/ Review

Development of New Synthetic Reactions Using Hetero-Heavy Atoms and Their Application to Synthesis of Biofunctional Molecules

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Novel reactions using hetero-heavy atoms (P, S, Si, Se, and Sn) were developed and applied to the synthesis of biofunctional molecules and some medicine-candidates, in which the following items are covered. 1) Development of introduction of C₁-unit using cyanophosphates (CPs). 2) Carbene-generation under neutral condition from CPs and its application to organic synthesis. 3) [3,3]Sigmatropic rearrangement-ring expansion reactions of medium-sized cyclic thionocarbonates containing a sulfur atom and their application to natural product synthesis. 4) Stereoselective synthesis of novel β -imidazole C-nucleosides *via* diazafulvene intermediates and their application to investigating ribozyme reaction mechanism. 5) Developments of novel histamine H₃- and H₄-receptor ligands using new synthetic methods involving Se or Sn atoms.

Key words cyanophosphate; carbene; sigmatropic rearrangement; ribozyme; histamine H₃ receptor; antagonist

1. Introduction

Organic synthesis has reached a high degree of perfection. However, reactions occurring within a living organism are much more mild, and more highly efficient, with specificity and selectivity. Thus, new efficient synthetic methods and reagents should be developed further. Although catalysts now occupy a central position in organic synthesis, reagents remain important, and many novel and improved reagents have been developed. The exploitation of useful reagents and methods can often make the impossible possible, and it is the starting point for the development of new fields in organic synthesis as well as their practical and industrial applications.

Diethyl phosphorocyanidate [(EtO)₂P(O)CN, DEPC] was developed by Shioiri in 1972 and is a useful and versatile reagent for organic synthesis,¹⁾ in particular as a condensing reagent for the synthesis of amides and peptides (Chart 1), and for its application in the total synthesis of macrolactams which are present in various marine natural products.¹⁾

The present author first encountered DEPC, which contains a phosphorus atom (a hetero-heavy atom), as a graduate student at Prof. Shioiri's laboratory of Nagoya City University. Since the DEPC structure contains a CN, the role of DEPC as a cyanating reagent under non-aqueous conditions could be expected. The author started to investigate novel cyanations using DEPC under his supervision, and it was my first job as a chemist. The study on cyanation using DEPC revealed the following non-aqueous reactions, that is, reaction of enamines

with DEPC to afford α -amino nitriles, the function of which is easily converted into α -amino acids²⁾ (Chart 2, Eq. 1); modified Strecker synthesis for the preparation of α -amino nitriles using DEPC from carbonyl compounds and amines in tetrahydrofuran (THF) under mild reaction conditions (Eq. 2)³⁾; modified Reissert–Henze reaction, in which DEPC reacts with the aromatic amine oxide in the presence of triethylamine to give the α -cyanated product (Eq. 3)⁴⁾; reaction of sodium sulfinates with DEPC to give thiocyanates, providing a novel route to thiocyanate synthesis (Eq. 4).^{5–7)} These reactions contrast to conventional cyanation procedures employing alkaline cyanides (NaCN/KCN) in aqueous solvents.

Through these studies, we addressed new reactions using hetero-heavy atoms (P, S, Si, Se, and Sn) having vacant d-orbital contributions. More importantly, the developed reactions were applied to the efficient synthesis of natural products, biofunctional molecules and some medicine-candidates. This review describes important aspects in the author's work over the last three decades, in which the following studies are covered: 1) C₁-unit introduction *via* cyanophosphates as well as carbene-generation from cyanophosphates under neutral condition and their application to organic synthesis. 2) [3,3]Sigmatropic rearrangement-ring expansion reaction of medium-sized cyclic thionocarbonates and application to synthesis of natural products. 3) Stereoselective synthesis of

Chart 1. Synthesis of Amides and Peptides Using DEPC

This review of the author's work was written by the author upon receiving the 2019 Pharmaceutical Society of Japan Award for Divisional Scientific Contribution.

Chart 2. Non-aqueous Cyanation Using DEPC

Fig. 1. Cyanohydrins **1**, TMS-Cyanohydrins **2** and CPs **3**

β -imidazole C-nucleosides and its application to investigating ribozyme reaction mechanism. 4) Development of novel histamine H_3 and H_4 receptor ligands using new reactions characterized by Se or Sn atoms.

2. C_1 -Unit Introduction Using Cyanophosphates

2.1. Formation of Cyanophosphates from Carbonyl Compounds Using DEPC Cyanohydrins **1** and *O*-trimethylsilyl (TMS)-protected derivatives **2** are well-known versatile synthetic intermediates in organic synthesis⁸⁾ (Fig. 1). To date, TMS-cyanohydrins **2** have been demonstrated useful precursors for the preparation of carbonyl anion synthons, cyanohydrins, β -amino alcohols, and other functional compounds.^{8,9)} However, the intrinsic instabilities of cyanohydrins and TMS derivatives have limited their potential application. We therefore investigated the formation of cyanohydrin-*O*-phosphates (cyanophosphates: CPs) **3** as novel protected cyanohydrins, which were expected to be diverse cyanation intermediates due to their high functional density and the electrophilic character of the phosphate leaving group. Examination of the literature revealed that CPs had been previously obtained by treating cyanohydrins with dialkyl phosphorochloridate, and some CPs were of particular use as agricultural chemicals, for example, as insecticides, fungicides, nematocides, and the

Chart 3. Cyanophosphorylation in the Presence of A Catalytic Amount of LDA

Chart 4. Cyanophosphorylation of Ketones **4** or Aldehydes **5** Using DEPC (3 eq.) and LiCN (3 eq.)

like.¹⁰⁾

In 1983, we first observed a cyanophosphorylation reaction on treatment of ketones or aldehydes with DEPC in the presence of a catalytic amount of lithium diisopropylamide (LDA) in THF¹¹⁾ (Chart 3). Although 1 eq. of DEPC was generally employed, 3 eq. DEPC was beneficial for sluggish reactions. Various ketones and aldehydes (**4** and **5**, respectively) react with DEPC to give CPs **3** in good yields.

A proposed mechanism for the cyanophosphorylation of ketones in the presence of LDA suggested that the treatment of ketones with DEPC in the presence of a catalytic amount of LiCN would produce CPs.^{1,10)} This working hypothesis therefore led to a convenient synthetic procedure for the formation of CPs **3** using DEPC (3 eq.) and LiCN (3 eq.) as outlined in

Biography

Shinya Harusawa was born in Nara City (Japan) in 1954. He received his education at OUPS. After graduation (1977), he began his graduate course at the Faculty of Pharmaceutical Sciences at Nagoya City University and obtained his Ph. D. in 1982 in studies on novel cyanation reactions using DEPC, under the supervision of Prof. Takayuki Shioiri. He returned to OUPS as a research fellow under the supervision of Prof. Takushi Kurihara and was appointed there as research associate in 1983. He was engaged in a postdoctoral position at Virginia Polytechnic Institute and State University (Virginia Tech) and Florida State University working on the total synthesis of taxol from 1984 to 1986 under the supervision of Prof. Robert A. Holton. In 1986, he returned to OUPS and was appointed as assistant professor in 1988. He was promoted to associate professor in 1991 and became a full professor in 2006. Shinya Harusawa received the PSJ (the Pharmaceutical Society of Japan) Kansai Branch Award for Young Scientists in 1992 and PSJ Award for Divisional Scientific Contribution in 2019. His present research fields include developments of histamine H_3 antagonists, growth inhibitors against breast cancer, chemical probes for ribozyme catalysis, and the exploitation of novel methods and reagents in organic synthesis. His hobbies are reading Japanese literature, visiting art museums and places of historical interest, and iaidō practice.

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Chart 4.¹²⁾

The LiCN method afforded CPs **3** from a range of ketones **4** and aldehydes **5** in excellent yields at room temperature (r.t.) under neutral conditions (*c.f.*, the basic conditions required for the LDA method).¹¹⁾ Indeed, this method has now been adopted in the majority of CP synthetic pathways.¹⁾ Furthermore, excess DEPC (3 eq.) and LiCN (3 eq.) are now used routinely for cyanophosphorylation,¹²⁾ although we have more recently demonstrated that the use of 1.2 eq. DEPC and 0.6 eq. LiCN is sufficient for the formation of CP from ketones, while 1.2 eq. DEPC and 0.1 eq. LiCN are required when aldehydes are employed, as shown in Table 1.¹³⁾

Since our early reports on the cyanophosphorylation of carbonyl compounds,^{11,12)} many synthetic procedures of CPs were reported by use of modified reagent systems; diethyl phosphorochloridate (DEPCl)-LiCN (Nájera *et al.*), DEPCl-NaCN (Shin *et al.*), DEPC-Et₃N in the solvent-free system (Nájera *et al.*), and DEPC-*N*-heterocyclic carbene (NHC) catalyst (Kondo *et al.* and Dai *et al.*).¹⁾ However, the DEPC-LiCN

method has been used as the simplest method for CPs in most cases.¹⁾ Furthermore, two groups of Nájera and Shibasaki reported independently the enantioselective cyanophosphorylation of aldehydes.¹⁾

2.2. Synthesis of α,β -Unsaturated Nitriles The majority of cyclic alkenenitriles are synthesized from cyclic ketone precursors *via* conversion to the corresponding cyanohydrins or their derivatives followed by dehydration.¹⁾ Due to the diethyl phosphate anion being a good leaving group, benzylic CPs **7** prepared from aromatic ketones **6** were readily converted into α,β -unsaturated nitriles **8** by treatment with boron trifluoride etherate (BF₃·OEt₂) in benzene at rt in high yields (Table 2), in which the transformation revealed an efficient synthetic route to α,β -unsaturated nitriles from aromatic ketones.¹²⁾ 4-Isobutylacetophenone (**6e**) afforded α -cyanostyrene **8e** (80%) (entry 5 in Table 2). As an application of this method, **8e** was subsequently subjected to hydrogenation followed by hydrolysis to give ($\pm$)-ibuprofen, an anti-inflammatory agent, in 70% overall yield from **6e**.¹²⁾ In the case of diketone **6f** bearing both aromatic and aliphatic ketones, the CP function derived from the aliphatic ketone remained intact under BF₃·OEt₂ treatment, giving **8f** in 92% yield (entry 6).¹²⁾

Kurihara *et al.* carried out cyanophosphorylation on Uhle's ketone (**9**) using DEPC and LiCN followed by treatment with BF₃·OEt₂ to afford α,β -unsaturated nitrile **11** (90%).^{14,15)} Compound **11** was then reduced using diisobutylaluminum hydride (DIBAL) to give the aldehyde **12**, which was successfully transformed into ($\pm$)-lysergic acid (**13**) over 4 steps, as outlined in Chart 5.^{14,15)}

Furthermore, in the synthetic applications of the cyanophosphorylation-dephosphorylation method, this method provided crucial unsaturated nitriles, which led to many bioactive compounds: A-80426 (an antidepressant combining α -2 antagonism with serotonin (5-HT) uptake inhibition); ($\pm$)-lunarine (a macrocyclic polyamine alkaloid); a dicyanofluorene dimer as an electron-accepting molecule; L-histidine with high ¹³C incorporation; spiroperidines using the formation of α,β -unsaturated nitrile by kinetic dephosphorylation.¹⁾

2.3. Reductive Cyanation of Ketones and Aldehydes

The transformation of carbonyl groups into nitriles represents a useful one-carbon homologation for the synthesis of a variety of carboxylic acid and amine derivatives. As one of the strategies, CPs have been employed as intermediates to achieve this transformation. For example, CPs **3** can be hydrogenated over Pd/C, treated with lithium and ammonia, or reduced mildly with samarium iodide (SmI₂) to give homologous nitriles **14**¹⁾ (Chart 6).

Synthesis of arylacetonitriles (R¹ = Ar) *via* hydrogenolysis over Pd/C of arylketone- or arylaldehyde-CPs in ethyl acetate was efficiently applied for the synthesis of a range of non-steroidal anti-inflammatory agents, including ibuprofen, naproxen, and clidanac.^{16,17)} Particularly, in the preparation of loxoprofen (**18**), diketone **15** was easily converted to bis-CP **16**, which was selectively dephosphorylated under standard hydrogenolysis conditions to give mono-CP **17**, which was then hydrolyzed to give loxoprofen (**18**).^{16,17)} In this case, the CP functional group in the cyclopentanone moiety played a significant role in protection of the carbonyl group (Chart 7).

In addition, we investigated dephosphorylation of CPs by reduction with lithium in liquid ammonia, as outlined in Table 3.^{18,19)} Reduction of a CP derived from citral **19** with lithium

Table 1. Cyanophosphorylation of Carbonyl Compounds Using DEPC (1.2 eq.) and LiCN (Cat)

$\text{R}^1-\text{C}(=\text{O})-\text{R}^2 \xrightarrow[\text{THF, rt, 30 min}]{\text{DEPC (1.2 eq.)}, \text{LiCN (cat)}} \text{EtO}-\text{P}(=\text{O})(\text{OEt})-\text{O}-\text{C}(\text{CN})(\text{R}^1)(\text{R}^2)$		
entry	carbonyl compound	CP: yield (%)
1		99 ^a
2		97 ^a
3		99 ^a
4		90 ^a
5		91 ^b
6		98 ^b
7		quant ^b
8		98 ^b

a) LiCN (0.6 eq); b) LiCN (0.1 eq)

Table 2. Synthesis of α,β -Unsaturated Nitriles from Ketones via Benzylic CPs

entry	aromatic ketone	8	yield (%)
1	(6a)	(8a)	74
2	(6b)	(8b)	61
3	(6c)	(8c)	94
4	(6d)	(8d)	78
5	(6e)	(8e)	80
6	$\text{CH}_3(\text{CH}_2)_2\text{C}(=\text{O})\text{C}_6\text{H}_4(\text{CH}_2)_2\text{C}(=\text{O})\text{CH}_3$ (6f)	(8f)	92

Chart 5. Synthesis of (±)-Lysergic Acid from Uhle's Ketone

Chart 7. Synthesis of Loxoprofen

Table 3. Reduction of CPs **3** with Lithium in Liquid Ammonia

$\text{R}^1\text{C}(=\text{O})\text{R}^2 \xrightarrow[\text{LiCN}]{\text{DEPC}} \left[\text{NC}-\text{C}(\text{R}^1)(\text{R}^2)\text{OP}(\text{OEt})_2 \right] \xrightarrow[2) \text{ quenching}]{1) \text{ Li, liq NH}_3, -78^\circ\text{C}} \text{Product}$				
entry	carbonyl compound	quenching	product	yield (%) ^{a)}
1		isoprene		86
2		Me(CH ₂) ₇ I		83
3		NH ₄ Cl ^{b)}		89

a) Isolated yields from the carbonyl compounds;

b) The reduction of CP was carried out at -33°C .

in liquid ammonia at -78°C and quenching of the reaction mixture with isoprene afforded β,γ -unsaturated nitrile **20** (86%, entry 1, Table 3), while quenching of the CP derived from α -ionone (**21**) using octyl iodide yielded the α -alkylated nitrile **22** (83%, entry 2). Furthermore, reduction of the CP derived from 4-isobutylacetophenone (**23**) under refluxing conditions (at -33°C) followed by quenching with ammonium chloride (NH₄Cl) yielded 1-ethyl-4-isobutylbenzene (**24**, 89%) (entry 3), thus giving a deoxygenated derivative of an aromatic ketone.¹⁹⁾ Interestingly, no isomerized or reduced alkenes were formed in the reduction stages.^{18,19)} Thus, these reductive dephosphorylation reactions have potential for application in the preparation of CPs derived from enones and aromatic ketones.¹⁾

We further developed the reductive cyanation of CPs using SmI₂, which is a moderate reducing agent^{20,21)} (Table 4). This method also yielded nitriles chemoselectively from various carbonyl compounds possessing multiple and sensitive functionalities *via* CPs (entries 1–6, Table 4). For example, cyanation of epoxy aldehyde **25** was conducted by a combination of cyanophosphorylation and SmI₂ reduction followed by recyclization of the oxirane ring-opened product with triethylamine, to give the epoxy nitrile **26** in 54% yield (entry 7).²⁰⁾ This reaction was successfully applied to α,β -unsaturated carbonyl compounds under neutral conditions to obtain the desired nitriles. Cholest-4-en-3-one (**27**) and α -ionone (**29**) gave the corresponding nitriles **28** and **30** (entries 9 and 10, Table 4), whereas under general tosylmethyl isocyanide (TosMIC)²²⁾ conditions in the presence of *t*-BuOK in DMF, nitriles **28** and **30** were detected only by TLC in trace amounts.²⁰⁾

The CPs-SmI₂ method was employed in a variety of organic syntheses,¹⁾ for instance, in the synthesis of the natural spongian pentacyclic diterpene, aplyrosoleol-1 and its related compounds; in the synthesis of hydroxyethene isosteres of human immunodeficiency virus (HIV) protease-catalyzed

Table 4. Reductive Cyanation of CPs with SmI₂

$\text{R}^1\text{C}(=\text{O})\text{R}^2 \xrightarrow[\text{LiCN}]{\text{DEPC}} \left[\text{NC}-\text{C}(\text{R}^1)(\text{R}^2)\text{OP}(\text{OEt})_2 \right] \xrightarrow[\text{THF, rt}]{\text{SmI}_2, t\text{-BuOH}} \text{R}^1\text{C}(\text{CN})\text{R}^2$			
entry	carbonyl compound	product	yield (%)
1			92
2			80
3			82
4			92
5			85
6			98
7			54
8			90
9			96 (trace) ^{a)}
10			97 (trace) ^{a)}

a) The product was prepared by TosMIC method.

Chart 8. Allylic Rearrangement of CPs to Give Allylic Phosphates

phenylalanine (Phe)-proline (Pro) hydrolysis transition state; in the transformation of aldol product from Boc-L-phenylalaninal into lactone; in the synthesis of sweroside aglycon; and in the preparation of nitriles from chiral ketone in the total synthesis of (±)-aphidicolin.

2.4. Allylic Rearrangement of CPs The CPs (**B**) derived from α,β -unsaturated ketones (**A**) were transformed into conjugated allylic phosphates (**C**) *via* BF₃·Et₂O-catalyzed allylic rearrangement^{23,24)} (Chart 8). In the case of 2-cyclohexen-

Chart 9. Stereochemistry of Allylic Rearrangement of CPs

Chart 10. Asymmetric Synthesis of Oseltamivir Phosphate Based on Allylic Rearrangement of CP

Chart 11. Synthesis of TzPD **43** from Pregnene-3,20-dione (**39**)

1-one (**31**), cyanophosphorylation followed by treatment of the resulting CP **32** with a catalytic amount of $\text{BF}_3 \cdot \text{OEt}_2$ (0.1 eq.) afforded 3-cyanocyclohex-2-enyl diethyl phosphate (**33**) in 81% yield^{23,24} (Chart 8).

From a stereochemical viewpoint, 6-methylbicyclo[4.4.0]dec-1-en-3-one (**34**) can be converted into CP **35** bearing a 3 α -cyano-3 β -diethylphosphoxy group. Subsequent treatment of **35** with $\text{BF}_3 \cdot \text{OEt}_2$ provided a diene nitrile **37** (90%), as shown in Chart 9.²³ Alternatively, heating CP **35** at reflux in benzene in the absence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$ produced **38** (46%), bear-

ing a 5 β -diethylphosphoxy group, along with the accompanying diene nitrile **37** (50%). Phosphate **38** could be converted into **37** in quantitative yield by treatment with $\text{BF}_3 \cdot \text{OEt}_2$. In addition, the formation of both **35** and **38** indicated that the rearrangement took place *via* a [3,3]-sigmatropic rearrangement in a suprafacial fashion, as shown at **36** in Chart 9.^{23,24}

Two decades after our initial report,²³ Shibasaki and co-workers achieved efficient asymmetric synthesis of oseltamivir phosphate (Tamiflu),²⁵ an important anti-influenza drug, *via* allylic rearrangement of a CP, as illustrated in Chart 10.²⁵

In continuation of our recent program on the use of CPs, we were encouraged to look once again at allylic rearrangements of CPs for a practicable synthetic method for key intermediate **41** for 3-(tetrazol-5-yl)-3,5-pregnadien-20-one (TzPD, **43**), a potent 5 α -reductase inhibitor (IC₅₀: 15.6 nM), from pregnene-3,20-dione (**39**) in 92% overall yield in four steps, as shown in Chart 11²⁶). Furthermore, it should be noted that the C₂₀-CP of bis-CP **40** also plays a significant role as a protecting group for the C₂₀-ketone.

3. Tetrazole-Fragmentation for Generation of Alkylidene Carbenes from CPs under Neutral Conditions and Its Application to Organic Synthesis²⁷⁾

3.1. Overview of Alkylidene Carbene-Generation Alkylidene carbenes (**44**) can be used in many synthetically valuable reactions including [1,2]-migration, ylide formation, alkyne cyclopropanation and [1,5]-C–H bond insertion.^{28,29)} Notably, [1,2]-migrations occurring with alkylidene carbenes (**44**) are useful for preparation of homologous alkynes (**45**), which are extensively used in organic synthesis^{28,29)} (Chart 12, Eq. 1). Meanwhile, alkylidene carbenes (**46**) having a long alkyl chain undergo regioselective and stereoselective [1,5]-C–H bond insertions, which are well suited for the synthesis of cyclopentenes (**47**, X = CH₂) (Eq. 2).^{28,29)} In the case that a heteroatom is present in the connecting chain, five-membered unsaturated heterocycles (**47**, X = O or N–R) are produced. The high reactivity of alkylidene carbenes necessitates *in situ* generation, typically *via* the use of diazo olefination reagents, haloalkenes, or alkynylodonium salts.^{28,29)} One of the most widely employed methods to access alkylidene carbenes is loss of N₂ from a double bond terminus, particularly from 1-diazoalkenes. These reactions employ dimethyl(diazomethyl)phosphonate esters [(MeO)₂P(O)CHN₂: DAMP] (Seyferth–Gilbert reagent)^{30–32)} or trimethylsilyldiazomethane (TMSCHN₂)³³⁾ (Chart 13, Eq. 1), but require strongly basic conditions, which is a potential drawback. Meanwhile, the Ohira–Bestmann procedure, in which DAMP is produced *in situ* from dimethyl-1-diazo-2-oxopropylphosphonate, has become the most popular method of transforming an aldehyde into the corresponding alkyne under mildly basic reaction condition (K₂CO₃/MeOH) (Eq. 2).^{34,35)} However, using this method, ketones cannot be transformed into internal alkynes, and α,β -unsaturated aldehydes do not yield enynes.³⁶⁾

3.2. Transformation of Ketones into Homologous Alkynes *via* CPs In continuation of our program of using CPs,

we have recently reported novel synthetic methods for homologous alkynes (**45**)¹³⁾ or five-membered unsaturated cyclic compounds (**47**)³⁷⁾ from carbonyl compounds (**4** and **5**) based on reactions of CPs (**3**) with trimethylsilyl azide (TMSN₃) in the presence of Bu₂SnO as catalyst (the Wittenberger method),³⁸⁾ as shown in Chart 14. In this two-step transformation, CPs (**3**) may form tetrazolylphosphates (**48**), which subsequently undergo successive fragmentation to generate alkylidene carbenes (**44** and **46**) that then undergo [1,2]-migration or [1,5]-C–H insertions to produce homologous alkynes (**45**) or five-membered cyclic compounds (**47**), respectively. The scope of these reactions, which occur under neutral conditions, could be extended towards a variety of alkynes and cyclopentene products that are not usually accessible from the corresponding carbonyl compounds *via* the lithium trimethylsilyldiazomethane [TMSC(Li)N₂] procedure^{39–41)} used currently in organic synthesis. In addition, the TMSC(Li)N₂

Chart 12. [1,2]-Migration and [1,5]-C–H Insertion Reactions of Alkylidene Carbenes

Shioiri modification

Ohira–Bestmann modification

Chart 13. Current Procedures for Homologous Alkyne Synthesis from Carbonyl Compounds

Chart 14. Proposed Mechanism for Formation of Alkynes and Cyclopentenes Mediated by Tetrazole-Fragmentation from CPs

Chart 15. Transformation of CPs **50** into Alkynes **52** under MW Conditions Chart 16. Transformation of Ketones **53** into Alkynes **52** via CPs **50**

Chart 17. Synthesis of the mGlu5 Receptor Antagonist, MPEP

The present method **Shioiri method**Chart 18. Transformation of β -Ketoester **55** to Its Corresponding Acetylenic Compound **56**

procedure requires basic conditions.

We first found that reaction of benzylic CPs **50** with sodium azide in the presence of $\text{Et}_3\text{N}\cdot\text{HCl}$ gave α -azidotetrazoles (ATs) **51**. Treatment of ATs **51** thus obtained under microwave (MW) irradiation gave the expected alkynes **52**, as shown in Chart 15.¹³⁾

The procedure outlined above still requires isolation of unstable ATs and relatively harsh conditions in the MW reactor. In the search for a milder variant, it was found that CPs **50** could be transformed directly into the corresponding alkyne **52** under Wittenberger conditions,³⁸⁾ *i.e.*, by using 1 equiv. of TMSN_3 in the presence of a catalytic amount of Bu_2SnO (0.1 eq.) at reflux in toluene, as shown in Chart 16.

Thus, many ketones **53** can be transformed into the corresponding alkynes **52** in moderate to high yields using this method.¹³⁾ The potential of the reaction methodology has been

further explored by applying it to the synthesis of a selective mGlu5 receptor antagonist, 2-methyl-6-(phenylethynyl)pyridine (**54**: MPEP).⁴²⁾ Starting from 6-methylpicolinaldehyde, MPEP was obtained in 68% overall yield over four steps, as shown in Chart 17.¹³⁾

Interestingly, β -ketoester **55**, which possesses readily enolizable α -protons, could also be converted into the corresponding alkyne **56** (68%) by the present method (Chart 18). In contrast, the reaction of **55** under Shioiri conditions [TMSCHN_2 /lithium diisopropylamide (LDA)]³⁹⁾ only resulted in the unreacted starting material.

Colvin and Hamill's original procedure used either TMSCHN_2 or DAMP, and its modifications cannot be applied to dialkyl ketones.^{30–32)} Furthermore, the Ohira–Bestmann reaction of ketones does not afford alkynes; instead, enol ethers are formed.^{34,35)} As shown in Chart 19, the method developed

The present method **Ohira-Bestmann reaction** **Shioiri method** Chart 19. Reactivity of Dialkyl Ketone **57** under Three Different Conditions

Table 5. Transformation of Aldehydes into Terminal Alkynes via CP

Method A : NaN_3 (3 eq), $\text{Et}_3\text{N}\cdot\text{HCl}$ (3 eq), THF, reflux, 16 h.

Method B : TMSN_3 (1 eq), Bu_2SnO (0.1 eq), toluene, reflux, 2 h.

entry	62	63 (%) ^a	Method	64	(%) ^a
1	 62a	 63a	91	 64a	68 47
2	 62b	 63b	99	 64b	57 55
3	 62c	 63c	98	 64c	29 71
4	 62d	 63d	98	 64d	17 80

a) Isolated yield. b) Reflux, 0.5 h. c) TMSN_3 (3 eq.), Bu_2SnO (0.1 eq.), reflux, 0.5 h.

in this work furnished the corresponding 1,6-diphenylhexen-3-yne (**58**) from 1,5-diphenylpentan-3-one (**57**) in 77% overall yield (Eq. 1). In contrast, the Ohira-Bestmann reaction of **57** gave methyl enol ether **59** in 40% yield (Eq. 2),^{34,35} whereas the Shioiri procedure yielded allenylsilane **60** (31%),⁴³ and a homologous aldehyde **61** (23%),⁴⁰ as well as unreacted **57** (46%) (Eq. 3). The formation of allenylsilanes or homologous

aldehydes from ketones using TMSCHN_2 has been reported independently by the Lee⁴³ and Shioiri groups.⁴⁰ These results indicate the versatility of our method.

3.3. Transformation of Aldehydes into Homologous Alkynes via CPs The present method was successfully extended to the transformation of aldehydes **62** into homologous terminal alkynes **64**, as shown in Table 5. In our studies on

Chart 20. Reactivity of β -D-Ribofuranosyl C₁-Carbaldehyde (**62e**) under Different Conditions Chart 21. Reactivity of Cinnamaldehyde **62d** Using the Present and Ohira–Bestmann Methods

the synthesis of novel triazole-containing RNA or DNA,⁴⁴⁾ we sought access to alkynes **64a** from β -D-ribofuranosyl aldehydes **62a**. The reaction of CP **63a** with NaN_3 (3 eq.) and $\text{Et}_3\text{N}\cdot\text{HCl}$ (3 eq.) gave β -ribofuranosyl alkyne **64a** in 68% yield (method A) (Table 5, entry 1). Alternatively, treatment of **62a** with TMSN_3 (1 eq.) in the presence of Bu_2SnO (0.1 eq.) gave **64a** in 47% yield (method B) (entry 1). Thus, method A is favorable for the preparation of homologous alkyne from the corresponding aldehyde. Both methods A and B may be employed equally for primary aldehydes (**62b**, entry 2), but method A is slightly more useful for aldehydes **62b** (entry 2). Conversely, in the case of the aromatic and α,β -unsaturated aldehyde CPs **62c** and **62d**, respectively, method B is favorable, providing alkynes **64c** and **64d** in 71% and 80% yields, respectively (entries 3 and 4). The reason for the preference towards either method A or B depending on the aldehyde is

unknown at present.

As shown in Chart 20, the transformation of aldehyde **62e** into alkyne β -64e in overall 99% yield is of particular interest, because the Ohira–Bestmann reaction and Shioiri modification procedures gave only inseparable 5:2 and 9:1 epimeric mixtures of β -64e and α -64e, respectively.⁴⁴⁾ The formation of the epimeric mixtures probably occurs because of the extraction of α -acidic proton of **62e** followed by β -elimination under basic conditions.

As mentioned before, the Ohira–Bestmann reaction does not give enynes from α,β -unsaturated aldehydes.^{34,35)} In contrast, it is possible to synthesize the corresponding enyne **64d** from cinnamaldehyde (**62d**) in 78% overall yield following the method described in this work (Chart 21). Furthermore, the Ohira–Bestmann reaction using **62d** as substrate gave (1-methoxybut-3-ynyl)benzene (**65**) in 10% yield, together with

Chart 22. Proposed Mechanism for the Formation of Alkynes **45** Mediated on Tetrazole-Fragmentation from CPs **3**Table 6. Transformation of Methyl Alkyl Ketones **70** into Five-Membered Products **72** via CPs **71**

entry	70	71 (%)	72 (%)	overall yields of 72 from 70 (%) ^g
1	 70a	 71a (96)	 72a ^b (69)	66 ^d
2	 70b	 71b (82)	 72b ^b (72)	59 ^d
3	 70c	 71c ^a (99)	 72c ^c (76)	75 ^d
4	 70d	 71d (97)	 72d ^c (92)	89 ^d (73 ^e , 68 ^f)

a) DEPC (3.0 eq), LiCN (3.0 eq.), 1 h, rt; b) TMSN₃ (1.0 eq.), Bu₂SnO (0.1 eq.), 2 h, reflux; c) TMSN₃ (2.0 eq), Bu₂SnO (0.2 eq), 4 h, reflux; d) 2 steps, overall yield from **70** by our method; e) Shiomi procedure using TMSCHN₂/BuLi; f) Gilbert procedure using DAMP/*tert*-BuOK; g) Figures in parentheses refer to the results of other methods.

dimethyl acetal (**66**, 67%).

The mechanism of Bu₂SnO-catalyzed cycloaddition of TMSN₃ on nitriles has been studied in detail by Kappe and colleagues.⁴⁵⁾ It was demonstrated that the active catalytic species is Bu₂Sn(OTMS)N₃ (**67**), and that regeneration of this catalyst occurs through S_N2 displacement at the silicon atom followed by fast ligand exchange at the tin atom. A plausible mechanism that would account for the formation of alkynes **45** from CPs **3** is shown in Chart 22. The proposed mecha-

nism also indicates that the C₁ unit of homologous alkynes **45** arises from the CN-carbon of DEPC.

3.4. Synthesis of Five-Membered Unsaturated Cyclic Compounds from Ketones via CPs Synthesis of five-membered unsaturated cyclic compounds from ketones by [1,5]-C–H insertion of alkylidene carbenes was carried out using (MeO)₂P(O)CHN₂ or TMSCHN₂ in the presence of a strong base.^{28,29)} Methyl alkyl ketones **70** with lower [1,2]-migratory aptitudes were thus chosen as substrates for the for-

Chart 23. Formation of 5-Alkylsilyl-2,3-dihydrofurans Using the Present Method

Cf. Lee and co-workers

Chart 24. Transformation of Cyclic Ketone **76** into Bicyclic Compound **77** Chart 25. Transformation of β -Keto Ester **80** into Cyclopentenyl Acetate **81**

mation of various five-membered compounds, as summarized in Table 6. In the case of methyl ketones **70a–d** (entries 1–4), cyclopentene **72a**, 2,5-dihydrofuran **72b**, and 2,5-dihydropyrroles **72c**, and **72d** were readily obtained in 66%, 59%, 75%, and 89% overall yields in two steps. Furthermore, the present method was successfully applied to the conversion of (4*S*)-2,2-dimethyl-4-(3-oxobutyl)-1,3-dioxolane (**70d**) into spiro[4.4]nonene (**72d**, 89% overall yield, entry 4), which is a versatile intermediate in the total synthesis of (–)-malynolidine⁴⁶ or (–)-frontalin⁴⁷ as reported by Ohira and co-workers. They employed Shioiri conditions (TMSCHN₂/*n*-BuLi)^{39–41} or the Seyferth–Gilbert procedure (DAMP/*t*-BuOK)^{30–32} for the preparation of **72d** from ketone **70d**, obtaining the products in 73 and 68% yields, respectively. The results summarized in Table 6 indicate that our method is preferable to existing methods.

The present method could further undergo [1,5]-O-SiMe₃ bond insertion⁴¹) to generate 5-trialkylsilyl-2,3-dihydrofurans **75a** (77%) and **75b** (81%) from the corresponding CPs **74a** (R = TMS) and **74b** (R = TBDMS), respectively (Chart 23).

Furthermore, the 16-membered cyclic ketone **76** yielded

bicyclo[12.2.1]heptadecene (**77**, 59%) via a CP together with cyclic alkyne homolog **78** (16%), in which [1,5]-C–H insertion mediated by alkylidene carbene from CP was preferred to the formation of cyclic alkyne homolog **78**, as shown in Eq. 1 of Chart 24. In contrast, Lee and co-workers reported that the reaction of small- to medium-sized cyclic ketones (**76**, *n* = 4–14) with TMSCHN₂ led to allenylsilanes (**79**, 10–81%) as per Eq. 2.⁴³)

Notably, β -keto ester **80**, which possesses readily enolizable α -protons, could be converted into cyclopentenyl acetate **81** in 95% overall yield using the present method (Chart 25, Eq. 1). In contrast, the reaction of **80** under Shioiri conditions (TMSCH₂/LDA) only resulted in the unreacted starting material (**80**), as per Eq. 2.

3.5. Synthesis of (–)-Neplanocin A through Tetrazole-Fragmentation from CPs⁴⁸ (–)-Neplanocin A (NPA, **91**) is a naturally occurring carbocyclic nucleoside⁴⁹) (Chart 26). NPA and other natural analogs have received considerable attention because of their interesting biological properties, such as their potent antiviral and antitumor properties.⁵⁰) NPA itself is cytotoxic to host cells,⁵¹) and this may account for its

Chart 26. Synthesis of (-)-Neplanocin A from CP 84

Fig. 2. Two Possible Pathways for the Formation of 87

reduced therapeutic potency. Therefore, the chemical synthesis and structural modification of NPA have been investigated extensively.⁵⁰⁾ Among them, protected cyclopentenone **89** and cyclopentenyl tetrol **90a** have been widely used as synthetic precursors not only for NPA, but also its analogs.^{52–54)} Based on these reports and to demonstrate the synthetic utility of the alkylidene carbenes generated from CPs, we attempted to apply them to the synthesis of NPA (-)-**91** and its synthetic precursors **89** and **90a** from ketone **83**,⁵³⁾ as shown in Chart 26.

Cyanophosphorylation of ketone **83** easily afforded CP **84** in 95% yield. The reaction of CP **84** with $\text{TMSN}_3/\text{Bu}_2\text{SnO}$ afforded an inseparable mixture of epimeric cyclopentenones **86 $\alpha\beta$** , which was the result of the C–H insertion reaction of alkylidene-carbene **85**, along with unexpected dihydropyran **87**.⁵³⁾ After the mixture was treated with tetrabutylammonium fluoride (TBAF) to remove the TBDMS group, deprotected cyclopentenones **88 $\alpha\beta$** (88 α /88 β = 1/3) and dihydropyran **87** were obtained in 65 and 20% yields, respectively, from CP **84**. As illustrated in Fig. 2, the formation of **87** may have been caused by [1,6]-O-Si bond insertion of the carbene **85a** or 1,2-TBDMS shift of ylide **85b**,⁵⁴⁾ as opposed to the usual [1,5]-O-Si insertion that generates dihydrofuran derivatives.^{28,41)}

4. Schematic Diagram on Applicability of CPs

A number of applications of CPs in organic synthesis are being developed continuously by researchers at home and abroad since the first author's report on CPs. These studies are described in detail in the recent review of DEPC,¹⁾ and the applicability of CPs in organic syntheses may be summarized in Figs. 3 and 4.¹⁾

5. [3,3]Sigmatropic Rearrangement of Cyclic Thionocarbonates of Medium Ring-Size⁵⁵⁾

5.1. Sigmatropic Rearrangement of Medium-Sized Thionocarbonates The exclusive *E*-selectivity of the double bond created by [3,3]sigmatropic rearrangement has been extensively used for natural product synthesis. However, few synthetic studies on the opposite (*Z*)-selective [3,3]sigmatropic rearrangement have so far been reported, probably due to lack of suitable methodology. Meanwhile, medium-ring compounds (those having a ring size in the range 8 to 11) are becoming increasingly important in organic chemistry, because they are discovered in an ever-growing number of natural products.⁵⁶⁾ In this context, we found that the [3,3]sigmatropic ring expansion of allylic 8-membered thionocarbonates induced the highly stereoselective synthesis of either *Z* or *E* olefins in 10-membered thiolcarbonates. In this chapter, we describe our results on the [3,3]sigmatropic rearrangement of cyclic thionocarbonates of medium ring-size and their application to the synthesis of natural products.

In our study of the formation of the 8-membered thionocarbonates,⁵⁷⁾ we found spontaneous ring expansion of the cyclic thionocarbonates *via* [3,3]sigmatropic rearrangements.^{58,59)} When a dry THF solution of $(\text{TMS})_2\text{NNa}$ was rapidly added to a THF solution of diol monothionocarbonate **92** at r.t., the

Fig. 4. Reaction of Allylic CPs

Chart 27. Ring Expansion via [3,3]Sigmatropic Rearrangement of Cyclic Thionocarbonate **93**

5.4. Synthesis of Medium-Sized Heterocyclic Allenes and Its Synthetic Application^{66,67)} Alternatively, treatment of acetylene diol monothionocarbonates **105** with $(\text{TMS})_2\text{NLi}$ gave medium-sized heterocyclic allenes **107** via spontaneous [3,3]sigmatropic ring expansion of alkynyl cyclic thionocarbonates **106**, as shown in Chart 31.⁶⁷⁾

The formation of the medium-sized heterocyclic allenes was applied to the synthesis of a racemic form ($\pm$)-**115** of (*R*)-(-)-methyl 8-hydroxy-5,6-octadienoate, which is a strong antifungal constituent in the leaves of a deciduous tree native to Japan, *Sapium japonicum*, as shown in Chart 32.⁶⁶⁾ This synthesis also features a novel application of the SmI_2 -HMPA reduction to a key intermediate, 10-membered thiol carbonate **111**. Reaction of diol monothionocarbonate **109**, prepared from aldehyde **108**, with $(\text{TMS})_2\text{NLi}$ afforded a 10-membered allene **111** in 85% yield via the [3,3]sigmatropic ring expansion of a cyclic thionocarbonate **110**. Subsequent treatment of **111** with

SmI_2 -HMPA reduction afforded an allenic alcohol **112** in 78% yield with the liberation of SCO. The bifunctional compound **112** was converted to ($\pm$)-**115** via four steps.

5.5. Synthesis of Strained 8-Membered Heterocyclic Allenes Decreasing the ring size of cyclic allenes results in deviation of both the normal $\text{C}=\text{C}=\text{C}$ linearity and the orthogonality of dihedral angle. Treatment of diol monothionocarbonate **116** having a *tert*-butyl alkyl group with $(\text{TMS})_2\text{NLi}$ in THF at r.t. afforded a 6-membered cyclic thionocarbonate **117**⁶⁷⁾ (Chart 33, Eq. 1). Rearrangement of **117** as expected proceeds upon refluxing in benzene solution for 1.5 h to give a pure 8-membered heterocyclic allene **118**. The structure of **118** was estimated from modified neglect of diatomic overlap (MNDO) calculation results (Eq. 2).⁶⁷⁾ The bond angle on the *sp* carbon, $\text{C1}-\text{C2}-\text{C3}$, is decreased from linearity to 170.1° . The dihedral angle $\text{CH}_3-\text{C1}-\text{C3}-t\text{-Bu}$, is twisted (64.3°) from vertical geometry

We then examined the behaviour of the 8-membered cyclic allenes **118** for a base or acid.⁶⁷⁾ Intriguing was the formation of 2*H*-thiopyran derivative **121** by treatment of **118** with 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU), inducing isomerization of the double bond of **118** and elimination of CO_2 from the resulting cyclic diene **120** (Chart 33, Eq. 3). However, no change was observed by treatment of **118** with *p*-toluenesulfonic acid in THF.

Chart 28. Relationship between Ring Size of Cyclic Thionocarbonates and *Z* or *E* Geometries of Products Chart 29. Highly Stereoselective Synthesis of *Z*- or *E*-Double Bonds with Conformational Control in [3,3]Sigmatropic Ring Expansion of 8-Membered Thionocarbonates **96** Fig. 5. Conformational Control in [3,3]Sigmatropic Ring Expansion of 8-Membered Thionocarbonates **96**

6. Synthetic Study of Imidazole C-Nucleosides and Their Application to Probing Catalytic Mechanisms of Ribozymes^{68,69)}

6.1. Stereoselective Synthesis of C-4 Linked β -Imidazole C-Ribonucleosides C-Ribonucleosides have attracted much attention in view of their remarkable anti-tumor and antiviral activities.^{70–72)} Azoles are biologically important heterocyclic compounds, and a variety of useful therapeutic agents containing the imidazole moiety have been developed.^{73,74)} Meanwhile, the synthetic method of imidazole C-nucleosides, in general, is limited and requires multiple steps.^{70–72)} We thus directed our attention to synthesizing 4(5)-(β -D-ribofuranosyl)imidazole (**127**) having the imidazole in place of nucleobase, as shown in Chart 34.^{75,76)}

The reaction of tribenzyl-D-ribose **122** with lithium salt **123** of bis-protected imidazole gave inseparable epimeric mixture **RS-124**, as illustrated in Chart 34. Hydrolysis of **RS-124** in refluxing 1.5N HCl afforded a 1:1 mixture **RS-125** of diols

having an unsubstituted imidazole. Treatment of the mixture **RS-125** with *N,N,N',N'*-tetramethylazodicarboxamide (TMAD)-Bu₃P at rt in benzene exclusively produced the desired β -anomer (**β -126**) in 92%, together with a small amount of α -anomer (**α -126**, 3.5%). The ratio of β - and α -anomer was 26.3:1. Debenzylation of **β -126** was carried out to achieve the synthesis of C-4 linked β -imidazole C-ribonucleoside **β -127** in four steps and 87% overall yield from the starting **122**.

Importantly, it is possible to synthesize the β -anomer without separation of the isomers (***R*-125** and ***S*-125**) or stereoselective synthesis of the *R*-isomer which can provide β -anomer through intramolecular *S_N2* reaction under standard Mitsunobu reaction. Accordingly, the unsubstituted-imidazole moiety is indispensable for exclusive formation of β -anomer. The β -stereoselectivity in this reaction can be rationalized as in Chart 35. Reaction of TMAD-Bu₃P adduct with ***R*-125** forms a zwitterion ***R*-128**. Preferential elimination of Bu₃P=O from **128** leads to diazafulvene **129a**. Spontaneous cyclization of **129a** gives **β -126**. Although ***S*-125** similarly leads to active species **129b**, it exclusively gives the β -anomer *via* rotamer **129a** being thermodynamically more stable. The remarkable stereoselectivity ($\beta/\alpha = 26/1$) of the β -ribofuranosylimidazole is facilitated by electron repulsion in **129b**.

The stereoselective synthetic method of C-nucleosides *via* diazafulvene intermediates was further applied to synthesis of various C-nucleosides: β -ribofuranosylimidazoles exhibiting antiulcer activity⁷⁷⁾; α -L-arabinofuranosylimidazoles⁷⁸⁾;

Chart 30. Synthesis of (-)-Yellow Scale Pheromone via [3,3]Sigmatropic Ring Expansion of 8-Membered Thionocarbonate Containing A Diene Moiety

Chart 31. Synthesis of Medium-Sized Heterocyclic Allenes

Chart 32. Synthesis of Antifungal Constituent of *Sapium Japonicum*

tetrahydrofuranylimidazoles^{79,80}; α -D-xylofuranosylimidazoles⁸¹; *trans*- or *cis*-4(5)-(5-aminomethyltetrahydrofuran-2-yl)imidazoles for histamine H₄-ligands⁸²; and pyrazole C-nucleosides.⁸³

6.2. Synthesis of Novel C4-Linked Imidazole Ribonucleoside Phosphoramidites for Probing General Acid and Base Catalysis in Ribozymes⁶⁹ The VS ribozyme is the largest of a group of nucleolytic ribozymes that include hammerhead, hairpin and HDV ribozymes, and catalyzes the

site-specific cleavage of a phosphodiester linkage to generate products containing 2',3'-cyclic phosphate and 5'-OH termini.⁸⁴ The VS ribozyme is the only member of the class of nucleolytic ribozymes for which there is no known crystal structure.⁸⁴ Lilley and colleagues recently indicated that the A730 loop of VS ribozyme is an important component of the active site in general acid and base catalysis⁸⁵ (Fig. 6). In particular, the adenine (A756) in the A730 loop is a critical base in the

Chart 33. Synthesis of Strained 8-Membered Heterocyclic Allene **118** and Its Derivative Chart 34. Stereoselective Synthesis of C-4 Linked β -Imidazole C-Nucleosides Chart 35. Mechanistic Consideration on β -Stereoselective Glycosidation

cleavage,⁸⁶) because sequence variants at the position 756 are especially impaired in the cleavage and ligation activity. In our collaborative studies with Lilley and colleagues, a *trans*-acting VS ribozyme, where a three-piece ribozyme-substrate system was used, have been designed, as illustrated in Fig. 6.

Given that imidazole with a pK_a of 7.1 is both a good donor and acceptor of proton, synthesis of an imidazole C-nucleoside would allow incorporation at the desired position to probe general acid and base catalysis. Therefore, synthetic study of the tribenzyl- β -ribofuranosylimidazole **β -126** led us to the development of a new chemical strategy for determining the role of acid-base catalysis in a ribozyme, in which C4-linked imidazole was placed into A756 of VS ribozyme covalently as a pseudonucleobase, as illustrated in Fig. 7.^{87,88}

In this study, a novel C4-linked imidazole 5'-*O*-dimethoxytrityl (DMT)-2'-*O*-*tert*-butyldimethylsilyl (TBDMS)-3'-*O*-cyanoethyl-diisopropylphosphoramidite **130** was designed as a synthetic unit of the A756Imz VS ribozyme, and synthesized starting from **β -126**, as shown in Chart 36. Investigation of protecting groups for the imidazole-*N* first indicated that pivaloyloxymethyl (POM) was adequate as an *N*-protecting group for the imidazole nucleoside **β -126** which could be readily removed under mild basic condition [aqueous ammonia-

MeOH (1:3, v/v), r.t., 3 h]. Therefore, treatment of **β -126** with POMCl followed by Pd(OH)₂-C/cyclohexene produced N-POM-imidazole C-nucleoside **131** (Chart 36). DM-tritylation of **131** afforded 5'-*O*-DMT-derivative **132**. Treatment of **132** with TBDMSOTf/Py led to an inseparable 1:1 mixture **133ab** (52%) of 2'-*O*-TBDMS and 3'-*O*-TBDMS isomers, together with a 2',3'-bis-*O*-substituted derivative **134** (17%). The mixture of **133ab** was subjected to phosphitylation with 2-cyanoethyl tetraisopropylphosphodiamidite to yield the desired 3'-phosphoramidite (PA) **130** (31%) and 2'-PA **135** (25%). However, the synthetic route of PA **130** led to problems of instability against the purification on silica gel column chromatography and low overall yield (<5%).

The imidazole PA **130** thus prepared was subjected to a TBDMS approach of RNA automated synthesis to provide an imidazole-substituted VS ribozyme (A756Imz) in an average 99% coupling yield⁸⁹) (Fig. 7). The A756Imz catalyzed the almost-complete cleavage of a substrate stem-loop at the correct position, as shown in Fig. 8.⁸⁹) Virtually complete cleavage has been obtained, with rates of 0.01 min⁻¹. The result powerfully supported a direct role of the nucleobase at position A756 in the chemistry of natural VS ribozyme.⁸⁹)

In another study, PA **130** was also used to replace G8 in the hairpin ribozyme with the imidazole base. The modified hairpin ribozyme (G8Imz) catalyzed both cleavage and ligation reactions, with a pH dependence consistent with a role of G8 in general acid-base catalysis.⁹⁰)

6.3. Accurate Molecular Weight Measurements of Nucleoside PAs: A Suitable Matrix of Mass Spectrometry^{91,92}
Nucleoside PAs are the most widely used building blocks in contemporary solid-phase synthesis of oligonucleotides. The novel imidazole PA **130** with POM group, which is easily removed under basic conditions and compatible with TBDMS synthesis of RNA, was first synthesized as a key ribonucleoside variant, as mentioned in chapter 6.2. However, in course of analytical investigation of **130**, we encountered difficulty in mass spectrometric characterization of **130**, which decomposed even on a standard TLC plate (Merck 60F₂₅₄). After

Fig. 6. Schematic of A Substrate and VS Ribozyme with Helices Numbered and Cleavage Position Arrowed

(A) The *trans*-acting VS ribozyme was used in our studies where the substrate and ribozyme were separated. (B) The A 730 loop is boxed.

Fig. 7. In Corporation of Imidazole into A756 of VS Ribozyme Using PA **130**

many investigations, a collaborative study with Fujitake clarified that the accurate molecular weight (MW) measurements of such molecules, which contain acid-labile moieties, may be easily determined by mass spectrometry using a new matrix system, triethanolamine (TEOA)-NaCl, on liquid secondary ion mass spectrometry (LSIMS) equipped with a double-focusing mass spectrometer, as illustrated in Fig. 9.⁹³⁾ In addition, this matrix has an advantage of permitting use on the alternative ionization method, FAB.⁹⁴⁾ The present method measures rapidly and easily the accurate MWs of various PAs as adduct ions $[M + Na]^+$ with average mass error smaller

than 0.4 ppm, allowing the formulas of various PAs in place of elemental analysis. The sodium-cationized molecule of PA130 appeared at m/z 953.4625 in the externally calibrated spectrum (polyethylene glycol) by high-resolution (HR)-MS and the error was only 0.4 ppm from the expected mass number, as shown in Fig. 10.⁹³⁾ Furthermore, all of more than 50 PAs investigated, which bear a variety of functional groups, gave sodium-cationized molecules using the present method.⁹³⁾ These observations successfully demonstrate the generality and potential of the method. Hence, the proposed method is a powerful tool for MS identification of PAs or fragile molecules.

6.4. Efficient Synthesis of 2'-O-Cyanoethylated Imidazole C-Ribonucleoside PAs We have described previously that a 2-O-TBDMS C4-linked imidazole ribonucleoside PA 130 can be applied as a key building block to investigate the role of general acid-base catalysis in VS and hairpin ribozyme RNA using solid-phase TBDMS chemistry implemented on an automated synthesizer. However, there is a serious hindrance to the general application of this approach. As shown in Chart 36, the synthetic route of PA 130 lacks the selective introduction of TBDMS group at 2'-hydroxy group of diol intermediate 132 owing to easy 2'-3' TBDMS-migration, leading to the inseparable 1:1 mixture 133ab. Thus, when the mixture 133ab was subjected to phosphorylation, PA 130 was only obtained in poor yield (31%) together with 2'-PA 135 (25%). Moreover, great care has to be taken for purification and isolation of 130 because of its acid-labile character and contamination by phosphorus residues. As a result, the overall yield of 130 is <5% through 8 steps from the starting tribenzyl D-ribose 122. In our systematic studies on the catalytic

Fig. 8. Cleavage Reaction of A756Imz VS Ribozyme

Fig. 9. Accurate Molecular Weight Measurement of PAs

Fig. 10. Positive Ionization LSIMS Spectrum of PA 130 Induced in a TEOA/NaCl Matrix

Chart 36. Synthesis of C4-Linked Imidazole Ribonucleoside PA 130

Chart 37. Synthesis of 2'-O-Cyanoethyl Imidazole C-Nucleoside PA 140

mechanism on ribozymes, we developed a variant PA **140**, in which the cyanoethyl (CE) group was used as the smallest available protecting group for the 2'-hydroxy function, as illustrated in Chart 37.⁹⁵⁾ It is particularly significant that a combination of CE and POM protecting groups inhibited 2'-3' migration between 2'- and 3'-hydroxy groups and provided additional stability for purification of the PA **140**. The total yield of **140** was 41% in ten steps from the commercially-available tribenzyl D-ribose **122**, demonstrating the effectiveness of this approach (Chart 37). This has resulted in a marked improvement of yield compared with unstable 2'-O-TBDMS PA **130**.⁹⁵⁾

The new synthon PA **140** enables the imidazole moiety to be incorporated into any RNA sequence in principle, and has been used as a practical building block for probing the catalytic mechanism of a ribozyme.

6.5. Synthesis of Novel C₂-Imidazole Ribonucleoside PA and Its Application to Probing the Catalytic Mechanism of Ribozymes In our studies of the catalytic mechanism of VS ribozyme, Lilley identified a guanine (G638) in the substrate loop of VS ribozyme as a second important nucleobase for general acid-base catalysis⁹⁶⁾ (Fig. 6). However, when we substituted C₀-linked imidazole nucleoside

Chart 38. Synthesis of ICN-C₂-PA 149Table 7. Comparison of Cleavage Rates between G638C₂Imz and G638C₀Imz

	Rates/min ⁻¹ natural	G638C ₂ Imz	G638C ₀ Imz	C ₂ Imz/C ₀ Imz
10mM Mg ²⁺ pH 8				
–Rz		6 × 10 ^{–5}	5 × 10 ^{–5}	
+1 μM Rz	0.61	0.001	7 × 10 ^{–5}	14
200mM Mg ²⁺ pH 6.5				
–Rz		1 × 10 ^{–5}	2 × 10 ^{–5}	
+1 μM Rz	6.2	0.008	5 × 10 ^{–4}	15

Fig. 11. Catalytic Mechanism of VS Ribozyme via G638 and A756 Nucleobases

Fig. 12. Structures of C4-Linked Imidazole- C_n -, and C5-Linked Tetrazole- C_n -Ribonucleoside PAs and Imidazole- C_n -2'-Deoxyribonucleoside PAs

Fig. 13. H_3 R Autoreceptors and Heteroreceptors

at position 638 using PA **130**, the modified VS ribozyme (G638C₀Imz) exhibited virtually no restoration of cleavage activity.⁹⁶⁾ This result suggests that the environment at the position 638 was more constrained, preventing the structural rearrangement that would be required to bring the imidazole base to the position normally occupied by the guanine N1. It might be anticipated that the manner of linking the imidazole group to the ribose would affect its ability to function in general acid base catalysis. If the linker is too short then its ring *N* cannot be superimposed with that of N1 of adenine or guanine. On the other hand, if it is too long then it may spend too little time in position required for its potential catalytic function. Furthermore, molecular graphics shows that the ring *N* atom of imidazole C_2 -nucleoside and N1 of guanine can readily be superimposed, whereas these atoms are separated by $\geq 2 \text{ \AA}$ for the C_0 -nucleoside in the absence of significant distortion.⁹⁶⁾ Consequently, we designed and synthesized a two-carbon-elongated homologue (Imz- C_2 -PA, **149**) that may be a better structural mimic of purine nucleobase.⁹⁶⁾ As shown in Chart 38, the synthesis of PA **149**, in which POM and 2-CE groups were used to protect imidazole-*N* and 2'-hydroxy function, was successfully achieved in 13 steps and 23% overall yield from starting tribenzyl D-ribose **122**.

The C_2 -imidazole nucleoside, a flexible structural mimic of a purine nucleobase, was successfully incorporated using the Imz- C_2 -PA **149** into position 638 of VS ribozyme through 2'-TBDMS chemistry to study the role of G638 in general acid-base catalysis.⁹⁶⁾

It is interesting to note that the cleavage rate of G638C₂Imz was 15-fold greater than that achieved with the ribozyme (G638C₀Imz) containing a C4-linked imidazole at the same position, as shown in Table 7.⁹⁶⁾

From these results, G638 is one of the nucleobases proposed to act in general acid-base catalysis in concert with A756 in the catalytic mechanism of the VS ribozyme.⁹⁶⁾ The chemical mechanism appears to involve general acid-base catalysis *via* the combination of G638 and A756 nucleobases which are juxtaposed with the scissile phosphate, as illustrated in Fig. 11.⁸⁴⁾

The VS ribozyme is the only member of the class of nucleolytic ribozymes for which there is no crystal structure, and mechanistic investigation cannot be guided by structural data.⁸⁴⁾ Because the whole series of ribose- $(CH_2)_n$ -Imz PAs ($n = 0-3$) could make an interesting series of compounds for systematically tracing out the geometry of the active site of ribozyme, Imz- C_1 - and C_3 -PAs **150** and **151** were prepared (Fig. 12A).⁹⁷⁾ Furthermore, starting from tetrazole(TeZ)- C_0 - and C_2 -

Fig. 14. Comparison between Histamine H₃R and H₄R Fig. 15. Folded and Extended Models as Bioactive Conformations of H₃R-Agonists

PAs **152** and **153** (Fig. 12B), C5-linked C₀- and C₂-tetrazoles were incorporated successfully into the VS ribozyme substrate to determine more specifically which nucleobases of the ribozymes function as the acid or base.⁹⁸ Ribose-(CH₂)_n-Imz or Tez species can, in principle, be incorporated at any site of RNA sequences through chemical synthesis. In addition, Imz-C₀- and C₂-deoxyribonucleoside phosphoramidites (dPAs, Fig. 12C) are successfully incorporated into the sequence of a 15-nt DNA, and the abilities of one or two imidazoles to pair with different bases are investigated through thermal melting (*T_m*) experiments on the resulting DNA duplexes.⁹⁹

7. Novel Synthetic Methods Using Se or Sn Atoms towards Histamine H₃ Receptor Ligands^{69,100}

7.1. Synthesis of Tetrahydrofuranylimidazoles Using a PhSe Group and Its Application to New Histamine H₃ Receptor Agonist, Imifuramine

Histamine receptors are a class of G protein-coupled receptors with histamine as their endogenous ligand. The histamine H₁ receptor (H₁R) is responsible for inflammation and allergic reactions, whereas the histamine H₂ receptor (H₂R) is responsible for the mechanism of gastric acid secretion. Both H₁R and H₂R antagonists are nowadays in widespread clinical use. The histamine H₃ receptors (H₃R), on the other hand, exist at the varicosities and endings of histaminergic fibers in the brain and modulate the synthesis and release of histamine as an autoreceptor, as illustrated in Fig. 13.¹⁰¹ Moreover, H₃Rs are heteroreceptors that modulate the release of a number of different neurotransmit-

Fig. 16. Four Stereoisomers of 4(5)-[5-(Aminomethyl)tetrahydrofuran-2-yl]imidazole

Chart 39. Synthesis of 1',2'-Anti-diol **162S**

ters. Therefore, histamine neurons play an important role in arousal, learning, and memory mechanisms, working together with other neuromodulatory systems. H₃R-antagonists are expected to be potential drugs for memory degenerative disorders such as Alzheimer's disease. This type of receptor can be also found in many peripheral tissues. Since H₃R-Agonist (*R*)- α -methylhistamine, imetit, and imepip were shown to pos-

Fig. 17. Anti-selectivity for **162S** by Chelation-Cyclic Model

sess inhibitory actions against airway smooth muscle contraction, H₃R-agonists are regarded as a target for new therapeutics of bronchial asthma.¹⁰²⁾

The most recently identified histamine H_4 receptor (H_4R) is mainly expressed in hematopoietic cells such as mast cells, basophils, eosinophils, T-cells, and dendritic cells, as shown in Fig. 14.¹⁰³⁾ It is therefore believed to play an important role in inflammation and immune responses, with possible applications in diseases such as inflammatory bowel disease, allergic asthma, and pruritus.¹⁰³⁾

A reoccurring issue in the development of H₃R antagonists is selectivity between H₃R and H₄R. These two receptors share the highest homology within the histamine receptor family (31% overall, 54% in the transmembrane region) (Fig. 14). Indeed, several classical H₃R antagonists, such as thio-peramide and clobenpropit,¹⁰⁴⁾ also have significant affinity for H₄R. To investigate the physiological and pathophysiological roles of H₃R and H₄R, the development of a range of selective

Fig. 18. Postulated Mechanism of Formation of **164a** and **164b**

Chart 40. Transformation of α -Anomer **164a** into *trans*-Isomer (–)-**157**

H₃R ligands is crucial.

Theoretical calculations of histamine or some H₃R-agonists have predicted the importance of an intramolecular hydrogen-bonding between the cationic primary amine and the *N* atom of the imidazole, leading to a folded model **A**, and an extended model **B** as bioactive conformations of H₃R-agonists was also reported^{105,106} (Fig. 15). However, they have not been experimentally demonstrated.

From the β -stereoselective synthesis of C-4 linked imidazole nucleosides, we envisioned that, while the *cis*-isomer **156** of 4(5)-[5-(aminomethyl)tetrahydrofuran-2-yl]imidazole could adopt a folded conformation through intramolecular hydrogen-bonding, the *trans*-isomer **157** would form the extended one (Fig. 16).

Therefore, we synthesized four isomers of novel THF derivatives **156** and **157** using a synthetic method characterized by use of a phenylselenenyl (PhSe) group for the formation of the THF ring.^{107,108} Introduction of a PhSe group into lactone (+)-**158** prepared from L-glutamic acid gave C2 α - and C2 β -selenolactone **160a** (52%) and **160b** (30%) (Chart 39). Both of which were used as substrates to prepare key intermediates **164a** and **164b**. Reduction of the major isomer **160a** with DIBAL followed by an addition of 5-lithioimidazole to the resulting lactol **162S** (73%) with a C1'S configuration, together with C1' epimer **162R** (10%). The *anti*-selectivity for **162S** may be account for by a chelation-cyclic model as indicated

in Fig. 17.

Deprotection of **162S** under reflux in HCl-THF afforded easily separable β - and α -nucleoside derivatives **164b** (42%) and **164a** (56%) (Fig. 18). The formation of **164b** and **164a** can be reasonably rationalized as shown in Fig. 18. The reaction of **162S** with HCl generates olefin **163** by a *trans*-stereospecific elimination of PhSeCl. Recombination of **163** and PhSeCl gives β - and α -anomers **164b** and **164a** through selenium-induced cyclization at both faces of double bond of **163**. Similar treatment of **162R** provided **164b** and **164a**. This synthetic method for the preparation of C-nucleoside derivatives using a combination of the elimination of PhSeCl and selenocyclization has not been reported.¹⁰⁸

Ethoxycarbonylation of the α -anomer **164a** gave **165a** which was then converted into **166a** by free radical deselenylation (Chart 40). Debenzylation of **166a** and subsequent phthaloylimination afforded 5'-substituted phthalimide **168a**. Double deprotection of **168a** with hydrazine hydrate yielded (–)-**157**. Alternatively, synthesis of (+)-**156** was attained in 62% overall yield from β -anomer **164b** by the same synthetic procedure. Their configuration counterparts, (–)-**156** and (+)-**157** were also synthesized starting from D-glutamic acid by the same methodology.¹⁰⁸ This synthetic approach should enable the supply of a variety of derivatives by which their biological activity can be assessed.

Using an *in vivo* microdialysis method¹⁰⁹ (Fig. 19), we examined whether the four synthesized stereoisomers had any effect on the release of histamine in the brain of rats. Among them, only (+)-**157**, imifuramine exhibited H₃R-agonistic activity.¹⁰⁷ Administration of 10 μ M imifuramine to the perfusion fluid decreased histamine release to about 30% of the basal release, as shown in Fig. 20. Furthermore, co-infusion of the H₃R-antagonist, clobenpropit (10 μ M),¹⁰⁴ fully antagonized this effect and increased histamine release to about 160% of basal levels¹⁰⁷ (Fig. 20). Activity of imifuramine measured by microdialysis was approximately equal to that of imipip.¹⁰⁴ These observations support the H₃R-agonistic activity of imifuramine. The other three isomers had no effect on histamine release at 10 μ M concentration. Therefore, imifuramine was indicated as a novel type of histamine H₃R-agonist, suggesting

Fig. 19. a) Schematic Model of Histaminergic System and Insertion Area of Microdialysis Probe

The histaminergic system has its cell bodies in the tuberomammillary nucleus (TM) and projects to several brain regions. b) Principle of the microdialysis probe. It can be used to recover and administer substances in living tissue. (Color figure can be accessed in the online version.)

Fig. 20. Effect of Imifuramine and Clobenpropit on Release of Histamine from Anterior Hypothalamic Area of Rats Using an *in Vivo* Microdialysis

Fig. 21. Selectivity of Imifuramine Derivatives for H₃ and H₄ Receptors Expressed in SK-N-MC Cells

Chart 41. Synthesis of Chiral 4(5)-(5-Aminotetrahydropyanyl-2-yl)imidazoles

the extended model **B** as bioactive conformations of the H₃-agonists^{107,108)} (Fig. 15).

7.2. Development of the First Selective Human H₄-Receptor Agonist OUP16¹¹⁰⁾ A new histamine receptor, H₄R was discovered by several groups in the quest for new G-protein-coupled receptors (GPCR).¹⁰³⁾ The overall amino acid sequence showed approximately 37% homology between H₃Rs and H₄Rs, as illustrated in Fig. 14. However, the distribution of human H₄R (hH₄R) mRNA was entirely different

from that of the H₃R. Although little is known about H₄R's *in vivo* function, its expression in bone marrow and eosinophils may suggest a potential new role for histamine in regulation of hemopoietic and immune functions (Fig. 14). To investigate the possible physiological function of H₄R, a specific ligand is required. Yet, most H₃R ligands are active at the H₄R as well. For example, the classical selective H₃R agonist, (*R*)- α -methylhistamine shows H₄R-agonistic activity, and the H₃R-antagonist prototype thioperamide has moderate affinities for

H₄R. No ligands have been reported that can selectively target H₄R.

In the study described above, we synthesized the respective four stereoisomers of the THFs (Fig. 16) and 2'- and 5'-dihydrofurans (DHF_s)¹⁰⁸ containing imidazole. Furthermore, a series of 16 compounds related to the chiral THFs were synthesized and examined *in vivo* by radioligand displacement studies and functional assays for H₃R_s and H₄R_s expressed in SK-N-MC cells.¹¹⁰ Among them, the (2*S*,5*S*)-isomer (–)-**157** of amino compounds showed approximately 300-fold higher selectivity at the H₃R than at the H₄R, as indicated in Fig. 21.

Fig. 22. Histamine Release of OUP-133 and OUP-153

Fig. 23. Development of Novel Non-imidazole H₃R Antagonists

On the other hand, (2*R*,5*S*)- and (2*R*,5*R*)-cyanoguanidine derivatives OUP-13 and OUP-16 bound to the H₄R with a pEC₅₀ value of 6.7 and 7.1, respectively, and exhibited full agonistic activities (α = 1.0) at the H₄R with 46- and 41-fold higher potency than at the H₃R, respectively. As such, OUP-13 and OUP-16 are the first selective H₄R agonists.¹¹⁰

In relation to the development of selective H₄R ligands, improved synthesis of imifuramine and its derivatives was achieved *via* cyclization of a diazafulvene intermediate generated by Bu₃P/TMAD treatment of a diol intermediate.⁸²

7.3. Development of Novel H₃R Antagonists OUP-133 and 153 The (2*R*,5*S*)-*trans*- and (2*S*,5*S*)-*cis*-stereoisomers **174a** and **174b** of 4(5)-(5-aminotetrahydropyanyl-2-yl)-imidazole, which have two chiral centers and adopt a stable chair conformation, were efficiently synthesized *via* cyclization of diol intermediate **170** using L-glutamine as starting material, as shown in Chart 41.¹¹¹ Their enantiomers, (2*S*,5*R*)-*trans*- and (2*R*,5*R*)-*cis*-isomers were synthesized by the same methodology from D-glutamine.¹¹¹ Their stereoisomers were converted into cyanoguanidines, and into *N*-isopropyl and *N*-3,3-dimethylbutyl derivatives, respectively.¹¹¹

The results of *in vivo* brain microdialysis of the derivatives apparently indicated that (2*S*,5*R*)-isomers increased release of neuronal histamine. Among the many (2*S*,5*R*)-*N*-alkyl derivatives, OUP-133 and OUP-153 increased histamine release to 180–190% and 180–200% of basal levels, respectively, and were novel histamine H₃ antagonists (Fig. 22).

7.4. Design and Synthesis of Novel Nonimidazole H₃R Antagonist OUP-186¹⁰⁰ Early imidazole-based H₃R antagonists are widely used in pharmacology as potent prototype H₃R antagonists such as thioperamide and clobenpropit.¹⁰² However, imidazole-derived ligands have an inhibitory effect on CYP enzymes, caused by complexation of the imidazole nitrogen atom to the iron center at the active site of the enzyme, thus leading to drug-drug interactions¹¹⁴ (Fig. 23). Moreover, because imidazole is a strong hydrogen-bond donor and acceptor, some ligands exhibit poor oral bioavailability

Chart 42. Efficient Synthesis of OUP-186

and difficulty in penetrating the blood brain barrier for entry into the central nervous system¹¹⁵⁾ (Fig. 23).

Recently, a number of new H₃R antagonists have been reported and are currently being evaluated in clinical trials.¹¹⁶⁾ These non-imidazole-based antagonists contain a range of different cyclic amines, such as piperidine, homopiperidine, and pyrrolidine. Therefore, the development of potent non-imidazole-based H₃R antagonists has become attractive in the search for potential drug candidates.¹¹⁷⁾

Meanwhile, an extremely potent H₃R agonist, clobenpropit is composed of an imidazole ring, a propylspacer and

an isothioureas central core that is connected to lipophilic 4-chlorobenzyl group, as indicated in Fig. 23. We designed novel non-imidazole-based H₃R antagonists by modification of clobenpropit, substituting the imidazole with typical secondary cyclic amines: pyrrolidine, morpholine, piperidine, methylpiperidine, and piperazine, and containing the different methylene-spacers ($n = 1-3$)^{112,113,117)} (Fig. 23).

As exemplified in Chart 42, a synthetic method of isothioureas was developed in this investigation. A H₃R antagonist target, *N*-[(4-chlorophenyl)butyl]-*S*-[3-(piperidin-1-yl)propyl]-isothiourea **178** (OUP-186), was synthesized from the starting amine **175** using 2-nitrophenylacetyl isothiocyanate (NPAI) which is a newly designed intercalating agent of SCN atoms.^{113,117)} Reaction of **175** with NPAI afforded thiourea **176** (92%). This thiourea **176** was subsequently converted into *S*-alkylisothiourea **177** (93%) *via* Mitsunobu *S*-alkylation.^{113,117)} Selective elimination of the acetyl moiety of 2-nitrophenylacetyl-protected isothioureas was achieved by catalytic reduction (10% palladium carbon (Pd-C)) of **177** under hydrogen atmosphere.¹¹³⁾ The final step proceeded through preferential cleavage of N-CO bond over cleavage of a fissile C-S bond, removing 1-hydroxy-2-oxindole from a hydroxylamine intermediate **179**, as shown in Chart 42.^{113,117)} The overall yield of **178** (OUP-186) was therefore 74% over three steps from amine

Fig. 24. Comparison of OUP-186 and OUP-181

Fig. 25. A and B: Predicted Molecular Structures of Antagonists in hH_3R and rH_3R TM Models

Molecular structures of **178** (OUP-186)- hH_3R and **180** (OUP-181)- rH_3R complex models are shown in A and B, respectively. C: Schematic diagram of predicted molecular structures of antagonists OUP-186 and OUP-181 in hH_3R and rH_3R TM models. (Color figure can be accessed in the online version.)

Fig. 26. Effects of H₃R and H₄R Ligands on Proliferation of Breast Cancer Cells

MDA-MB-231 and MCF7 cells were treated with various concentrations of H₃R and H₄R ligands for 48 h. Cell proliferation was determined by WST-1 assay. Results expressed as mean $\pm$ S.D. ($n = 3$).

175.¹¹³⁾

7.5. OUP186: High Affinity and Human/Rat Species-Selective Histamine H₃R Antagonist¹¹²⁾ The synthetic method provided many analogs, in which **178** (OUP-186) showed a potent and selective H₃R antagonistic activity ($pA_2 = 9.6$, $pIC_{50} = 8.2$) against *in vitro* hH₃R, while being inactive against hH₄R (Fig. 24). However, surprisingly, *In vivo* rat brain microdialysis was completely inactive. This result suggested that **178** was inactive as H₃R antagonist in rats, but potent in humans, suggesting differences in antagonist affinities between species. On the other hand, homologue **180** (OUP-181, $n = 2$), which was tethered to the chlorophenyl group by a C₂ chain, gave hH₃R antagonistic activity ($pA_2 = 7.4$, $pIC_{50} = 8.1$), whereas *in vivo* rat brain microdialysis increased the histamine release by 140–150% of basal release (Fig. 24).

This discrepancy of **178** between *in vitro* and *in vivo* studies prompted us to investigate the structural differences in the ligand-binding cavities of hH₃R and rat H₃R (rH₃R) using molecular modeling.¹¹²⁾ Among the amino acids that showed variation between the two species, only Thr119/Ala119 and Ala122/Val122 were found situated in the inner site of transmembrane (TM) region. *In silico* docking studies of **178** and the C₂-shorted homolog **180** with h/rH₃Rs were carried out.¹¹²⁾ As shown in Fig. 25, three hydrophilic interactions were observed in the **178**-hH₃R model (Fig. 25A). Similar stable interaction was observed in **180**-rH₃R model, as shown in Fig. 25B. Because **180** has a more compact structure than **178**, it could bind to the shorter cavity of rH₃R, filling up by the side-chain of Val122. We next attempted to construct the complex of **178** with rH₃R, but failed to produce a stable model, considering the theoretical potential energy of the whole molecular system, because of the van der Waals repulsion between chlorophenylbutyl group of **178** and Val122 side-chain.¹⁰⁰⁾ From these findings, it revealed that this species-selectivity of **178** is caused by an Ala122/Val122 mutation between the antagonist-docking cavities in hH₃Rs and rH₃Rs. In view of the observation that drug development for many human diseases relies heavily on rodent-based models, the discovery that **178** (OUP-186) behaves so differently in the two species is of

great significance.

7.6. Human Breast Cancer Cell Proliferation Inhibition of OUP-186¹¹⁸⁾ H₃R is expressed in a variety of cancers, suggesting a possible role for histamine in tumorigenesis. H₃R expression in breast carcinomas correlates with increases in levels of proliferating cell nuclear antigen and malignancy. Activation of H₃R by its agonist induces proliferation of breast carcinoma MDA-MB-231 cells.¹¹⁹⁾ As described above, these data suggest that H₃R antagonists may be effective therapeutic agents against breast cancer. OUP-186 exhibited potent and selective H₃R antagonistic activity as well as no activity against the hH₄R. Therefore, we compared the effects of OUP-186 on proliferation of estrogen receptor negative (ER-) breast cancer cells (MDA-MB-231) and ER+ breast cancer cells (MCF7) *versus* clobenpropit.¹¹⁸⁾ OUP-186 and clobenpropit suppressed the proliferation of breast cancer cells, as shown in Fig. 26. The IC₅₀ values at 48 h for OUP-186 and clobenpropit were approximately 10 μ M and 50 μ M, respectively. Furthermore, OUP-186 potently induced cell death by activating caspase-3/7.¹¹⁸⁾ These results indicate that OUP-186 potently suppresses proliferation and induces caspase-dependent apoptotic death in ER+ and ER- breast cancer cells.

Conclusion

1) CPs having a phosphate moiety are useful and versatile intermediates for introduction of a C₁ unit in synthesis of medicines and natural products. Alternatively, the generation of alkylidene carbenes from CPs under neutral condition was developed and its usefulness in organic synthesis was demonstrated.

2) A highly stereoselective synthesis of *Z*- or *E*-double bonds in 10-membered thiolcarbonates was achieved by [3,3]-sigmatropic rearrangement of 8-membered thionocarbonates which proceeds through the chair-like *versus* boat-like transition states. The utility of this methodology was demonstrated by unique and stereoselective synthesis of (–)-yellow scale pheromone.

3) β -Stereoselective synthesis of C4-linked imidazole C-nucleosides *via* diazafulvene intermediates was developed. Furthermore, imidazole C₀- or C₂-ribonucleoside PAs with a

combination of POM and CE groups were synthesized and thereby incorporated the imidazole nucleosides into the specific position of VS and hairpin ribozymes, successfully probing their catalytic mechanism.

4) Tetrahydrofuranylimidazoles using PhSe group were efficiently synthesized and applied to new hH₃R agonist, imifuramine, and the first selective hH₄R agonist, OUP-16. Furthermore, S-Alkyl-N-alkylisothiourea compounds were synthesized using NPAI to discover novel non-imidazole H₃R antagonists. Among the synthesized compounds, OUP-186 exhibited potent and selective antagonism against hH₃R but not hH₄R, *in vitro*. Of particular interest, it did not show antagonism for histamine release in rat brain microdialysis *in vivo*, suggesting species-selective differences in antagonist affinities. Furthermore, *in silico* docking studies of OUP-186 and its C₂-homolog (OUP-181) in human/rat H₃Rs indicated that the structural difference of antagonist-docking sites between human and rat H₃Rs was attributable to Ala122/Val122 mutation. In addition, OUP-186 exhibited a potent suppressive activity against proliferation of human breast cancer cells.

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