=C cross-coupling reaction.<sup>41</sup> Analysis of the <sup>1</sup>H-NMR clearly showed that the (*E*)-vinylphosphonate had been produced as the major compound and it was not possible to detect the corresponding (*Z*)-isomer. A number of other minor products were formed in this reaction which might be the products of benzylidene transfer from the catalyst 68 to 65 and 66, the cross-metathesis of 65 with itself and the cross-metathesis of 66 with itself. In order to confirm these suspicions, a series of control experiments were performed to examine the behaviour of each of the cross-metathesis partners 65 and 66 under the reaction conditions.



**Scheme 40.** Olefin cross-metathesis reaction between vinylphosphonate 66 (1.25 eq) and alkene 65 (1.0 eq). (i) Catalyst 68 (0.2 eq), DCM, 35°C, 16 h, 58% (70% based on recovered 65), 1:1 mixture of diastereomers at phosphorus.

Firstly, terminal alkene 65 was exposed to the metathesis conditions and two products were formed (Scheme 41). The major product was the homo-dimer 70 (5:1 *E:Z*), produced by cross metathesis of 65 with itself in 72% yield and the minor product was the alkene 71 produced by transfer of the benzylidene group from the catalyst 68. As 20 mol% of catalyst was used, this latter product represents a quantitative transfer of the benzylidene moiety during catalyst activation.



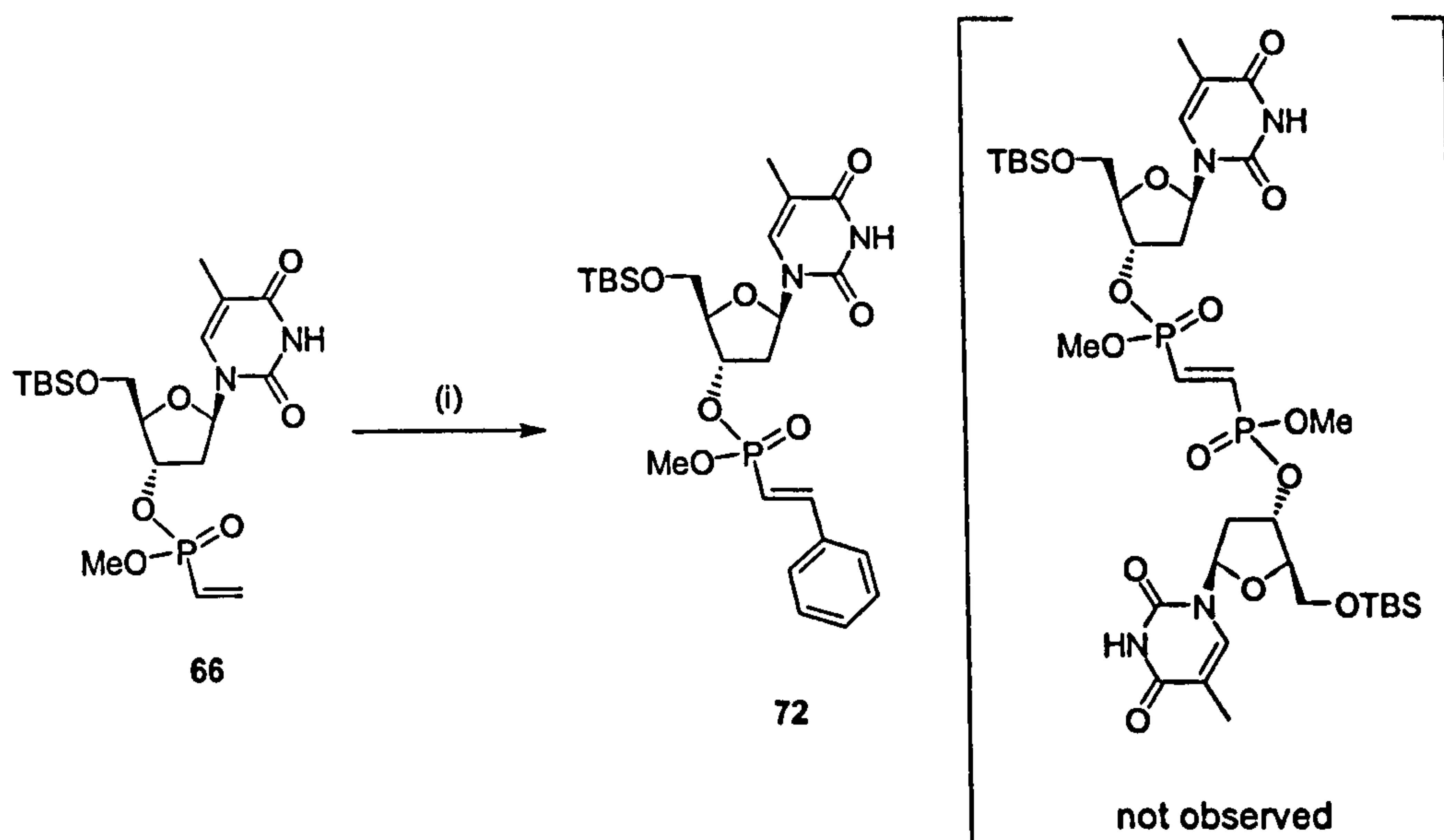
**Scheme 41.** Terminal alkene 65 under the metathesis conditions. (i) Catalyst 68 (0.2 eq), DCM, 35°C, 14 h, 72% yield of 70 and 20% yield of 71.

Vinylphosphonate 66 was next exposed to the cross-metathesis conditions and in this case no cross-metathesis of 66 with itself was observed, and instead the vinylphosphonate 72 was formed as a 1:1 mixture of diastereomers at phosphorus in



20% yield (Scheme 42). The mass balance of the reaction was unreacted **66** and as seen previously, the formation of **72** in 20% yield represents quantitative transfer of the benzylidene group during catalyst activation.

With this study in hand, it was possible to identify the minor products formed in the cross-metathesis reaction shown in Scheme 40, and these were **70** (10%), **71** (15-20%) and **72** (<5%).

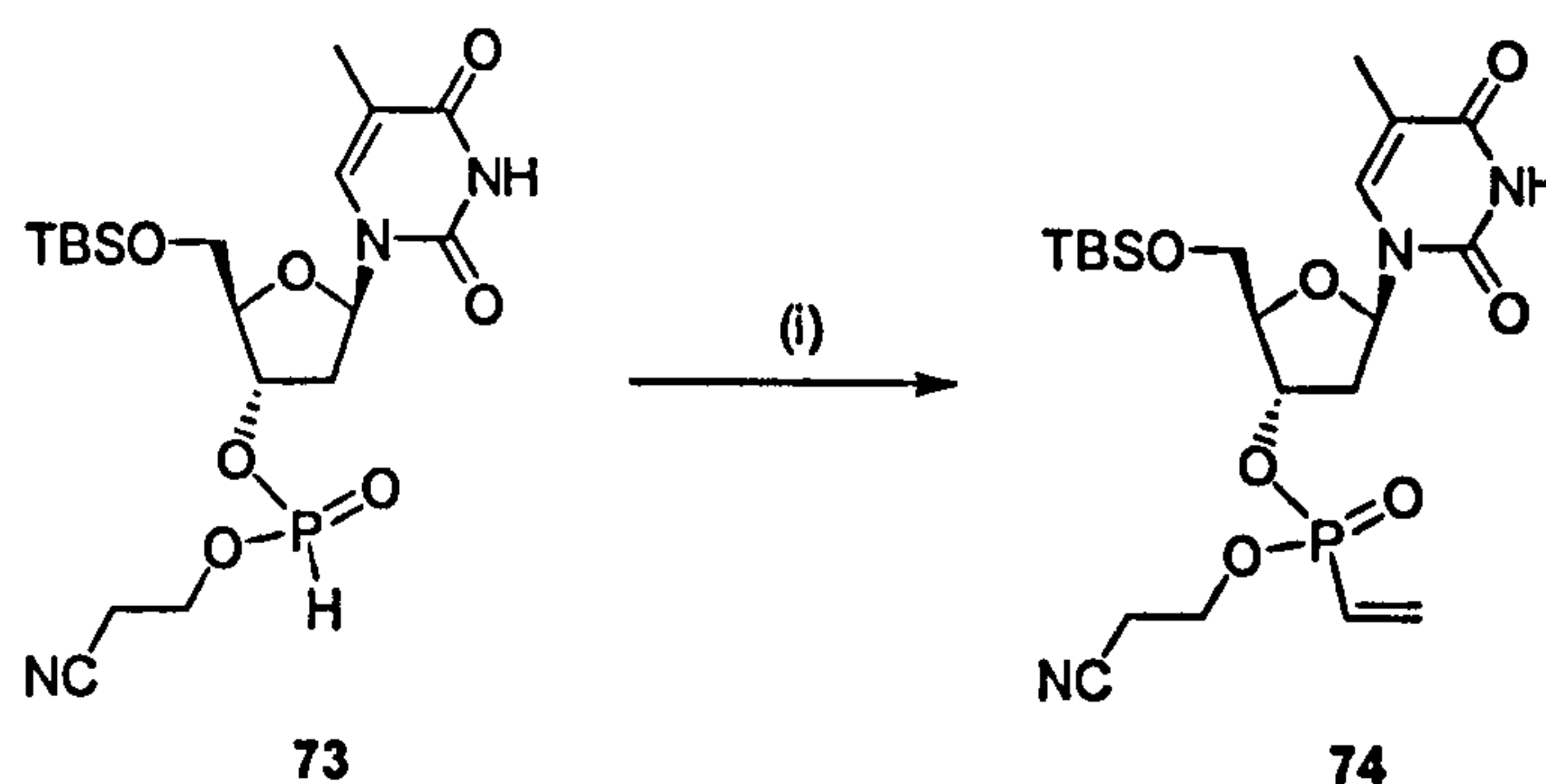


Scheme 42. Vinylphosphonate **66** under the metathesis conditions. (i) Catalyst **68** (0.2 eq), DCM, 35°C, 14 h, 20% yield of **72**.

At this point, the possibility of increasing the yield in the cross-metathesis of **65** and **66** was examined using lower amounts of the benzylidene catalyst **68** with the aim of reducing the amount of the phenyl substituted alkenes **71** and **72**, however when this reaction was performed using 10 mol%, 5 mol% and 2 mol% lower yields were obtained. It was possible to see from the crude  $^1\text{H}$ -NMR spectra that in this reaction the yield is directly dependent of the amount of catalyst used, thus lower amounts of catalyst afforded lower yields. This fact was attributed to the formation of relatively stable chelated structures similar to those presented in Figure 23 which cause catalyst

inhibition. A second way to improve the yield of **69** could be to use a larger excess (2-3 eq) of either of the metathesis partners **65** or **66**. This approach has found application in a number of studies reported in the literature, but is only practical when one of the alkenes is readily available in quantity. In our case, both alkenes are produced using multistep routes and in this situation it is desirable to use them in near equimolar amounts. When the reaction was attempted using a slight excess of the terminal alkene **65** (1.5 eq) and vinylphosphonate **66** (1.0 eq), the desired dinucleotide **69** was obtained in a similar yield (53%) compared with the reaction in which an excess of vinylphosphonate **66** (1.25 eq) was used (58%) (Scheme 40).

The use of a cyanoethyl-phosphate protecting group in the olefin cross-metathesis reaction was also examined due to its compatibility with the solid-phase oligonucleotide synthesis. Firstly, *H*-phosphonate **73** was synthesised by the same methodology used for the synthesis of *H*-phosphonates **17** and **62** (Schemes 13 and 33). Secondly, vinylphosphonate **74** containing this group was prepared in an analogous manner to that described for the methyl-protected analogue **66** (Scheme 43).

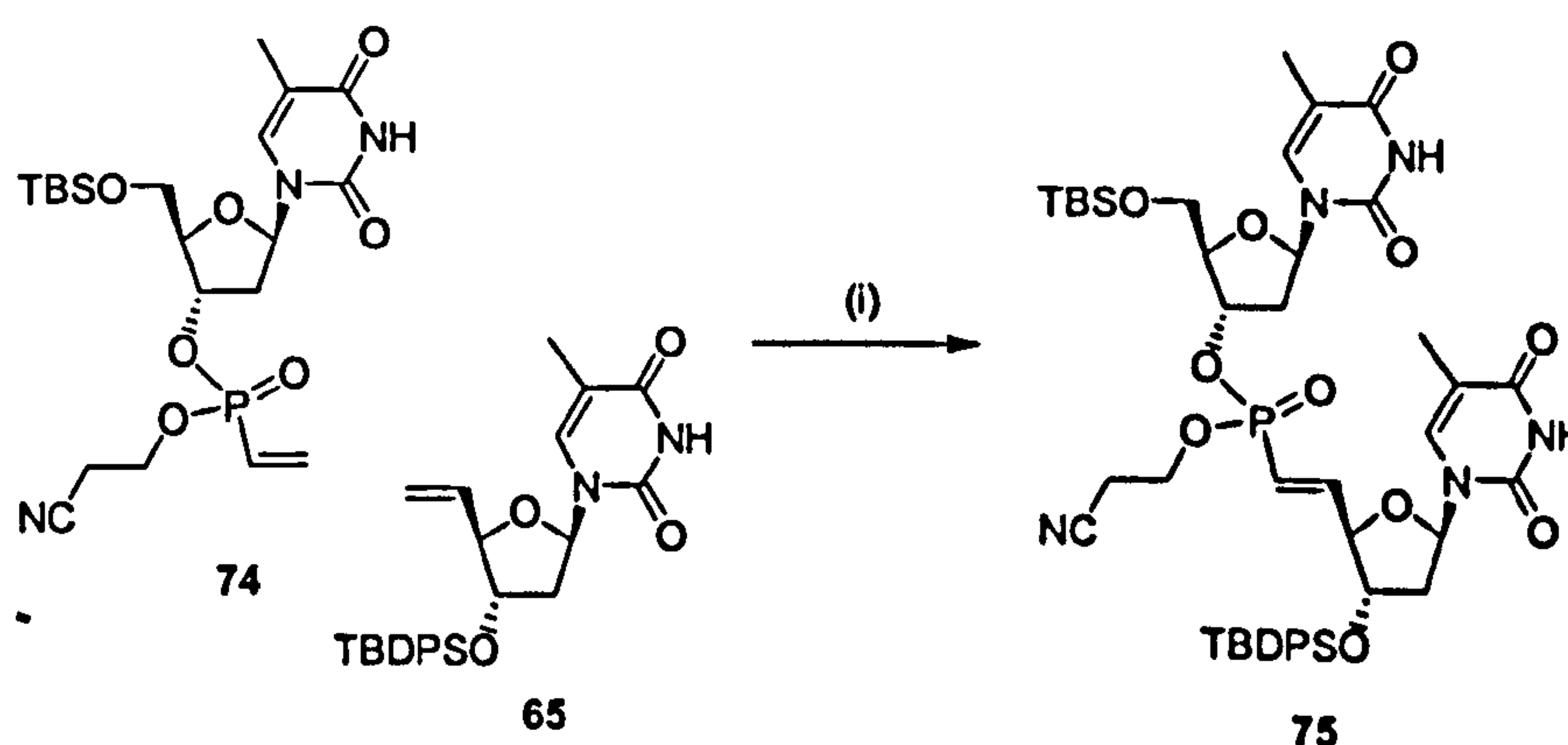


**Scheme 43.** Synthesis of the vinylphosphonate coupling partner **74**. (i) Pd(OAc)<sub>2</sub> (0.1 eq), Ph<sub>3</sub>P (0.4 eq), vinylbromide (4.1 eq), propylene oxide (15 eq), THF, 70°C, 15 h, 25%, 1:1 mixture of diastereomers at phosphorus.

Vinylphosphonate **74**, under identical conditions used previously for the successful cross-metathesis of **65** and **66**, gave the desired cross-metathesis product **75** in 19%

yield, with the mass balance consisting largely of unreacted starting materials and **71** (Scheme 44).<sup>60</sup>

It was suspected that the 19% yield represented just under one catalytic turnover and in order to confirm this, the same reaction was performed using 40 mol% of catalyst **68**. Under these conditions the desired dimer **75** was obtained in 32% yield, with the mass balance once again being unreacted starting materials and **71**. From these results, it was assumed that the introduction of the cyanoethyl group adversely affected catalyst turnover. This fact was probably due to the Lewis-base characteristics of the nitrile group which could be involved in the formation of chelated structures deactivating the catalyst.

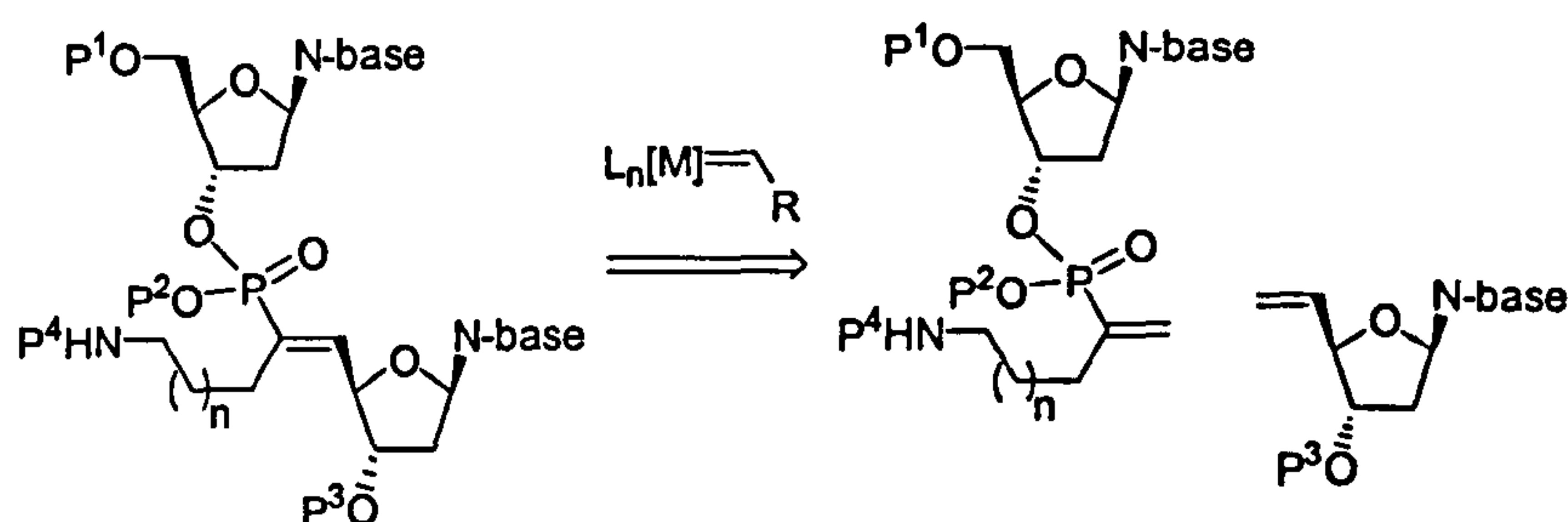


Scheme 44. Olefin cross-metathesis reaction between vinylphosphonate **74** (1.25 eq) and alkene **65** (1.0 eq). (i) Catalyst **68** (0.2 eq), DCM, 35°C, 16 h, 19%, 1:1 mixture of diastereomers at phosphorus. When 0.4 eq of catalyst **68** were used 32% yield of **75**.

#### 2.2.2.2 Attempts to Synthesise Building Block II by Olefin Cross-Metathesis

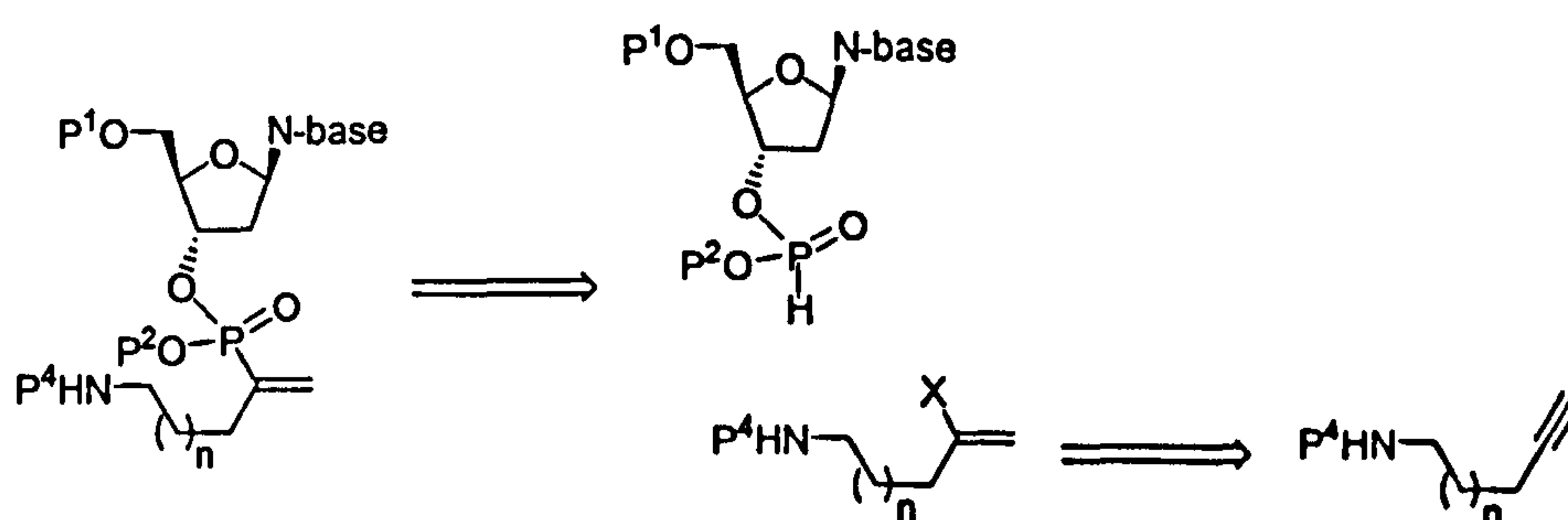
After showing that an olefin cross-metathesis reaction can be used to access vinylphosphonate-linked nucleotide dimers, the synthesis of building block II was re-examined. This building block can be disconnected across the C=C of the vinylphosphonate moiety *via* a retro cross-metathesis reaction (Scheme 45).





Scheme 45. Retrosynthetic analysis proposed for building block II *via* an olefin cross-metathesis reaction.

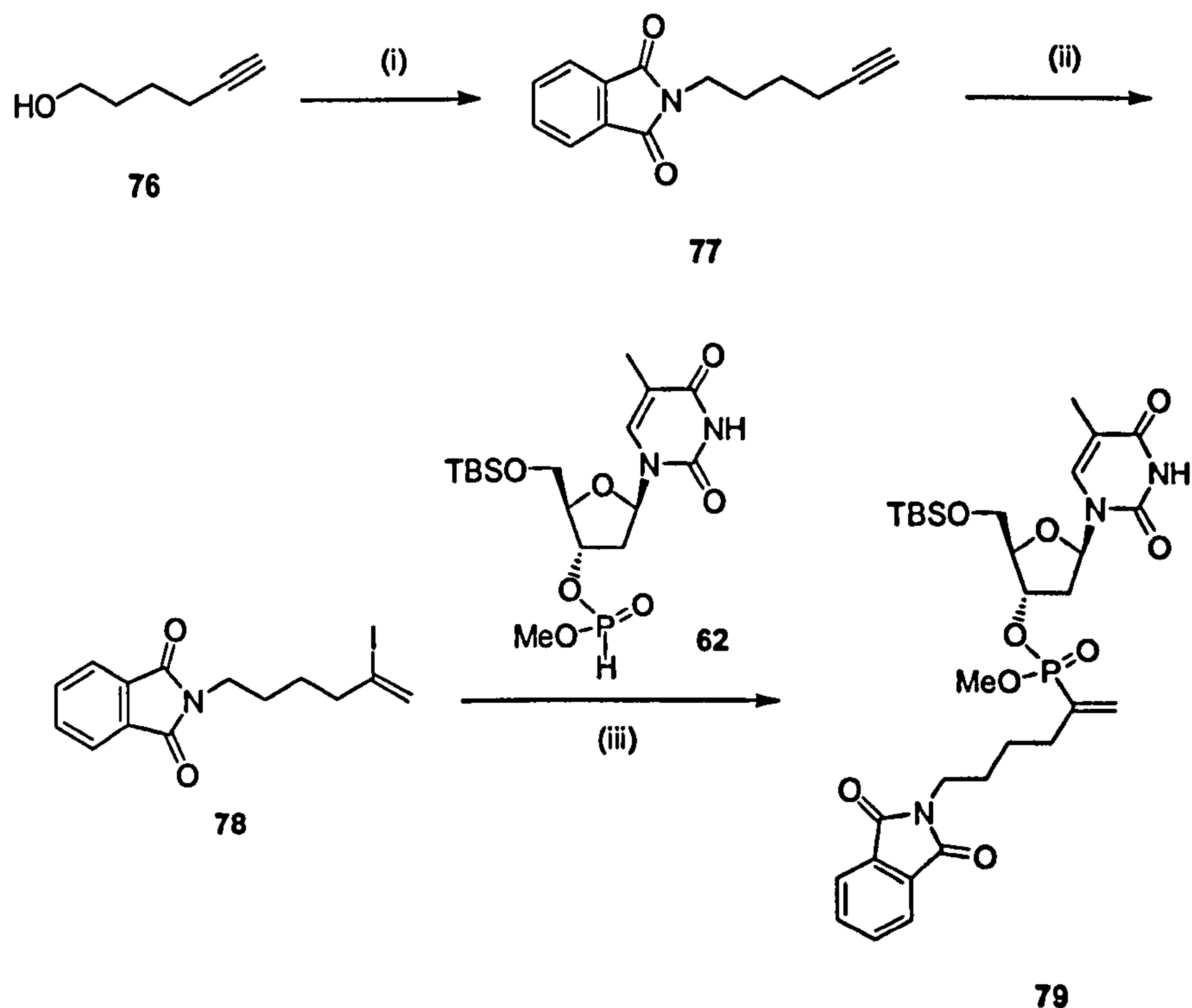
In order to attempt this reaction, it was necessary to synthesise a vinylphosphonate containing a linker and a protected amine. These new vinylphosphonates could potentially be synthesised *via* a palladium catalysed cross-coupling reaction between a vinylhalide and an *H*-phosphonate. Internal vinylhalides can be prepared from the corresponding alkynes with iodo- or bromo-BBN<sup>61</sup> (Scheme 46).



Scheme 46. Retrosynthetic analysis proposed for the synthesis of a vinylphosphonate containing a linker and a protected amine.

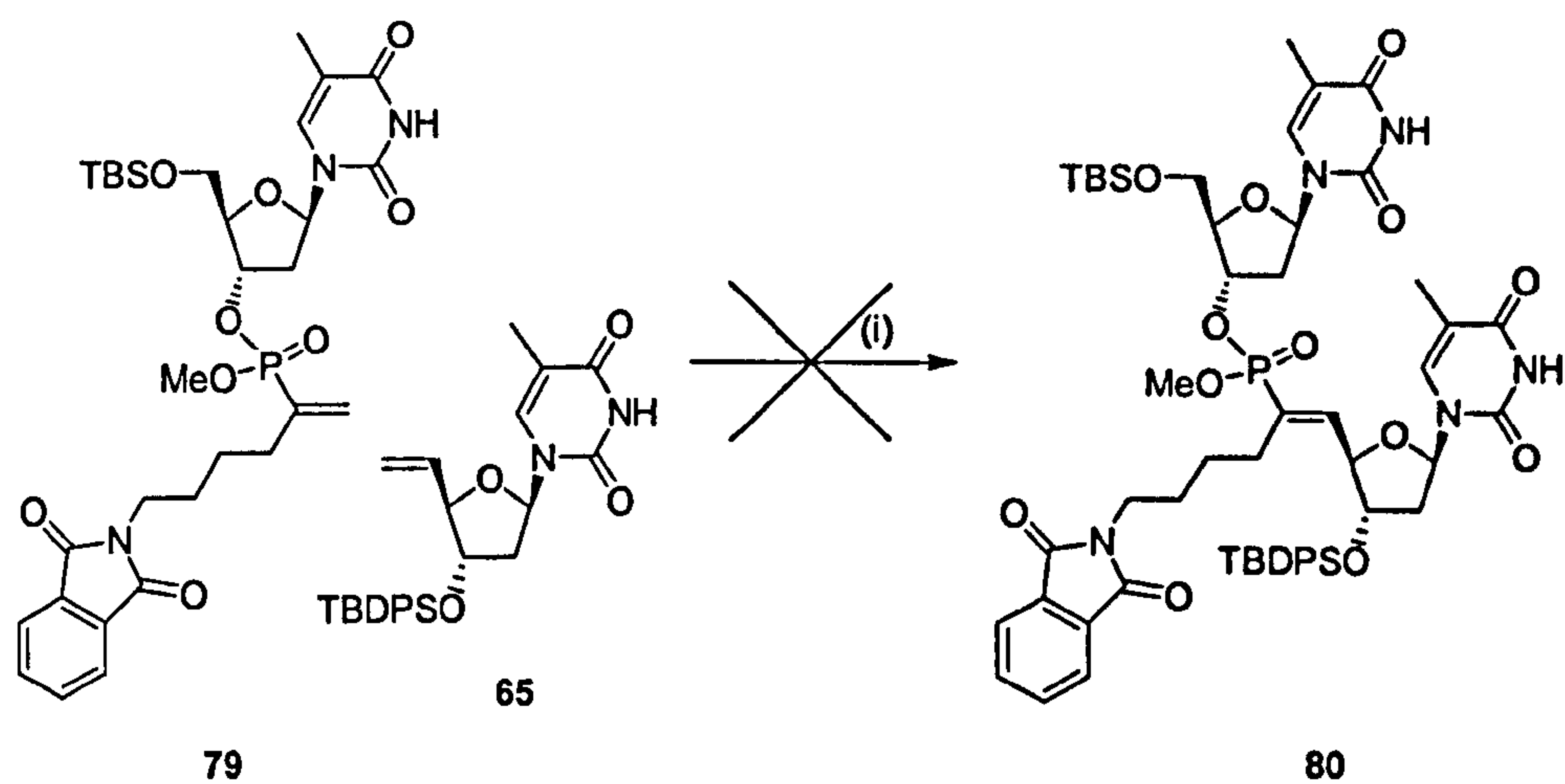
Vinyliodide **78** was synthesised by a Mitsunobu reaction from 5-hexyn-1-ol **76**,<sup>43</sup> followed by an iodination reaction of alkyne **77** using iodo-BBN.<sup>60</sup> With vinyliodide **78** in hand, the palladium catalysed cross-coupling reaction using *H*-phosphonate **62** (1:1 mixture of diastereomers at phosphorus) was attempted, and pleasingly, the

desired vinylphosphonate **79** was obtained in 43% yield as a 1:1 mixture of diastereomers at phosphorus (Scheme 47).



**Scheme 47.** Synthesis of vinylphosphonate **79**. (i) DIAD (2.0 eq),  $\text{Ph}_3\text{P}$  (2.0 eq), phthalimide (2.0 eq), THF, 0°C to RT, 18 h, 60%. (ii) Iodo-BBN (1.3 eq), DCM, -10°C, 1 h, AcOH (Xs.), -10°C, 1 h, NaOH/H<sub>2</sub>O<sub>2</sub> (Xs.), -10°C to RT, 30 min, 33%. (iii)  $\text{Pd}(\text{OAc})_2$  (0.1 eq), dppf (0.2 eq), propylene oxide (3.0 eq), *H*-phosphonate **62** (1.5 eq), THF, 70°C, 15 h, 43%, 1:1 mixture of diastereomers at phosphorus.

With both coupling partners in hand, the reaction shown in Scheme 48 using identical conditions as those used in the successful cross-metathesis reaction (Scheme 40) was attempted, but unfortunately only starting materials were recovered, suggesting that the presence of the linker and the protected amine in vinylphosphonate **79** makes this process much more difficult to be achieved probably due to steric effects, although chelate formation cannot be ruled out.

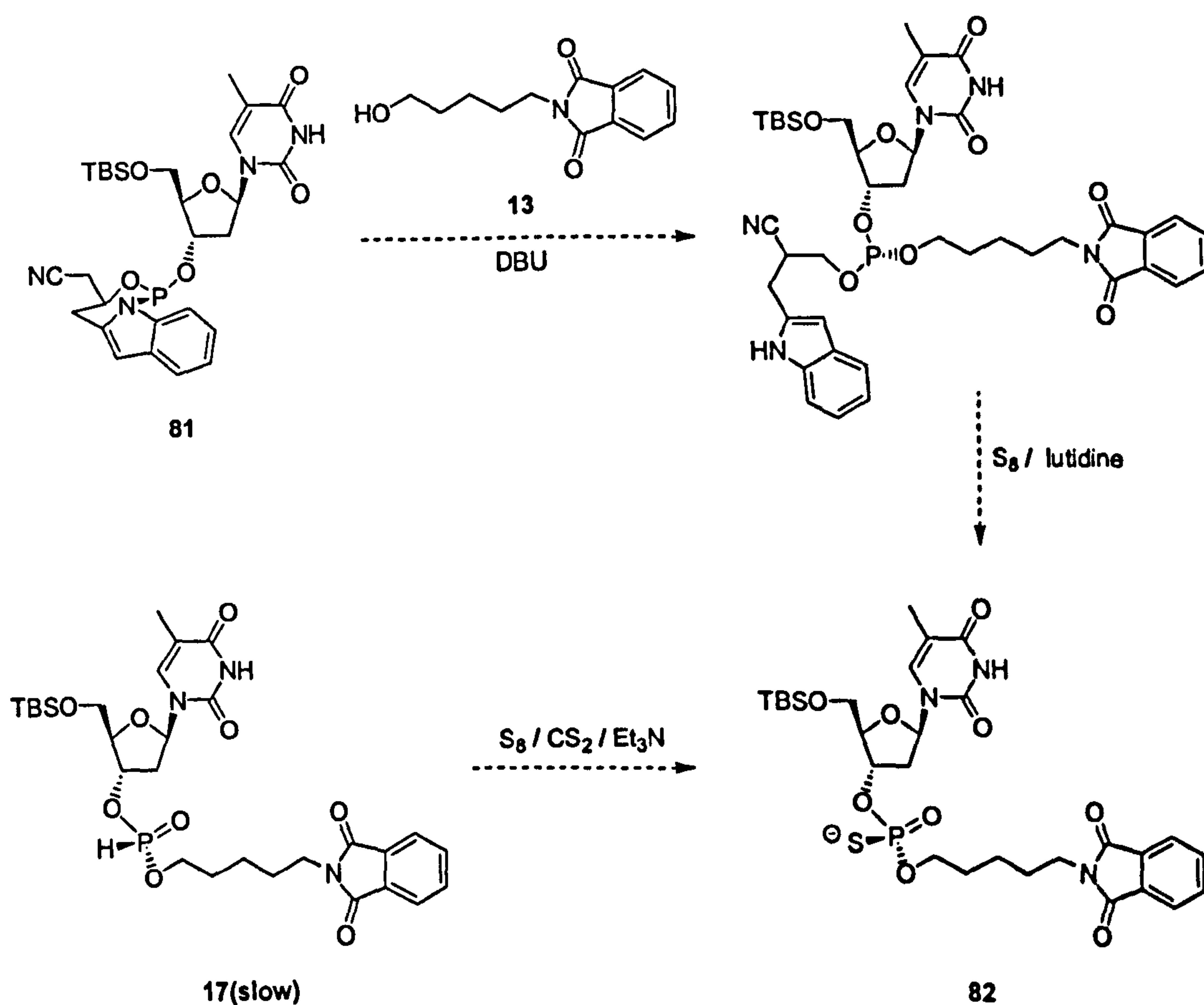


**Scheme 48.** Attempt to synthesise building block II 80 by olefin cross-metathesis. (i) Catalyst 68 (0.20 eq), vinylphosphonate 79 (1.25 eq), alkene 65 (1.00 eq). DCM, 35°C, 16 h.



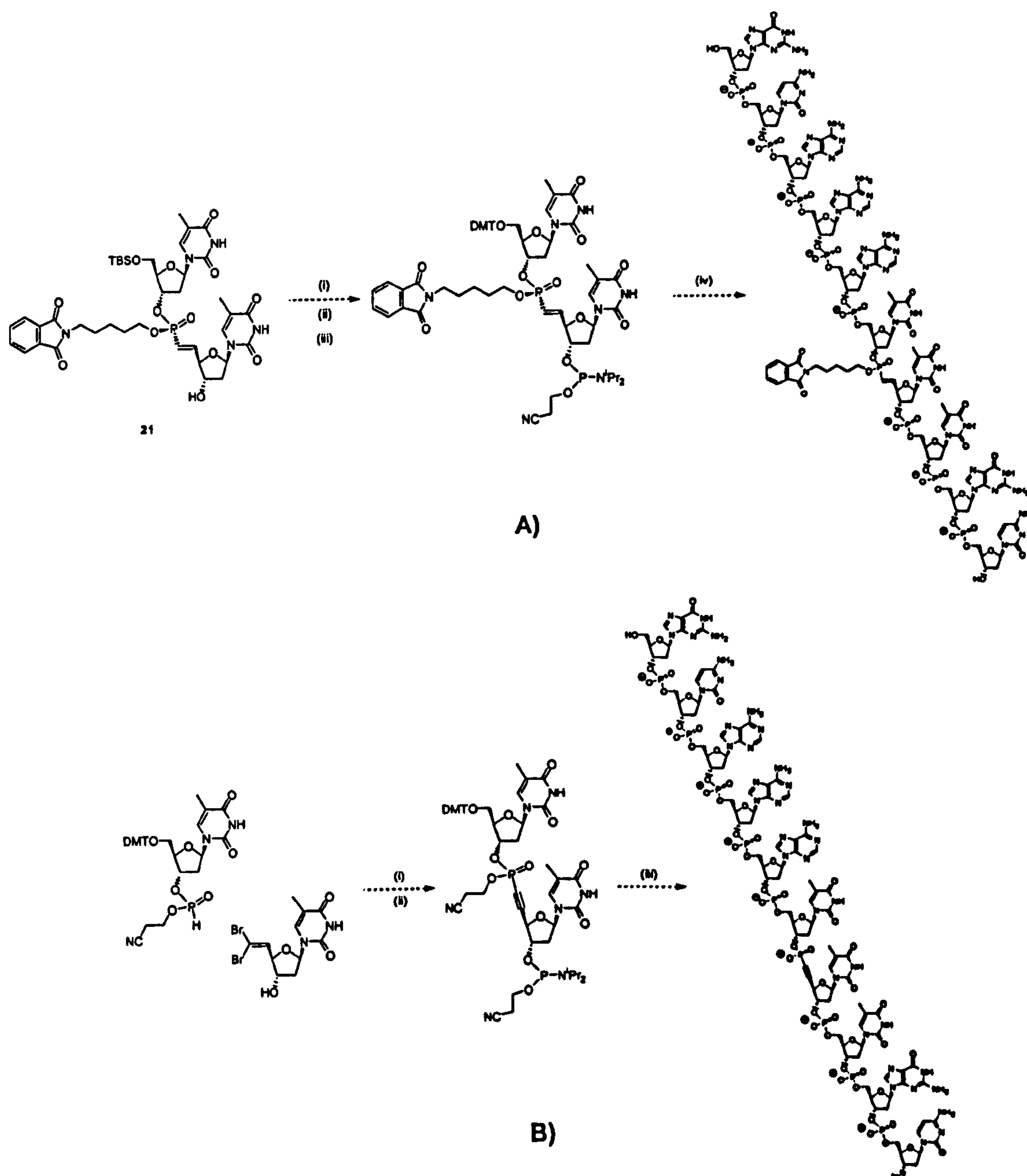
### 3. Future Work

- The stereochemistry at phosphorus in *H*-phosphonates **17(fast)** and **17(slow)**, and in all the dinucleotides synthesised from them, remains to be determined. One possibility to resolve this problem could be to synthesise phosphorothioate **82** from phosphoroamidite **81** which has a known stereochemistry<sup>62</sup> and compare it with the phosphorothioates derived from *H*-phosphonates **17(fast)** and **17(slow)** (Scheme 49).



Scheme 49

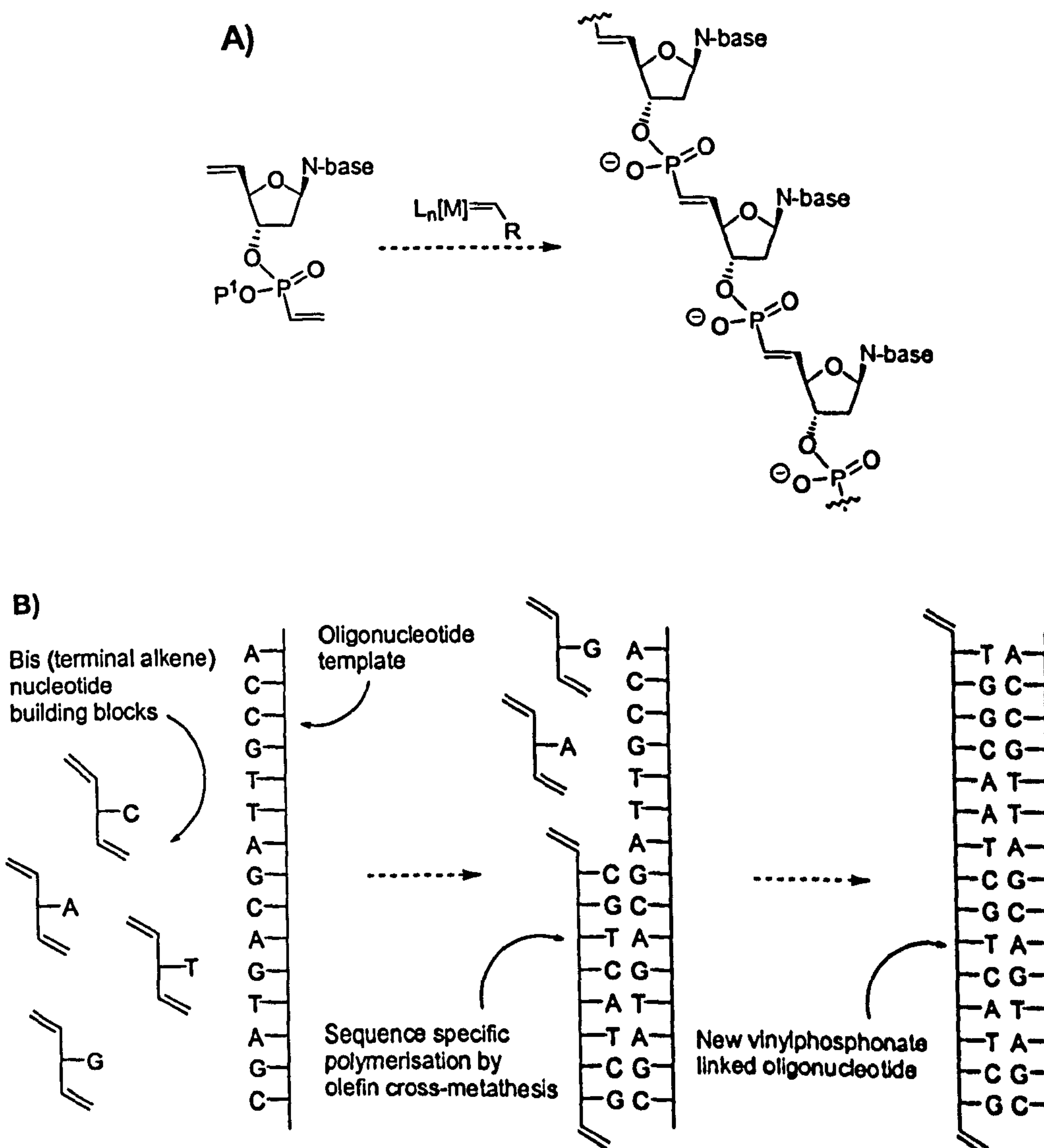
- It is important to determine if the functionality present in dimers **21** and **63** is tolerated in solid supported DNA synthesis, and if they can be incorporated into an oligonucleotide sequence. For this purpose, the synthesis of the corresponding 5'-O-DMT protected dimers need to be accomplished and, possible routes are presented in Scheme 50.



**Scheme 50.** A) (i) HF; (ii) DMTCl, base; (iii) *Bis*(disopropylamino)cynoethylphosphite; (iv) DNA synthesiser. B) (i)  $\text{Pd}(\text{OAc})_2$ , ligand, propylene oxide; (ii) *Bis*(disopropylamino)cynoethylphosphite; (iii) DNA synthesiser.

- The palladium catalysed cross-coupling reaction with 1,1-dibromoalkenes needs to be studied to try to find the conditions for the selective formation of monocoupled products. This may be achieved by using different solvents, bases, ligands, or palladium sources, and would be of importance not only to re-examine the synthesis of building block II, but also from a methodology point of view.

- The successful synthesis of vinylphosphonate-linked dinucleotides by olefin cross-metathesis creates many interesting possibilities. One example is to attempt the polymerisation of a building block containing two terminal olefins, one at the 3'- and another at the 5'-position, by olefin cross-metathesis to synthesise vinylphosphonate-linked oligonucleotides (Scheme 51A). It would be even more interesting to expose the four building blocks containing the four different bases: A, G, C, and T to the metathesis conditions in the presence of an oligonucleotide that could act as a template to investigate the preparation of sequence specific oligonucleotides (Scheme 51B).



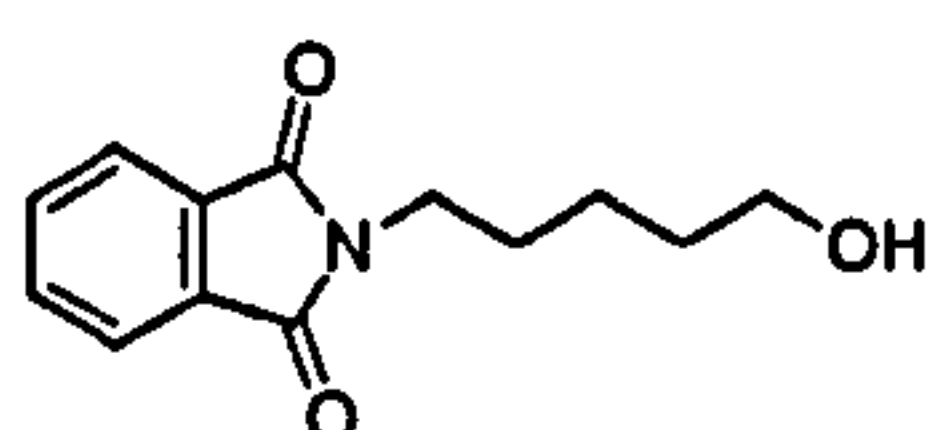
**Scheme 51.** A) Polymerisation of a nucleotide building block by olefin cross-metathesis. B) Polymerisation of a nucleotide building block by olefin cross-metathesis in the presence of a template.



## 4. Experimental

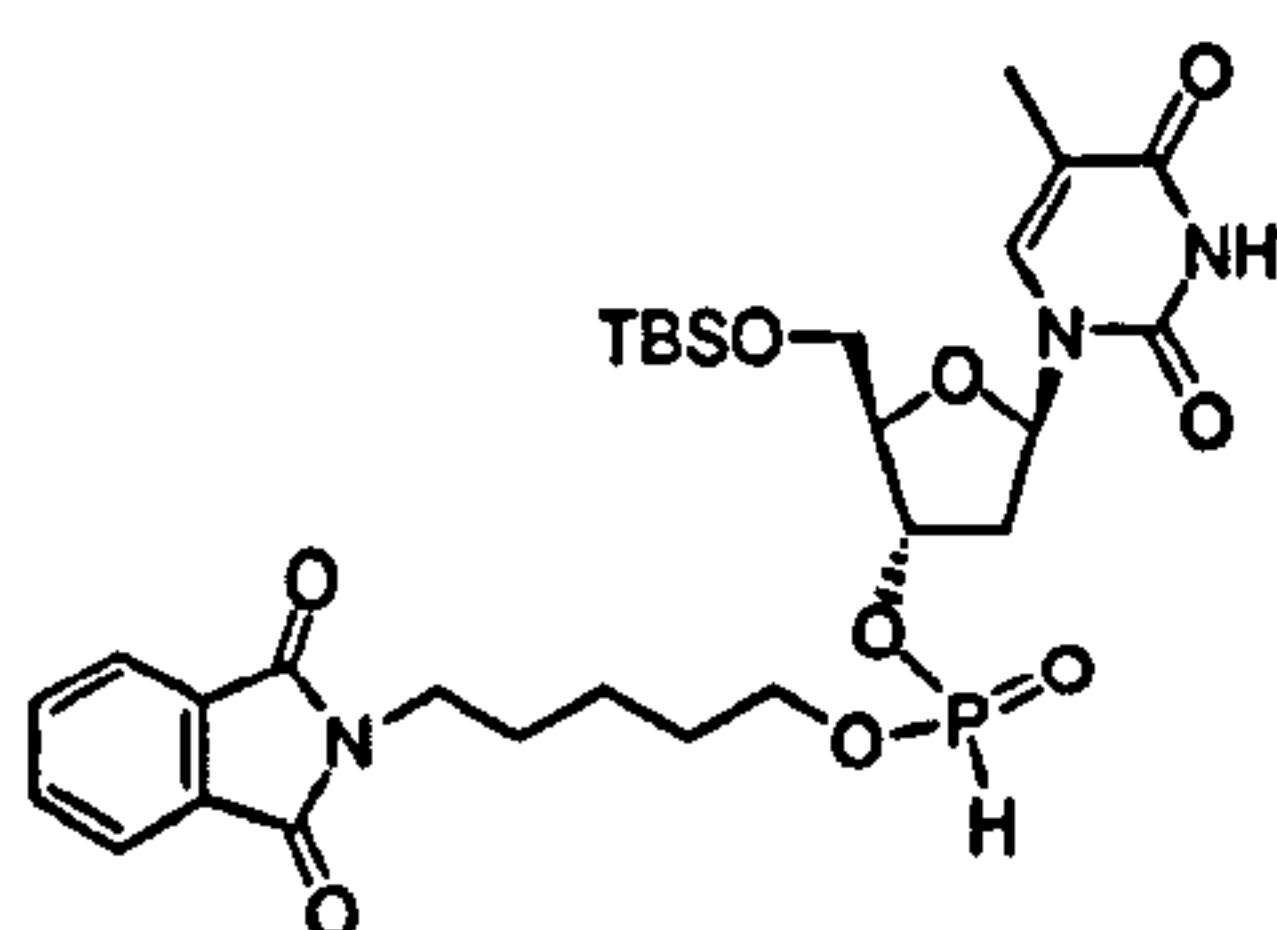
*General Details.*- As in the experimental of the synthesis of azatriquinacene (Part I).

### N-(5-Hydroxypentyl)phthalimide 14.



DIAD (6.7 mL, 0.034 mol) was dropwise added to a stirred solution of  $\text{Ph}_3\text{P}$  (8.90 g, 0.034 mol) in THF (110 mL) at  $0^\circ\text{C}$  under a nitrogen atmosphere. The mixture was stirred for 20 min and a solution of 1,5-pentanediol (3 mL, 0.029 mol) in THF (40 mL) was added followed by phthalimide (5.06 g, 0.034 mol) and stirring was continued for 15 h. The solvent was removed *in vacuo* and the residue purified by column chromatography (AcOEt:Pentane 1:1) to give 14 (3.42 g, 51%) as a white solid; mp  $44\text{--}46^\circ\text{C}$ , (Found: C, 66.5; H 6.4; N, 5.9.  $\text{C}_{13}\text{H}_{15}\text{NO}_3$  requires C, 66.9; H, 6.5; N, 6.0 %); (Found: (HRMS FAB)  $\text{M}^+\text{+H}$  234.1136,  $\text{C}_{13}\text{H}_{16}\text{NO}_3$  requires 234.1131);  $\nu_{\text{max}}/\text{cm}^{-1}$  3625, 2942, 2864, 1772, 1711, 1616, 1468, 1398, 1366, 1051, 961, 876;  $\delta_{\text{H}}$ (400 MHz) 7.76 (2H, dd,  $J$  5.4, 3.1), 7.65 (2H, dd,  $J$  5.5, 3.0), 3.62 (2H, t,  $J$  6.5), 3.57 (2H, t,  $J$  6.5), 2.47 (1H, br s), 1.65 (2H, app quint,  $J$  7.4), 1.56 (2H, app quint,  $J$  6.7), 1.36 (2H, m);  $\delta_{\text{C}}$ (100 MHz) 168.3 (C), 133.8 (CH), 131.8 (C), 123.0 (CH), 62.1 ( $\text{CH}_2$ ), 37.7 ( $\text{CH}_2$ ), 32.0 ( $\text{CH}_2$ ), 28.2 ( $\text{CH}_2$ ), 22.9 ( $\text{CH}_2$ );  $m/z$  (FAB) 234 ( $\text{M}^+\text{+H}$ , 29) 133 (29), 160 (19), 155 (20), 154 (100), 138 (29), 137 (60), 136 (72), 107 (22), 91 (15), 90 (11), 89 (18), 77 (22), 71 (10), 69 (18), 57 (29), 55 (24).

### Pentylphthalimide *H*-phosphonate 17.



A solution of 5'-O-(*tert*-butyldimethylsilyl)- $\alpha$ -thymidine 2 (0.50 g, 1.40 mmol) in THF (15 mL) was added dropwise to a stirring solution of  $\text{PCl}_3$  (0.56 mL, 6.42 mmol) and pyridine (0.56 mL, 6.92 mmol) in THF (14 mL) at  $-78^\circ\text{C}$  under an argon atmosphere

over a period of 22 min. The mixture was stirred at this temperature for 10 min, at RT for 1 h and then heated at reflux for 2 h. The mixture was cooled to RT and the volatiles removed *in vacuo*. The residue was coevaporated with DCM (2 x 20 mL) to remove the excess of  $\text{PCl}_3$  and then dissolved in THF (20 mL) under an argon atmosphere. Pyridine (0.56 mL, 6.92 mmol) was added followed by a solution of 14 (0.324 g, 1.391 mmol) in THF (10 mL). The mixture was heated at reflux for 1 h and then cooled to RT. Water (10 mL) was added and the mixture was stirred for 5 min. DCM (200 mL) was then added and the mixture washed with a saturated aqueous solution of sodium bicarbonate. The layers were separated and the aqueous layer extracted with DCM (2 x 100 mL). The combined organic phase was dried, the solvent removed *in vacuo* and the residue purified by column chromatography (AcOEt:Pentane 4:1) to give 17 (0.678 g, 76%) as a 1:1 mixture of the separable diastereomers 17(fast) and 17(slow).

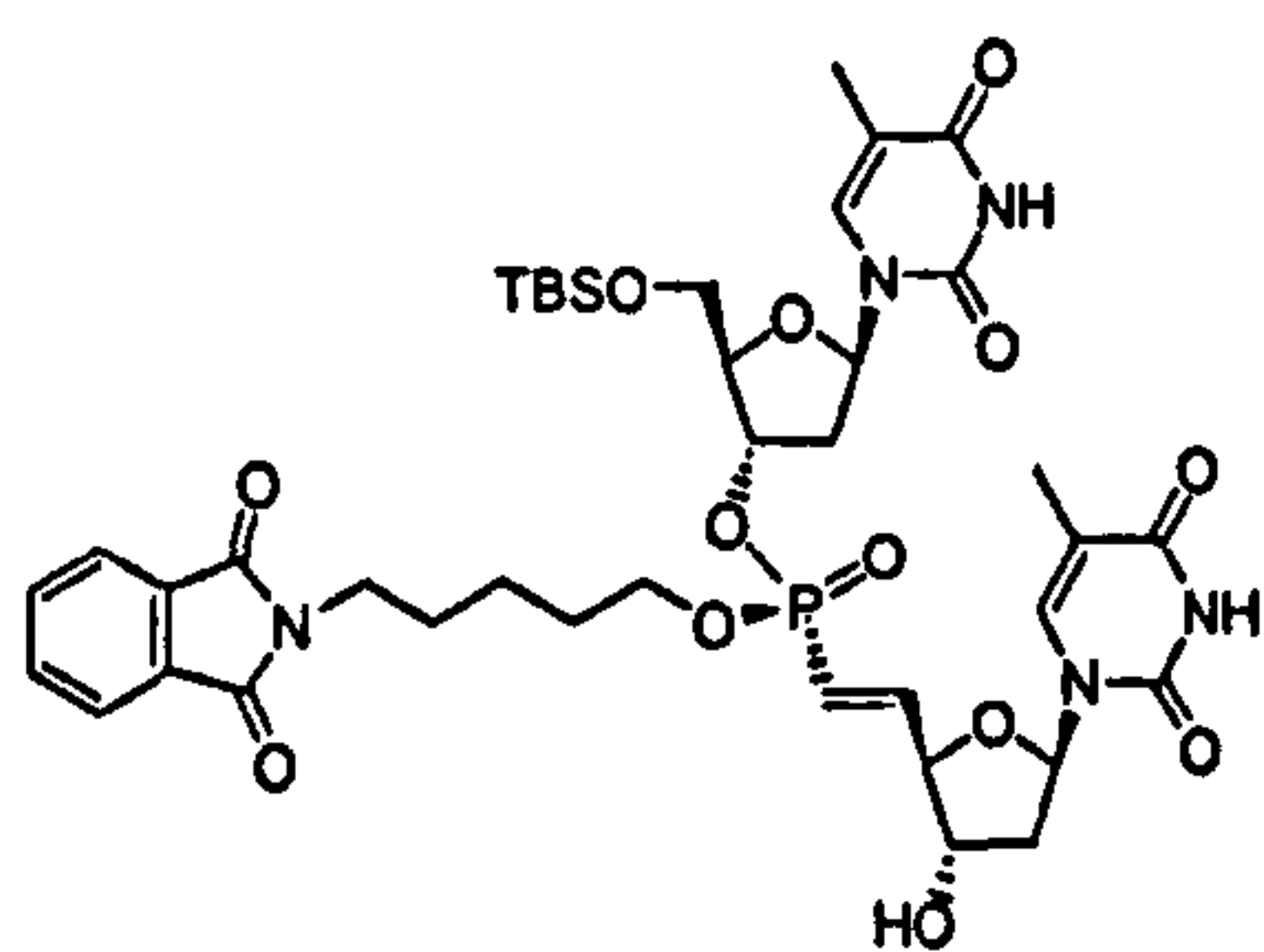
Data for the least polar isomer 17(fast): (Found: (HRMS ES)  $\text{M}^+ + \text{Na}$  658.2322,  $\text{C}_{29}\text{H}_{42}\text{N}_3\text{O}_9\text{PSiNa}$  requires 658.2326);  $[\alpha]_{\text{D}} +6.0$  (c 1.3 in  $\text{CHCl}_3$ );  $\nu_{\text{max}}/\text{cm}^{-1}$  3632, 3393, 3188, 2931, 2860, 1772, 1714, 1462, 1397, 1363, 1322, 1292, 1277, 1128, 1068, 978, 906, 838, 648;  $\delta_{\text{H}}$ (400 MHz) 9.33 (1H, br s), 7.82 (2H, dd,  $J$  5.5, 3.0), 7.69 (2H, dd,  $J$  5.5, 3.0), 7.45 (1H, m), 6.86 (1H, d,  $J$  708.6), 6.36 (1H, dd,  $J$  8.9, 5.3), 5.09 (1H, app dd,  $J$  7.6, 6.1), 4.23 (1H, m), 4.09 (2H, m), 3.87 (2H, m), 3.68 (2H, t,  $J$  7.1), 2.51 (1H, ddd,  $J$  13.9, 5.5, 1.4), 2.15 (1H, ddd,  $J$  14.3, 8.9, 5.8), 1.90 (3H, d,  $J$  1.1), 1.74 (4H, m), 1.44 (2H, m), 0.90 (9H, s), 0.11 (3H, s), 0.11 (3H, s);  $\delta_{\text{C}}$ (100 MHz) 168.4 (C), 163.9 (C), 150.4 (C), 135.0 (CH), 134.0 (CH), 132.1 (C), 123.2 (CH), 111.2 (CH), 85.8 (d,  $^3J_{\text{CP}}$  5.3) (CH), 84.5 (CH), 76.9 (d,  $^2J_{\text{CP}}$  6.3) (CH), 66.0 (d,  $^2J_{\text{CP}}$  5.7) ( $\text{CH}_2$ ), 63.1 ( $\text{CH}_2$ ), 39.6 (d,  $^3J_{\text{CP}}$  3.2) ( $\text{CH}_2$ ), 37.6 ( $\text{CH}_2$ ), 29.8 (d,  $^3J_{\text{CP}}$  6.3) ( $\text{CH}_2$ ), 28.0 ( $\text{CH}_2$ ),



25.9 (CH<sub>3</sub>), 22.8 (CH<sub>2</sub>), 18.3 (C), 12.5 (CH<sub>3</sub>), -5.4 (CH<sub>3</sub>), -5.5 (CH<sub>3</sub>);  $\delta_P$ (161.98 MHz) 8.2.

Data for the most polar isomer 17(slow): (Found: (HRMS ES)  $M^+ + Na$  658.2307, C<sub>29</sub>H<sub>42</sub>N<sub>3</sub>O<sub>9</sub>PSiNa requires 658.2326);  $[\alpha]_D$  +8.0 (c 0.9 in CHCl<sub>3</sub>);  $\nu_{max}/cm^{-1}$  3632, 3392, 2931, 2860, 1772, 1712, 1693, 1463, 1397, 1363, 1322, 1276, 1129, 1067, 977, 908, 837;  $\delta_H$ (400 MHz) 9.22 (1H, br s), 7.83 (2H, dd,  $J$  5.4, 3.0), 7.70 (2H, dd,  $J$  5.4, 3.0), 7.46 (1H, m), 6.89 (1H, d,  $J$  705.4), 6.37 (1H, dd,  $J$  8.9, 5.3), 5.08 (1H, app t,  $J$  6.4), 4.25 (1H, m), 4.09 (2H, m), 3.88 (2H, m), 3.69 (2H, t,  $J$  7.1), 2.50 (1H, app dd,  $J$  13.6, 4.9), 2.13 (1H, ddd,  $J$  14.3, 8.5, 5.8), 1.91 (3H, s), 1.74 (4H, m), 1.44 (2H, m), 0.91 (9H, s), 0.11 (3H, s), 0.11 (3H, s);  $\delta_C$ (100 MHz) 168.4 (C), 163.8 (C), 150.4 (C), 135.0 (CH), 134.0 (CH), 132.1 (C), 123.3 (CH), 111.2 (CH), 86.1 (d,  $^3J_{CP}$  3.5) (CH), 84.7 (CH), 76.8 (d,  $^2J_{CP}$  4.9) (CH), 65.9 (d,  $^2J_{CP}$  5.8) (CH<sub>2</sub>), 63.1 (CH<sub>2</sub>), 39.5 (d,  $^3J_{CP}$  4.6) (CH<sub>2</sub>), 37.6 (CH<sub>2</sub>), 29.8 (d,  $^3J_{CP}$  6.4) (CH<sub>2</sub>), 28.0 (CH<sub>2</sub>), 25.9 (CH<sub>3</sub>), 22.8 (CH<sub>2</sub>), 18.3 (C), 12.5 (CH<sub>3</sub>), -5.4 (CH<sub>3</sub>), -5.4 (CH<sub>3</sub>);  $\delta_P$ (161.98 MHz) 8.4.

#### 5'-O-(*tert*-Butyldimethylsilyl)pentylphthalimidephosphonate T\*T dimer 21.

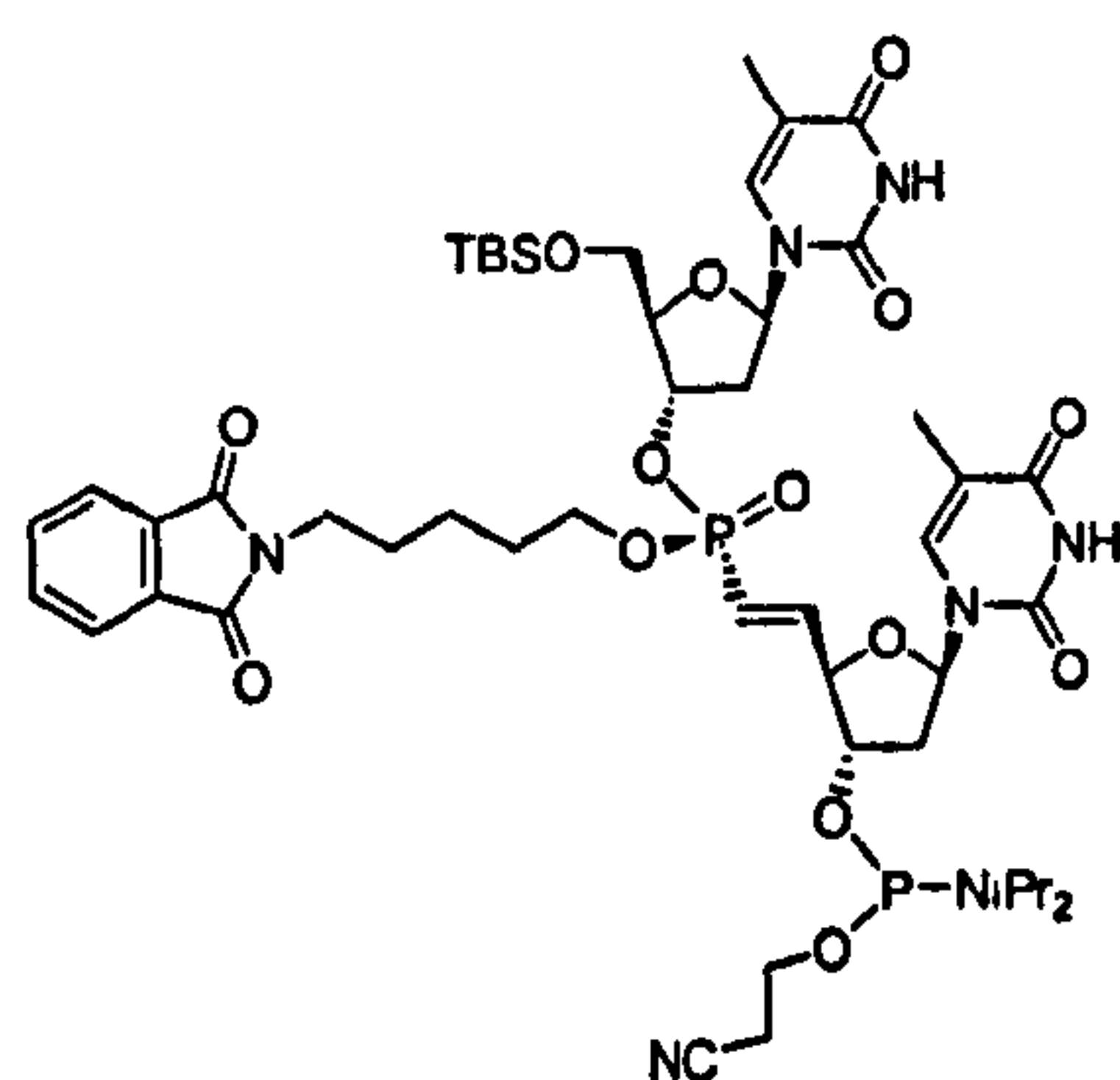


A 2.5 mL screw cap reaction vial was charged with Pd(OAc)<sub>2</sub> (0.007 g, 0.031 mmol, 0.1 eq), Ph<sub>3</sub>P (0.033 g, 0.126 mmol, 0.4 eq), *H*-phosphonate 17(fast) (0.250 g, 0.394 mmol, 1.25 eq), vinylbromide 8 (0.100 g, 0.315 mmol), propylene oxide (0.44 mL, 6.3 mmol, 20 eq) and THF (1.8 mL). The stirring mixture was heated at 70°C (oil bath temperature) for 15 h. After cooling to RT, the volatiles were removed *in vacuo* and the residue was purified by column chromatography (AcOEt:MeOH 20:1) to give 21 (0.221 g, 80%) as a pale yellow solid; mp 93-95°C, (Found: (HRMS ES)  $M^+ + Na$  894.3141, C<sub>40</sub>H<sub>54</sub>N<sub>5</sub>O<sub>13</sub>PSiNa



requires 894.3123);  $[\alpha]_D +4.6$  (c 0.8 in  $\text{CHCl}_3$ );  $\nu_{\text{max}}/\text{cm}^{-1}$  3391, 2931, 1772, 1711, 1464, 1398, 1363, 1276, 1128, 1074, 1002, 976, 908;  $\delta_{\text{H}}(400 \text{ MHz})$  9.49 (1H, br s), 7.83 (2H, dd,  $J$  5.4, 3.0), 7.71 (2H, dd,  $J$  5.4, 3.0), 7.48 (1H, m), 7.13 (1H, m), 6.98 (1H, ddd,  $J$  22.7, 17.1, 4.2), 6.36 (1H, dd,  $J$  9.0, 5.1), 6.29 (1H, t,  $J$  6.5), 6.06 (1H, ddd,  $J$  20.6, 18.4, 1.2), 5.02 (1H, app t,  $J$  6.4), 4.47 (2H, m), 4.28 (1H, br s), 4.06 (2H, dt,  $J$  6.8, 6.5), 3.87 (2H, m), 3.69 (2H, t,  $J$  7.1), 2.51 (1H, dd,  $J$  13.4, 5.0), 2.43 (1H, m), 2.32 (1H, m), 2.14 (1H, m), 1.92 (3H, s), 1.91 (3H, s), 1.73 (4H, m), 1.44 (2H, m), 0.91 (9H, s), 0.12 (6H, s);  $\delta_{\text{C}}(100 \text{ MHz})$  168.6 (C), 163.9 (C), 150.7 (C), 150.5 (C), 149.5 (CH), 135.9 (CH), 135.1 (C), 134.1 (CH), 132.1 (CH), 123.3 (CH), 117.6 (d,  $^1J_{\text{CP}}$  188.8) (CH), 111.6 (C), 111.4 (C), 86.0 (d,  $^3J_{\text{CP}}$  4.4) (CH), 85.8 (CH), 85.8 (d,  $^3J_{\text{CP}}$  21.4), 84.7 (CH), 77.3 (d,  $^2J_{\text{CP}}$  5.1) (CH), 74.2 (CH), 66.4 ( $\text{CH}_2$ ), 63.4 ( $\text{CH}_2$ ), 39.5 ( $\text{CH}_2$ ), 39.2 ( $\text{CH}_2$ ), 37.7 ( $\text{CH}_2$ ), 29.9 (d,  $^3J_{\text{CP}}$  4.8) ( $\text{CH}_2$ ), 28.1 ( $\text{CH}_2$ ), 26.0 ( $\text{CH}_3$ ), 22.9 ( $\text{CH}_2$ ), 18.4 (C), 12.7 ( $\text{CH}_3$ ), 12.6 ( $\text{CH}_3$ ), -5.3 ( $\text{CH}_3$ ), -5.4 ( $\text{CH}_3$ );  $\delta_{\text{P}}(161.98 \text{ MHz})$  18.8.

**5'-O-(*tert*-Butyldimethylsilyl)-3'-O-(cyanoethyldiisopropylaminophosphine) pentylphthalimidephosphonate T\*T dimer 22.**



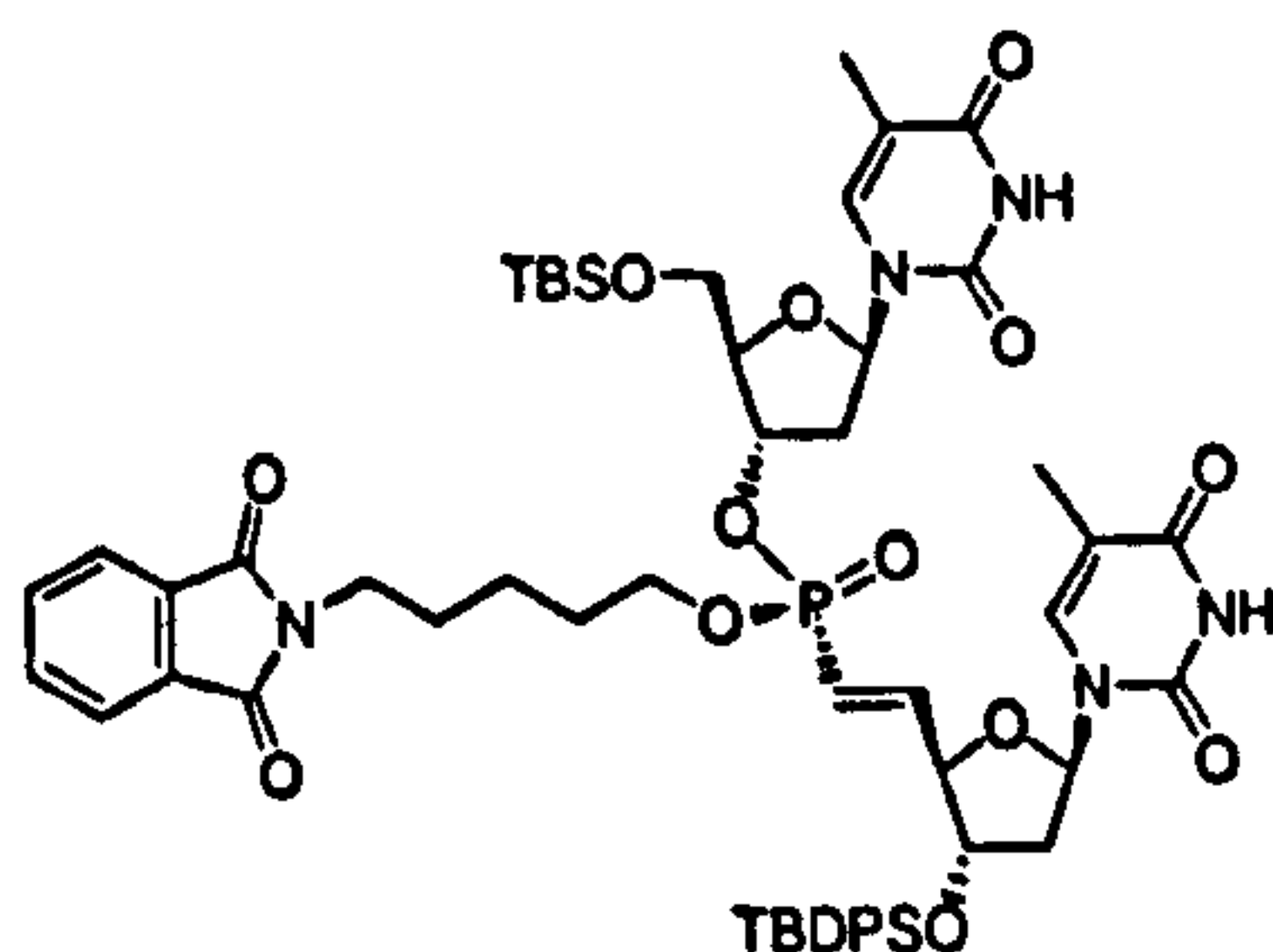
Cyanoethylbisdiisopropylamino phosphine (0.10 mL, 0.31 mmol, 1.52 eq) was dropwise added to a mixture of dimer 21 (0.178 g, 0.204 mmol), *1H*-tetrazole (0.014 g, 0.200 mmol, 1.02 eq) in DCM (2 mL) at RT under an argon atmosphere and then, stirred for 1 h. The mixture

was diluted with DCM (20 mL) and washed with a saturated aqueous solution of sodium bicarbonate (20 mL). The layers were separated and the aqueous layer extracted with DCM (2 x 30 mL). The combined organic phase was dried, the solvent removed *in vacuo* and the residue purified by column chromatography (AcOEt) to

give **22** (0.176 g, 80%) as a 1:1 mixture of diastereoisomers. (Data given for the mixture of diastereoisomers): (Found: (HRMS ES)  $M^+ + Na$  1094.4279,  $C_{49}H_{71}N_7O_{14}P_2SiNa$  requires 1094.4201);  $[\alpha]_D +11.7$  (c 2.2 in  $CHCl_3$ );  $\nu_{max}/cm^{-1}$  3697, 3600, 3391, 2954, 2931, 2862, 2359, 2339, 2256, 1772, 1711, 1692, 1602, 1463, 1397, 1366, 1266, 1128, 1075, 977, 908, 838;  $\delta_H$ (400 MHz) 8.88 (2H, m), 7.83 (2H, dd,  $J$  5.5, 3.0), 7.71 (2H, dd,  $J$  5.4, 3.1), 7.48 (1H, m), 7.09 (1H, m), 6.98 (1H, ddd,  $J$  22.8, 17.1, 4.3), 6.90 (1H, ddd,  $J$  22.7, 17.0, 4.3), 6.36 (1H, dd,  $J$  8.4, 5.1), 6.30 (1H, app q,  $J$  7.2), 6.06 (1H, ddd,  $J$  20.1, 17.1, 1.7), 6.03 (1H, ddd,  $J$  19.8, 17.0, 1.5), 5.04 (1H, app t,  $J$  6.3), 4.56 (0.5H, m), 4.50 (1.5H, m), 4.23 (1H, m), 4.04 (2H, m), 3.87 (3H, m), 3.79-3.56 (5H, m), 2.80-2.66 (1H, m), 2.65 (1H, t,  $J$  6.1), 2.55-2.41 (2H, m), 2.34 (1H, m), 2.13 (1H, m), 1.92 (6H, m), 1.73 (4H, m), 1.44 (2H, m), 1.19 (12H, m), 0.92 (4.5H, s), 0.92 (4.5H, s), 0.12 (6H, m);  $\delta_C$ (100 MHz) 168.5 (C), 163.8 (C), 163.7 (C), 150.4 (C), 150.4 (C), 150.3 (C), 148.8 (d,  $^2J_{CP}$  6.0)/148.5 (d,  $^2J_{CP}$  5.3) (CH), 135.7/135.5 (CH), 135.1 (CH), 134.0 (CH), 132.1 (CH), 123.3 (CH), 118.4 (d,  $^1J_{CP}$  189.5)/117.9 (d,  $^1J_{CP}$  189.8) (CH), 118.0/117.7 (C), 111.8 (C), 111.2 (C), 86.0 (d,  $^3J_{CP}$  4.3) (CH), 85.4 (d,  $^3J_{CP}$  15.1) (CH), 84.6 (CH), 76.9 (d,  $^2J_{CP}$  6.1)/76.8 (d,  $^2J_{CP}$  6.1) (CH), 76.0 (d,  $^2J_{CP}$  15.3)/75.5 (d,  $^2J_{CP}$  16.8) (CH), 66.2 (d,  $^2J_{CP}$  5.5) (CH<sub>2</sub>), 63.3 (CH<sub>2</sub>), 58.2 (d,  $^2J_{CP}$  13.5)/58.0 (d,  $^2J_{CP}$  13.3) (CH<sub>2</sub>), 43.4 (d,  $^2J_{CP}$  2.6)/43.3 (d,  $^2J_{CP}$  3.1) (CH), 39.6 (CH<sub>2</sub>), 38.4/38.4 (CH<sub>2</sub>), 37.7 (CH<sub>2</sub>), 29.9 (d,  $^3J_{CP}$  5.9) (CH<sub>2</sub>), 28.1 (CH<sub>2</sub>), 26.0 (CH<sub>3</sub>), 24.6 (d,  $^3J_{CP}$  6.2)/24.6 (d,  $^3J_{CP}$  7.0) (CH<sub>3</sub>), 22.9 (CH<sub>2</sub>), 20.5 (d,  $^3J_{CP}$  3.0)/20.5 (d,  $^3J_{CP}$  3.1) (CH<sub>2</sub>), 18.4 (C), 12.6 (CH<sub>3</sub>), 12.5 (CH<sub>3</sub>), -5.3 (CH<sub>3</sub>), -5.4 (CH<sub>3</sub>);  $\delta_P$ (161.98 MHz) 150.6, 150.5, 19.1, 18.8.



**5'-O-(*tert*-Butyldimethylsilyl)-3'-O-(*tert*-butyldiphenylsilyl)pentylphthalimide phosphonate T\*T dimer 18a.**

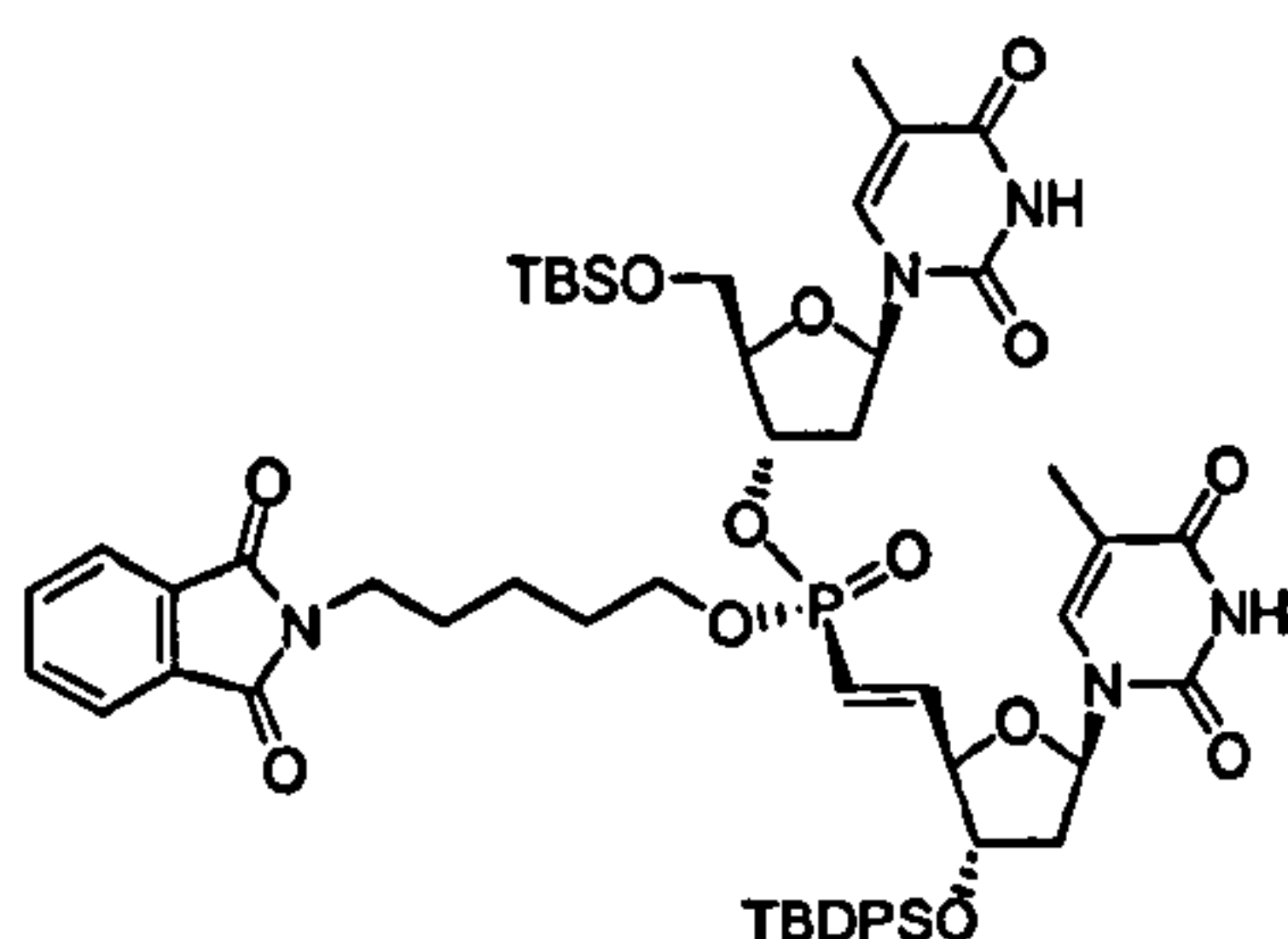


A 2.5 mL screw cap reaction vial was charged with Pd(OAc)<sub>2</sub> (0.004 g, 0.018 mmol, 0.13 eq), dppf (0.016 g, 0.029 mmol, 0.21 eq), *H*-phosphonate 17(fast) (0.090 g, 0.142 mmol, 1.01 eq), vinylbromide 7 (0.078 g, 0.141 mmol), propylene oxide (0.20 mL, 2.86 mmol, 20.3 eq) and THF (1.6 mL). The stirring mixture was heated at 70°C (oil bath temperature) for 15 h. After cooling to RT, the volatiles were removed *in vacuo* and the residue was purified by column chromatography (AcOEt:Petrol:MeOH 1:1:0.1) to give 18a (0.078 g, 50%) as a pale yellow waxy solid; (Found: (HRMS ES) M<sup>+</sup>+Na 1132.4279, C<sub>56</sub>H<sub>72</sub>N<sub>5</sub>O<sub>13</sub>PSi<sub>2</sub>Na requires 1132.4301); [α]<sub>D</sub> −9.8 (c 0.9 in CHCl<sub>3</sub>); ν<sub>max</sub>/cm<sup>−1</sup> 3666, 3392, 2931, 2859, 1772, 1713, 1693, 1589, 1462, 1396, 1366, 1322, 1294, 1276, 1113, 1066, 999, 976, 886, 838; δ<sub>H</sub>(400 MHz) 9.24 (1H, br s), 9.16 (1H, br s), 7.81 (2H, dd, *J* 5.4, 3.1), 7.69 (2H, dd, *J* 5.4, 3.1), 7.63 (4H, m), 7.50-7.36 (7H, m), 6.96 (1H, m), 6.52 (1H, ddd, *J* 22.3, 17.1, 4.9), 6.40 (1H, dd, *J* 7.9, 5.9), 6.36 (1H, dd, *J* 9.1, 5.3), 5.70 (1H, ddd, *J* 19.7, 17.1, 1.6), 4.99 (1H, app t, *J* 6.6), 4.47 (1H, m), 4.28 (1H, m), 4.14 (1H, d, *J* 1.0), 3.94 (2H, m), 3.85 (1H, dd, *J* 11.7, 2.0), 3.79 (1H, dd, *J* 11.5, 2.1), 3.66 (2H, t, *J* 7.2), 2.49 (1H, dd, *J* 13.5, 4.9), 2.28 (1H, ddd, *J* 13.7, 6.0, 2.9), 2.10 (1H, ddd, *J* 14.3, 9.1, 5.7), 1.97 (1H, m), 1.92 (3H, d, *J* 0.9), 1.86 (3H, d, *J* 0.8), 1.68 (4H, m), 1.39 (2H, m), 1.09 (9H, s), 0.91 (9H, s), 0.11 (3H, s), 0.10 (3H, s); δ<sub>C</sub>(100 MHz) 168.4 (C), 163.8 (C), 163.6 (C), 150.4 (C), 150.4 (C), 148.7 (d, <sup>2</sup>*J*<sub>CP</sub> 5.4) (CH), 135.8 (CH), 135.7 (CH), 135.3 (CH), 135.0 (CH), 134.0 (CH), 132.7 (CH), 132.1 (C), 130.4 (CH), 130.3 (CH), 128.1 (CH), 128.1 (CH), 123.2 (CH), 118.2 (d, <sup>1</sup>*J*<sub>CP</sub> 189.7) (CH), 111.6 (C), 111.2 (C), 86.2 (d, <sup>3</sup>*J*<sub>CP</sub> 22.5) (CH), 86.0 (d, <sup>3</sup>*J*<sub>CP</sub> 4.6) (CH), 85.7 (CH), 84.6 (CH), 77.1 (d, <sup>2</sup>*J*<sub>CP</sub>



5.0) (CH), 76.1 (CH), 66.1 (d,  $^2J_{CP}$  5.6) (CH<sub>2</sub>), 63.3 (CH<sub>2</sub>), 39.7 (d,  $^3J_{CP}$  3.2) (CH<sub>2</sub>), 39.1 (CH<sub>2</sub>), 37.6 (CH<sub>2</sub>), 29.9 (d,  $^3J_{CP}$  6.3) (CH<sub>2</sub>), 28.1 (CH<sub>2</sub>), 26.9 (CH<sub>3</sub>), 26.0 (CH<sub>3</sub>), 22.8 (CH<sub>2</sub>), 19.1 (C), 18.3 (C), 12.6 (CH<sub>3</sub>), 12.6 (CH<sub>3</sub>), -5.3 (CH<sub>3</sub>), -5.4 (CH<sub>3</sub>);  $\delta_P$ (161.98 MHz) 18.8.

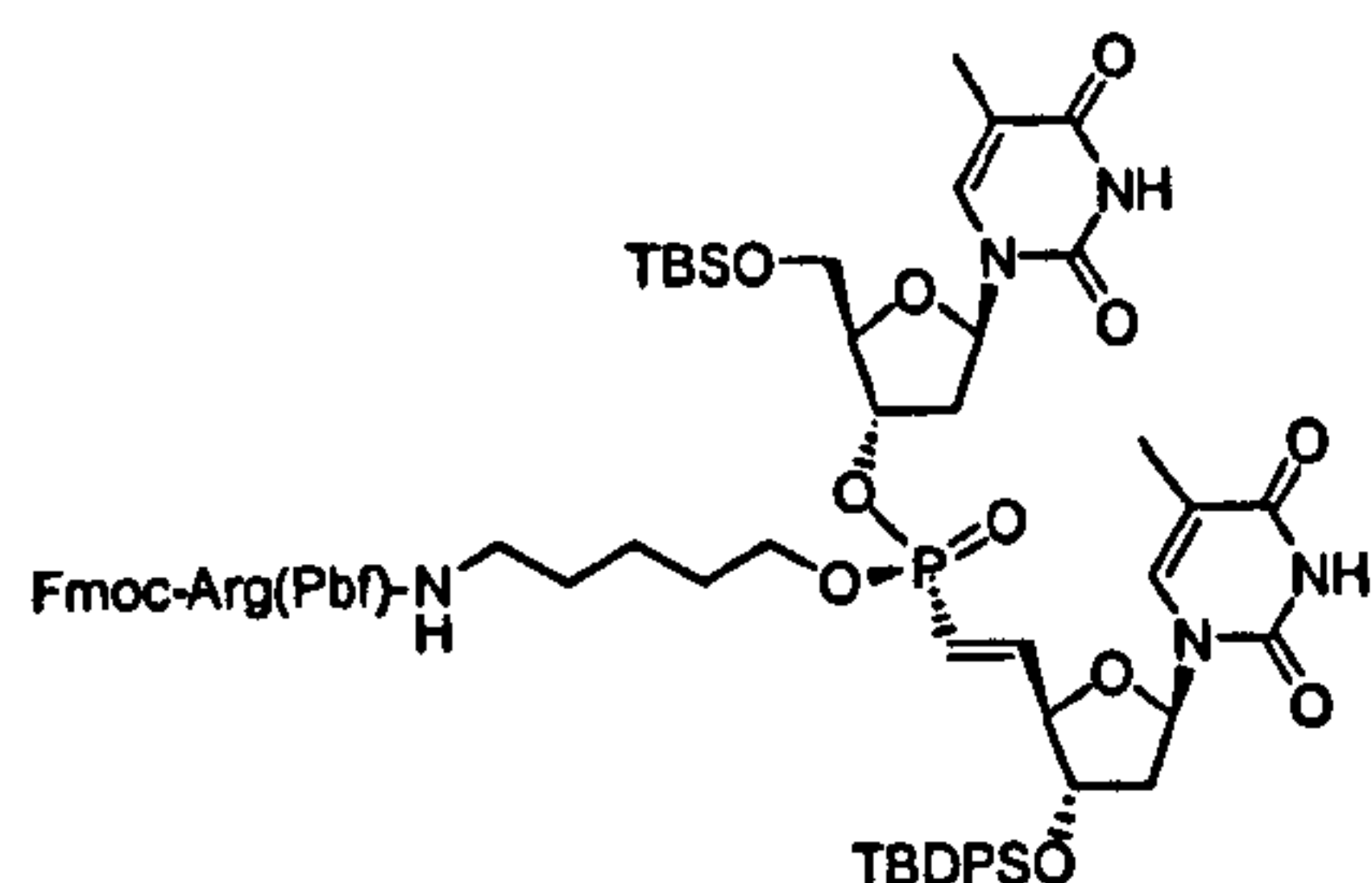
**5'-O-(*tert*-Butyldimethylsilyl)-3'-O-(*tert*-butyldiphenylsilyl)pentylphthalimide phosphonate T\*T dimer 18b.**



A 2.5 mL screw cap reaction vial was charged with Pd(OAc)<sub>2</sub> (0.006 g, 0.027 mmol, 0.10 eq), dppf (0.029 g, 0.052 mmol, 0.20 eq), *H*-phosphonate 17(slow) (0.182 g, 0.287 mmol, 1.08 eq), vinylbromide 7 (0.147 g, 0.266 mmol), propylene oxide (0.36 mL, 5.14 mmol, 19.3 eq) and THF (2.0 mL). The stirring mixture was heated at 70°C (oil bath temperature) for 13 h. After cooling to RT, the volatiles were removed *in vacuo* and the residue was purified by column chromatography (AcOEt:Petrol:MeOH 1:1:0.1) to give 18b (0.195 g, 67%) as a white waxy solid; (Found: (HRMS ES) M<sup>+</sup>+Na 1132.4296, C<sub>56</sub>H<sub>72</sub>N<sub>5</sub>O<sub>13</sub>PSi<sub>2</sub>Na requires 1132.4301); [ $\alpha$ ]<sub>D</sub> +8.9 (c 1.0 in CHCl<sub>3</sub>);  $\nu_{max}/cm^{-1}$  3385, 2932, 2851, 1711, 1692, 1463, 1397, 1363, 1265, 1076, 1000, 908;  $\delta_H$ (400 MHz) 9.28 (1H, br s), 9.11 (1H, br s), 7.83 (2H, dd, *J* 5.4, 3.1), 7.70 (2H, dd, *J* 5.4, 3.1), 7.63 (4H, m), 7.47-7.36 (7H, m), 7.01 (1H, m), 6.55 (1H, ddd, *J* 22.0, 17.1, 4.7), 6.32 (1H, dd, *J* 5.0, 3.4), 6.30 (1H, dd, *J* 6.2, 2.9), 5.73 (1H, ddd, *J* 19.2, 17.1, 1.7), 4.97 (1H, app t, *J* 6.3), 4.48 (1H, m), 4.32 (1H, m), 4.20 (1H, d, *J* 1.0), 3.93 (2H, dd, *J* 12.6, 6.0), 3.89 (1H, dd, *J* 11.4, 1.8), 3.84 (1H, dd, *J* 11.4, 2.0), 3.67 (2H, t, *J* 7.2), 2.43 (1H, dd, *J* 13.7, 5.2), 2.24 (1H, ddd, *J* 13.6, 6.1, 3.0), 2.09 (1H, m), 2.01 (1H, m), 1.91 (3H, d, *J* 0.8), 1.87 (3H, d, *J* 0.9), 1.69 (4H, m), 1.39 (2H, m), 1.08 (9H, s), 0.91 (9H, s), 0.11 (3H, s), 0.10 (3H, s);  $\delta_C$ (100

MHz) 168.4 (C), 163.7 (C), 150.4 (C), 150.3 (C), 148.9 (d,  $^2J_{CP}$  5.9) (CH), 136.1 (CH), 135.8 (CH), 135.7 (CH), 135.0 (CH), 134.0 (CH), 132.8 (C), 132.1 (C), 130.3 (CH), 130.3 (CH), 128.1 (CH), 128.0 (CH), 123.3 (CH), 118.2 (d,  $^1J_{CP}$  188.6) (CH), 111.5 (C), 111.3 (C), 86.5 (CH), 86.3 (d,  $^3J_{CP}$  18.1) (CH), 86.2 (CH), 84.7 (CH), 77.1 (d,  $^2J_{CP}$  5.0) (CH), 76.1 (CH), 65.8 (d,  $^2J_{CP}$  5.5) (CH<sub>2</sub>), 63.4 (CH<sub>2</sub>), 39.4 (d,  $^3J_{CP}$  2.9) (CH<sub>2</sub>), 38.9 (CH<sub>2</sub>), 37.6 (CH<sub>2</sub>), 29.9 (d,  $^3J_{CP}$  6.3) (CH<sub>2</sub>), 28.1 (CH<sub>2</sub>), 26.9 (CH<sub>3</sub>), 26.0 (CH<sub>3</sub>), 22.8 (CH<sub>2</sub>), 19.1 (C), 18.4 (C), 12.6 (CH<sub>3</sub>), -5.3 (CH<sub>3</sub>), -5.4 (CH<sub>3</sub>);  $\delta_P$ (161.98 MHz) 18.9.

**5'-O-(*tert*-Butyldimethylsilyl)-3'-O-(*tert*-butyldiphenylsilyl)-pentylamino-N-(Fmoc-Arg(Pbf))phosphonate T\*T dimer 25a.**



Hydrazine monohydrate (0.11 mL, 2.27 mmol, 32.0 eq) was added dropwise to a stirring solution of the dimer 18a (0.079 g, 0.071 mmol) in MeOH (3.8 mL) at RT under an argon atmosphere and the

mixture was stirred for 2 h. DCM (30 mL) was added and this organic layer was washed with water (20 mL) and brine (20 mL), then was dried and the volatiles removed *in vacuo* to give the crude free amine 23a (0.065 g) as a colourless waxy solid; (Found: (HRMS ES)  $M^+ + H$  980.4415, C<sub>48</sub>H<sub>71</sub>N<sub>5</sub>O<sub>11</sub>PSi<sub>2</sub> requires 980.4426);  $[\alpha]_D +5.3$  (c 1.3 in CHCl<sub>3</sub>);  $\nu_{max}/cm^{-1}$  3392, 2931, 2859, 1692, 1463, 1363, 1277, 1067, 999, 975, 908, 837;  $\delta_H$ (400 MHz) 7.65 (4H, m), 7.40 (7H, m), 6.93 (1H, m), 6.55 (1H, m), 6.40-6.20 (2H, m), 5.72 (1H, m), 4.99 (1H, m), 4.49 (1H, m), 4.45-4.32 (1H, m), 4.18 (1H, m), 4.11-3.74 (6H, m), 2.53 (1H, m), 2.25 (1H, m), 2.19-1.98 (2H, m), 1.90 (3H, s), 1.86 (3H, s), 1.65 (4H, m), 1.39 (2H, m), 1.09 (9H, s), 0.91 (9H, s), 0.10 (3H, s), 0.10 (3H, s).

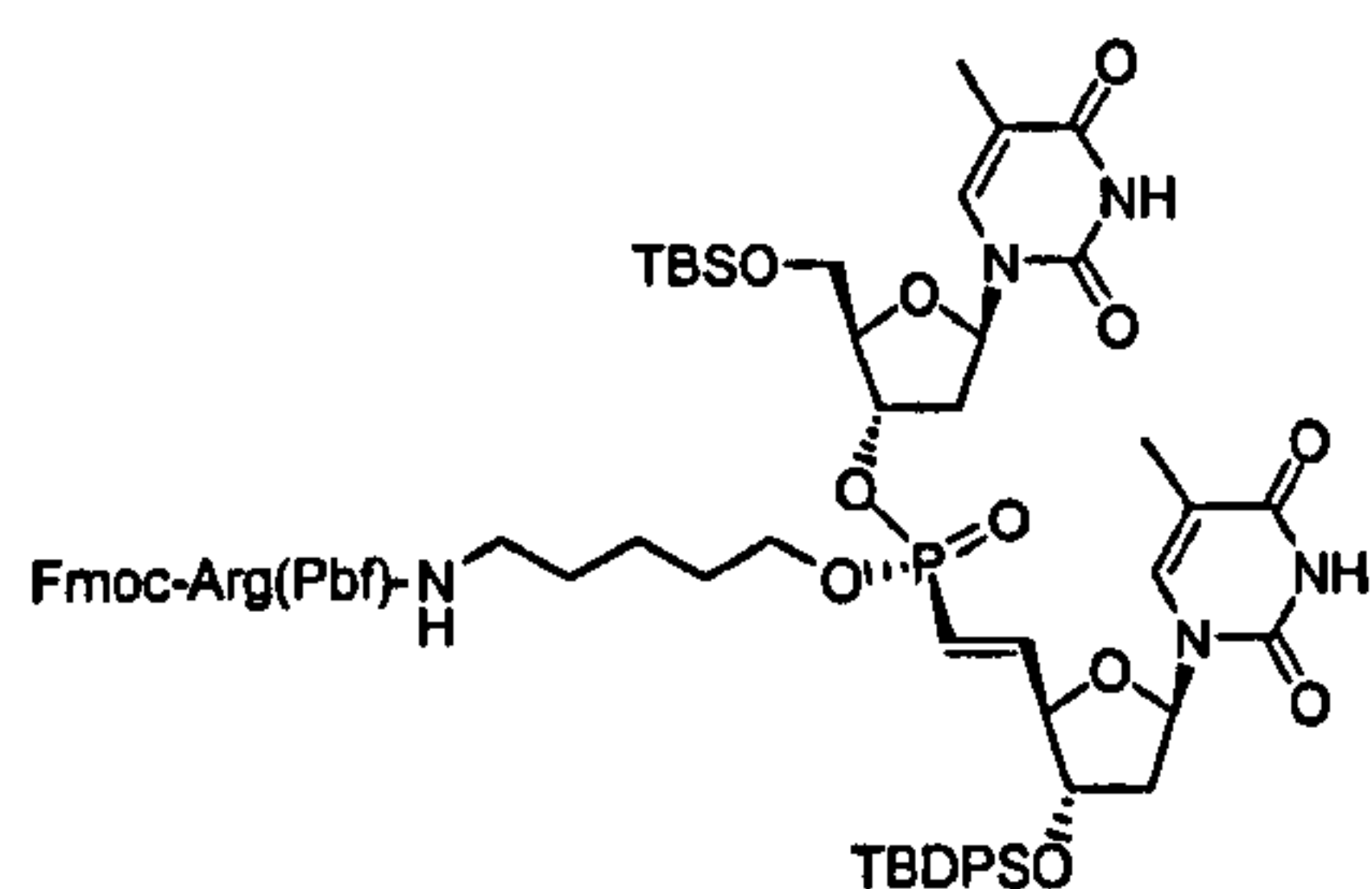


EDC (0.017 g, 0.089 mmol, 1.44 eq), BtOH (0.015 g, 0.111 mmol, 1.79 eq) and NMM (0.011 mL, 0.100 mmol, 1.61 eq) were added to a solution of Fmoc-Arg(Pbf)-OH (0.043 g, 0.066 mmol, 1.06 eq) in DCM (2.8 mL) at 0°C under an argon atmosphere. The mixture was stirred for 20 min and a solution of the crude free amine **23a** (0.061 g, 0.062 mmol) in DCM (1.5 mL) was added dropwise. The mixture was stirred for 15 h allowing it to warm to RT. Water (5 mL) was then added and the mixture stirred for 5 min. The mixture was diluted with DCM (30 mL) and washed with water (10 mL). The layers were separated and the aqueous layer extracted with DCM (2 x 20 mL). The combined organic phase was dried, the solvent removed *in vacuo* and the residue purified by column chromatography (AcOEt:MeOH 20:1) to give **25a** (0.052 g, 49%) as a white solid, mp 129-131°C; (Found: (MS FAB)  $M^+ + Na$  1633,  $M^+ + H$  1611  $C_{82}H_{109}N_9O_{17}PSSi_2Na$  requires 1633,  $C_{82}H_{110}N_9O_{17}PSSi_2$  requires 1611);  $\nu_{max}/cm^{-1}$  3350, 2931, 2859, 1892, 1558, 1462, 1363, 1292, 1105, 998, 974, 907, 838, 641;  $\delta_H$ (400 MHz) 9.71 (2H, m), 7.73 (2H, d,  $J$  7.5), 7.67-7.56 (6H, m), 7.51-7.31 (9H, m), 7.25 (2H, m), 6.96 (1H, m), 6.55 (1H, ddd,  $J$  22.2, 17.0, 4.5), 6.34 (2H, m), 6.23 (2H, m), 5.76 (1H, m), 4.99 (1H, m), 4.47 (1H, m), 4.35 (4H, m), 4.17 (1H, t,  $J$  7.0), 4.12 (1H, br s), 3.99-3.71 (4H, m), 3.33 (2H, m), 3.16 (2H, m), 2.91 (2H, s), 2.58 (3H, s), 2.54 (1H, m), 2.51 (3H, s), 2.22 (2H, m), 2.12 (1H, m), 2.06 (3H, s), 1.91 (3H, d,  $J$  0.9), 1.86 (3H, br s), 1.84 (2H, m), 1.66 (2H, m), 1.53 (4H, m), 1.43 (6H, s), 1.39 (2H, m), 1.09 (9H, s), 0.91 (9H, s), 0.10 (6H, m);  $\delta_C$ (100 MHz) 172.4 (C), 164.0 (C), 163.4 (C), 158.8 (C), 156.7/156.6 (C), 150.8 (C), 150.3 (C), 149.1 (d,  $^2J_{CP}$  5.9) (CH), 143.9 (C), 143.8 (C), 141.3 (C), 138.4 (CH), 137.0 (CH), 135.8 (CH), 135.7 (CH), 135.0 (CH), 133.1 (C), 133.0 (C), 132.7 (C), 132.7 (C), 132.4 (C), 130.4 (CH), 130.3 (CH), 130.3 (CH), 128.1 (CH), 128.1 (CH), 128.0 (CH), 127.7 (CH), 127.1 (CH), 125.3 (CH), 124.7 (C), 120.0 (CH), 117.8 (d,  $^1J_{CP}$  188.6) (CH), 117.5 (C), 111.5 (C), 111.2



(C), 86.5 (d,  $^3J_{CP}$  22.2) (CH), 86.4 (C), 86.2 (d,  $^3J_{CP}$  3.1) (CH), 86.1/86.0 (CH), 84.8/84.8 (CH), 77.1 (d,  $^2J_{CP}$  7.5) (CH), 76.3 (CH), 67.2 (CH<sub>2</sub>), 66.5 (d,  $^2J_{CP}$  3.2) (CH<sub>2</sub>), 63.4 (CH<sub>2</sub>), 54.1 (CH), 47.2 (CH), 43.3 (CH<sub>2</sub>), 40.1 (CH<sub>2</sub>), 39.9 (CH<sub>2</sub>), 39.4 (CH<sub>2</sub>), 39.1 (CH<sub>2</sub>), 38.5 (CH<sub>2</sub>), 29.8 (CH<sub>2</sub>), 28.6 (CH<sub>3</sub>), 26.9 (CH<sub>3</sub>), 26.0 (CH<sub>3</sub>), 23.0 (CH<sub>2</sub>), 19.4 (CH<sub>3</sub>), 19.1 (CH<sub>3</sub>), 18.4 (CH<sub>3</sub>), 18.0 (CH<sub>3</sub>), 12.6 (CH<sub>3</sub>), 12.6 (CH<sub>3</sub>), -5.3 (CH<sub>3</sub>), -5.4 (CH<sub>3</sub>);  $\delta_P$ (161.98 MHz) 19.0.

**5'-O-(*tert*-Butyldimethylsilyl)-3'-O-(*tert*-butyldiphenylsilyl)-pentylamino-N-(Fmoc-Arg(Pbf))phosphonate T\*T dimer 25b.**



Hydrazine monohydrate (0.19 mL, 3.92 mmol, 31.9 eq) was added dropwise to a stirring solution of the dimer 18b (0.136 g, 0.123 mmol) in MeOH (6.5 mL) at RT under an argon atmosphere and the mixture

was stirred for 2 h. DCM (50 mL) was added and this organic layer was washed with water (50 mL) and brine (50 mL), then was dried and the volatiles removed *in vacuo* to give the crude free amine 23b (0.130 g) as a colourless waxy solid; (Found: (HRMS ES)  $M^+ + H$  980.4459, C<sub>48</sub>H<sub>71</sub>N<sub>5</sub>O<sub>11</sub>PSi<sub>2</sub> requires 980.4426);  $[\alpha]_D$  +2.5 (c 1.3 in CHCl<sub>3</sub>);  $\nu_{max}/cm^{-1}$  3391, 2932, 2859, 1692, 1463, 1363, 1276, 1112, 1076, 999, 975, 908, 838;  $\delta_H$ (400 MHz) 7.62 (4H, m), 7.42 (7H, m), 6.98 (1H, m), 6.54 (1H, m), 6.41-6.22 (2H, m), 5.73 (1H, m), 4.96 (1H, m), 4.46 (1H, m), 4.32 (1H, m), 4.20 (3H, m), 4.01-3.80 (4H, m), 2.40 (1H, m), 2.22 (1H, m), 2.20-1.97 (2H, m), 1.89 (3H, s), 1.85 (3H, s), 1.64 (4H, m), 1.57-1.32 (2H, m), 1.08 (9H, s), 0.90 (9H, s), 0.11 (3H, s), 0.10 (3H, s).

EDC (0.031 g, 0.162 mmol, 1.41 eq), BtOH (0.018 g, 0.133 mmol, 1.16 eq) and NMM (0.020 mL, 0.182 mmol, 1.58 eq) were added to a solution of Fmoc-Arg(Pbf)-OH

(0.076 g, 0.118 mmol, 1.03 eq) in DCM (5.4 mL) at 0°C under an argon atmosphere. The mixture was stirred for 20 min and a solution of the crude free amine **23b** (0.113 g, 0.115 mmol) in DCM (2.7 mL) was added dropwise. The mixture was stirred for 15 h allowing it to warm to RT. Water (10 mL) was then added and the mixture stirred for 5 min. The mixture was diluted with DCM (50 mL) and washed with water (20 mL). The layers were separated and the aqueous layer extracted with DCM (2 x 30 mL). The combined organic phase was dried, the solvent removed *in vacuo* and the residue purified by column chromatography (AcOEt:MeOH 20:1) to give **25b** (0.115 g, 67%) as a white solid, mp 133-135°C; (Found: (MS FAB)  $M^+ + Na$  1633,  $M^+ + H$  1611  $C_{82}H_{109}N_9O_{17}PSSi_2Na$  requires 1633,  $C_{82}H_{110}N_9O_{17}PSSi_2$  requires 1611);  $\nu_{max}/cm^{-1}$  3696, 3605, 3349, 2932, 2859, 1892, 1602, 1562, 1462, 1362, 1291, 1105, 998, 975, 908, 837;  $\delta_H$ (400 MHz) 9.65 (1H, br s), 9.20 (1H, br s), 7.74 (2H, d,  $J$  7.5), 7.66-7.55 (6H, m), 7.49-7.33 (9H, m), 7.26 (2H, m), 6.99 (1H, m), 6.51 (1H, m), 6.36 (1H, m), 6.29 (1H, dd,  $J$  8.8, 5.3), 6.10 (2H, m), 5.70 (1H, ddd,  $J$  20.2, 16.7, 1.4), 4.92 (1H, m), 4.46 (1H, m), 4.35 (4H, m), 4.22 (1H, br s), 4.17 (1H, t,  $J$  7.0), 3.91 (2H, m), 3.84 (2H, m), 3.40 (2H, m), 3.14 (2H, m), 2.92 (2H, s), 2.60 (3H, s), 2.52 (3H, s), 2.39 (2H, m), 2.24 (1H, m), 2.07 (3H, s), 2.06 (1H, m), 1.91 (3H, d,  $J$  0.8), 1.87 (3H, br s), 1.86 (2H, m), 1.64 (2H, m), 1.53 (4H, m), 1.43 (6H, s), 1.38 (2H, m), 1.08 (9H, s), 0.91 (9H, s), 0.11 (3H, s), 0.10 (3H, s);  $\delta_C$ (100 MHz) 172.3 (C), 164.1 (C), 163.6 (C), 158.8 (C), 156.6 (C), 150.6 (C), 150.2 (C), 149.1 (d,  $^2J_{CP}$  5.8) (CH), 144.0 (C), 143.8 (C), 141.3 (C), 138.4 (CH), 137.8 (CH), 135.8 (CH), 135.8 (CH), 135.1 (CH), 133.1 (C), 132.9 (C), 132.8 (C), 132.4 (C), 130.4 (CH), 130.3 (CH), 128.1 (CH), 128.1 (CH), 127.8 (CH), 127.1 (CH), 125.3 (CH), 124.7 (C), 120.0 (CH), 117.6 (d,  $^1J_{CP}$  187.8) (CH), 117.5 (C), 111.6 (C), 111.1 (C), 86.8 (CH), 86.7 (d,  $^3J_{CP}$  20.9) (CH), 86.4 (C), 86.2 (CH), 86.2 (CH), 84.9 (CH), 77.4 (CH), 76.4 (CH), 67.1 (CH<sub>2</sub>), 66.4 (d,  $^2J_{CP}$  4.6)

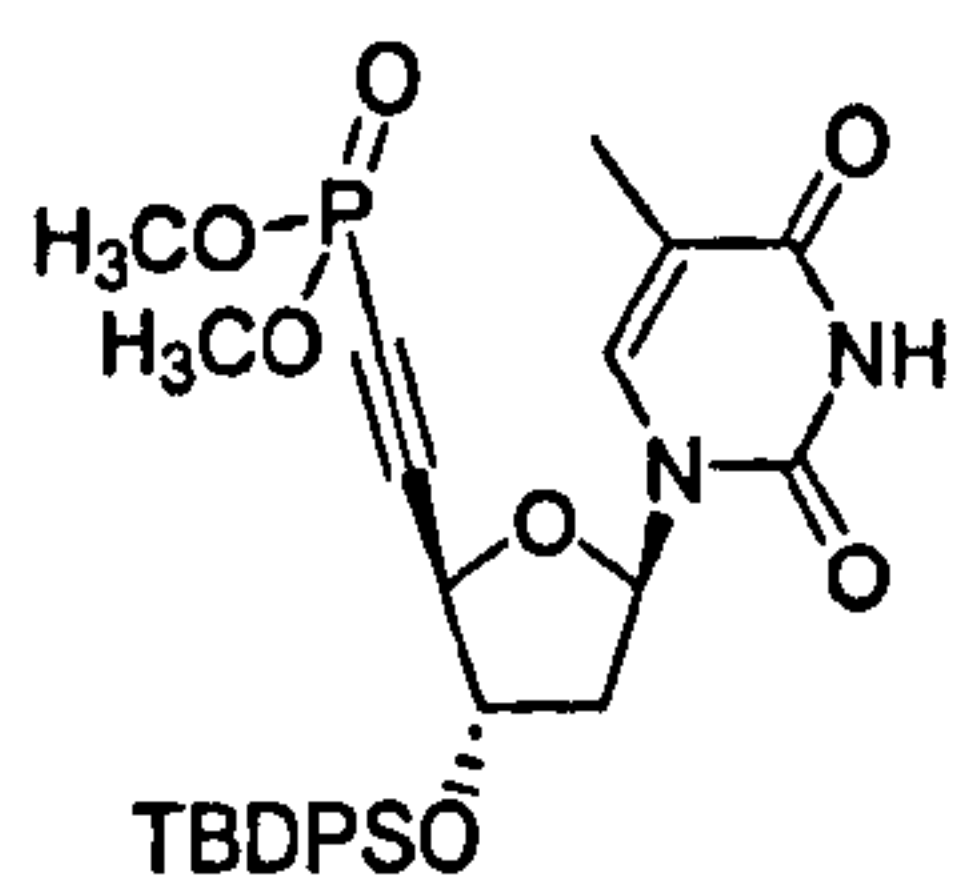


(CH<sub>2</sub>), 63.5 (CH<sub>2</sub>), 53.9 (CH), 47.2 (CH), 43.3 (CH<sub>2</sub>), 39.9 (d, <sup>3</sup>J<sub>CP</sub> 4.4) (CH<sub>2</sub>), 39.3 (CH<sub>2</sub>), 39.2 (CH<sub>2</sub>), 38.4 (CH<sub>2</sub>), 29.8 (CH<sub>2</sub>), 28.7 (CH<sub>3</sub>), 26.9 (CH<sub>3</sub>), 26.0 (CH<sub>3</sub>), 22.8 (CH<sub>2</sub>), 19.4 (CH<sub>3</sub>), 19.1 (CH<sub>3</sub>), 18.4 (CH<sub>3</sub>), 18.1 (CH<sub>3</sub>), 12.6 (CH<sub>3</sub>), 12.6 (CH<sub>3</sub>), -5.3 (CH<sub>3</sub>), -5.4 (CH<sub>3</sub>); δ<sub>P</sub>(161.98 MHz) 18.8; *m/z* (FAB) 1633 (M<sup>+</sup>+Na, 6), 1611 (M<sup>+</sup>+H, 10), 441 (12), 419 (17), 189 (16), 179 (12), 176 (25), 155 (14), 154 (52), 149 (37), 138 (17), 137 (33), 136 (48), 135 (11), 127 (14), 109 (14), 107 (24), 105 (15), 97 (23), 95 (28), 93 (12), 91 (25), 89 (21), 85 (35), 83 (35), 81 (33), 79 (17), 77 (26), 73 (29), 71 (64), 70 (10), 69 (64), 67 (33), 57 (100), 55 (85).

#### General procedure A for the preparation of alkynylphosphonates:

A mixture of Pd(OAc)<sub>2</sub> (0.2 eq) and dppf (0.4 eq) or TFP (0.8 eq) in DMF was flushed with dry nitrogen and stirred at RT for 20 min. A solution of the 1,1-dibromoalkene (1.0 eq) in DMF, propylene oxide (3.0 eq) and dimethyl phosphite (2.0 eq) were added and the mixture was heated at 80°C (bath temperature) for 14 h and then cooled to RT. Removal of the solvent *in vacuo* left a residue which was purified by column chromatography.

**[(2R, 3S, 5R)-3-(*tert*-Butyl-diphenyl-silanyloxy)-5-(5-methyl-2,4-dioxo-3,4-dihydro-2*H*-pyrimidin-1-yl)-tetrahydro-furan-2ylethynyl]-phosphonic acid dimethyl ester 59.**

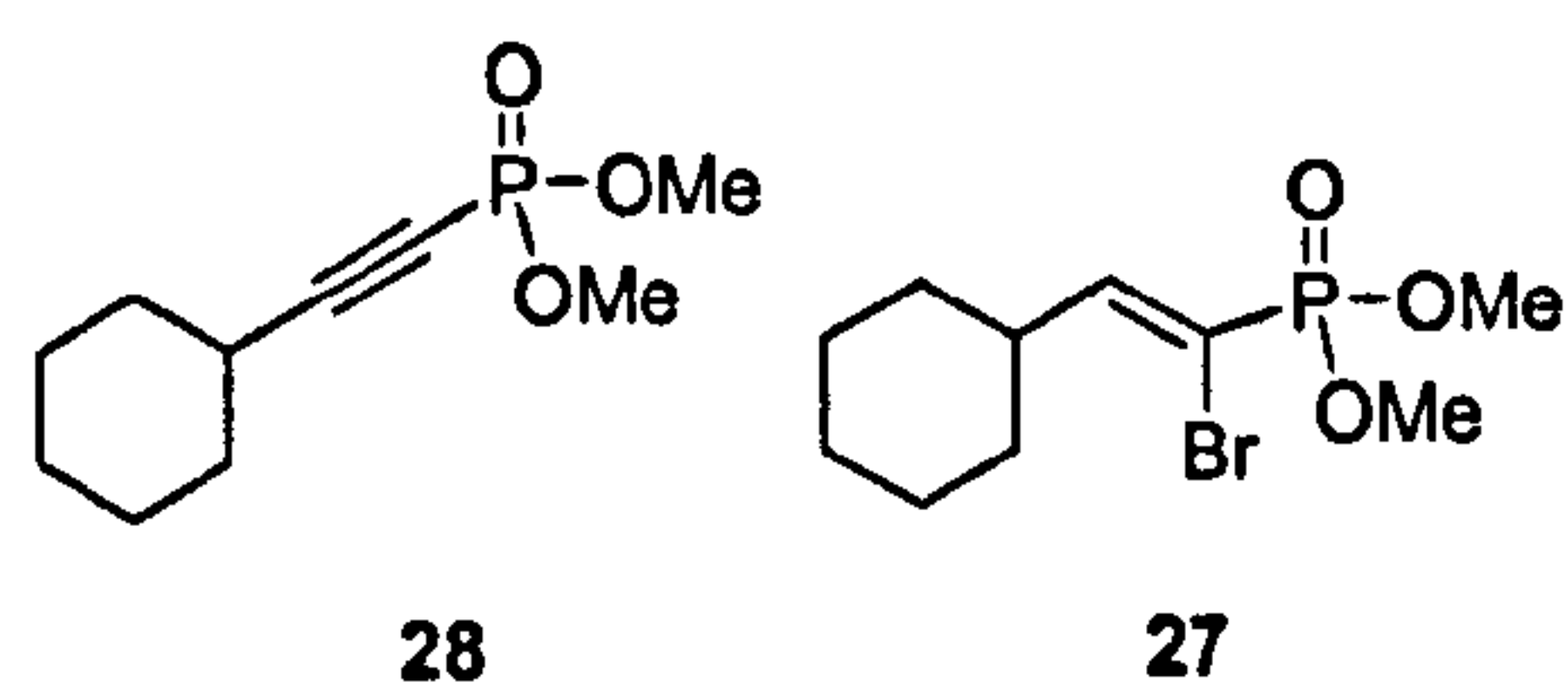


According to the general procedure A using Pd(OAc)<sub>2</sub> (7 mg, 0.033 mmol) and dppf (36 mg, 0.066 mmol) in DMF (1 mL), dibromo 6 (104 mg, 0.165 mmol) in DMF (1.5 mL), propylene oxide (35 μL, 0.50 mmol) and dimethyl phosphite (30 μL, 0.33 mmol) and after purification by column chromatography (AcOEt: Pentane 3:1) was



obtained **59** (60 mg, 63%) as a white solid, mp 98-100°C; (Found (HRMS FAB)  $M^+ + H$ , 583.2047,  $C_{29}H_{36}O_7N_2PSi$  requires 583.2029);  $\nu_{\max}/\text{cm}^{-1}$  3391, 2955, 2933, 2205, 1694, 1463, 1394, 1365, 1309, 1294, 1278, 1105, 1053, 980, 955, 908, 843;  $\delta_H$ (400 MHz) 7.64 (4H, m), 7.44 (6H, m), 6.62 (1H, dd,  $J$  8.4, 5.6), 4.64 (1H, d,  $J$  3.2), 4.57 (1H, d,  $J$  4.2), 3.74 (3H, d,  $J$  12.2), 3.73 (3H, d,  $J$  12.2), 2.46 (1H, m), 2.05 (1H, m), 1.92 (3H, s), 1.09 (9H, s);  $\delta_C$ (100 MHz,  $C_6D_6$ ) 164.0 (C), 150.9 (C), 136.0 (CH), 135.9 (CH), 134.8 (CH), 132.8 (C), 130.6 (CH), 128.4 (CH), 128.4 (CH), 111.8 (C), 96.3 (d,  $^2J_{CP}$  48.2) (C), 86.9 (CH), 78.5 (CH), 78.4 (d,  $^1J_{CP}$  290.8) (C), 76.4 (d,  $^3J_{CP}$  3.4) (CH), 52.9 (d,  $^2J_{CP}$  5.2) ( $CH_3$ ), 39.8 ( $CH_2$ ), 26.8 (3 x  $CH_3$ ), 19.1 (C), 12.7 ( $CH_3$ );  $\delta_P$ (161.98 MHz) -3.8;  $m/z$  (FAB) 583 ( $M^+ + H$ , 33), 525 (21), 307 (20), 201 (45), 176 (32), 165 (27), 155 (28), 154 (94), 147 (22), 138 (32), 137 (68), 136 (100), 135 (30), 121 (23), 107 (39), 105 (27), 95 (23), 91 (47), 90 (34), 89 (36), 81 (33), 79 (25), 78 (20), 77 (47), 73 (66), 69 (39), 67 (25), 57 (46), 55 (48).

**Cyclohexylethynyl-phosphonic acid dimethyl ester **28** and ((Z)-1-Bromo-2-cyclohexyl-vinyl)-phosphonic acid dimethyl ester **27**.**



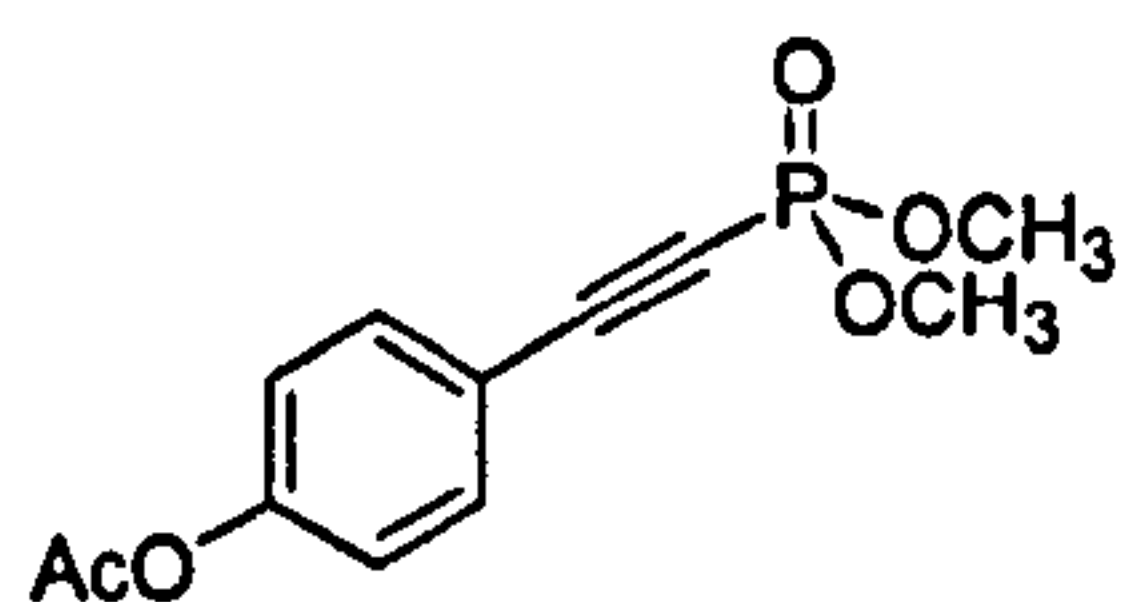
According to the general procedure A using  $Pd(OAc)_2$  (21 mg, 0.094 mmol) and dppf (102 mg, 0.184 mmol) in DMF (1 mL), dibromo **26** (123 mg, 0.459 mmol) in DMF (1.5 mL), propylene oxide (96  $\mu$ L 1.37 mmol) and dimethyl phosphite (84  $\mu$ L, 0.92 mmol) and after purification by column chromatography (AcOEt:Petroleum ether 1:1) was obtained **28** (88 mg, 89%) as an orange oil. When  $Pd(OAc)_2$  (16 mg, 0.071 mmol) and dppf (90 mg, 0.162 mmol) in THF (1 mL), dibromo **26** (85 mg, 0.32 mmol) in THF (1 mL), propylene oxide (70  $\mu$ L, 1.00 mmol) and dimethyl phosphite (60  $\mu$ L, 0.65 mmol) were used and after

purification by column chromatography (AcOEt:Petroleum ether 1:1) was obtained **28** (28 mg, 41%) and **27** (23 mg, 24%) as a separable mixture.

Data for **28**: (Found: C, 55.8; H 7.8.  $C_{10}H_{17}O_3P$  requires C, 55.6; H, 7.9); (Found (HRMS FAB)  $M^+ + H$ , 217.1012,  $C_{10}H_{18}O_3P$  requires 217.0994);  $\nu_{\max}/\text{cm}^{-1}$  2937, 2857, 2201, 1450, 1272, 1048, 842;  $\delta_H$ (400 MHz) 3.78 (6H, d,  $J$  12.3), 2.54 (1H, m), 1.83 (2H, m), 1.71 (2H, m), 1.52 (3H, m), 1.32 (3H, m);  $\delta_C$ (100 MHz,  $C_6D_6$ ) 106.2 (d,  $^2J_{CP}$  51.0) (C), 70.9 (d,  $^1J_{CP}$  300.5) (C), 52.5 (d,  $^2J_{CP}$  5.3) ( $CH_3$ ), 31.4 ( $CH_2$ ), 29.3 (d,  $^3J_{CP}$  3.7) (CH), 25.6 ( $CH_2$ ), 24.5 ( $CH_2$ );  $\delta_P$ (161.98 MHz)  $-1.3$ ;  $m/z$  (FAB) 218 ( $M^+ + H$ , 11), 217 (100), 154 (8), 136 (10).

Data for **27**: (Found (HRMS FAB)  $M^+ + H$ , 297.0264,  $C_{10}H_{19}O_3PBr$  requires 297.0255);  $\nu_{\max}/\text{cm}^{-1}$  2932, 2854, 1614, 1450, 1278, 1042, 952, 881, 838;  $\delta_H$ (400 MHz) 6.99 (1H, dd,  $J$  14.4, 9.0), 3.77 (6H, d,  $J$  11.2), 2.61 (1H, m), 1.74 (5H, m), 1.35-1.20 (5H, m);  $\delta_C$ (67.5 MHz) 155.9 (d,  $^2J_{CP}$  13.3) (CH), 108.5 (d,  $^1J_{CP}$  208.6) (C), 53.3 (d,  $^2J_{CP}$  4.9) ( $CH_3$ ), 41.2 (d,  $^3J_{CP}$  12.1) (CH), 30.7 ( $CH_2$ ), 25.6 ( $CH_2$ ), 25.2 ( $CH_2$ );  $\delta_P$ (161.98 MHz) 4.0;  $m/z$  (FAB) 297 ( $M^+ + H$ , 100), 154 (52), 138 (21), 137 (49), 136 (40).

#### Acetic acid 4-(dimethoxy-phosphorylethynyl)-phenyl ester **44**.

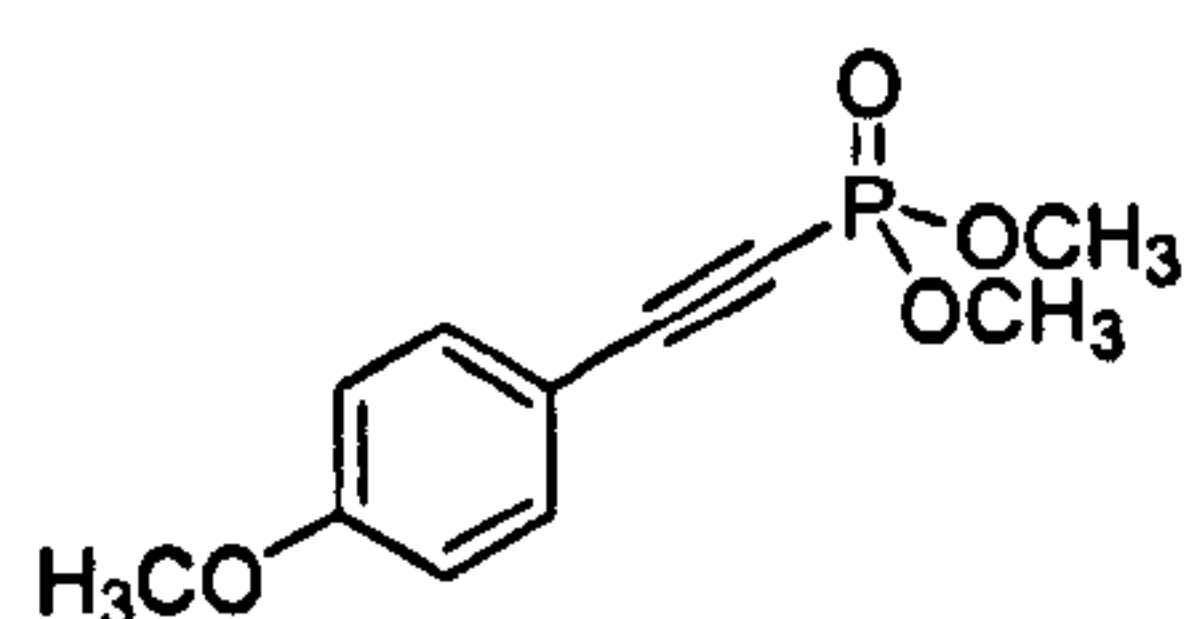


According to the general procedure A using  $Pd(OAc)_2$  (13 mg, 0.058 mmol) and dppf (61 mg, 0.110 mmol) in DMF (1 mL), dibromo **37** (87 mg, 0.27 mmol) in DMF (1.5 mL), propylene oxide (57  $\mu\text{L}$ , 0.81 mmol) and dimethyl phosphite (50  $\mu\text{L}$ , 0.55 mmol) and after purification by column chromatography (AcOEt:Petroleum ether 3:1) was obtained **44** (50 mg, 68%) as a colourless oily solid; (Found (HRMS ES)  $M^+ + H$ , 269.0563,  $C_{12}H_{14}O_5P$  requires 269.0579);  $\nu_{\max}/\text{cm}^{-1}$  2955, 2854, 2190, 1766, 1600, 1504, 1371,



1275, 1165, 1046, 911, 871, 843;  $\delta_{\text{H}}$ (400 MHz) 7.59 (2H, d,  $J$  8.6), 7.13 (2H, d,  $J$  8.6), 3.85 (6H, d,  $J$  12.3), 2.31 (3H, s);  $\delta_{\text{C}}$ (67.5 MHz) 168.7 (C), 152.4 (C), 134.0 (d,  $^4J_{\text{CP}}$  2.4) (CH), 122.1 (CH), 116.7 (d,  $^3J_{\text{CP}}$  4.9) (C), 99.0 (d,  $^2J_{\text{CP}}$  52.2) (C), 77.0 (d,  $^1J_{\text{CP}}$  300.8) (C), 53.4 (d,  $^2J_{\text{CP}}$  6.0) (CH<sub>3</sub>), 21.0 (CH<sub>3</sub>);  $\delta_{\text{P}}$ (161.98 MHz, CDCl<sub>3</sub>) -1.7;  $m/z$  (ES) 269 (M<sup>+</sup>+H, 100), 254 (2).

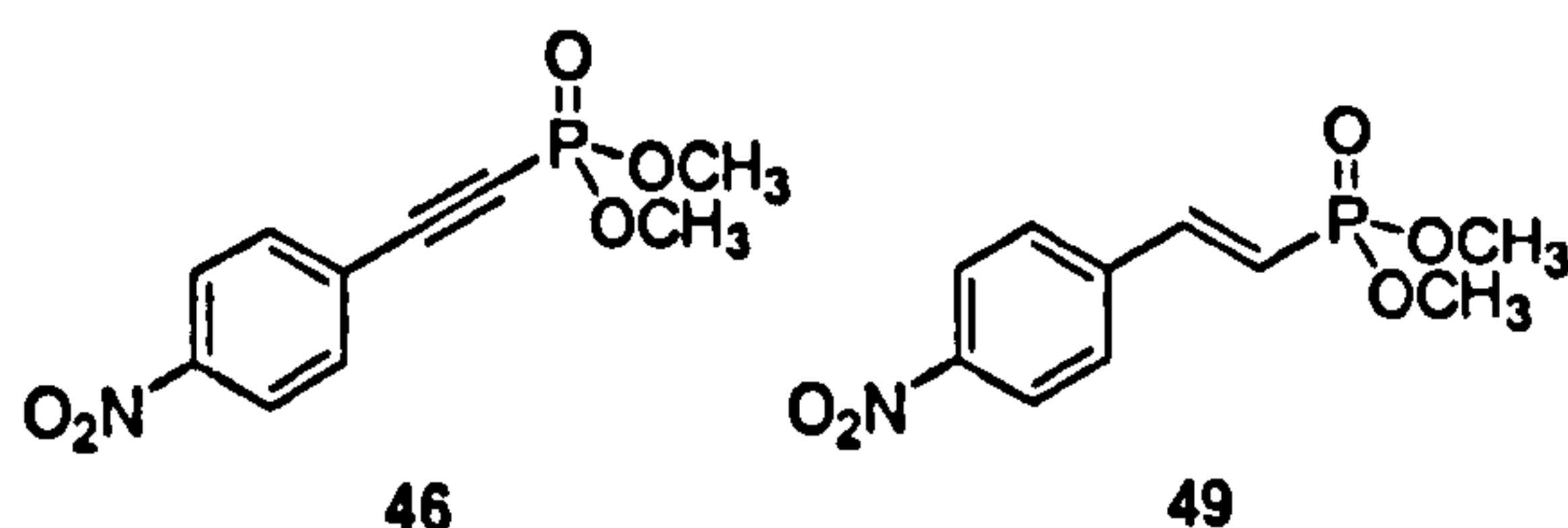
#### (4-Methoxy-phenylethynyl)-phosphonic acid dimethyl ester 43.



According to the general procedure A using Pd(OAc)<sub>2</sub> (20 mg, 0.089 mmol) and dppf (93 mg, 0.17 mmol) in DMF (1 mL), dibromo 36 (122 mg, 0.418 mmol) in DMF (1.5 mL), propylene oxide (88  $\mu$ L, 1.26 mmol) and dimethyl phosphite (77  $\mu$ L, 0.84 mmol) and after purification by column chromatography (AcOEt:Petroleum ether 1:1) was obtained 43 (73 mg, 73%) as an orange oil; (Found (HRMS EI) M<sup>+</sup>, 240.0558, C<sub>11</sub>H<sub>13</sub>O<sub>4</sub>P requires 240.0552);  $\nu_{\text{max}}$ /cm<sup>-1</sup> 2954, 2184, 1605, 1459, 1298, 1276, 1050, 872;  $\delta_{\text{H}}$ (400 MHz) 7.48 (2H, d,  $J$  8.8), 6.85 (2H, d,  $J$  8.8), 3.81 (6H, d,  $J$  12.3), 3.80 (3H, s);  $\delta_{\text{C}}$ (100 MHz, C<sub>6</sub>D<sub>6</sub>) 161.6 (C), 134.6 (CH), 114.4 (CH), 111.7 (d,  $^3J_{\text{CP}}$  5.7) (C), 99.8 (d,  $^2J_{\text{CP}}$  53.1) (C), 78.0 (d,  $^1J_{\text{CP}}$  299.9) (C), 54.8 (CH<sub>3</sub>), 52.7 (d,  $^2J_{\text{CP}}$  5.3) (CH<sub>3</sub>);  $\delta_{\text{P}}$ (161.98 MHz) -0.9;  $m/z$  (EI) 240 (M<sup>+</sup>, 9), 181 (13), 169 (9), 132 (59), 131 (52), 119 (23), 117 (13), 100 (11), 93 (14), 89 (21), 69 (100), 63 (10), 62 (10), 47 (13).



(4-Nitro-phenylethynyl)-phosphonic acid dimethyl ester **46** and ((E)-2-(4-nitrophenyl-vinyl)-phosphonic acid dimethyl ester **49**.



According to the general procedure A using Pd(OAc)<sub>2</sub> (18 mg, 0.080 mmol) and dppf (82 mg, 0.15 mmol) in DMF (1 mL), dibromo **39** (113 mg, 0.368 mmol) in DMF (1.5 mL), propylene oxide (77 μL, 1.10 mmol) and dimethyl phosphite (68 μL, 0.74 mmol) and after purification by column chromatography (AcOEt:Petroleum ether 1:1) a separable mixture of **46** (25 mg, 27%) and **49** (10 mg, 11%) was obtained. When Pd(OAc)<sub>2</sub> (16 mg, 0.071 mmol) and TFP (65 mg, 0.28 mmol) in DMF (1 mL), dibromo **39** (102 mg, 0.332 mmol) in DMF (1.5 mL), propylene oxide (70 μL, 1.00 mmol) and dimethyl phosphite (61 μL, 0.67 mmol) were used and after purification by column chromatography (AcOEt:Petroleum ether 1:1) acetylene **46** (26 mg, 31%) was obtained.

Data for **46**: Orange crystals, mp 97-99°C; (Found: C, 47.2; H, 3.9; N, 5.4. C<sub>10</sub>H<sub>10</sub>NO<sub>5</sub>P requires C, 47.1; H 4.0; N 5.5); (Found (HRMS EI) M<sup>+</sup>+H, 256.0385, C<sub>10</sub>H<sub>11</sub>O<sub>5</sub>NP requires 256.0375); ν<sub>max</sub>/cm<sup>-1</sup> 2955, 2195, 1596, 1528, 1488, 1458, 1348, 1277, 1047, 871, 857; δ<sub>H</sub>(400 MHz) 8.28 (2H, d, *J* 8.7), 7.76 (2H, d, *J* 8.7), 3.90 (6H, d, *J* 12.3); δ<sub>C</sub>(100 MHz) 148.8 (C), 133.7 (CH), 126.0 (d, <sup>3</sup>*J*<sub>CP</sub> 5.8) (C), 123.9 (CH), 96.4 (d, <sup>2</sup>*J*<sub>CP</sub> 52.4) (C), 81.7 (d, <sup>1</sup>*J*<sub>CP</sub> 297.9) (C), 53.8 (d, <sup>2</sup>*J*<sub>CP</sub> 5.6) (CH<sub>3</sub>); δ<sub>P</sub>(161.98 MHz) -3.0; *m/z* (EI) 256 (M<sup>+</sup>+H, 36), 154 (100), 138 (35), 137 (64), 136 (72), 107 (25), 89 (21).

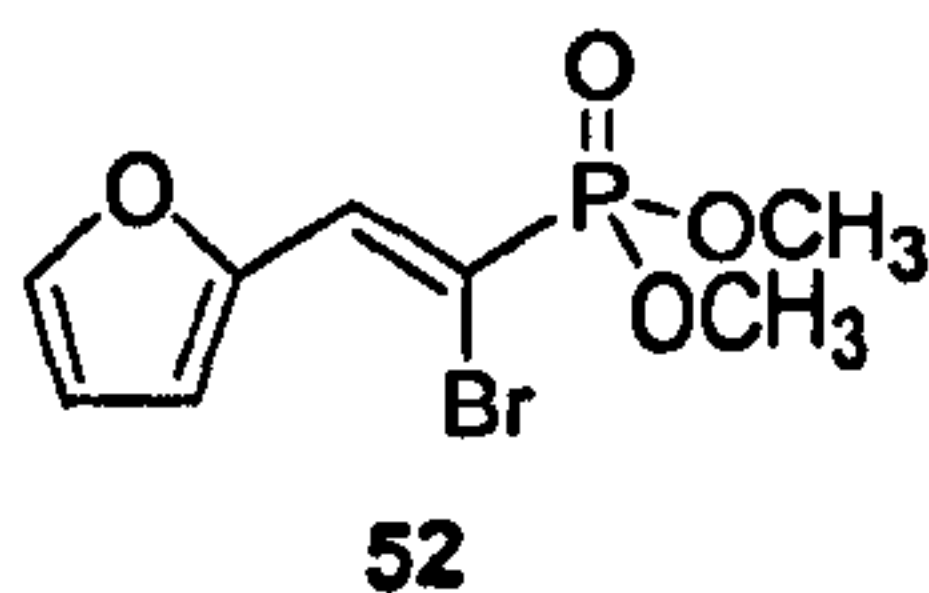
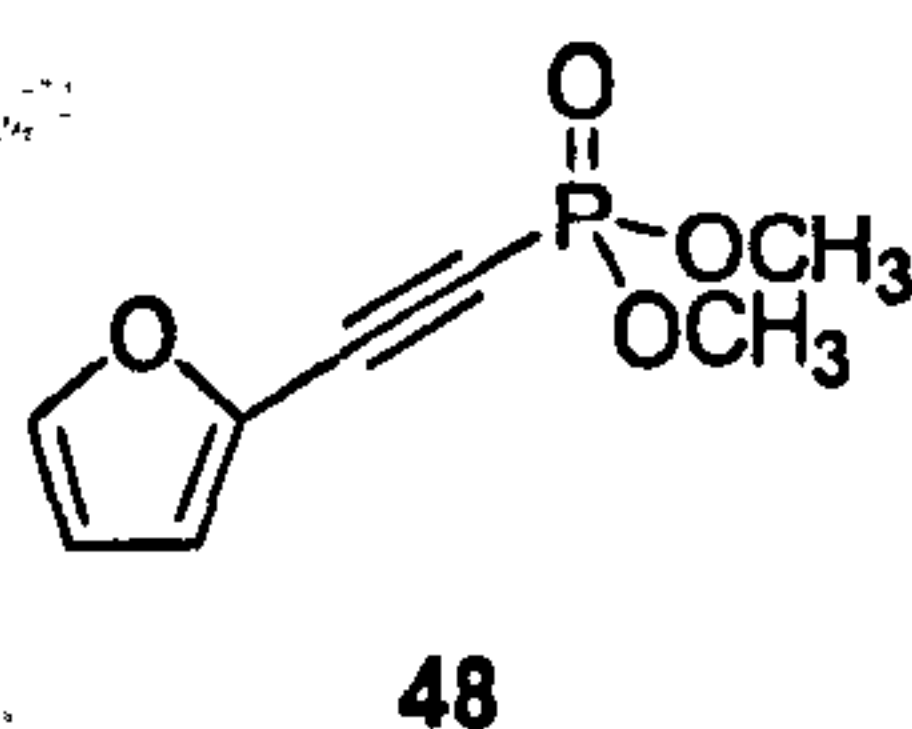
Data for **49**: White crystals, mp 102-104°C; (Found (HRMS EI) M<sup>+</sup>, 257.0457, C<sub>10</sub>H<sub>12</sub>O<sub>5</sub>NP requires 257.0453); ν<sub>max</sub>/cm<sup>-1</sup> 2955, 2852, 1596, 1347, 1110, 1059, 1042, 987, 859, 838; δ<sub>H</sub>(400 MHz) 8.27 (2H, d, *J* 8.8), 7.66 (2H, d, *J* 8.8), 7.57 (1H, dd, *J* 22.3, 17.6), 6.41 (1H, dd, *J* 17.5, 16.4), 3.82 (6H, d, *J* 11.1); δ<sub>C</sub>(100 MHz) 149.0

(C), 146.9 (d,  $^2J_{CP}$  6.5) (CH), 141.0 (d,  $^3J_{CP}$  23.7) (C), 128.8 (CH), 124.6 (CH), 118.3 (d,  $^1J_{CP}$  191.2) (CH), 53.1 (d,  $^2J_{CP}$  5.6) (CH<sub>3</sub>);  $\delta_P$ (161.98 MHz) 21.3;  $m/z$  (EI) 257 ( $M^+$ , 30), 240 (17), 181 (11), 119 (19), 117 (11), 116 (100), 110 (48).

### Phenylethynyl-phosphonic acid dimethyl ester 45.

According to the general procedure A using Pd(OAc)<sub>2</sub> (19 mg, 0.085 mmol) and dppf (94 mg, 0.17 mmol) in DMF (1 mL), dibromo 38 (113 mg, 0.431 mmol) in DMF (1.5 mL), propylene oxide (91  $\mu$ L, 1.30 mmol) and dimethyl phosphite (79  $\mu$ L, 0.86 mmol) and after purification by column chromatography (AcOEt:Petroleum ether 1:1) 45 (57 mg, 63%) was obtained as an orange oily solid; (Found (HRMS EI)  $M^+$ , 210.0452, C<sub>10</sub>H<sub>11</sub>O<sub>3</sub>P requires 210.0446);  $\nu_{max}/cm^{-1}$  2995, 2955, 2854, 2189, 1490, 1459, 1273, 1047, 865, 842, 645;  $\delta_H$ (400 MHz) 7.57 (2H, m), 7.45 (1H, m), 7.37 (2H, m), 3.85 (6H, d,  $J$  12.3);  $\delta_C$ (100 MHz, C<sub>6</sub>D<sub>6</sub>) 132.7 (d,  $^4J_{CP}$  1.4) (CH), 130.6 (CH), 128.6 (CH), 119.8 (d,  $^3J_{CP}$  5.3) (C), 99.1 (d,  $^2J_{CP}$  52.0) (C), 79.0 (d,  $^1J_{CP}$  296.9) (C), 52.8 (d,  $^2J_{CP}$  5.2) (CH<sub>3</sub>);  $\delta_P$ (161.98 MHz) -1.6;  $m/z$  (EI) 210 ( $M^+$ , 13), 115 (47), 102 (100), 89 (27), 86 (20), 84 (31), 63 (19), 49 (92), 47 (25).

### Furan-2-ylethynyl-phosphonic acid dimethyl ester 48 and ((Z)-1-Bromo-2-furyl-vinyl)-phosphonic acid dimethyl ester 52.



According to the general procedure A using Pd(OAc)<sub>2</sub> (20 mg, 0.089 mmol) and dppf (96 mg, 0.17 mmol) in DMF (1 mL), dibromo 41 (110 mg, 0.437 mmol) in DMF (1.5 mL), propylene oxide (92  $\mu$ L, 1.31 mmol) and dimethyl phosphite (80  $\mu$ L, 0.87 mmol) and after purification by column

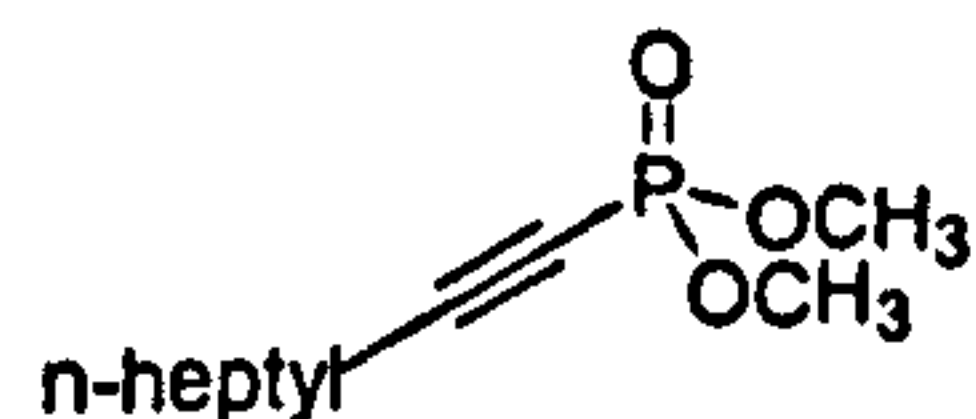


chromatography (AcOEt:Petroleum ether 1:1) a mixture of **48** (22 mg, 18%) as a colourless oil and **52** (22 mg, 25%) as a colourless oil was obtained. When Pd(OAc)<sub>2</sub> (20 mg, 0.089 mmol) and TFP (82 mg, 0.35 mmol) in DMF (1 mL), dibromo **41** (112 mg, 0.444 mmol) in DMF (1.5 mL), propylene oxide (94 µL, 1.34 mmol) and dimethyl phosphite (82 µL, 0.89 mmol) were used and after purification by column chromatography (AcOEt:Petroleum ether 1:1) acetylene **48** (53 mg, 60%) was obtained.

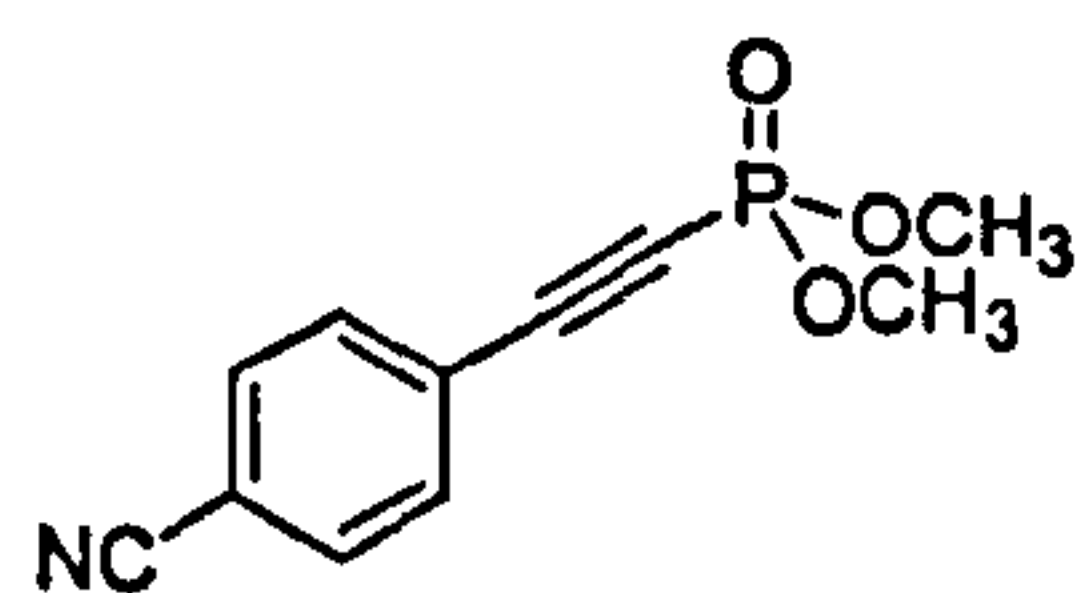
Data for **48**: (Found (HRMS EI)  $M^+$ , 200.0240, C<sub>8</sub>H<sub>9</sub>O<sub>4</sub>P requires 200.0239);  $\nu_{\max}/\text{cm}^{-1}$  2955, 2854, 2192, 2177, 1613, 1458, 1277, 1048, 964, 942, 886, 842;  $\delta_{\text{H}}$ (400 MHz) 7.47 (1H, d,  $J$  1.6), 6.89 (1H, dd,  $J$  3.5, 0.5), 6.44 (1H, dd,  $J$  3.5, 1.8), 3.83 (6H, d,  $J$  12.3);  $\delta_{\text{C}}$ (100 MHz, C<sub>6</sub>D<sub>6</sub>) 145.8 (CH), 134.9 (d,  $^3J_{\text{CP}}$  6.4) (C), 120.2 (CH), 111.3 (CH), 88.5 (d,  $^2J_{\text{CP}}$  52.5) (C), 84.3 (d,  $^1J_{\text{CP}}$  292.6) (C), 52.8 (d,  $^2J_{\text{CP}}$  5.3) (CH<sub>3</sub>);  $\delta_{\text{P}}$ (161.98 MHz) -2.2;  $m/z$  (EI) 200 ( $M^+$ , 14), 92 (100), 77 (12), 64 (16), 63 (37), 62 (11), 51 (19), 47 (28).

Data for **52**: (Found (HRMS EI)  $M^+$ , 279.9506, C<sub>8</sub>H<sub>10</sub>O<sub>4</sub>PBr requires 279.9500);  $\nu_{\max}/\text{cm}^{-1}$  2986, 2955, 2853, 1614, 1574, 1462, 1266, 1148, 1045, 964, 947, 908, 886, 839;  $\delta_{\text{H}}$ (400 MHz) 8.00 (1H, d,  $J$  16.3), 7.61 (1H, d,  $J$  0.7), 7.37 (1H, d,  $J$  3.5), 6.57 (1H, dd,  $J$  3.5, 1.7), 3.83 (6H, d,  $J$  11.4);  $\delta_{\text{C}}$ (67.5 MHz) 149.9 (d,  $^3J_{\text{CP}}$  22.9) (C), 144.8 (CH), 133.9 (d,  $^2J_{\text{CP}}$  18.9) (CH), 116.4 (CH), 112.3 (CH), 104.3 (d,  $^1J_{\text{CP}}$  211.7) (C), 53.6 (d,  $^2J_{\text{CP}}$  4.8) (CH<sub>3</sub>);  $\delta_{\text{P}}$ (161.98 MHz) 15.0;  $m/z$  (EI) 280 ( $M^+$ , 4), 282 (4), 280 (4), 202 (8), 201 (100), 109 (45), 92 (23), 84 (6), 79 (12), 65 (9), 64 (18), 63 (40), 62 (12), 52 (10), 51 (8), 49 (12), 47 (28).



**Non-1-ynyl-phosphonic acid dimethyl ester 42.**

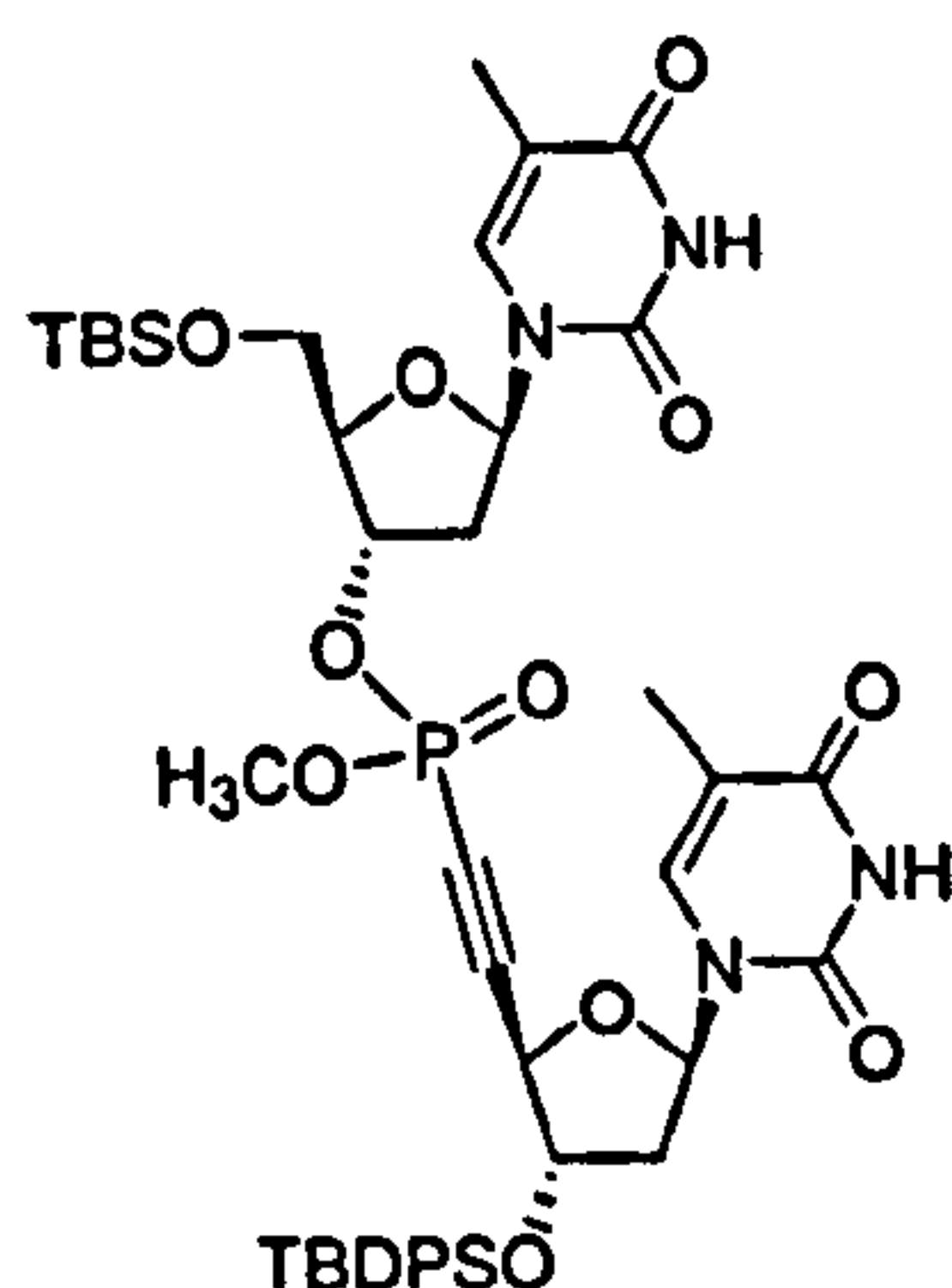
According to the general procedure A using Pd(OAc)<sub>2</sub> (18 mg, 0.080 mmol) and dppf (81 mg, 0.15 mmol) in DMF (1 mL), dibromo **35** (105 mg, 0.370 mmol) in DMF (1.5 mL), propylene oxide (78  $\mu$ L, 1.11 mmol) and dimethyl phosphite (68  $\mu$ L, 0.74 mmol) and after purification by column chromatography (AcOEt:Petroleum ether 1:1) **42** (57 mg, 66%) was obtained as a pale yellow oil; (Found (HRMS ES) M<sup>+</sup>+H, 233.1320, C<sub>11</sub>H<sub>22</sub>O<sub>3</sub>P requires 233.1307);  $\nu_{\max}/\text{cm}^{-1}$  2954, 2930, 2856, 2207, 1459, 1273, 1048, 841;  $\delta_{\text{H}}$ (400 MHz) 3.76 (6H, d,  $J$  12.2), 2.34 (2H, m), 1.58 (2H, m), 1.38 (2H, m), 1.26 (4H, m), 0.88 (3H, m);  $\delta_{\text{C}}$ (100 MHz) 104.3 (d,  $^2J_{\text{CP}}$  53.2) (C), 69.1 (d,  $^1J_{\text{CP}}$  305.6) (C), 53.2 (d,  $^2J_{\text{CP}}$  5.4) (CH<sub>3</sub>), 31.6 (CH<sub>2</sub>), 28.8 (CH<sub>2</sub>), 28.6 (CH<sub>2</sub>), 27.4 (CH<sub>2</sub>), 22.6 (CH<sub>2</sub>), 19.2 (CH<sub>2</sub>), 14.1 (CH<sub>3</sub>);  $\delta_{\text{P}}$ (161.98 MHz) -1.7.

**(4-Cyano-phenylethynyl)-phosphonic acid dimethyl ester 47.**

According to the general procedure A using Pd(OAc)<sub>2</sub> (19 mg, 0.085 mmol) and TFP (78 mg, 0.34 mmol) in DMF (1 mL), dibromo **40** (120 mg, 0.418 mmol) in DMF (1.5 mL), propylene oxide (88  $\mu$ L, 1.26 mmol) and dimethyl phosphite (77  $\mu$ L, 0.84 mmol) and after purification by column chromatography (AcOEt:Pentane 1:1) **47** (28 mg, 29%) was obtained as white crystals, mp 77-79°C; (Found: C, 55.8; H, 4.6; N, 5.6. C<sub>11</sub>H<sub>10</sub>NO<sub>3</sub>P requires C, 56.2; H 4.3; N 6.0); (Found (HRMS FAB) M<sup>+</sup>+H, 236.0488, C<sub>11</sub>H<sub>11</sub>O<sub>3</sub>NP requires 236.0477);  $\nu_{\max}/\text{cm}^{-1}$  2955, 2854, 2232, 2193, 1605, 1501, 1459, 1275, 1046, 865, 841;  $\delta_{\text{H}}$ (400 MHz) 7.68 (4H, s), 3.87 (6H, d,  $J$  12.2);  $\delta_{\text{C}}$ (100 MHz) 133.2 (CH), 132.3 (CH), 124.1 (d,  $^3J_{\text{CP}}$  5.5) (C), 117.8 (C), 114.4 (C), 96.8 (d,  $^2J_{\text{CP}}$  52.2) (C), 81.0 (d,  $^1J_{\text{CP}}$  298.1) (C) 53.7 (d,  $^2J_{\text{CP}}$  5.4) (CH<sub>3</sub>);  $\delta_{\text{P}}$ (161.98 MHz) -2.8;  $m/z$  (FAB) 236

( $M^+ + H$ , 30), 154 (30), 137 (18), 136 (24), 109 (16), 107 (13), 97 (26), 95 (35), 93 (15), 91 (18), 85 (25), 83 (42), 81 (48), 79 (16), 77 (12), 71 (48), 69 (80), 67 (33), 57 (100), 55 (92).

**5'-O-(*tert*-Butyldimethylsilyl)-3'-O-(*tert*-butyldiphenylsilyl)methyl-alkynyl phosphonate T\*T dimer 63.**



According to the general procedure A using  $Pd(OAc)_2$  (11 mg, 0.049 mmol) and dppf (54 mg, 0.097 mmol) in DMF (0.6 mL), dibromo 6 (144 mg, 0.370 mmol) in DMF (1.5 mL), propylene oxide (48  $\mu$ L, 0.69 mmol) and *H*-phosphonate 62 (137 mg, 0.316 mmol) and after purification by column chromatography (AcOEt:Pentane:MeOH 10:10:1) 63 (105 mg, 51%) was

obtained as a separable 1:1 mixture of diastereoisomers.

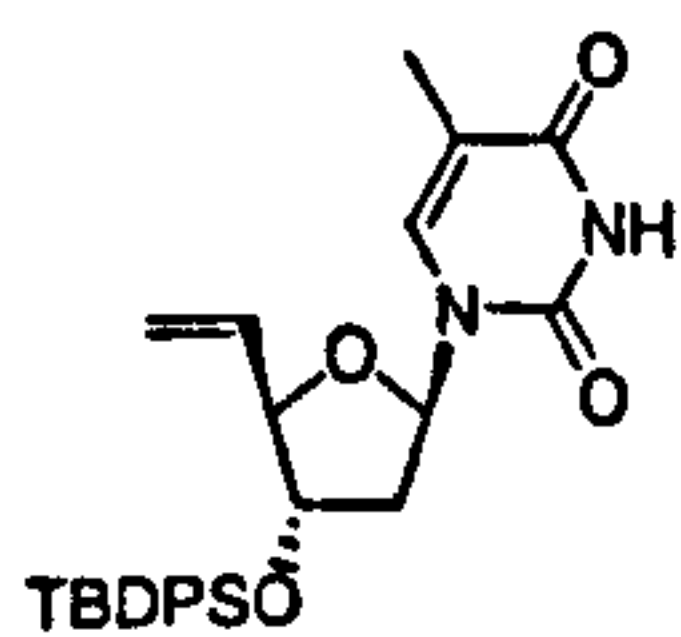
Data for the least polar diastereomer: Yellow solid, mp 112-114°C; (Found (HRMS ES)  $M^+ + H$ , 907.3546,  $C_{44}H_{60}O_{11}N_4PSi_2$  requires 907.3535);  $\nu_{max}/cm^{-1}$  3392, 2954, 2932, 2896, 2859, 2205, 1693, 1463, 1363, 1293, 1275, 1128, 1104, 1049, 1006, 979, 956, 908, 838, 611;  $\delta_H$ (400 MHz) 8.46 (1H, br s), 8.28 (1H, br s), 7.64 (4H, m), 7.46 (6H, m), 6.56 (1H, dd,  $J$  8.5, 5.8), 6.32 (1H, dd,  $J$  9.2, 5.1), 5.05 (1H, app t,  $J$  6.9), 4.68 (1H, d,  $J$  3.4), 4.61 (1H, d,  $J$  4.6), 4.22 (1H, d,  $J$  1.2), 3.88 (1H, dd,  $J$  11.5, 2.1), 3.83 (1H, dd,  $J$  11.5, 2.1), 3.74 (3H, d,  $J$  12.5), 2.48 (2H, m), 2.16-2.01 (2H, m), 1.93 (6H, s), 1.10 (9H, s), 0.92 (9H, s), 0.12 (3H, s), 0.11 (3H, s);  $\delta_C$ (100 MHz) 163.4 (C), 150.2 (C), 135.9 (CH), 135.3 (CH), 134.8 (CH), 132.5 (C), 132.3 (C), 130.8 (CH), 128.5 (CH), 111.9 (C), 111.4 (C), 96.8 (d,  $^2J_{CP}$  50.0) (C), 87.2 (CH), 86.0 (CH), 84.6 (CH), 78.8 (CH), 78.2 (CH), (doublet corresponding to the quaternary C  $\alpha$  to P was occluded by the  $CDCl_3$ ), 76.4 (CH), 63.2 (CH<sub>2</sub>), 53.7 (d,  $^2J_{CP}$  5.4) (CH<sub>3</sub>), 40.2 (CH<sub>2</sub>),



39.2 (CH<sub>2</sub>), 27.0 (3x CH<sub>3</sub>), 26.0 (3 x CH<sub>3</sub>), 19.1 (C), 18.4 (C), 12.7 (CH<sub>3</sub>), 12.6 (CH<sub>3</sub>), -5.3 (CH<sub>3</sub>), -5.4 (CH<sub>3</sub>);  $\delta_P$ (161.98 MHz) -5.8;  $m/z$  (ES) 907 (M<sup>+</sup>+H, 43), 569 (8), 340 (8), 339 (100).

Data for the most polar diastereomer: Yellow solid, mp 108-110°C; (Found (HRMS ES) M<sup>+</sup>+H, 907.3521, C<sub>44</sub>H<sub>60</sub>O<sub>11</sub>N<sub>4</sub>PSi<sub>2</sub> requires 907.3535);  $\nu_{\max}/\text{cm}^{-1}$  3390, 2954, 2932, 2204, 1692, 1463, 1363, 1274, 1105, 1063, 979;  $\delta_H$ (400 MHz) 8.57 (1H, br s), 7.65 (4H, m), 7.45 (6H, m), 6.58 (1H, dd,  $J$  8.6, 5.7), 6.36 (1H, dd,  $J$  9.2, 5.2), 5.06 (1H, app t,  $J$  7.1), 4.66 (1H, d,  $J$  3.3), 4.60 (1H, d,  $J$  4.5), 4.23 (1H, d, 1.3), 3.86 (1H, dd,  $J$  11.5, 2.1), 3.80 (1H, dd,  $J$  11.5, 2.1), 3.77 (3H, d,  $J$  12.5), 2.48 (2H, m), 2.16-2.01 (2H, m), 1.93 (6H, s), 1.10 (9H, s), 0.91 (9H, s), 0.12 (3H, s), 0.11 (3H, s);  $\delta_C$ (100 MHz) 163.6 (C), 163.4 (C) 150.3 (C), 135.7 (CH), 135.0 (CH), 134.8 (CH), 132.4 (C), 132.2 (C), 130.5 (CH), 128.2 (CH), 112.0 (C), 111.4 (C), 96.6 (d,  $^2J_{CP}$  50.3) (C), 87.0 (CH), 85.7 (d,  $^3J_{CP}$  5.0) (CH), 84.7 (CH), 78.6 (d,  $^3J_{CP}$  4.9) (CH), 78.1 (CH), (doublet corresponding to the quaternary C  $\alpha$  to P was occluded by the CDCl<sub>3</sub>), 76.3 (CH), 63.2 (CH<sub>2</sub>), 53.8 (d,  $^2J_{CP}$  5.5) (CH<sub>3</sub>), 40.1 (CH<sub>2</sub>), 39.4 (CH<sub>2</sub>), 26.8 (3 x CH<sub>3</sub>), 26.0 (3 x CH<sub>3</sub>), 19.1 (C), 18.4 (C), 12.6 (CH<sub>3</sub>), 12.6 (CH<sub>3</sub>), -5.3 (CH<sub>3</sub>), -5.4 (CH<sub>3</sub>);  $\delta_P$ (161.98 MHz) -5.8;  $m/z$  (ES) 907 (M<sup>+</sup>+H, 33), 709 (10), 576 (20), 339 (38).

### Alkene 65.

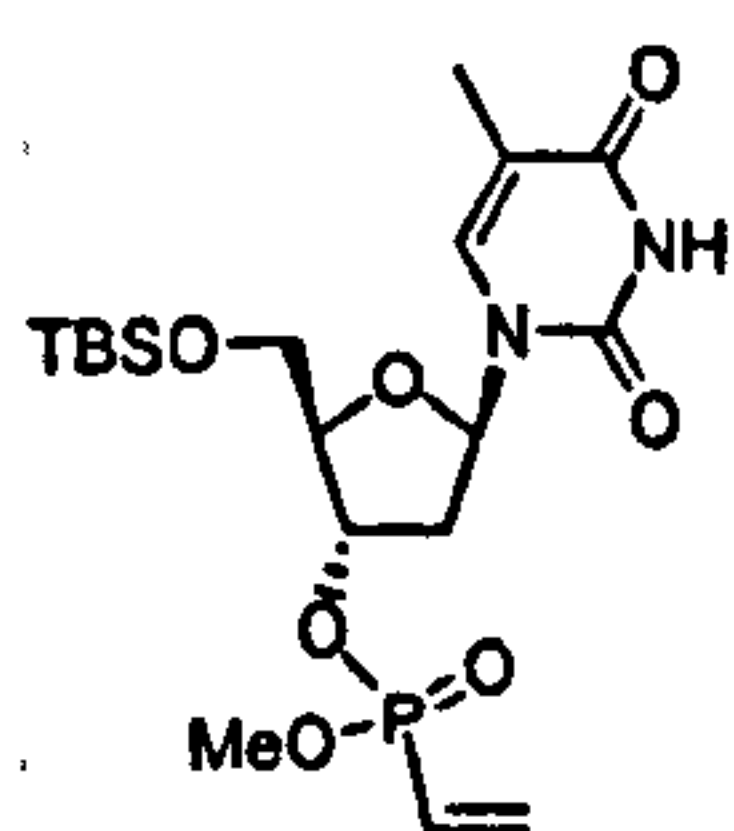


A solution of n-butyllithium in hexanes (2.22 M, 2.70 mL, 5.99 mmol) was dropwise added to a stirred suspension of methyl-triphenylphosphonium bromide in THF (10 mL) at 0°C under nitrogen. This mixture was stirred for 20 min and then added dropwise to a stirred solution of aldehyde 5 (715 mg, 1.49 mmol) in THF (10 mL) at 0°C under nitrogen. The mixture was then stirred for 18 h during which it was allowed to come to RT. Sat ammonium chloride solution (25 mL) was added and the mixture was stirred for a further 5 min.



The mixture was extracted with DCM (3 x 40 mL), the combined organic phase was dried, the solvent removed *in vacuo* and the residue was purified by column chromatography to give 65 (174 mg, 24%) as a pale yellow solid, mp 54-56°C; (Found (HRMS FAB)  $M^+ + H$ , 477.2183,  $C_{27}H_{33}O_4N_2Si$  requires 477.2210);  $\nu_{\max}/\text{cm}^{-1}$  3392, 2932, 2860, 1690, 1464, 1363, 1272, 1113, 1053, 997, 895;  $\delta_H$ (400 MHz) 8.08 (1H, br s), 7.67-7.63 (4H, m), 7.49-7.38 (6H, m), 7.05 (1H, q,  $J$  1.2), 6.37 (1H, dd,  $J$  7.4, 6.0), 5.58 (1H, ddd,  $J$  17.0, 10.5, 6.4), 5.15 (2H, m), 4.38 (1H, m), 4.20 (1H, dt,  $J$  6.5, 3.4), 2.35 (1H, ddd,  $J$  13.6, 6.0, 3.4), 1.88 (3H, d,  $J$  1.2), 1.82 (1H, ddd,  $J$  13.6, 7.5, 6.1), 1.10 (9H, s);  $\delta_C$ (100 MHz) 164.1 (C), 150.5 (C), 135.8 (CH), 135.7 (CH), 135.1 (CH), 135.0 (CH), 133.0 (C), 133.0 (C), 130.0 (CH), 127.8 (CH), 127.8 (CH), 117.9 (CH<sub>2</sub>), 111.0 (C), 87.4 (CH), 85.0 (CH), 76.2 (CH), 40.0 (CH<sub>2</sub>), 26.8 (3 x CH<sub>3</sub>), 19.0 (C), 12.6 (CH<sub>3</sub>);  $m/z$  (FAB) 477 ( $M^+ + H$ , 8), 267 (14), 247 (21), 239 (9), 199 (28), 197 (43), 189 (9), 183 (17), 181 (9), 176 (11), 165 (10), 155 (15), 154 (20), 149 (13), 145 (14), 139 (12), 137 (36), 136 (39), 135 (100), 127 (66), 121 (15), 117 (11), 115 (13), 107 (18), 105 (17), 95 (38), 93 (12), 91 (31), 90 (11), 89 (28), 83 (16), 81 (22), 79 (15), 77 (24), 75 (20), 73 (50), 71 (15), 69 (35), 67 (22), 57 (40), 55 (47).

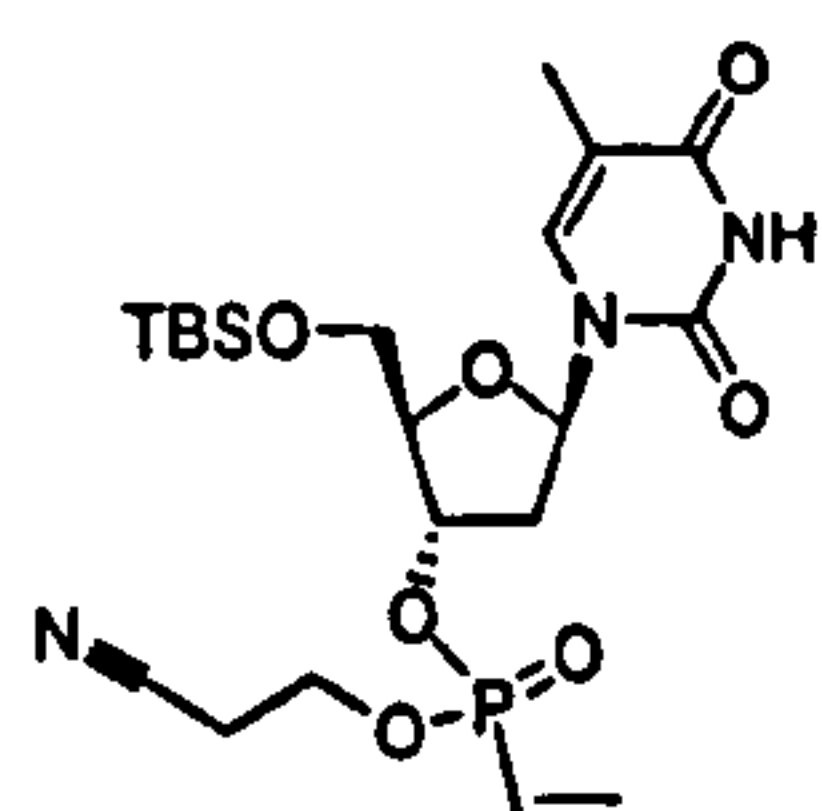
### Vinylphosphonate 66.



A 4.5 mL screw cap reaction vial was charged with  $\text{Pd}(\text{OAc})_2$  (0.019 g, 0.085 mmol, 0.1 eq),  $\text{Ph}_3\text{P}$  (0.044 g, 0.17 mmol, 0.2 eq), *H*-phosphonate 62 (0.367 g, 0.846 mmol), vinylbromide (3.5 mL, 1.0 M soln in THF, 4.1 eq) and propylene oxide (1.0 mL, 14.3 mmol, 17 eq). The mixture was heated at 70°C (oil bath temperature) for 15 h. After cooling to RT, the volatiles were removed *in vacuo* and the residue was purified by column chromatography (AcOEt:MeOH 3:1) to give 66 (0.248 g, 64%) as a 1:1 mixture of diastereoisomers.

(Data given for the mixture of diastereomers): (Found (HRMS ES)  $M^+ + Na$ , 483.1669,  $C_{19}H_{33}O_7N_2PSiNa$  requires 483.1692);  $\nu_{max}/cm^{-1}$  3393, 3186, 2954, 2930, 2858, 1694, 1463, 1400, 1385, 1363, 1322, 1292, 1277, 1128, 1065, 1050, 1005, 977, 892, 838;  $\delta_H$ (400 MHz) 9.96 (1H, br s), 7.42 (1H, s), 6.38-6.20 (2.5H, m), 6.13-5.95 (1.5H, m), 5.02 (0.5H, app t,  $J$  6.5), 4.96 (0.5H, app t,  $J$  6.5), 4.22 (0.5H, m), 4.17 (0.5H, m), 3.84 (0.5H, m), 3.82 (0.5H, m), 3.70 (1.5H, d,  $J$  11.2), 3.69 (1.5H, d,  $J$  11.3), 2.50 (0.5H, dd,  $J$  13.8, 6.3), 2.43 (0.5H, dd,  $J$  13.8, 6.3), 2.12-2.01 (1H, m), 1.86 (3H, s), 0.87 (9H, s), 0.07 (6H, s);  $\delta_C$ (100 MHz) 164.1 (C), 150.6 (C), 137.0/136.8 (CH<sub>2</sub>), 134.9 (CH), 124.9 (d,  $^1J_{CP}$  185.2)/124.8 (d,  $^1J_{CP}$  184.2)(CH), 111.1 (C), 86.0 (d,  $^2J_{CP}$  3.2)/85.9 (d,  $^2J_{CP}$  4.8)(CH), 84.4 (CH), 76.6/76.5 (CH), 52.6 (d,  $^2J_{CP}$  5.5)/52.4 (d,  $^2J_{CP}$  5.5)(CH<sub>3</sub>), 39.5 (d,  $^3J_{CP}$  3.3)/39.4 (d,  $^3J_{CP}$  4.6)(CH<sub>2</sub>), 25.8 (CH<sub>3</sub>), 18.2 (C), 12.4 (CH<sub>3</sub>), -5.5/-5.6 (CH<sub>3</sub>);  $\delta_P$ (161.98 MHz) 20.4/20.1.

### Vinylphosphonate 74.

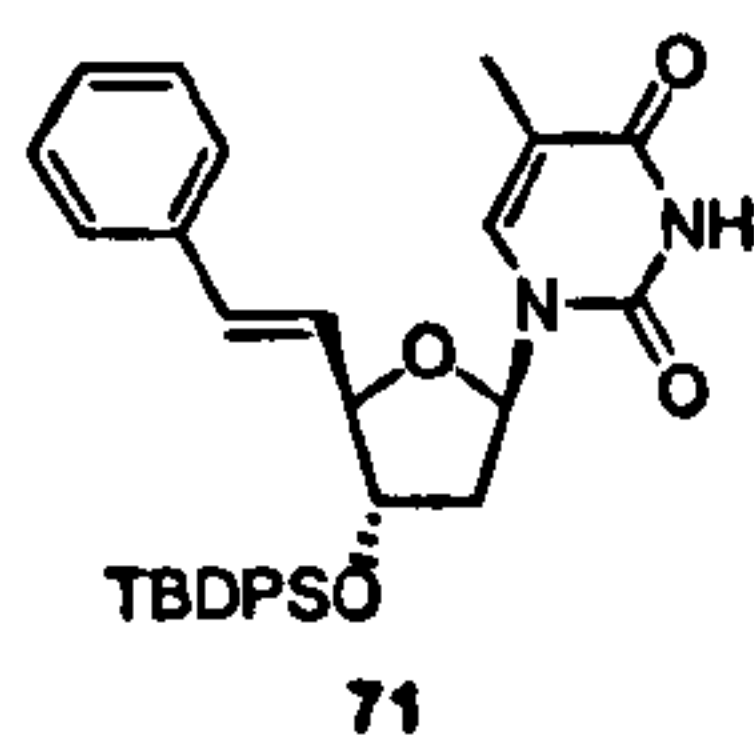
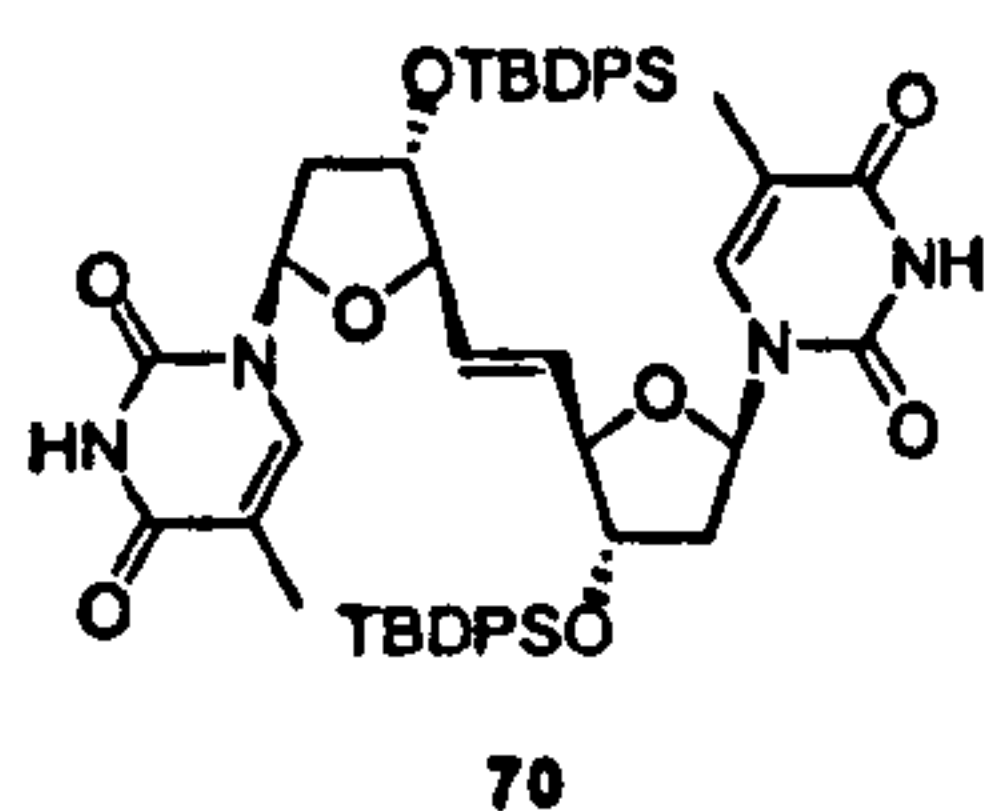


A 4.5 mL screw cap reaction vial was charged with  $Pd(OAc)_2$  (0.025 g, 0.111 mmol, 0.1 eq),  $Ph_3P$  (0.098 g, 0.374 mmol, 0.4 eq), *H*-phosphonate 73 (0.440 g, 0.930 mmol), vinylbromide (3.5 mL, 1.0 M soln in THF, 4.1 eq) and propylene oxide (1.0 mL, 14.3 mmol, 15 eq) and the mixture was heated at 70°C (oil bath temperature) for 15 h. After cooling to RT, the volatiles were removed *in vacuo* and the residue was purified by column chromatography (AcOEt) to give 74 (0.115 g, 25%) as a 1:1 mixture of diastereomers. (Data for the mixture of diastereomers): (Found (HRMS ES)  $M^+ + Na$ , 522.1829,  $C_{21}H_{34}O_7N_3PSiNa$  requires 522.1801);  $\nu_{max}/cm^{-1}$  3393, 3187, 2954, 2931, 2859, 2257, 1694, 1462, 1400, 1385, 1363, 1322, 1292, 1126, 1075, 978, 905, 838;  $\delta_H$ (400 MHz) 9.25 (0.5H, br s), 9.19 (0.5H, br s), 7.47 (0.5H, q,  $J$  1.3), 7.46 (0.5H, q,  $J$  1.2), 6.49-



6.30 (2.5H, m), 6.26-6.04 (1.5H, m), 5.08 (0.5H, app t,  $J$  5.9), 5.05 (0.5H, app t,  $J$  7.6), 4.30-4.17 (3H, m), 3.90 (1H, m), 3.89 (0.5H, dd,  $J$  11.5, 2.3), 3.84 (0.5H, dd,  $J$  11.5, 2.3), 2.81-2.74 (2H, m), 2.58 (0.5H, dd,  $J$  13.9, 5.2), 2.50 (0.5H, dd,  $J$  13.8, 6.4), 2.20-2.06 (1H, m), 1.91 (3H, d,  $J$  1.2), 0.92 (9H, s), 0.12 (3H, s), 0.12 (3H, s);  $\delta_c$ (100 MHz) 163.8 (C), 150.5/150.5 (C), 138.1/137.7 (CH<sub>2</sub>), 134.9 (CH), 124.7 (d,  $^1J_{CP}$  185.5)/124.6 (d,  $^1J_{CP}$  184.5)(CH), 116.5/116.5 (C), 111.3 (C), 86.0 (d,  $^2J_{CP}$  3.5)/85.9 (d,  $^2J_{CP}$  5.2)(CH), 84.6 (CH), 77.3/77.2 (CH), 63.3/63.2 (CH<sub>2</sub>), 60.5 (d,  $^2J_{CP}$  4.7)/60.3 (d,  $^2J_{CP}$  4.2)(CH<sub>2</sub>), 39.6 (d,  $^3J_{CP}$  3.8)/39.5 (d,  $^3J_{CP}$  4.6)(CH<sub>2</sub>), 26.0 (CH<sub>3</sub>), 20.0 (2 x CH<sub>2</sub>), 18.4 (C), 12.6 (CH<sub>3</sub>), -5.4/-5.4 (CH<sub>3</sub>);  $\delta_p$ (161.98 MHz) 19.8/19.6.

### Alkenes 70 and 71.



A flask charged with alkene 65 (0.046 g, 0.097 mmol), catalyst 68 (0.017 g, 0.020 mmol) and dichloromethane (2.0 mL) under an argon

atmosphere was heated at reflux for 14 h. The solvent was removed under reduced pressure and the residue purified by column chromatography (hexane:AcOEt 1:1) to give 70 (0.032 g, 72%, *E:Z* 5:1) and 71 (0.010 g, 20%).

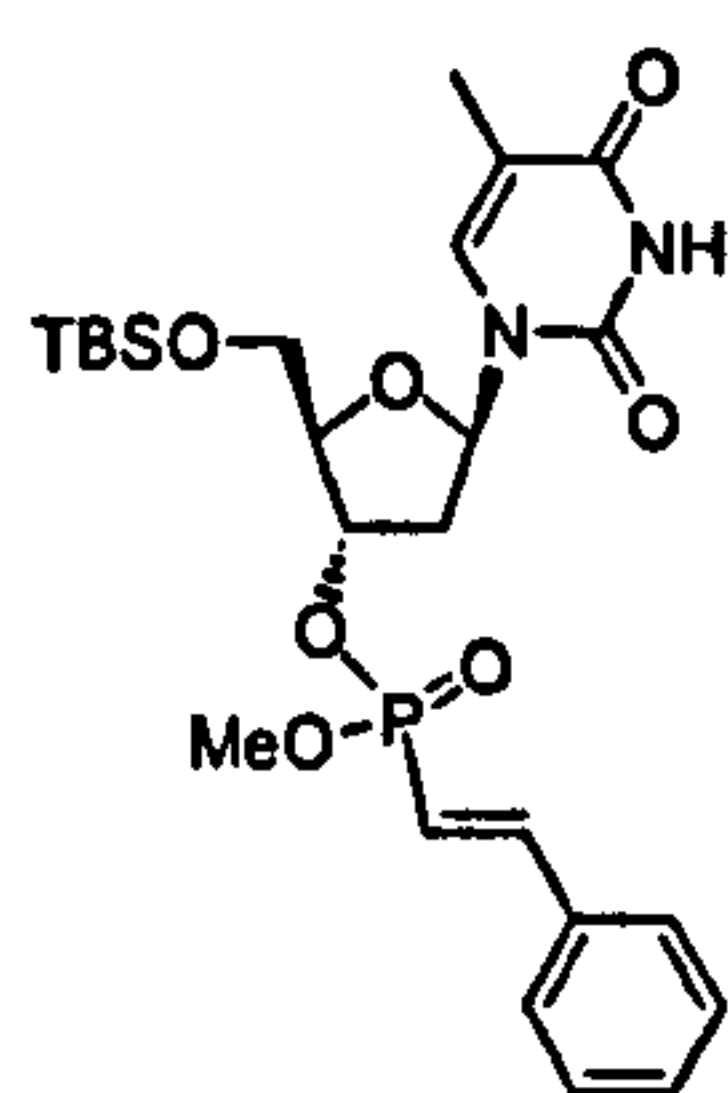
Data for 70: (Found (HRMS FAB)  $M^+ + Na$ , 947.3930, C<sub>52</sub>H<sub>60</sub>O<sub>8</sub>N<sub>4</sub>P<sub>2</sub>Si<sub>2</sub>Na requires 947.3847);  $\nu_{max}/cm^{-1}$  3392, 2932, 2895, 2859, 1694, 1463, 1364, 1276, 1112, 1051, 990, 908; *Trans*-isomer:  $\delta_H$ (400 MHz) 8.42 (2H, br s), 7.65-7.57 (8H, m), 7.45-7.30 (12H, m), 6.92 (2H, br s), 6.32 (2H, app t,  $J$  6.8), 5.19 (2H, dd,  $J$  3.6, 1.6), 4.18 (2H, m), 4.06 (2H, m), 2.32 (2H, ddd,  $J$  13.3, 5.8, 2.6), 1.85 (2H, m), 1.81 (6H, s), 1.08 (18H, s);  $\delta_c$ (100 MHz) 163.3 (C), 150.1 (C), 135.9 (CH), 135.8 (CH), 135.2 (CH), 133.1 (C), 133.0 (C), 130.3 (CH), 130.2 (CH), 128.0 (CH), 127.9 (CH), 111.3 (C), 86.4 (CH), 85.3 (CH), 76.4 (CH), 39.6 (CH<sub>2</sub>), 26.9 (CH<sub>3</sub>), 19.1 (C), 12.6 (CH<sub>3</sub>); *Cis*-



isomer (where signals not occluded by the *trans*-isomer):  $\delta_{\text{H}}$ (400 MHz) 6.13 (2H, app t,  $J$  6.5), 5.44 (2H, m), 4.69 (2H, m).  $m/z$  (FAB) 947 ( $\text{M}^+ + \text{Na}$ , 3), 307 (14), 199 (19), 197 (39), 183 (12), 154 (14), 137 (22), 136 (28), 135 (100), 127 (13), 121 (11), 107 (10), 91 (11), 77 (12), 73 (12), 69 (11), 57 (14), 55 (13).

Data for **71**: (Found (HRMS ES)  $\text{M}^+ + \text{Na}$ , 575.2323,  $\text{C}_{33}\text{H}_{36}\text{O}_4\text{N}_2\text{SiNa}$  requires 575.2342);  $\nu_{\text{max}}/\text{cm}^{-1}$  3391, 2932, 2859, 1690, 1464, 1364, 1273, 1112, 1052, 965, 908;  $\delta_{\text{H}}$ (400 MHz) 8.29 (1H, br s), 7.66-7.63 (4H, m), 7.48-7.22 (11H, m), 7.07 (1H, s), 6.45 (1H, d,  $J$  15.8), 6.38 (1H, app t,  $J$  6.6), 5.84 (1H, dd,  $J$  15.8, 7.0), 4.51 (1H, ddd,  $J$  6.6, 4.0, 1.0), 4.27 (1H, m), 2.44 (1H, ddd,  $J$  13.6, 6.6, 4.0), 1.93 (1H, app dt,  $J$  13.6, 6.7), 1.87 (3H, s), 1.10 (9H, s);  $\delta_{\text{C}}$ (100 MHz) 163.4 (C), 150.1 (C), 136.0 (CH), 135.8 (CH), 135.3 (CH), 133.5 (CH), 133.1 (C), 133.1 (C), 130.2 (CH), 130.2 (CH), 128.7 (CH), 128.3 (CH), 128.0 (CH), 128.0 (CH), 126.6 (CH), 125.8 (CH), 111.1 (C), 87.4 (CH), 85.0 (CH), 76.4 (CH), 40.2 ( $\text{CH}_2$ ), 26.9 ( $\text{CH}_3$ ), 19.1 (C), 12.7 ( $\text{CH}_3$ ).

### Vinylphosphonate **72**.



A flask charged with the vinylphosphonate **66** (0.046 g, 0.100 mmol), catalyst **68** (0.016 g, 0.019 mmol) and dichloromethane (2.0 mL) under an argon atmosphere was heated at reflux for 14 h. The solvent was removed under reduced pressure and the residue was

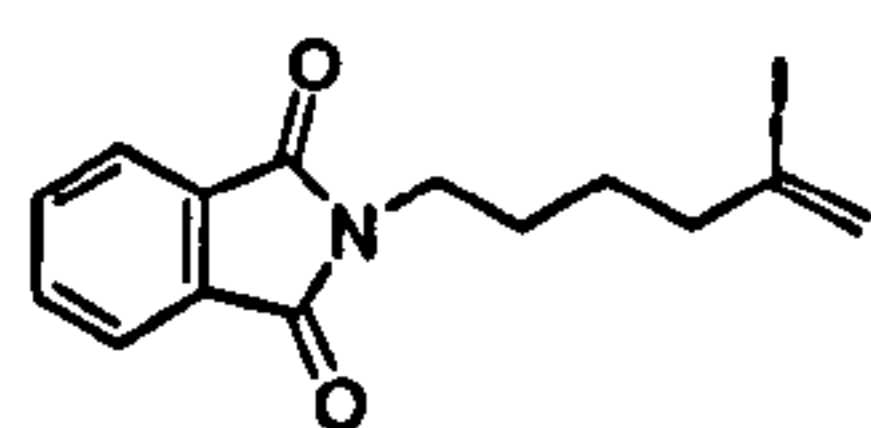
purified by column chromatography (AcOEt) to give **72** (0.011 g, 20%) as a 1:1 mixture of separable diastereomers.

Data for the least polar diastereomer: (Found (HRMS FAB)  $\text{M} + \text{H}^+$ , 537.2158,  $\text{C}_{25}\text{H}_{38}\text{O}_7\text{N}_2\text{PSi}$  requires 537.2186);  $\nu_{\text{max}}/\text{cm}^{-1}$  3392, 2954, 2930, 2858, 1689, 1615, 1463, 1353, 1322, 1275, 1128, 1049, 1007, 976, 908, 863, 836;  $\delta_{\text{H}}$ (400 MHz) 8.16 (1H, br s), 7.61-7.41 (7H, m), 6.41 (1H, dd,  $J$  9.1, 5.3), 6.25 (1H, dd,  $J$  17.7, 17.7),

5.06 (1H, app t,  $J$  6.3), 4.33 (1H, m), 3.93 (2H, m), 3.78 (3H, d,  $J$  11.3), 2.51 (1H, ddd,  $J$  14.0, 5.7, 0.9), 2.12 (1H, m), 1.92 (3H, s), 0.92 (9H, s), 0.13 (3H, s), 0.13 (3H, s);  $\delta_{\text{C}}$ (100 MHz) 163.4 (C), 150.4 (CH, d,  $^2J_{\text{CP}}$  7.0), 150.1 (C), 135.1 (CH), 134.5 (C, d,  $^3J_{\text{CP}}$  23.8), 130.8 (CH), 129.1 (CH), 128.0 (CH), 112.6 (CH, d,  $^1J_{\text{CP}}$  192.2), 111.2 (C), 86.3 (CH, d,  $^2J_{\text{CP}}$  3.6), 84.7 (CH), 76.6 (CH), 63.3 (CH<sub>2</sub>), 52.6 (CH<sub>3</sub>, d,  $^2J_{\text{CP}}$  5.6), 39.7 (CH<sub>2</sub>, d,  $^3J_{\text{CP}}$  4.8), 26.0 (CH<sub>3</sub>), 18.4 (CH<sub>3</sub>), 12.6 (CH<sub>3</sub>), -5.3 (CH<sub>3</sub>), -5.4 (CH<sub>3</sub>);  $\delta_{\text{P}}$ (161.98 MHz) 22.9.  $m/z$  (FAB) 537 ( $\text{M}^+ + \text{H}$ , 21), 339 (29), 307 (13), 199 (57), 176 (15), 155 (29), 154 (83), 139 (18), 138 (33), 137 (58), 136 (68), 127 (13), 123 (11), 121 (17), 120 (13), 119 (15), 115 (15), 111 (14), 109 (23), 107 (38), 106 (11), 105 (20), 97 (34), 95 (43), 93 (20), 91 (33), 90 (18), 89 (52), 85 (24), 83 (47), 81 (86), 79 (28), 78 (14), 77 (32), 75 (12), 73 (64), 71 (46), 69 (82), 67 (46), 65 (13), 57 (97), 55 (100), 53 (13).

Data for the most polar diastereomer: (Found (HRMS ES)  $\text{M}^+ + \text{Na}$ , 559.2022,  $\text{C}_{25}\text{H}_{37}\text{O}_7\text{N}_2\text{PSiNa}$  requires 559.2005);  $\nu_{\text{max}}/\text{cm}^{-1}$  3392, 2954, 2930, 2858, 1689, 1616, 1463, 1362, 1322, 1276, 1128, 1064, 1048, 1006, 976, 908, 864, 838;  $\delta_{\text{H}}$ (400 MHz) 8.28 (1H, br s), 7.71-7.40 (7H, m), 6.41 (1H, dd,  $J$  8.9, 5.3), 6.25 (1H, dd,  $J$  18.0, 18.0), 5.11 (1H, m), 4.26 (1H, m), 3.89 (1H, dd,  $J$  11.4, 2.1), 3.85 (1H, dd,  $J$  11.4, 2.1), 3.79 (3H, d,  $J$  11.2), 2.60 (1H, ddd,  $J$  14.3, 5.3, 1.0), 2.16 (1H, m), 1.93 (3H, s), 0.91 (9H, s), 0.11 (3H, s), 0.11 (3H, s);  $\delta_{\text{C}}$ (100 MHz) 163.4 (C), 150.2 (C), 150.1 (CH, d,  $^2J_{\text{CP}}$  7.7), 135.1 (CH), 134.5 (C, d,  $^3J_{\text{CP}}$  23.3), 130.8 (CH), 129.1 (CH), 127.9 (CH), 112.7 (CH, d,  $^1J_{\text{CP}}$  193.4), 111.2 (C), 86.2 (CH), 84.7 (CH), 76.7 (CH), 63.3 (CH<sub>2</sub>), 52.7 (CH<sub>3</sub>, d,  $^2J_{\text{CP}}$  5.2), 39.8 (CH<sub>2</sub>, d,  $^3J_{\text{CP}}$  3.8), 26.0 (CH<sub>3</sub>), 18.4 (CH<sub>3</sub>), 12.6 (CH<sub>3</sub>), -5.3 (CH<sub>3</sub>), -5.4 (CH<sub>3</sub>);  $\delta_{\text{P}}$ (161.98 MHz) 22.7.



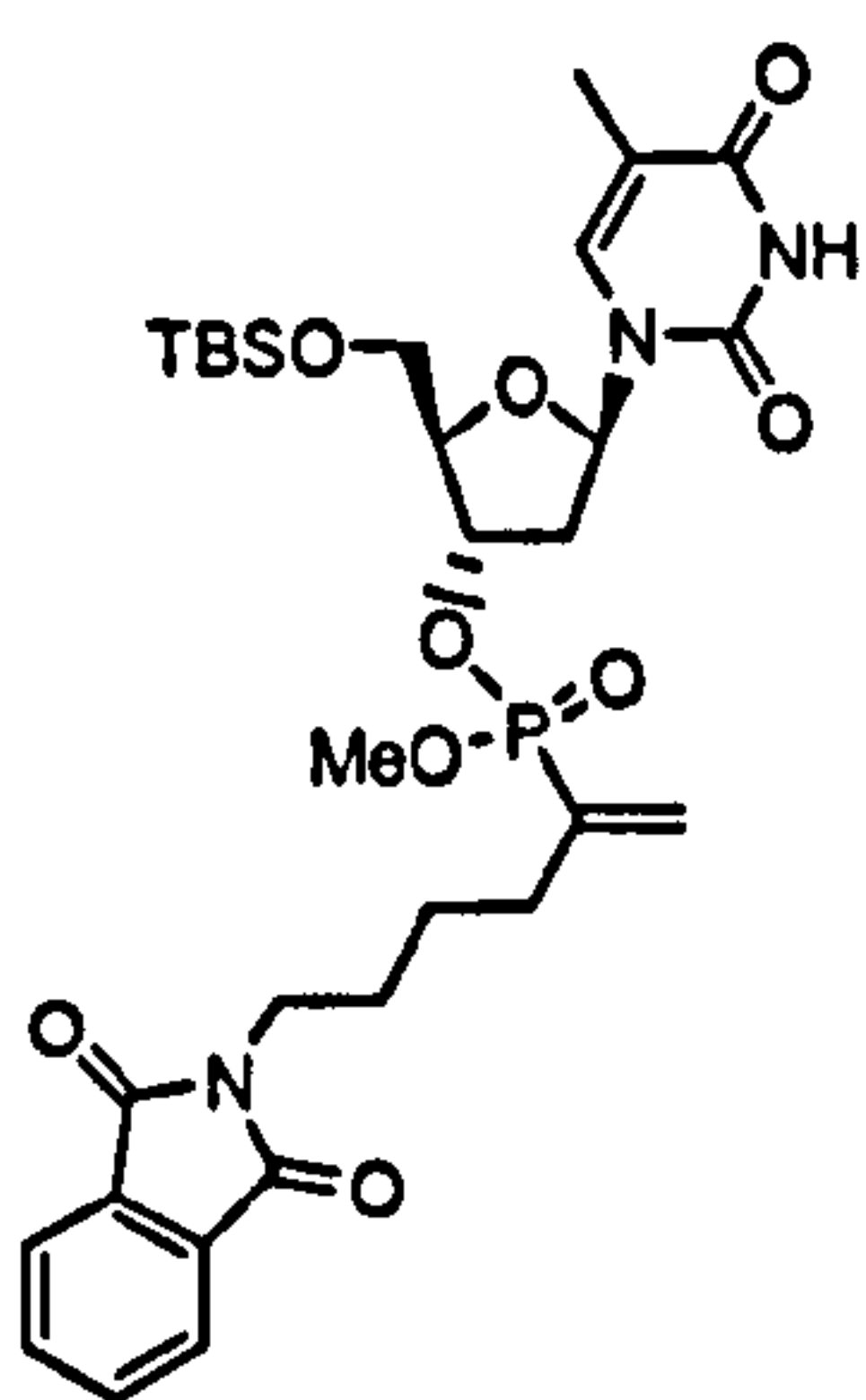
**Vinyl iodide 78.**

A solution of iodoborabicyclononane in hexanes (1.0 M, 2.0 mL, 2.0 mmol) was added dropwise to a stirred solution of **77** (352 mg,

1.55 mmol) in DCM (11 mL) at  $-10^{\circ}\text{C}$  under nitrogen. The reaction was stirred at this temperature for 1 h and acetic acid (1.35 mL, 23.6 mmol) was dropwise added. The mixture was stirred for a further 1 h and a mixture of 3 M NaOH (16 mL) and 30% hydrogen peroxide (2.7 mL) was added dropwise and then the mixture stirred for 30 min. DCM (100 mL) and water (80 mL) were added and after vigorous stirring for 5 min the layers were separated and the aqueous layer extracted with DCM (2 x 50 mL). The combined organic phase was washed with sat sodium thiosulphate (150 mL), water (100 mL), sat sodium hydrogencarbonate (100 mL) and brine (100 mL), then dried and the solvent removed *in vacuo* to give a residue which was purified by column chromatography (AcOEt:Petroleum ether 1:4) to afford **78** (179 mg, 33%) as a pale yellow solid, mp  $40\text{--}42^{\circ}\text{C}$ ; (Found: (HRMS FAB)  $\text{M}^{\text{+}}\text{+H}$  356.0139,  $\text{C}_{14}\text{H}_{15}\text{NO}_2\text{I}$  requires 356.0148);  $\nu_{\text{max}}/\text{cm}^{-1}$  3307, 2940, 2862, 1770, 1713, 1616, 1468, 1397, 1374, 1153, 1088, 1039, 898;  $\delta_{\text{H}}$ (400 MHz) 7.83 (2H, dd,  $J$  5.7, 3.1), 7.71 (2H, dd,  $J$  5.5, 2.9), 6.03 (1H, app q,  $J$  1.4), 5.68 (1H, m), 3.69 (2H, t,  $J$  7.0), 2.42 (2H, dt  $J$  7.3, 0.9), 1.67 (2H, m), 1.56 (2H, m);  $\delta_{\text{C}}$ (100 MHz) 168.4 (C), 134.0 (CH), 132.1 (C), 126.0 (CH<sub>2</sub>), 123.2 (CH), 111.6 (C), 44.5 (CH<sub>2</sub>), 37.6 (CH<sub>2</sub>), 27.0 (CH<sub>2</sub>), 26.2 (CH<sub>2</sub>);  $m/z$  (FAB) 356 ( $\text{M}^{\text{+}}\text{+H}$ , 3), 154 (15), 136 (10), 123 (13), 111(17), 109 (25), 107 (14), 97 (35), 95 (42), 93 (17), 91 (21), 85 (27), 83 (49), 81 (50), 79 (21), 77 (15), 73 (16), 71 (46), 69 (77), 67 (42), 57 (90), 55 (100).



## Vinyl phosphonate 79.



A mixture of  $\text{Pd}(\text{OAc})_2$  (8 mg, 0.04 mmol) and dppf (31 mg, 0.056 mmol) in THF (0.5 mL) was flushed with dry nitrogen and stirred at RT for 20 min. A solution of 78 (90 mg, 0.25 mmol) and *H*-phosphonate 62 (180 mg, 0.41 mmol) in THF (2.5 mL) and propylene oxide (53  $\mu\text{L}$ , 0.76 mmol) were added and the mixture was heated at 70°C (bath temperature) for 15 h and then cooled to

RT. Removal of the solvent *in vacuo* left a residue which was purified by column chromatography (AcOEt) to give 79 (73 mg, 43%) as a 1:1 mixture of diastereomers. (Data given for the mixture of diastereomers): (Found (HRMS ES)  $\text{M}^+ + \text{Na}$ , 684.2442,  $\text{C}_{31}\text{H}_{44}\text{O}_9\text{N}_3\text{PSiNa}$  requires 684.2482);  $\nu_{\text{max}}/\text{cm}^{-1}$  3668, 3392, 2952, 2931, 2859, 1770, 1713, 1463, 1397, 1363, 1322, 1292, 1276, 1128, 1065, 1046, 1004, 974, 908, 838;  $\delta_{\text{H}}$ (400 MHz) 8.51 (0.5H, br s), 8.47 (0.5H, br s), 7.84 (2H, dd,  $J$  5.4, 3.1), 7.71 (2H, dd,  $J$  5.5, 3.0), 7.49 (0.5H, d,  $J$  1.2), 7.48 (0.5H, d,  $J$  1.2), 6.37 (0.5H, dd,  $J$  8.9, 5.3), 6.36 (0.5H, dd,  $J$  9.1, 5.2), 6.10 (1H, dd,  $J$  23.5, 11.3), 5.91 (0.5H, dd,  $J$  10.1, 1.3), 5.79 (0.5H, dd,  $J$  10.1, 1.3), 5.05 (0.5H, app t,  $J$  4.8), 4.98 (0.5H, app t,  $J$  6.5), 4.28 (0.5H, m), 4.22 (0.5H, m), 3.89 (1H, t,  $J$  2.1), 3.89 (0.5H, dd,  $J$  11.6, 2.0), 3.84 (0.5H, dd,  $J$  11.6, 2.1), 3.72 (2H, obscured t), 3.72 (1.5H, d,  $J$  11.2), 3.71 (1.5H, d,  $J$  11.1), 2.55 (0.5H, ddd,  $J$  14.3, 6.3, 1.0), 2.46 (0.5H, ddd,  $J$  12.9, 5.4, 1.1), 2.30 (2H, app quint,  $J$  7.7), 2.11 (1H, m), 1.92 (3H, s), 1.70 (2H, m), 1.60 (2H, m), 0.92 (4.5H, s), 0.92 (4.5H, s), 0.13 (1.5H, s), 0.12 (1.5H, s), 0.12 (1.5H, s), 0.12 (1.5H, s);  $\delta_{\text{C}}$ (100 MHz) 168.5 (C), 163.6 (C), 150.2 (C), 137.9 (d,  $^1J_{\text{CP}}$  171.8)/137.8 (d,  $^1J_{\text{CP}}$  170.9) (C), 135.1 (CH), 134.0 (CH), 132.2 (C), 131.2 (d,  $^2J_{\text{CP}}$  9.7)/130.8 (d,  $^2J_{\text{CP}}$  9.7) ( $\text{CH}_2$ ), 123.3 (CH), 111.2/111.2 (C), 86.3 (d,  $^3J_{\text{CP}}$  3.2)/86.0 (d,  $^3J_{\text{CP}}$  4.8) (CH), 84.7/84.6 (CH), 76.7 (d,  $^2J_{\text{CP}}$  5.8)/76.6 (d,  $^2J_{\text{CP}}$  5.2) (CH), 63.3/63.2 ( $\text{CH}_2$ ), 52.7 (d,  $^2J_{\text{CP}}$  5.5)/52.5 (d,  $^2J_{\text{CP}}$

5.6) (CH<sub>3</sub>), 39.8 (d,  $^3J_{CP}$  3.3)/39.5 (d,  $^3J_{CP}$  4.9) (CH<sub>2</sub>), 37.6 (CH<sub>2</sub>), 31.7 (d,  $^2J_{CP}$  3.7)/31.5 (d,  $^2J_{CP}$  3.6) (CH<sub>2</sub>), 28.1 (CH<sub>2</sub>), 26.0 (3 x CH<sub>3</sub>), 25.2 (d,  $^3J_{CP}$  4.7)/25.2 (d,  $^3J_{CP}$  4.5) (CH<sub>2</sub>), 18.4 (C), 12.6 (CH<sub>3</sub>), -5.3 (CH<sub>3</sub>), -5.4 (CH<sub>3</sub>);  $\delta_P$ (161.98 MHz) 22.7/22.4.

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## Appendix

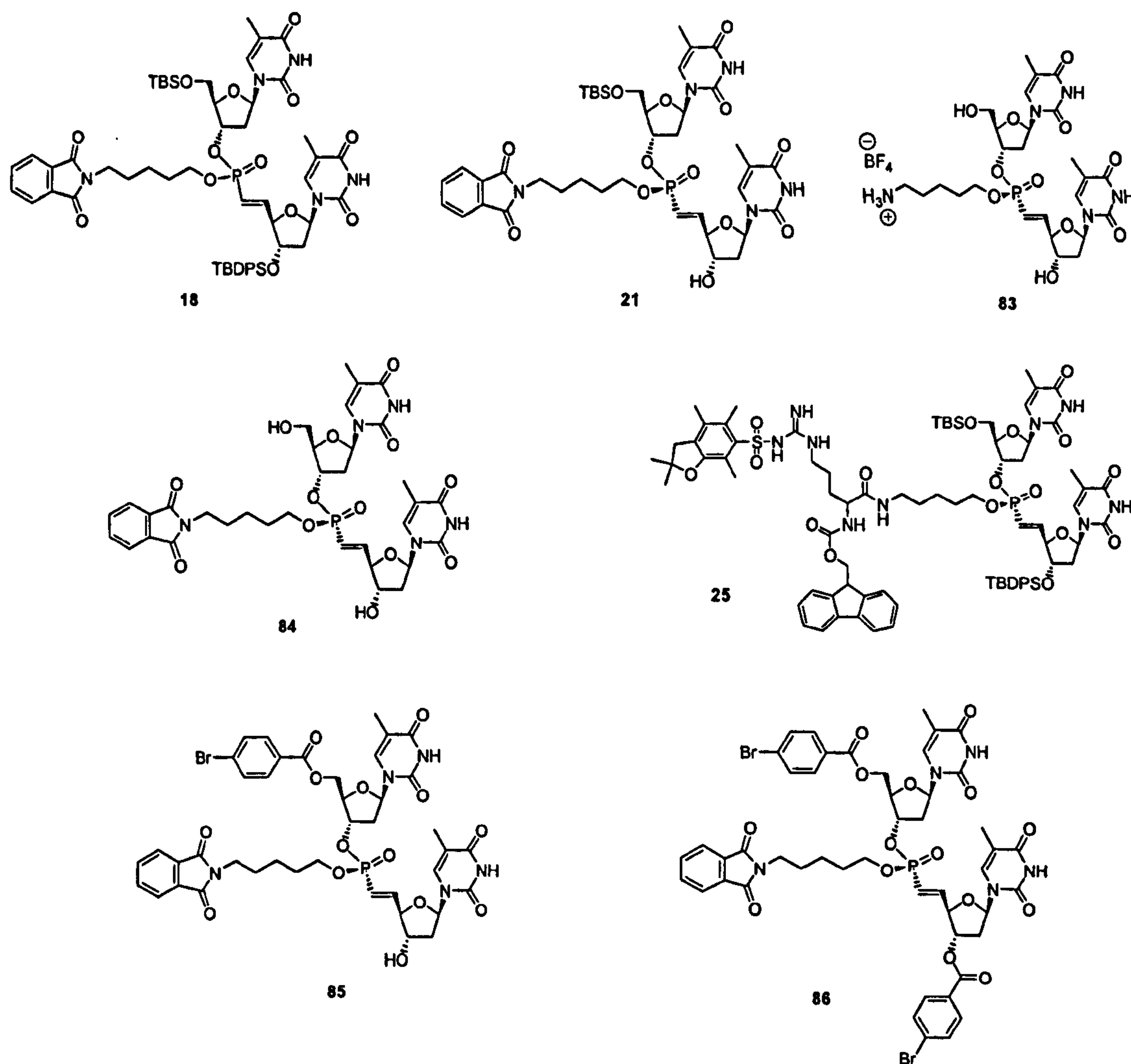


Attempts to determine the stereochemistry at phosphorus of *H*-phosphonates  
17(fast) and 17(slow)

Growing crystal experiments using slow evaporation or diffusion methods have been performed with the solvent systems presented in Table 3 and the compounds shown in Figure 25.

Compound	Method		
	Solvent	Diffusion	
		Solvent in	Solvent out
18	DCM , AcOEt	DCM	Ether
21	DCM, AcOEt	DCM	Ether
83	DCM / MeOH,*  <sup>i</sup> PrOH	DCM / MeOH*	Ether
84	DCM / MeOH*  <sup>i</sup> PrOH	DCM / MeOH*	Ether
25	DCM, AcOEt	DCM  AcOEt	Ether  Ether
85	DCM, AcOEt	-	-
86	DCM, AcOEt	-	-

Table 3. Different conditions used in the growing crystal experiments. \* Mixture of solvents.



**Figure 25.** Different compounds used in the growing crystal experiments.