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CONTRIBUTORS TO THIS ISSUE (本特集号の寄稿者)

Shiroh Futaki
二木 史朗, Kyoto University

Azusa Kondoh and Masahiro Terada*
近藤 梓, 寺田 眞浩, Tohoku University

Subrata Mukherjee, Shintaro Kawamura,* and Mikiko Sodeoka*
スブラタ ムカジー, 河村 伸太郎, 袖岡 幹子, RIKEN

The Japanese phoenix

Since the 1950s, world-class Japanese chemists inside and outside of Japan have contributed immensely to the advancement of the science. What chemist worth the name is not familiar with the Suzuki–Miyaura, the Negishi, or the Sonogashira cross-coupling, to name just a few contributions? Likewise, Japanese chemical institutions; academic, industrial, and governmental; have played a crucial role in propelling Japanese science to its current preeminent position.

At Merck, we applaud the giant strides and contributions that Japanese science has made in all areas of research, in particular life science and chemistry research. This research has resulted in discoveries and products that have greatly benefited all of mankind. We look forward to continuing and growing our strong collaborations with Japanese scientists to make their inventions more accessible for the purpose of improving human health worldwide.

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Head of Life Science Chemistry

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General Correspondence
Editor: Sharbil J. Firsan, Ph.D.
Sharbil.Firsan@milliporesigma.com

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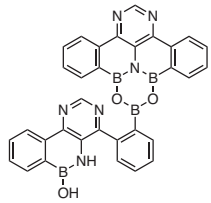

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ABOUT OUR COVER

What immediately comes to mind when you see a row of cherry trees in bloom? For us it is Japan! ***Cherry Blossom Viewing*** (ink and color on silk panel, 37.6 x 64.8 cm) is attributed to Katsushika Hokusai* (葛飾北斎,1760–1849). He was a renowned, prodigious, and much sought-after artist in Japan. Hokusai initially trained in woodblock prints of actors and courtesans. He later shifted his interest to depictions of landscapes, plants, animals, and the daily lives of the Japanese. Possibly the most famous of his landscapes are *Under the Great Wave Off Kanagawa* and his numerous depictions of Mount Fuji. Hokusai did not achieve international fame and influence until after his death when Japan became more accessible to the rest of the world.

Detail from ***Cherry Blossom Viewing***. Photo courtesy The Freer Gallery of Art, Washington, DC.

Another type of year-round bloom in Japan is scientific research, in particular chemistry research. Is it any wonder that in the past two decades over half a dozen Japanese researchers working at home and abroad have been awarded the Nobel Prize in Chemistry? This issue celebrates the achievements of Japanese chemistry research by showcasing vignettes from prestigious research groups at three renowned Japanese scientific institutions.

Katsushika Hokusai / Freer Gallery of Art, Smithsonian Institution, Washington, DC: Gift of Charles Lang Freer, F1902.2.

* *Interestingly, the artist Hokusai was known by many other names. To find out more, visit* ***SigmaAldrich.com/acta***

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Peptide-Mediated Strategies for Intracellular Protein Delivery

ペプチドを利用した細胞内へのタンパク質送達戦略

Prof. S. Futaki

Shiroh Futaki†
二木 史朗

Institute for Chemical Research
Kyoto University
Uji, Kyoto 611-0011, Japan

Email: futaki@scl.kyoto-u.ac.jp

Keywords. cell-penetrating peptide; arginine; cell membrane; intracellular delivery; endocytosis; macropinocytosis; membrane lysis; endosomal escape; attenuated cationic amphiphilic lytic (ACAL) peptide; antibody.

キーワード. 細胞透過ペプチド; アルギニン; 細胞膜; 細胞内送達; エンドサイトーシス; マクロピノサイトーシス; 膜溶解; エンドソーム脱出; 弱毒型カチオン性両親媒性膜溶解ペプチド (ACALペプチド); 抗体.

Abstract. Establishing methodologies that allow protein delivery into cells has an impact both on the design of membrane-interacting molecular systems and on their practical applications to modulate cellular functions. Here we introduce our approaches for intracellular delivery using peptides that have unique modes of membrane interaction and perturbation.

細胞内へのタンパク質導入のための方法論の確立は、膜と相互作用する分子システムの設計ならびにこれらの細胞機能調節への応用の双方の観点から大きな意味を持ちます。ここでは、ユニークな膜相互作用様式と膜構造破壊能を有するプチドを用いた私達の細胞内送達の試みを紹介します.

Outline

1. Introduction
2. Arginine-Rich, Cell-Penetrating Peptides
3. Attenuated Cationic Amphiphilic Lytic (ACAL) Peptides for Intracellular Protein Delivery
4. Combination Approach of Macropinocytosis-Inducing Peptides and Endosomolytic Peptides
5. Conclusion and Outlook
6. References

1. Introduction

The cell membrane (plasma membrane) acts as a barrier to separate the interior from the exterior of cells. Thus, cellular components needed for cellular activity such as proteins and peptides as well as nucleic acids are retained inside cells without leaking out to the outside. On the other hand, there is a strong demand for delivering such biologically relevant molecules into cells because of their potential therapeutic effects. The approaches that enable intracellular delivery of such molecules are also beneficial for basic studies on the elucidation and modulation of cellular functions. Mechanical means to deliver such molecules into cells, such as microinjection, electroporation, and glass-bead transfection[1] have been employed for these purposes. However, such means are often accompanied by severe damage to cells, or suffer from inefficiency in handling. Therefore, more efficient and safer approaches, preferably ones that can be extended to therapeutic applications, are needed. Numerous approaches based on polymers and lipid nanoparticles have been reported.[2-4] These approaches often yield satisfactory results at least at the cellular level for gene and nucleic acid delivery. However, in terms of protein and peptide delivery, the incorporation of these biomolecules into such particles is generally not easy.

As a new means to achieve facile intracellular delivery, an approach to employ peptides having membrane permeability (cell-penetrating peptides or CPPs) has been introduced.[5-8] Examples of such intracellular delivery include formation of conjugates or stable complexes of CPPs with the proteins of interest to obtain the desired activities of the proteins (**Figure 1**, Part (a)).[9] This approach is not limited to protein delivery, but has been extended to delivery of various peptides,

nanoparticles, and nucleic acids. Our group is interested in understanding the internalization processes of CPPs, especially those rich in arginine (arginine-rich peptides),[2,9] and creating more efficient delivery peptides based on this understanding. Complementary approaches have been developed for delivering relatively large proteins such as antibodies (i.e., immunoglobulin G, IgG) with the assistance of endosomolytic peptides (Figure 1, Part (b)).[10–12] These and related topics are reviewed and discussed in this article.

2. Arginine-Rich, Cell-Penetrating Peptides

The origin of CPPs goes back to the finding of cellular internalization of the Tat protein of the human immunodeficiency virus type 1 (HIV-1).[13,14] The Tat protein is involved in the transcription regulation of the virus. Since the protein is endogenous, a control experiment to examine the effect of externally added Tat protein on transcription was carried out by Frankel and Pabo.[13] Although no effect was originally expected, activation of transcription was observed, which suggested the internalization of externally added Tat protein. A similar effect was also observed by Green and Loewenstein.[14] It bears mentioning that the Tat protein does not play any role in HIV-1 infection of host cells. A later study by Fawell et al. demonstrated that delivery of proteins into cells was feasible through chemical conjugation of the partial sequences of the Tat protein with exogenous proteins.[15] Lebleu and co-workers demonstrated that the RNA-binding segment of the Tat protein corresponding to positions 48–60 (GRKKRRQRRRPPQ, designated as Tat peptide in this article) plays a crucial role in the delivery of the protein to the cell.[16] As is often observed with DNA- and RNA-binding segments, the Tat sequence is rich in basic amino acids (i.e., arginine and lysine) and is hydrophilic. In spite of this fact, there have been many reports that demonstrated the practicality of using Tat for intracellular peptide and protein delivery to yield the expected activities.[6,17] This raised the question of why such a segment, possessing basic and hydrophilic properties, can penetrate through the hydrophobic lipid bilayer core.

Through the synthesis of the D-form of Tat, oligoarginine, and branched-chain oligoarginines, our group demonstrated the importance of clusters of arginine or guanidino functions for membrane translocation.[18] A comparison of the translocation ability of different lengths of oligoarginine identified marked translocation abilities of the 6- to 12-mers of arginine. Similar findings were independently reported by Wender's research group at Stanford University.[19,20] Comparing the cellular uptake efficacy among unmodified mono-methylated and di-methylated arginine peptides, the Stanford group clearly established that the guanidino structure—which can potentially form two hydrogen bonds with phosphate, carboxylate, sulfate, and other functional groups—is more important than basicity (**Figure 2**).[21] The same group suggested that the membrane potential (difference in voltage on both sides of the membrane) also plays an important role in the transport across the membrane.[21] Several peptides having different physicochemical characteristics but sharing membrane permeability have also been reported. Examples of such peptides are penetratin[7] and transportan.[22] The former peptide is derived from the DNA-binding region of the antennapedia homeobox protein and has a potentially amphiphilic structure.[7] The latter peptide is a chimera of the

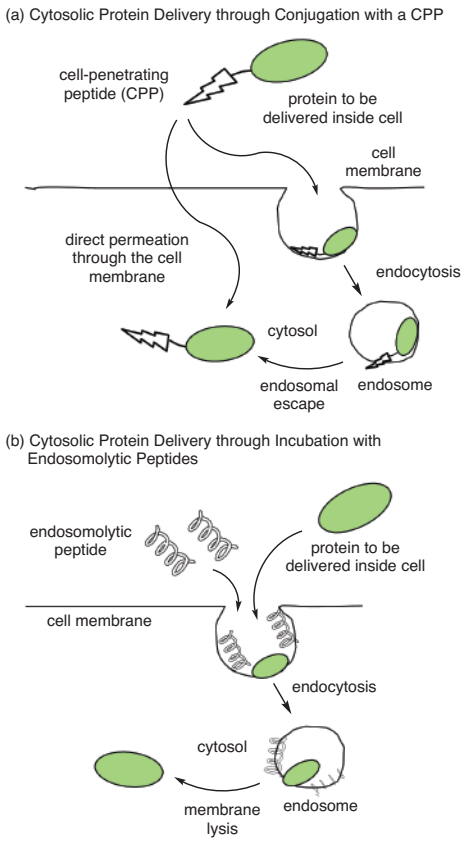

Figure 1. Two Representative Approaches of Cytosolic Protein Delivery. (a) By Conjugation with a Cell-Penetrating Peptide (CPP): (i) Protein May Permeate through the Cell Membrane, or (ii) Is Taken Up by the Cell via Endocytosis, Followed by Endosomal Escape to Reach the Cytosol. (b) Cytosolic Protein Delivery Is Also Possible by Incubating the Protein with Endosomolytic Peptides. *(Ref. 9,10)*

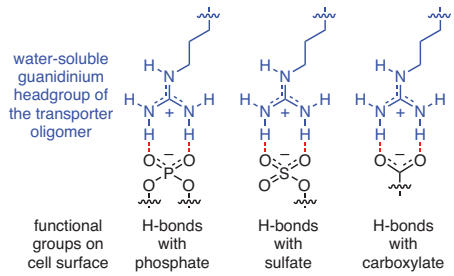

Figure 2. Possible Divalent Hydrogen Bond Formation by the Side-Chain Guanidino Moiety of Arginine with Phosphate, Sulfate, and Carboxylate Functional Groups in Cell-Membrane-Associated Molecules. *(Ref. 21)*

neuropeptide "galanin" and a bee venom "mastoparan", having a primary amphipathic structure.[22] On the other hand, arginine-rich peptides do not include a notable hydrophobic part in their sequence.[23] Since each peptide has membrane permeability and is capable of intracellular delivery by conjugation or by complex formation with cargo molecules, it would be reasonable to consider different methods for internalization.

Later studies demonstrated that, depending on administration conditions, arginine-rich peptides and their conjugates with cargos can penetrate a cell membrane (plasma membrane) directly, especially when cargos are peptides or small molecules and when a high concentration of peptides accumulates on cell surfaces.[24,25] The addition of appropriate hydrophobic moieties (e.g., a hexanoyl group or a tetra(phenylalanine) sequence) to arginine-rich peptides enhances their direct-penetration ability.[26–28] It is worth noting that an excessive increase in hydrophobicity may increase membrane perturbation ability and make the peptides rather toxic. The increase in hydrophobicity may also increase the serum binding of the peptides, and thus reduce the effective peptide concentration on cell surfaces leading to reduced permeation efficacy.

Lebleu's study of the Tat peptide indicated that the internalization process is not inhibited at 4 °C,[16] and that endocytosis—cellular uptake machinery of extracellular materials and solutes into cells using intracellular vesicular transport—is not involved. However, later studies demonstrated that this was due to an artifact in microscopic observation and that Tat and the conjugates are also taken up by the cells via endocytosis.[29] We and others have reported the involvement of micropinocytosis in the cellular uptake of arginine-rich peptides including Tat.[30,31] Macropinocytosis is actin-driven fluid-phase endocytosis, and interaction of arginine-rich peptides with the cell surface activates this form of endocytosis (**Figure 3**).[32] The interaction of arginine-rich peptides with membrane-associated proteoglycans (membrane proteins decorated with sulfated polysaccharides) is critical for the activation.[30,33] The extracellular materials are delivered into cells while encapsulated in vesicular compartments (i.e., endosomes). Endocytosed materials are usually delivered into lysosomes to be digested. Therefore, they must escape from endosomes to exert the expected activity. Endocytosed arginine-rich peptides and their conjugates escape from endosomes, but it is thought that only a small proportion of endocytosed arginine-rich peptides and the conjugates can go into the cytosol.[34] Although a detailed understanding of the methods of endosomal escape should contribute to the creation of more efficient delivery systems, little is known about the actual mechanism. In this context, a potential role has been proposed for bis(monoacylglycero)-phosphate (BMP), a negatively charged phospholipid specifically localized in endosomal membranes, in the escape of CPPs into the cytosol.[35]

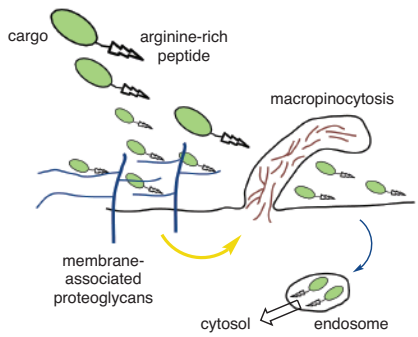

Figure 3. The Interaction of Arginine-Rich CPPs with Membrane-Associated Proteoglycans Displayed on Cell Surfaces Leads to Macropinocytosis, Which Involves Actin Reorganization, Membrane Ruffling, and Fluid-Phase Massive Uptake of Extracellular Liquid and Solutes. Leakage through the Endosomal Membranes Achieves Cytosolic Translocation. *(Ref. 32)*

3. Attenuated Cationic Amphiphilic Lytic (ACAL) Peptides for Intracellular Protein Delivery

Although arginine-rich peptides are a useful tool for delivering extracellular materials into cells, the efficacy of endosomal escape may not be very high especially when the peptide is conjugated with large proteins (e.g., >30 kDa) or other macromolecules. As described in the preceding section, arginine-rich peptides do not have marked hydrophobic domains. On the other hand, cationic amphiphilic peptides often exhibit membrane lytic activity, and the interaction of the membrane with the hydrophobic domain of the cationic peptides is important for the activity (**Figure 4**, Part (a)).[36] If one were to suppress the hydrophobic interaction of the lytic peptides on cell surfaces but allow it inside endosomes, effective perturbation of the endosomal membrane could be achieved. It is known that the interior of endosomes has a reduced pH (~5) compared with that (neutral) on the outside of cells.[37] Aiming at utilizing this pH difference as a switch of the lytic activity, many pH-responsive peptides and polymers have been reported.[38] However, in our view, the resultant lytic activities in endosomes may not be high enough. We thus considered using instead peptides of high lytic activity by replacing the hydrophobic amino acids on the potential hydrophobic face of the amphiphilic lytic peptide with glutamic acid (negatively charged amino acid bearing a carboxy moiety), which would reduce the membrane lytic activity on cell surface (Figure 4, Part (b)). In this case, since the cell surface is negatively charged by the presence of proteoglycans or sialic acid, if we could keep the net positive charges of the peptide, the peptide would effectively adsorb on the cell surface and would then be endocytosed. Under the reduced pH in endosomes, the carboxy group of the glutamic acid may become more protonated, and the lytic activity of the peptide is then recovered. This may lead to rupture of the endosomal membrane, stimulating the endosomal escape of the molecules of interest (cargo molecules).

To ascertain the validity of the above idea, we selected the Carolina wolf spider derived amphiphilic peptide, M-lycotoxin (M-LCTX),[39] as the template. We then substituted a series of amino acids on the potential hydrophobic face of the peptide with glutamic acid, and compared the cytotoxicity of the

resulting lytic peptides. We found a mutant L17E (leucine at position 17 of M-lycotoxin was replaced with glutamic acid: IWLTALKFLGKHAAKHEAKQQLSKL-amide) to have the desired activity.[10] By placing glutamic acid at position 17, the

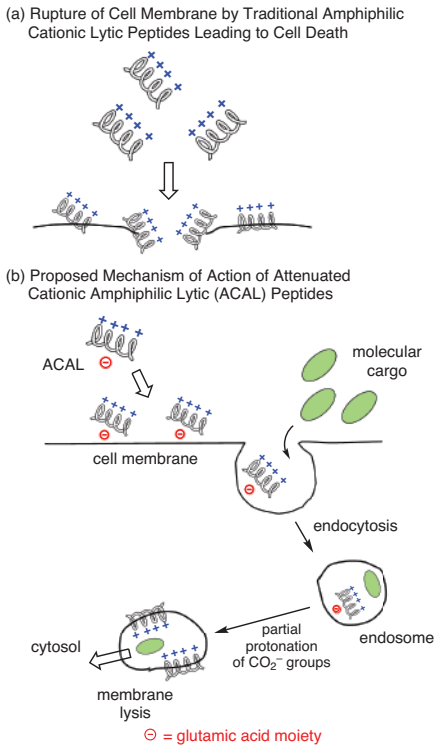

Figure 4. (a) Rupture of Cell Membrane by Amphiphilic Cationic Lytic Peptides, Leading to Cell Death. (b) Design Concept of Attenuated Cationic Amphiphilic Lytic (ACAL) Peptides. By Placing a Negatively Charged Amino Acid (e.g., Glu) in Cationic Amphiphilic Lytic Peptides, the Lytic Activity toward the Cell Membrane Is Greatly Suppressed and the Peptides Are Effectively Adsorbed on the Cell Surface, Leading to Endocytic Uptake of the Peptides and Cargo Molecules. The Reduced pH in Endosomes Should Reduce the Negative Charges of ACAL at Low pH, Resulting in the Recovery of the Lytic Activity of ACAL and Cytosolic Delivery of the Cargo. *(Ref. 10)*

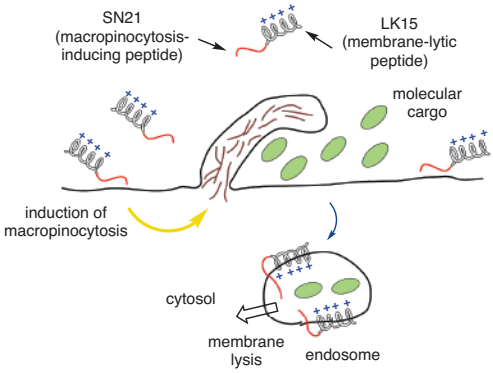

Figure 5. Combination of a Macropinocytosis-Inducing Peptide (SN21, Stimulating Cellular Uptake) and a Membrane-Lytic Peptide (LK15, Rupturing Endosomal Membrane) for Cytosolic Delivery of Bioactive Cargoes. *(Ref. 12)*

EC_{50} (concentration that yields 50% cell death) of L17E was reduced to >40 μM compared to that of M-lycotoxin (1.36 μM). On the other hand, treatment of cells with the model macromolecules polydextran (10 kDa) and immunoglobulin G (IgG, ~150 kDa) in the presence of L17E yielded apparent cytosolic appearance of these molecules in 50% of cells.[10] A later study demonstrated that the cytosolic appearance was accomplished in 5 min, suggesting cytosolic translocation at the very early stages of endocytosis.[40] A design modification that was conducted to increase hydrophobicity and enhance the helical structure at low pH yielded an improved peptide HAad [IWLTALKFLGKAAAKAXAKQXLSKL-amide; X = L-2-aminoadipic acid (Aad)], and this achieved a cytosolic appearance of IgG in 75% of cells, ~25% higher than that obtained by L17E.[11] Using L17E and HAad, successful cytosolic protein delivery was attained, which was confirmed by protein activity exerted in the cell. It is worth mentioning that, at the current stage, proteins and delivery peptides are administrated to cells simply by mixing them together; however, conjugation or complex formation should be considered in order to extend this approach further.

4. Combination Approach of Macropinocytosis-Inducing Peptides and Endosomolytic Peptides

To achieve intracellular delivery via endocytosis, promoting endosomal escape is of course important; however, as a prerequisite, the molecules of interest have to be taken up into the endosomes. To recruit molecules of interest in endosomes, we thought that massive-uptake methods of macropinocytosis may be preferable. Our laboratory had previously identified CXCR4 as a receptor that induces macropinocytosis.[41] Stromal cell-derived factor 1α (SDF-1α) is a natural ligand of CXCR4. We thus prepared the peptides corresponding to the N-terminal sequence of SDF-1α and found that the peptide corresponding to the N-terminal 21 residues (SN21: KPVSLSYRCPCRFFESHVARA-amide) induces macropinocytosis.[12] It is known that Tat and R8 have the ability to induce macropinocytosis, but, in terms of stimulation effect of 70 kDa polydextran uptake, SN21 has a higher macropinocytosis induction ability than that of Tat and R8. LK15 is a cationic membrane-lytic peptide comprised of only leucine and lysine (KLLKLLLKLLLKLLK-amide). A tandem peptide bearing sequences of SN21 and LK15 was prepared (KPVSLSYRCPCRFFESHVARA-GG-KLLKLLLKLLLKLLK-amide) and found to have a marked ability to deliver bioactive proteins and IgGs into cells (**Figure 5**).[12,42] The needed amounts of the proteins are much smaller than those that are needed for the delivery of L17E and HAad. Moreover, SN21–LK15 was able to deliver plasmid and siRNA to an extent comparable to that of lipofectamine, a commercially available, efficient transfection agent. Therefore, the validity of this approach was confirmed. Although similar combinations of macropinocytosis-inducing peptides and endosomolytic peptides have been reported,[31,43] SN21–LK15 has a higher ability than these peptides presumably because of the more potent macropinocytosis induction and membrane-lytic abilities of SN21 and LK15, respectively.

5. Conclusion and Outlook

We introduced in this short review some of our strategies for intracellular delivery of biomacromolecules, in particular bioactive peptides and proteins. With respect to direct penetration through cell membranes, we have proposed the possible transient membrane permeabilization or lipid packing loosening induced by peptide–membrane interaction. This permeabilization may take place either by a physicochemical or physiological process of membrane structural alteration. As is seen in the internalization of arginine-rich peptides, cellular internalization via direct cell-membrane penetration and endocytic uptake followed by endosomal escape can be conducted simultaneously, although one aspect may become more apparent depending on conditions. One possible explanation for the factors that make each pathway more dominant is the kinetics of membrane penetration. If the molecules of interest have relatively small molecular sizes and appropriate physicochemical properties, they tend to go through membranes. However, if they lack high efficacy in permeation, they are eventually trapped in endosomes and endocytosed. Based on these insights, the design of more suitable systems for intracellular delivery should be of great interest.

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(†) The author declares no conflict of interest.
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About the Author

Shiroh Futaki obtained his Ph.D. degree in 1989 from Kyoto University, Japan. Following his appointment as Research Associate and Associate Professor at the University of Tokushima, he moved to Kyoto University in 1997. Meanwhile, he spent 16 months (1989–1991) in the U.S. as Postdoctoral Associate in the Department of Biochemistry, Rockefeller University. He has been Professor of Biochemistry at the Institute of Chemical Research, Kyoto University, since 2005. His research interests include the design of bioactive peptides that possess unique functions (cell penetration, membrane structural organization, and so on). Shiroh has been honored with, among others, The Japanese Peptide Society Award for Young Scientists (1997), The Pharmaceutical Society of Japan Award for Young Scientists (1998), and The Pharmaceutical Society of Japan (PSJ) Award (2020). He was appointed Invited Professor at Pierre and Marie Curie University in 2010 and has been Honorary Member of the Hungarian Academy of Sciences since 2019.

Chiral Organosuperbase Catalysts as Useful Tools for Developing Enantioselective Reactions

キラル超強塩基性有機触媒を用いた不斉反応の開発


Prof. A. Kondoh


Prof. M. Terada

Azusa Kondoh[a] and Masahiro Terada[*,b]
近藤 梓, 寺田 眞浩

[a] Research and Analytical Center for Giant Molecules
Graduate School of Science
Tohoku University
Aoba-ku, Sendai 980-8578, Japan

[b] Department of Chemistry
Graduate School of Science
Tohoku University
Aoba-ku, Sendai 980-8578, Japan

Email: mterada@tohoku.ac.jp

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Abstract. In the field of chiral Brønsted base catalysis, a long-standing challenge has been the expansion of the scope of pronucleophiles that can be applied in enantioselective reactions. In this review, we summarize our recent efforts to overcome this challenge and expand the scope of viable enantioselective transformations that are available under this type of catalysis. We accomplished this by developing two types of chiral organosuperbase catalyst and applying them in enantioselective reactions.

Outline

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3. Applications of Chiral Bis(guanidino)iminophosphorane Catalysts
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4. Development of Chiral Cooperative Binary Base Catalysts
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1. Introduction

Brønsted base catalysis is a fundamental and reliable methodology in the field of synthetic chemistry. Over the past decades, the development of enantioselective reactions by using chiral uncharged organobase catalysts has attracted considerable attention because the methodology enables the direct transformation of pronucleophiles into enantio-enriched compounds in a highly atom-economical fashion under mild reaction conditions.[1] Traditionally, chiral tertiary amines have been employed as the chiral organobase catalyst. Cinchona alkaloid based catalysts and bifunctional catalysts consisting of tertiary amines and hydrogen-bond donors, such as (thio)ureas and squaramides, are widely used, and a large number of enantioselective reactions have been developed so far.[2] However, diversity of the applied pronucleophiles is lacking: the scope of the pronucleophiles applicable to the reactions is highly dependent on the basicity of the catalyst molecules, and the basicity of tertiary amines is considerably low. Recently, chiral uncharged organobases—such as chiral guanidines,[3] P1-phosphazenes,[4] iminophosphoranes,[5] and cyclopropenimines[6]—possessing higher basicities than those of tertiary amines have emerged as efficient chiral Brønsted base catalysts, and considerable progress has been achieved in the development of a variety of enantioselective reactions by

employing these catalysts.[7] However, even with these "strong" organobase catalysts, the applicable pronucleophiles are still limited to compounds that have a highly acidic proton, such as 1,3-dicarbonyl compounds and nitroalkanes. In order to expand the scope of viable enantioselective transformations that are available under Brønsted base catalysis, we have focused on the development of chiral uncharged organosuperbase catalysts that possess much higher basicities than those of the aforementioned conventional chiral catalysts (**Figure 1**).[3–8] In the rest of this article, we present our recent studies on the development of two types of chiral organosuperbase catalyst and their applications to enantioselective reactions.

2. Development of Chiral Bis(guanidino)-iminophosphorane Catalysts

For the purpose of developing chiral organobase catalysts possessing exceptionally high basicity, we envisioned utilizing a higher order phosphazene as a new motif of the chiral catalyst. Phosphazenes are pentavalent phosphorus compounds that contain a P=N double bond and have a high basicity. Whereas the basicity of P1-phosphazenes—which have secondary amine groups attached to the iminophosphorane core—is similar to that of guanidines, replacing the secondary amine groups with phosphazene or guanidine subunit(s) enhances the basicity owing to better delocalization of the positive charge formed through protonation. Thus, the higher order phosphazenes would exhibit a much higher basicity than that of P1-phosphazenes and other conventional organobases (Figure 1).[8] We anticipated that the development of chiral organobases in which a higher order phosphazene is embedded as a core structure would open up a new avenue in asymmetric Brønsted base catalysis by exploiting their high basicity. Specifically, we designed and synthesized pseudo-C_2-symmetric bis(guanidino)-iminophosphoranes (*M*)-**1**, in which two guanidine subunits were introduced to the central iminophosphorane core, as a novel family of chiral organosuperbases (**Figure 2**).[9]

The characteristic feature of the newly designed catalysts (*M*)-**1** is underscored by their helical chirality that is based on the 7,7-memberd spirocyclic system along with their carbon center based chirality resulting from (1*S*,2*S*)-1,2-diphenyl-1,2-ethanediamine ((*S*,*S*)-DPEN). In this catalyst design, we arranged the hydrogen-bond donor and acceptor sites around the central phosphorus atom: the nitrogen atom of the iminophosphorane moiety (P=N) functions as the hydrogen-bond acceptor, while the N–H moiety attached to the iminophosphorane core functions as the hydrogen-bond donor. The side-by-side arrangement of the donor and acceptor sites has been proven to be a fundamental approach to designing efficient chiral organobase catalysts, such as chiral guanidines and P1-phosphazenes, to achieve high enantioselectivity.[3,4] Importantly, in this case, the conjugate acid of the catalyst generated through deprotonation of a pronucleophile can simultaneously interact with an anionic nucleophile (Nu) and an electrophile (X=Y) through hydrogen bonding by using two adjacent N–H moieties to form a cyclic transition state. To validate the catalytic performance of bis(guanidino)iminophosphoranes (*M*)-**1** as chiral organosuperbases, a series of (*M*)-**1** were tested in the electrophilic amination of 2-alkyltetralone (e.g., **2a**; as a less acidic pronucleophile) with azodicarboxylate **3**. (*M*)-**1a**, possessing methyl groups on the nitrogen of the guanidine moieties, efficiently catalyzed the reaction of **2a** with **3**, and

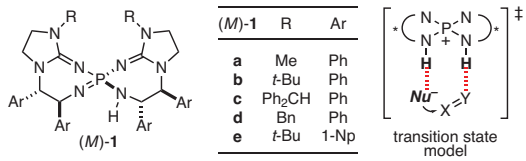


Figure 2. Chiral, Pseudo-C_2-Symmetric Bis(guanidino)iminophosphoranes as Novel, Brønsted Organosuperbase Catalysts. *(Ref. 9)*

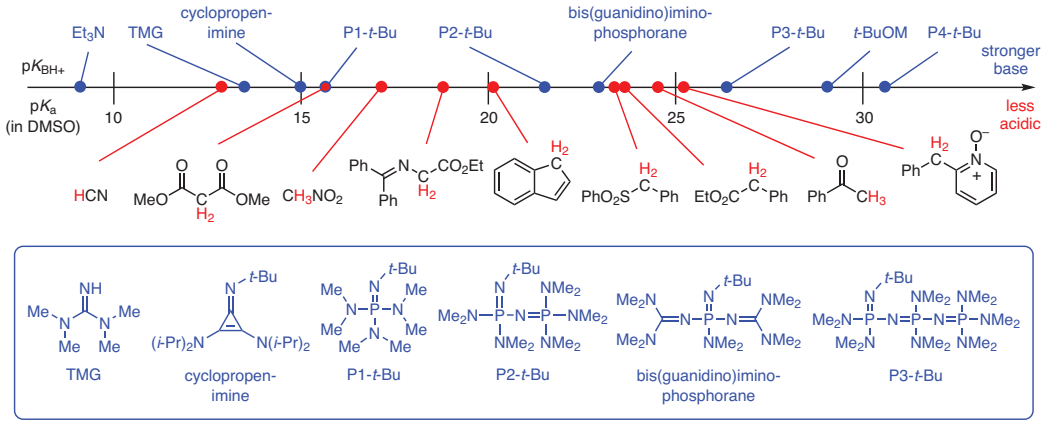


Figure 1. Relationship between Basicity of Uncharged Organobases and Acidity of Representative Pronucleophiles. *(Ref. 3–8)*

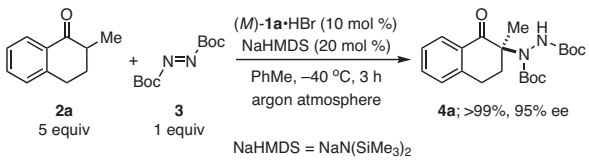
the corresponding adduct **4a** was obtained in high yield and with high enantioselectivity (**eq 1**).[9a] Thus, the higher-order phosphazene-based (*M*)-**1** were confirmed as a superb class of uncharged chiral organosuperbase catalysts that facilitate the enantioselective reactions of less acidic pronucleophiles.

3. Applications of Chiral Bis(guanidino)-iminophosphorane Catalysts

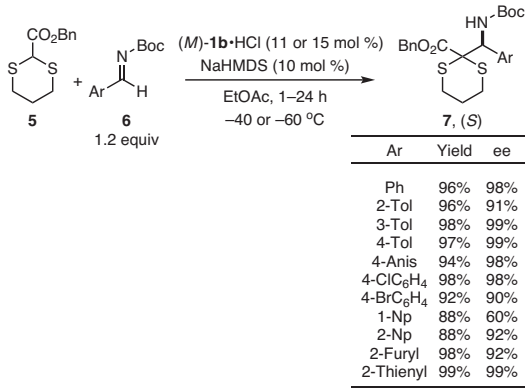
3.1. Enantioselective Addition of Less Acidic Pronucleophiles

In order to extend the utility of chiral bis(guanidino)-iminophosphoranes, particularly in synthetically useful transformations, we first focused our attention on 2-alkoxycarbonyl-1,3-dithianes as a less acidic pronucleophile class. The anion of 1,3-dithiane is a useful synthetic intermediate which can be regarded as an acyl anion equivalent.[10] Generally, a stoichiometric amount of a strong Brønsted base is employed for generating the dithiane anion prior to its reaction with electrophiles because of the low acidity of the hydrogen at C-2, even when it is α to an ester moiety.[11] In particular, the enantioselective addition reactions of the dithiane anion have not been reported.[12] Thus, we envisioned that the direct enantioselective addition of 2-alkoxycarbonyl-1,3-dithianes to imines by using our newly developed catalyst class would provide enantio-enriched α-amino-1,3-dithiane derivatives, which are known as valuable versatile building blocks. Currently, the synthesis of these compounds relies on methods involving asymmetric additions in which stoichiometric amounts of the achiral Brønsted base and a chiral auxiliary are required.[13] As a result of our investigation, chiral bis(guanidino) iminophosphorane (*M*)-**1b** (R = *t*-Bu, Ar = Ph) promoted the addition of 2-benzyloxycarbonyl-1,3-dithiane (**5**) to *N*-Boc imines **6** in a highly enantioselective manner (**eq 2**).[14] Imines with various functionalities on the benzene ring as well as heteroaromatic imines were applicable to the reaction, and the resulting adducts **7** were obtained in high yields and with high enantioselectivities.

Next, we envisioned utilizing chiral bis(guanidino)-iminophosphoranes to develop diastereo- and enantioselective addition reactions for the construction of vicinal quaternary stereogenic centers. The catalytic asymmetric construction of vicinal quaternary stereogenic centers, which are commonly found as a core structure in bioactive compounds, is a topic of profound interest in synthetic chemistry. This is because of the difficulty encountered in not only forming the carbon–carbon bond in the presence of steric congestion, but also in controlling the requisite stereochemical outcome.[15] The nucleophilic 1,2 addition of trisubstituted carbon pronucleophiles to ketones and ketimines is one of the potential straightforward methods for constructing vicinal quaternary stereogenic centers under Brønsted base catalysis.[16] Thus, we investigated the enantioselective direct Mannich-type reaction of α-imino esters with α-alkyl-substituted thionolactones as less acidic pronucleophiles by employing chiral bis(guanidino)-iminophosphoranes (**eq 3**).[17] The screening of the reaction conditions revealed that the substituents on the nitrogen of the guanidine moieties of the catalyst and the benzoyl moiety of the α-imino ester highly influence the stereoselectivity, in particular the diastereoselectivity, of the reaction. Using *para*-trifluoromethylbenzoyl-substituted α-imino esters as electrophiles, we achieved the construction of vicinal quaternary stereogenic centers in a highly diastreo- and enantioselective manner by employing (*M*)-**1c** (R = Ph_2CH, Ar =Ph), which incorporates sterically hindered diphenylmethyl groups on the two nitrogens of the guanidino group. The newly developed enantioselective Mannich-type reaction provides efficient access to densely functionalized and complex amino acid derivatives, such as **11**, which are difficult to prepare by prior methods.

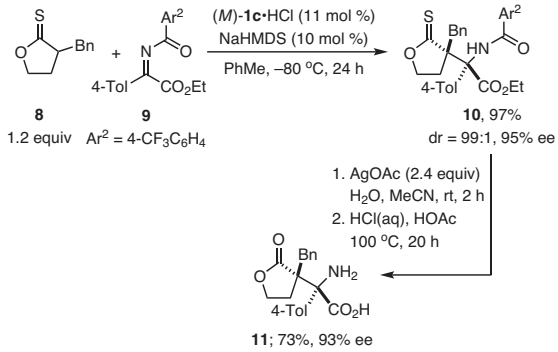


eq 1 *(Ref. 9a)*



Ar	Yield	ee
Ph	96%	98%
2-Tol	96%	91%
3-Tol	98%	99%
4-Tol	97%	99%
4-Anis	94%	98%
4-ClC_6H_4	98%	98%
4-BrC_6H_4	92%	90%
1-Np	88%	60%
2-Np	88%	92%
2-Furyl	98%	92%
2-Thienyl	99%	99%

eq 2 *(Ref. 14)*



eq 3 *(Ref. 17)*

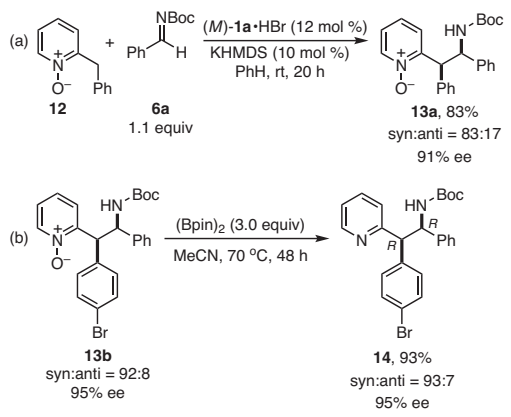
Most enantioselective reactions under Brønsted base catalysis, including our previous reactions, had involved enolate formation from the corresponding carbonyl-based pronucleophiles. We envisioned the development of an enantioselective reaction involving direct α deprotonation of 2-alkylazaarenes,[18] which is a potentially useful approach because it can directly provide 2-substituted azaarenes with a stereogenic center at the α position, which are commonly encountered motifs in natural products and pharmaceuticals. However, the main obstacle to developing the reaction is the low acidity of the α protons in the 2-alkyl substituents, which impedes the deprotonation required for initiation of the catalytic process. To overcome this difficulty, we utilized azaarene *N*-oxides as azaarene surrogates possessing higher electron-deficient properties[19] (**Scheme 1**, Part (a)).[20] Control experiments suggested that the primary role of the *N*-oxide moiety is not limited to enhancing the acidity of the α protons to facilitate the requisite deprotonation. It also acts as an additional coordination site for the chiral bis(guanidino)-iminophosphorane catalyst, where the orientation of the nucleophilic 2-benzylpyridine *N*-oxides would be well-organized in the chiral environment created by the catalyst. The *N*-oxide moiety of the products can be readily removed under mild conditions without any loss in the diastereomeric ratio and enantiomeric excess (Scheme 1, Part (b)).[20] This reaction is a rare example of the direct construction of a stereogenic center at the less acidic α position of an azaarene derivative under Brønsted base catalysis.

3.2. Enantioselective Protonation

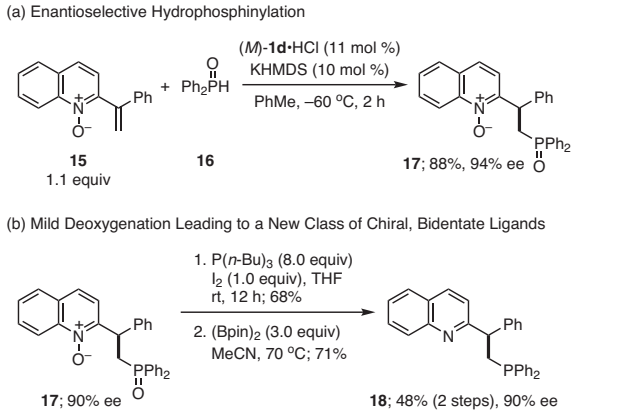
In addition to the enantioselective carbon–carbon bond forming reactions, we have applied chiral bis(guanidino)-iminophosphoranes to the construction of stereogenic centers through the enantioselective protonation of transient prochiral carbanions. In the past decade, the enantioselective protonation of transient enolates under Brønsted base catalysis has received considerable attention.[21] The transient enolate can be readily generated in a catalytic fashion by 1,4 addition of anionic nucleophiles to α,β-unsaturated carbonyl compounds. Moreover, the reaction can be performed under metal-free conditions and utilizing a simple procedure. In light of this, we reasoned that enantioselective protonation under the influence of chiral organosuperbase catalysts could markedly broaden the utility of the methodology by expanding the scope of possible transient carbanions resulting from the deprotonation of less acidic pronucleophiles. Toward this aim, we investigated the hydrophosphinylation reaction of diarylphosphine oxides with 2-vinyl azaheterocyclic *N*-oxides.[22] We found that chiral catalyst (*M*)-**1d** (R = Bn, Ar = Ph) facilitated the reaction of 2-(1-arylvinyl)quinoline *N*-oxides with diarylphosphine oxides—represented by **15** and **16**, respectively—to provide the desired adducts in high yields and high enantioselectivities (**Scheme 2**, Part (a)).[22] Some experimental results strongly suggested that the weak conjugate acid of the chiral organosuperbase, not the diarylphosphine oxides, acts as the active protonating agent in this reaction system. Enantio-enriched phosphine oxide **17** thus obtained could be easily deoxygenated under mild conditions to produce trivalent phosphine **18** (Scheme 2, Part (b)),[22] thereby affording a new class of chiral bidentate P,N ligands for forming chiral transition-metal complexes.

3.3. Enantioselective Formal [3 + 2] Cycloaddition of Epoxides

The [3 + 2] cycloaddition is a powerful method for the synthesis of densely functionalized five-membered-ring heterocyclic compounds containing oxygen.[23] Recently, the development of the enantioselective formal variant of the reaction has been advanced by using either transition-metal or Lewis acid catalysts with chiral ligands.[24,25] However, few catalytic systems exist that assemble multiple stereogenic centers in a highly stereoselective manner by utilizing this approach. In this context, we anticipated that the development of a catalytic



Scheme 1. Enantioselective Direct Mannich-Type Reaction of Azaarene *N*-Oxides as Azaarene Surrogates with Enhanced Acidity of the α Protons. *(Ref. 20)*



Scheme 2. (a) Representative Enantioselective Protonation through Hydrophosphinylation of a 2-Vinylquinoline *N*-Oxide. (b) Deoxygenation under Mild Conditions, Producing Trivalent Phosphine and a New Class of Chiral Bidentate P,N Ligands for Transition Metals. *(Ref. 22)*

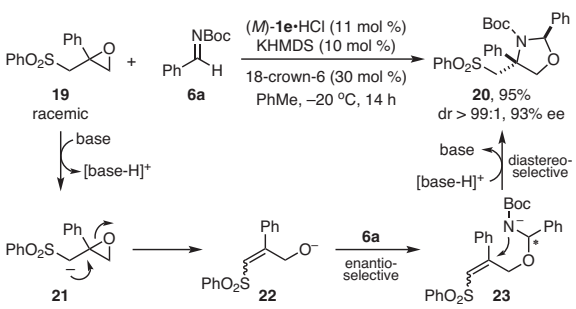
system employing a chiral Brønsted base would expand the range of the methodology. Therefore, we investigated the enantioselective reaction of β,γ-epoxy sulfone **19** with *N*-Boc imine **6a** by using chiral bis(gunanidino)iminophosphoranes. Screening of these organosuperbases revealed that (*M*)-**1e** (R = *t*-Bu, Ar = 1-Np) is the best catalyst for providing 1,3-oxazolidine **20** as a single diastereomer in an enantioselective formal [3 + 2] cycloaddition (**Scheme 3**).[26]

The reaction is initiated by the catalytic ring-opening of racemic β,γ-epoxy sulfone **19** leading to alkoxide **22**, which possesses an electron-deficient alkene moiety. Intermediate **22** formally serves as the synthetic equivalent of a 1,3-dipole, and its stereoselective cycloaddition with imine **6a** proceeds in a stepwise fashion: The enantioselective addition of **22** to **6a** is followed by a diastereoselective intramolecular aza-Michael addition of the hemiaminal ether anion **23** to afford enantioenriched 1,3-oxazolidine **20**.[27] The main challenge of the overall reaction is stereocontrol of the two stereogenic centers one of which is a quaternary one. Control experiments indicated that, in this tandem catalytic process, the key roles the chiral bis(guanidino)iminophosphorane plays are: (i) facilitating the reaction with its high basicity, (ii) controlling the enantioselectivity in the addition of alkoxide intermediate **22** to the *N*-Boc imine, and (iii) assisting the diastereocontrol of the intramolecular aza-Michael addition of anion **23**.

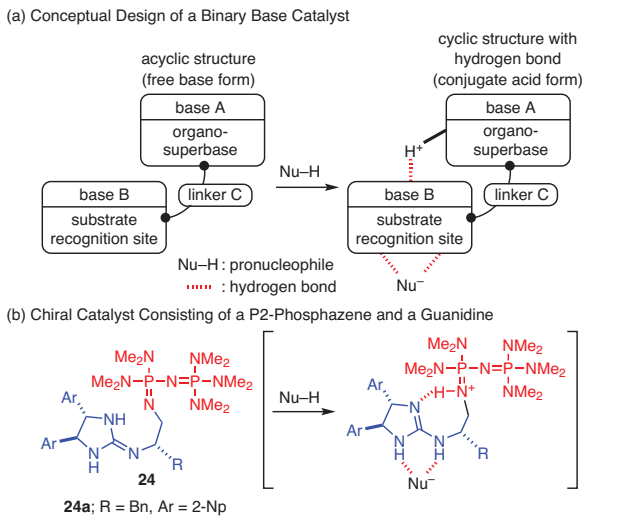
4. Development of Chiral Cooperative Binary Base Catalysts

Our preceding studies with chiral bis(guanidino)-iminophosphorane catalysts revealed the benefit of employing chiral organosuperbases possessing high basicity in developing new catalytic enantioselective reactions and in expanding the scope of pronucleophiles. However, the development of chiral organosuperbase catalysts and related chiral strong Brønsted base catalysts, which would lead to significant progress in the field of enantioselective catalysis, is still less advanced because of the difficulty in designing catalyst molecules that possess both high basicity and high stereocontrol ability.[28] In this regard, we have developed a conceptually new molecular design of a "chiral cooperative binary base" for chiral organosuperbases: a chiral acyclic organic molecule having two different organobase functional groups, one of which functions as an organosuperbase and the other as the substrate recognition site (**Scheme 4**, Part (a)).[29]

The key feature of this design is the distinctive cooperative function of the two organobases in a single chiral catalyst molecule. We expected that the conjugate acid of the catalyst, which is the actual key species in the stereochemistry-determining step of the enantioselective reaction, would form a chiral cyclic structure with an intramolecular hydrogen bond between the two organobase functionalities.[30] Thus, these functional groups would work cooperatively to form an effective chiral environment around the substrate recognition site by limiting the conformational flexibility in the conjugate acid form. The newly designed catalyst molecule **24** possesses a P2-phosphazene as an organosuperbase and a chiral guanidine as a hydrogen-bond donor for substrate recognition (Scheme 4, Part (b)). The two organobase functional groups are connected by a chiral two-carbon linker derived from an α-amino acid. A series of such binary molecules were prepared in a convergent manner from three readily accessible components: (i) a triaminophosphonium salt as a P2-phosphazene precursor, (ii) a chiral cyclic thiourea as a guanidine precursor, and (iii) a chiral 1,2-diaminoethane derivative as a linker. This approach would make it easy to optimize the catalyst structure to suit a variety of enantioselective reactions. The prominent catalytic activity of the chiral cooperative binary base catalyst was demonstrated in the unprecedented enantioselective direct Mannich-type reaction of an α-(phenythio)acetate as a less acidic pronucleophile with *N*-Boc imines to construct, in a highly enantioselective manner, a β-amino-α-thiocarbonyl scaffold, which is a synthetically useful building block incorporated into a number of sulfur-containing biologically active compounds (**eq 4**).[29]



Scheme 3. Representative Example of the Enantioselective Formal [3 + 2] Cycloaddition of Epoxides with Imines under Brønsted Base Catalysis. *(Ref. 26)*



Scheme 4. Chiral Cooperative Binary Base Catalyst. *(Ref. 29)*

5. Conclusion

In the field of chiral Brønsted base catalysis, a long-standing issue has been the expansion of the scope of pronucleophiles that can be applied to enantioselective reactions. To address this issue, we have developed two new types of chiral organosuperbase catalyst: chiral bis(guanidino)iminophosphorane catalysts and chiral cooperative binary base catalysts, both of which possess a much higher basicity than conventional chiral organobase catalysts as well as a high stereocontrol ability. Their prominent catalytic activity was demonstrated in several enantioselective transformations, including the direct enantioselective addition of less acidic pronucleophiles, the enantioselective protonation, and the enantioselective formal [3 + 2] cycloaddition of epoxides. Our results provide, not only new methods for the synthesis of enantio-enriched compounds which are difficult to prepare by using other methods, but a new guiding principle for the design and development of new chiral Brønsted base catalysts that would broaden their usefulness in organic synthesis. Further studies are in progress to develop novel catalytic enantioselective transformations with less acidic pronucleophiles and to develop highly efficient catalysts based on our molecular design.

6. Acknowledgments

The authors thank all their co-workers for their contributions to the chemistry described in this article. This research was supported by a Grant-in-Aid for Scientific Research on Innovative Areas "Advanced Molecular Transformations by Organocatalysts" (No. 23105002) and "Hybrid Catalysis for Enabling Molecular Synthesis on Demand" (No. 17H06447) from MEXT (Japan), and a Grant-in-Aid for Scientific Research (No. 16H06354, No. 16K05680, and No. 25810057) from the JSPS.

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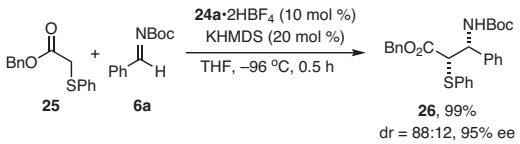

25 **6a** **26**, 99%
dr = 88:12, 95% ee

eq 4 *(Ref. 29)*

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About the Authors

Azusa Kondoh was born in 1982 in Osaka, Japan. He earned his B.S. and Ph.D. degrees in 2005 and 2010, respectively, from Kyoto University under the supervision of Prof. Koichiro Oshima. He then joined the group of Prof. Alois Fürstner at the Max-Plank-Institut für Kohlenforschung as a postdoctoral fellow. In 2012, he became Assistant Professor at Tohoku University, working with Prof. Masahiro Terada, and was promoted to Associate Professor in 2020. His current research program focuses on the development of new synthetic methods on the basis of organocatalysis as well as transition-metal catalysis. He received the Chemical Society of Japan Award for Young Chemists (2018) and the Young Scientists' Award, The Commendation for Science and Technology by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) (2020).

Masahiro Terada received his B.S. degree in 1986 from the Department of Applied Chemistry, and completed his Ph.D. degree in 1993 at the Tokyo Institute of Technology. During his Ph.D. study, he was appointed Assistant Professor at the Tokyo Institute of Technology (1989–2001). Between 1999 and 2000, he was a postdoctoral fellow at Harvard University and, in 2001, he accepted a position as Associate Professor at Tohoku University. In 2006, he was promoted to Professor of Chemistry at the Graduate School of Science, Tohoku University, and was appointed Dean of the Graduate School of Science and Faculty of Science in 2017. His current research interests focus on the development of useful synthetic methods by designing novel chiral Brønsted acid and base catalysts as well as the utilization of transition-metal catalysts. He is the recipient of The incentive Award in Synthetic Organic Chemistry, Japan (2003), The Chemical Society of Japan Award for Creative Work (2008), the Mukaiyama Award (2010), the Daiichi-Sankyo Award for Medicinal Organic Chemistry (2011), The Nagoya Silver Medal (2012), the Molecular Chirality Award (2015), and the Synthetic Organic Chemistry Award, Japan (2017).

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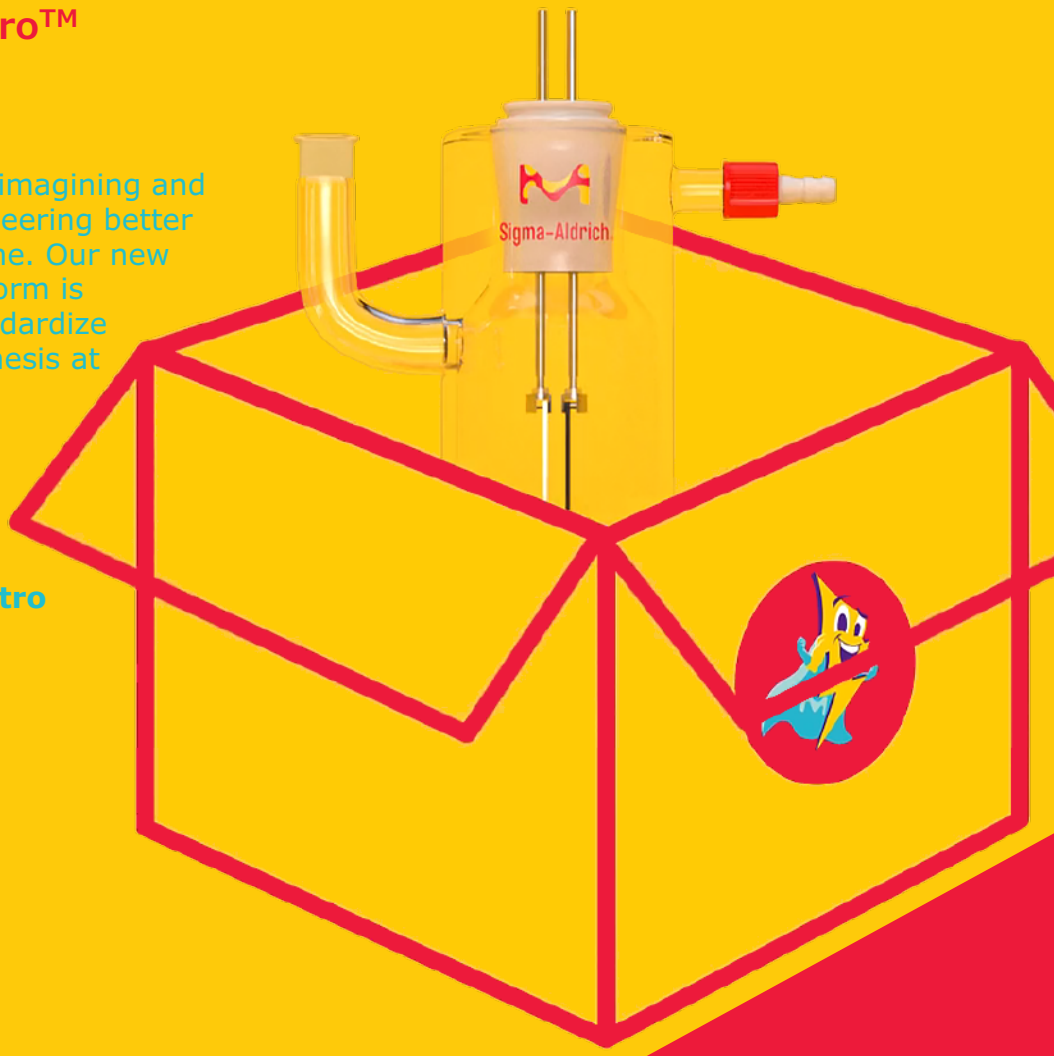

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Difunctionalization-Type Fluoroalkylations of Alkenes via Intramolecular Carbo- or Heterocycle Formation

炭素環およびヘテロ環の分子内形成を伴うアルケンの二官能基化型フルオロアルキル化反応


Dr. S. Mukherjee


Dr. S. Kawamura


Prof. M. Sodeoka

Subrata Mukherjee,[a] Shintaro Kawamura,*[,a,b] and Mikiko Sodeoka*[,a,b]
スブラタ ムカジー, 河村 伸太郎, 袖岡 幹子

[a] Catalysis and Integrated Research Group
RIKEN Center for Sustainable Resource Science
2-1 Hirosawa, Wako, Saitama, 351-0198, Japan

[b] Synthetic Organic Chemistry Laboratory
RIKEN Cluster for Pioneering Research
2-1 Hirosawa, Wako, Saitama 351-0198, Japan

Email: skawamura@riken.jp, sodeoka@riken.jp

Keywords. perfluoroalkylation; trifluoromethylation; radical reaction; difunctionalization of alkenes; intramolecular cyclization; Togni reagent; perfluorocarboxylic anhydride; copper catalyst; carbocycle; heterocycle.

キーワード. ペルフルオロアルキル化; トリフルオロメチル化; ラジカル反応; アルケンの二官能基化; 分子内環化; Togni試薬; ペルフルオロカルボン酸無水物; 銅触媒; 炭素環; ヘテロ環.

Abstract. Fluoroalkylation is often an effective strategy for improving the drug-like properties and bioactivities of compounds such as pharmaceuticals and agrochemicals. Efficient and practical fluoroalkylation methods are therefore important tools in the synthetic chemist's tool chest. In particular, difunctionalization-type fluoroalkylations of alkenes—installing a fluoroalkyl group and constructing a cyclic skeleton simultaneously—pose a significant challenge for synthetic organic chemists. Focusing mainly on work in our laboratory, this mini-review summarizes intramolecular difunctionalization-type fluoroalkylations of alkenes with Togni reagent and fluorine-containing carboxylic anhydrides. These reactions lead to a variety of fluoroalkyl-group-containing carbo- and heterocycles as potentially bioactive molecules and building blocks suitable for further elaboration.

Outline

1. Introduction
2. Trifluoromethylation with Togni Reagent
   2.1. Synthesis of CF_3-Containing Carbocycles
   2.2. Synthesis of CF_3-Containing Heterocycles
3. Fluoroalkylation with Fluorine-Containing Carboxylic Anhydrides
   3.1. Fluorine-Containing Carboxylic Anhydrides as Fluoroalkyl Sources
   3.2. Catalytic Fluoroalkylations
   3.3. Transition-Metal-Free Fluoroalkylations
4. Conclusion and Outlook
5. Acknowledgments
6. References and Notes

1. Introduction

Introduction of fluoroalkyl groups is a common strategy in the development of drug candidates and agrochemicals (**Figure 1**).[1] For example, a fluorinated thymidine analogue, trifluridine, gained FDA approval for the treatment of metastatic colorectal cancer in 2015.[2a,b] Efavirenz, containing a tertiary stereogenic center, is used to treat human immunodeficiency virus (HIV) patients.[2c] Fulvestrant (trade name: Faslodex®) is a pentafluoroethylated drug molecule used for the treatment

of breast cancer.[2d] Fluralaner, containing two trifluoromethyl groups and marketed as Bravecto®, is a pesticide that is employed to treat companion animals and poultry for fleas, ticks, and mites.[2e,f] Flupoxam, bearing a pentafluoroethyl group, is a herbicide that acts by inhibiting cellulose biosynthesis.[2g]

A major reason for the remarkable increase in fluoroalkyl-containing drugs and agrochemicals is that the introduction of fluoroalkyl groups into bioactive molecules often improves their lipophilicity, membrane permeability, metabolic stability, and pharmacokinetics.[1] Moreover, the presence of the strongly electronegative fluorine atom in the fluoroalkyl group sometimes enables new hydrogen bonding, dipole–electrostatic, or dipole–dipole interactions that lead to enhanced or novel biological activities.

While there is strong interest in fluoroalkyl compounds as drug candidates and agrochemicals, only a few naturally occurring fluorine-containing organic molecules are known.[3] Consequently, electrophilic fluoroalkylation methods have rapidly advanced in the past decade to expand the chemical space of fluoroalkyl compounds. Among them, the intramolecular difunctionalization-type fluoroalkylation of alkenes—involving electrophilic addition of a fluoroalkyl group and intramolecular carbo- or heterocycle formation—is an efficient synthetic strategy (**eq 1**).[4] In the rest of this article, we cover these fluoroalkylation reactions that employ Togni reagent and fluorine-containing carboxylic anhydrides, focusing mainly on our work in this field.[5]

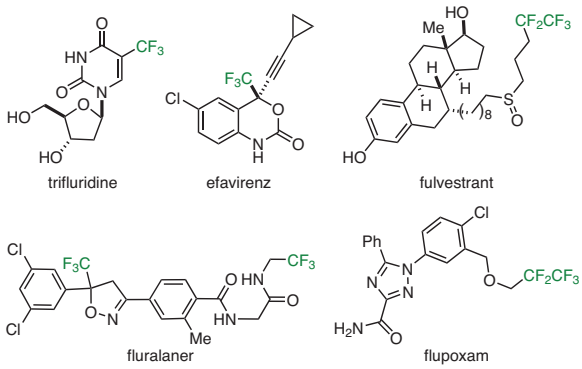
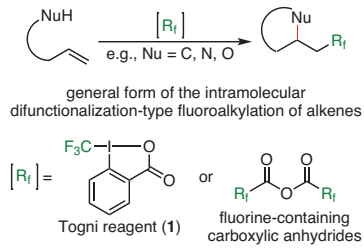


Figure 1. Examples of Drugs and Agrochemicals Incorporating One or More Fluoroalkyl Groups. *(Ref. 1,2)*



eq 1 *(Ref. 4,5)*

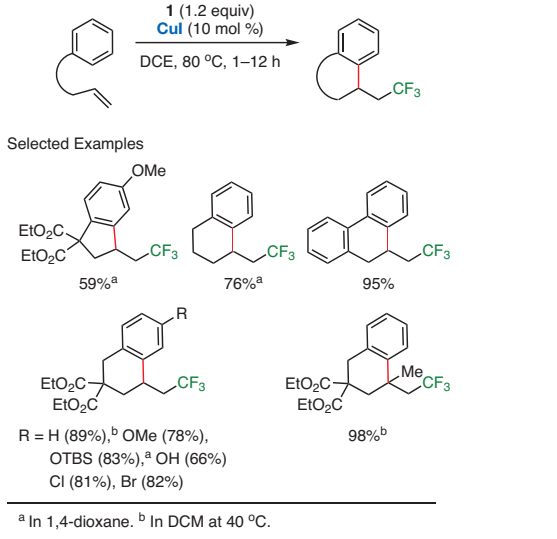
2. Trifluoromethylation with Togni Reagent

In 2010, Togni's group[6a] and our group[6b] independently discovered that zinc and copper salts can activate Togni reagent and catalyze C–CF_3 bond formation in the electrophilic trifluoromethylation of indoles.[7] Later, it was reported that the combination of Togni reagent and transition-metal catalysts, mostly copper catalysts, can promote various difunctionalization-type trifluoromethylation reactions of alkenes.[4,5]

2.1. Synthesis of CF_3-Containing Carbocycles

In 2013, we reported the first example of intramolecular carbo-trifluoromethylation of alkenes with Togni reagent in the presence of a Cu(I) catalyst. In this transformation, alkenes bearing an aromatic ring as the nucleophilic motif at an appropriate position of the carbon side chain, afforded CF_3-containing benzo-fused carbocycles in high yields (**eq 2**).[8] This was an interesting result, since related previous work had found that the reaction of alkenes with Togni reagent in the presence of copper catalyst resulted in allylic trifluoromethylation.[9] We found that the use of CuI as the catalyst, together with a judicious choice of solvent, such as 1,2-dichloroethane (DCE), 1,4-dioxane, or dichloromethane (DCM), was key to the success of this novel intramolecular carbo-trifluoromethylation. Since then, a variety of conditions for alkene trifluoromethylations with Togni reagent have been uncovered by many research groups, enabling the synthesis of a diverse array of CF_3-containing carbocycles.[4,5]

We recently developed a method for synthesizing CF_3-containing tetrahydrocarbazoles by carbo-trifluoromethylation



eq 2 *(Ref. 8)*

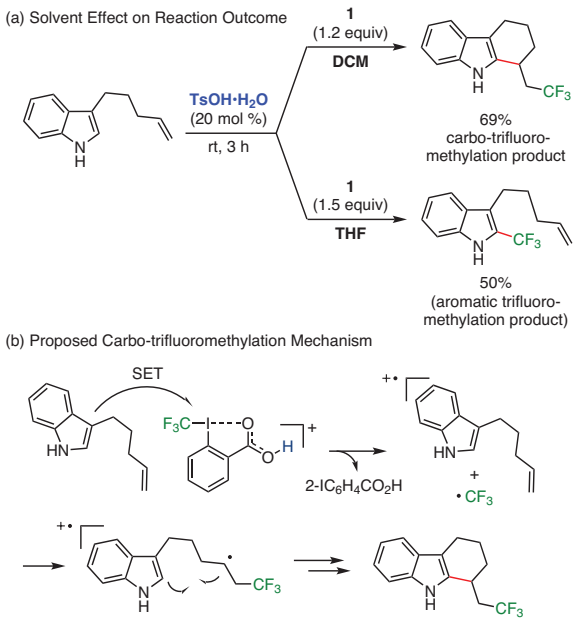
of indoles bearing a pentenyl group at the C-3 position with Togni reagent.[10] In this reaction, a simple Brønsted acid, TsOH, exhibited a higher catalytic reactivity than CuI.Notably, this was the first synthesis of these compounds, although some related work on trifluoromethylation of indoles bearing an alkene side-chain had been reported.[11] The resulting CF_3-substituted compounds are potential drug candidates since the tetrahydrocarbazole motifs are often found in bioactive molecules.[12] In addition, it was observed that the site-selectivity of the trifluoromethylation could be controlled by changing the reaction solvent; specifically, carbo-trifluoromethylation proceeded in dichloromethane, while aromatic trifluoromethylation products were mostly formed in THF. Mechanistic studies suggested that the tetrahydrocarbazole-forming reaction proceeds via single-electron transfer (SET) between the indole substrate and the protonated Togni reagent; addition of the trifluoromethyl radical to the alkene double bond leads to a key radical cation intermediate which undergoes the carbocyclization (**Scheme 1**).[10]

2.2. Synthesis of CF_3-Containing Heterocycles

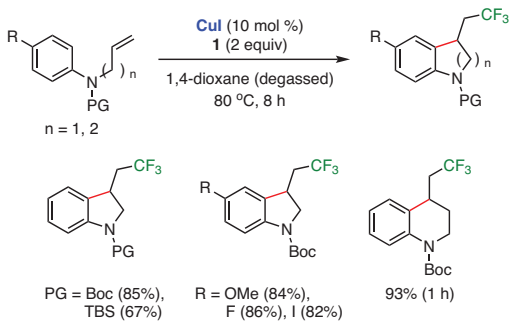
Heterocycles are privileged motifs of bioactive molecules and have a vast range of applications in medicinal chemistry and the agrochemicals industry.[13] More than 85% of all biologically active chemicals contain a heterocyclic fragment, and thus trifluoromethylated heterocycles are of interest in drug discovery and medicinal chemistry.

In this context, we applied our carbo-trifluoromethylation of alkenes with Togni reagent to the synthesis of CF_3-containing heterocycles. We employed alkenes possessing a heteroatom in the side chain in order to construct benzo-fused aliphatic heterocyclic skeletons.[8] Indeed, the reactions of N-protected allylaniline and homoallylaniline derivatives furnished CF_3-containing indolines and tetrahydroquinolines, respectively (**eq 3**).[8] We also extended our protocol to the synthesis of CF_3-containing oxindole derivatives from acryloanilides under mild reaction conditions (**eq 4**).[14,15] Later, further progress in intramolecular carbo-trifluoromethylation reactions for the construction of CF_3-containing heterocycles was made by other research groups.[4,5]

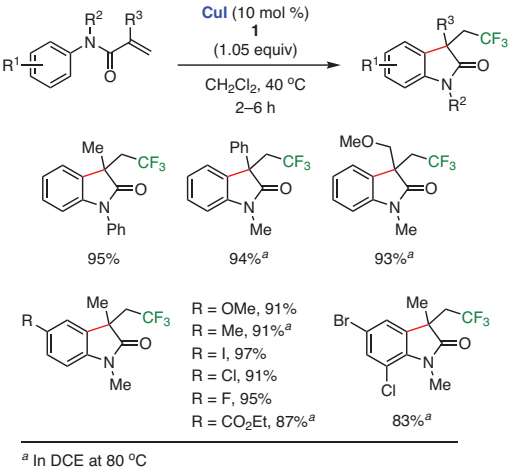
Carbo-trifluoromethylations of alkenes possessing a heteroatom in the side chain are useful for providing the corresponding heterocycles; however, these are limited to benzo-fused compounds. To broaden the range of available CF_3-containing aza-heterocyclic compounds, we envisioned an analogous intramolecular amino-trifluoromethylation reaction of aminoalkenes. In 2013, our group described the first amino-trifluoromethylation of alkenyl amines with Togni reagent to synthesize CF_3-containing aza-heterocycles.[16,17] The reaction of allylamines with Togni reagent in the presence of 1–5 mol % CuI gave the corresponding CF_3-containing



Scheme 1. Formation of Trifluoromethyl-Containing Tetrahydrocarbazoles by Carbo-trifluoromethylation. *(Ref. 10)*



eq 3 *(Ref. 8)*

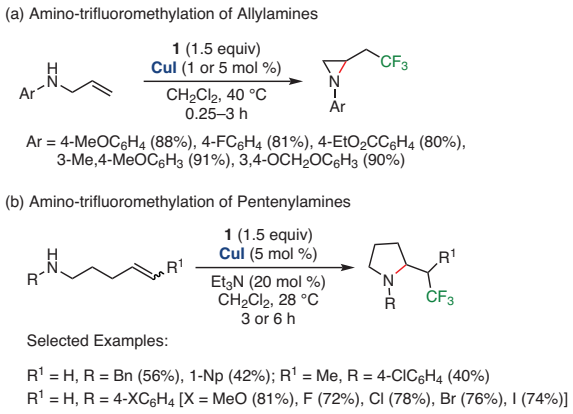


eq 4 *(Ref. 14)*

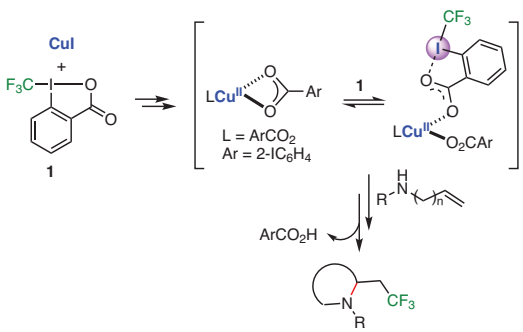
aziridines in excellent yields (**Scheme 2**, Part (a)).[16] We also found that the use of Et_3N as a co-catalyst enabled the smooth amino-trifluoromethylation of pentenylamines, affording CF_3-containing pyrrolidines (Scheme 2, Part (b)).[18]

Our mechanistic experiments, including kinetic studies, ^{19}F NMR, and ESI-MS analyses, showed that a Cu(II) species generated in situ from CuI serves as a Lewis acid to activate Togni reagent (**Scheme 3**).[18] The synthesis of CF_3-containing aza-heterocycles has also been achieved under a variety of conditions by several other research groups.[4,5]

We next focused on the synthesis of trifluoromethylated oxazolines (**Scheme 4**),[19] because the oxazoline motif is frequently found in natural products, and thus their trifluoromethyl analogues are expected to show interesting bioactivity.[20,21] We initially examined our previously reported conditions for the Cu-catalyzed intermolecular oxy-trifluoromethylation reaction of alkenes.[22] Specifically, we expected to construct the oxazoline ring together with introduction of the CF_3 group by intramolecular cyclization of *N*-allylamides with Togni reagent in the presence of $[Cu(MeCN)_4]PF_6$ as catalyst. Unfortunately, this was not successful with the model compound *N*-allylbenzamide; a complex mixture was formed containing only a small amount of the desired oxazoline. Next, we considered another approach based on our iodotrifluoromethylation reaction;[23] the oxazoline was expected to be formed by the iodotrifluoromethylation of the alkene followed by nucleophilic cyclization. When KI was used as an electron donor to activate Togni reagent, the desired 2-phenyl-5-(2,2,2-trifluoro)ethyloxazoline was formed in 75% yield. We examined various *N*-allyl arylamides as well as *N*-allyl alkylamides as substrates under these reaction conditions, and synthesized a wide range of oxazoline derivatives (see Scheme 4).[19] Moreover, this methodology was also applied to the late-stage trifluoromethylation of telmisartan and lithocholic acid analogues.



Scheme 2. Amino-trifluoromethylation Reaction of Allylamines and Alkenylamines with **1**. *(Ref. 16,18)*

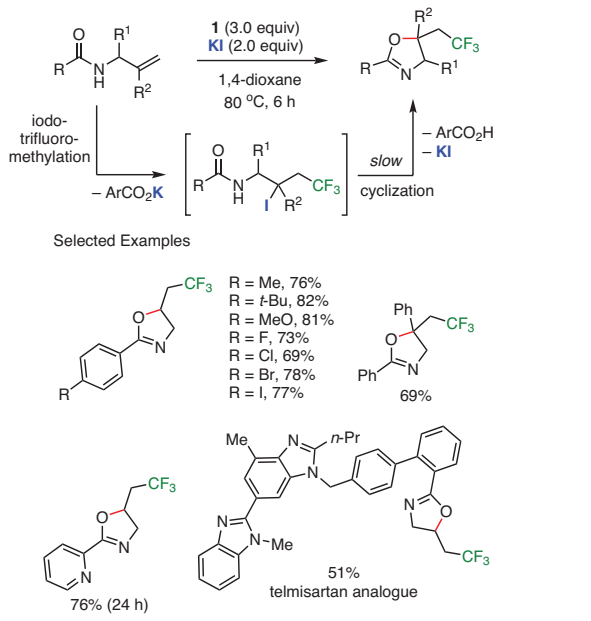


Scheme 3. Mechanism of the Copper-Catalyzed Amino-trifluoromethylation Reaction. *(Ref. 18)*

3. Fluoroalkylation with Fluorine-Containing Carboxylic Anhydrides

3.1. Fluorine-Containing Carboxylic Anhydrides as Fluoroalkyl Sources

Sophisticated electrophilic trifluoromethylating reagents such as Togni, Umemoto, and Langlois reagents have enabled the development of various difunctionalization-type trifluoromethylations of alkenes;[4,5] however, high cost and/or multistep preparation of these reagents sometimes limit their synthetic applications, particularly in large-scale synthesis. To address this issue, we focused on the class of fluorine-containing carboxylic anhydrides as a fluoroalkyl source. In addition to their low cost, various fluorine-containing carboxylic anhydrides—possessing not only a trifluoromethyl group, but also other fluoroalkyl groups such as C_2F_5, C_3F_7, and CF_2Cl—are commercially available. Nevertheless, it has been a long-standing challenge to utilize them as fluoroalkyl sources in fluoroalkylations of unactivated alkenes.[24] Recently, we



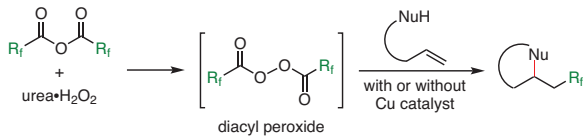
Scheme 4. Oxazoline-Forming Trifluoromethylation Using **1**. *(Ref. 19)*

achieved difunctionalization-type fluoroalkylations of alkenes via intramolecular carbo/heterocycle formation with fluorine-containing carboxylic anhydrides (**Scheme 5**).[5b,c] The key to success in this reaction was the in situ generation of diacyl peroxide from the carboxylic anhydride and urea-hydrogen peroxide, as well as precise control of the reactivity of the radical species formed during the reaction with the aid of copper catalysts or appropriate substrate structures.

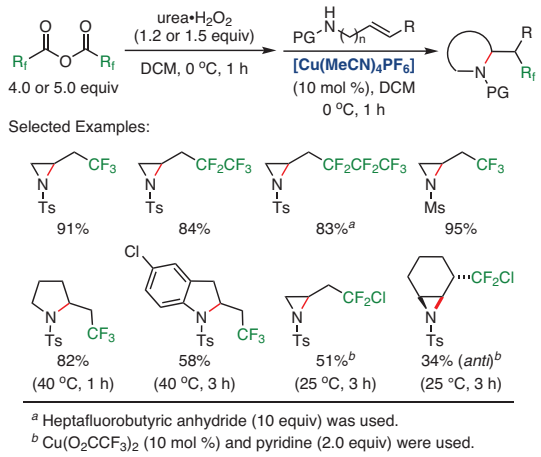
3.2. Catalytic Fluoroalkylations

In 2017, we achieved the first amino-perfluoroalkylation of alkenylamines by using perfluorocarboxylic anhydrides.[25] Specifically, diacyl peroxide—generated in situ from the perfluorocarboxylic anhydride (R_f = CF_3, C_2F_5, C_3F_7) and urea•H_2O_2—was reacted with sulfonyl-protected allylamines or pentenylamines in the presence of a catalytic amount of $[Cu(MeCN)_4]PF_6$, affording the desired aziridines or pyrrolidines in up to 95% yields (**Scheme 6**).[25,26] Notably, the amino-trifluoromethylation with trifluoroacetic anhydride (TFAA) showed higher reactivity than that with Togni reagent: the reaction of *N*-tosyl allylamine with TFAA gave the CF_3-containing aziridine in 91% yield at 0 °C after 1 h, whereas only 17% yield was obtained in the reaction with Togni reagent even at 40 °C. Furthermore, we also developed an amino-chlorodifluoromethylation by using chlorodifluoroacetic anhydride (CDFAA).[26] CDFAA is also an inexpensive fluoroalkyl source, and the CF_2Cl-containing products would be useful building blocks for CF_2-containing molecules via substitution of the chlorine atom. However, the relatively unstable diacyl peroxide prepared from CDFAA, in contrast to that containing CF_3, makes it difficult to control the reactivity and selectivity of the reaction with the copper catalyst due to undesired background radical reactions. Finally, we found that the use of $Cu(O_2CCF_3)_2$ as catalyst instead of $[Cu(MeCN)_4]PF_6$ and pyridine as an additive improved the yield of the reaction.

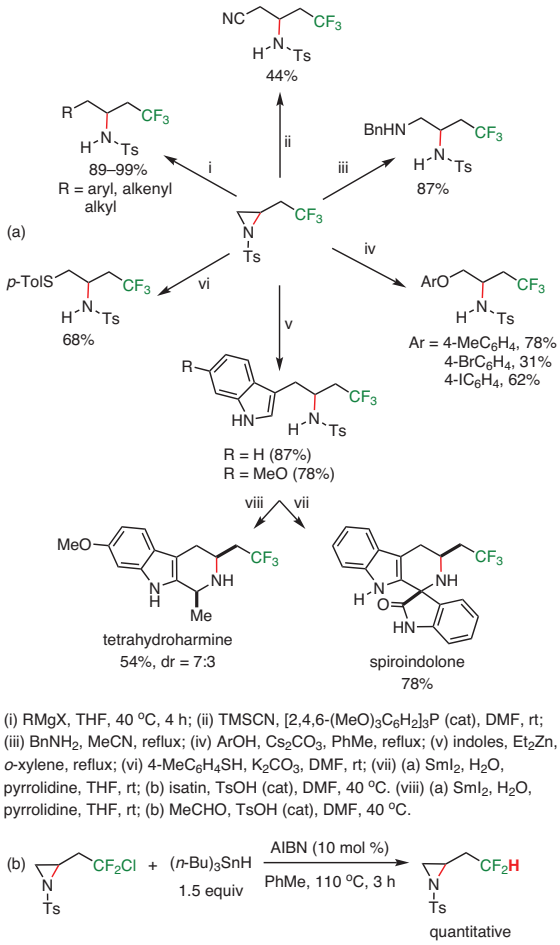
To demonstrate the practical utility of this method, we synthesized bench-stable CF_3-containing *N*-tosyl aziridines on a gram scale and derivatized them to various CF_3-containing amines by means of ring-opening reactions with nucleophiles: (i) RMgX, THF, 40 °C, 4 h; (ii) TMSCN, $[2,4,6\text{-}(MeO)_3C_6H_2]_3P$ (cat), DMF, rt; (iii) $BnNH_2$, MeCN, reflux; (iv) ArOH, Cs_2CO_3, PhMe, reflux; (v) indoles, Et_2Zn, *o*-xylene, reflux; and (vi) 4-MeC_6H_4SH, K_2CO_3, DMF, rt (**Scheme 7**, Part (a)).[25] Among the products, we were particularly interested in the tryptamine derivatives. Trifluoromethylated tetrahydroharmine and



Scheme 5. Intramolecular Difunctionalization-Type Fluoroalkylation Reactions Mediated by Diacyl Peroxide Generated in Situ from Fluorine-Containing Carboxylic Anhydrides. *(Ref. 5b,c)*



Scheme 6. Copper-Catalyzed Amino-fluoroalkylation Reaction Using Fluorine-Containing Carboxylic Anhydrides. *(Ref. 25,26)*



Scheme 7. (a) Derivatizations of Fluoroalkyl-Group-Containing Aziridines via Ring-Opening Reactions with Nucleophiles. (b) Reduction of CF_2Cl to CF_2H. *(Ref. 25,26)*

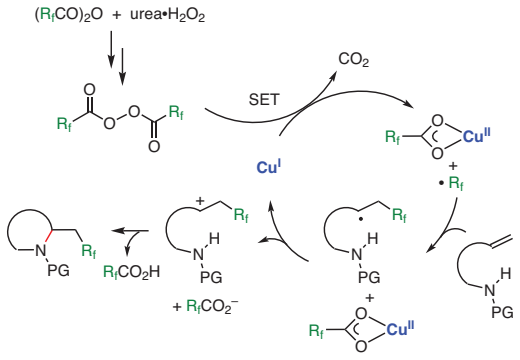
spiroindolone were easily synthesized via the Pictet–Spengler reaction with acetaldehyde and isatin after cleavage of the Ts protecting group in the presence of SmI_2.[25] We also transformed the CF_2Cl-containing aziridine into CF_2H-containing aziridine by reduction with (n-Bu)$_3$SnH/AIBN (Scheme 7, Part (b)).[26]

In the proposed reaction mechanism (**Scheme 8**),[25] single-electron transfer (SET) from Cu(I) to the diacyl peroxide forms the perfluoroalkyl (R_f) radical after decarboxylation. Reaction of the alkene with the perfluoroalkyl (R_f) radical generates an alkyl radical. Oxidation of the alkyl radical with the Cu(II) species affords the corresponding carbocation intermediate and regenerates the Cu(I) species. This oxidation step is considered the most important step for the success of the reaction because, without any treatment, the alkyl radical would give rise to undesired products. The carbocation smoothly produces the desired compound via nucleophilic cyclization.

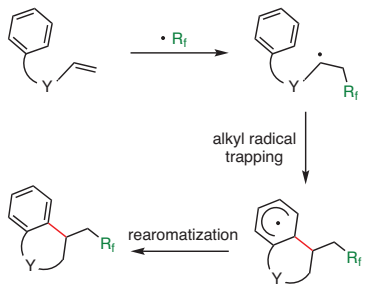
3.3. Transition-Metal-Free Fluoroalkylations

Fluorine-containing diacyl peroxide produces a fluoroalkyl radical upon heating, and reaction of the latter with an alkene readily generates an alkyl radical. In the absence of a transition-metal catalyst, however, the highly reactive alkyl radical affords a complex mixture. We hypothesized that if an aromatic ring were present at an appropriate position on the side chain of the alkene, the alkyl radical would be trapped, and subsequent aromatization would yield the benzo-fused product (**Scheme 9**).[25–27] Gratifyingly, we successfully effected the carbofluoroalkylation reaction of unactivated alkenes bearing a pendant aromatic group by using a fluorine-containing carboxylic anhydride and urea•H_2O_2 without any catalyst or additive (**Scheme 10**).[25–27] By applying this approach, we efficiently constructed various fluoroalkyl-group-containing five-membered-ring and six-membered-ring carbocycles and heterocycles, including oxindoles, benzothiazinane dioxides, indolines and dihydroisoquinolinones. Moreover, addition of a copper catalyst enables carrying out a divergent synthesis by efficiently leading to a different heterocyclic product. For example, the reaction of *N*-tosylallylamine in the presence and absence of a catalytic amount of [Cu(MeCN)$_4$]PF$_6$ selectively afforded CF_3-containing aziridine via amino-trifluoromethylation and benzothiazinane dioxide via carbo-trifluoromethylation, respectively.

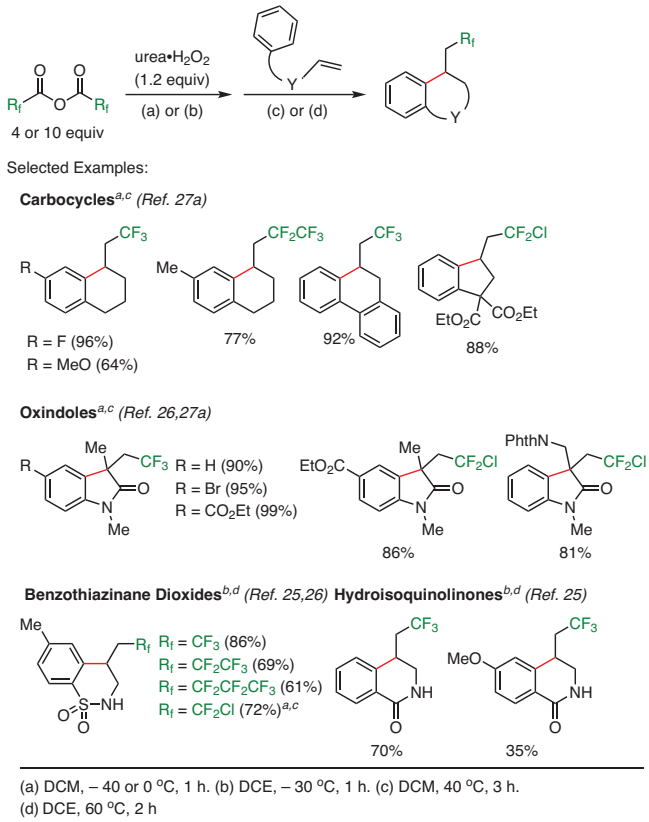
Furthermore, as another approach to the transition-metal-free reaction, the intramolecular aminoperfluoroalkylation was achieved by taking advantage of the unique redox activity of styrenes (**Scheme 11**).[28] The transition-metal-free amino-fluoroalkylation is extremely rare[29] because of the difficulty



Scheme 8. Proposed Mechanism of the Amino-fluoroalkylation with Fluorine-Containing Carboxylic Anhydrides. *(Ref. 25)*



Scheme 9. Conceptual Metal-Free Carbo-fluoroalkylation Reaction with Fluorine-Containing Carboxylic Anhydrides. *(Ref. 25–27)*

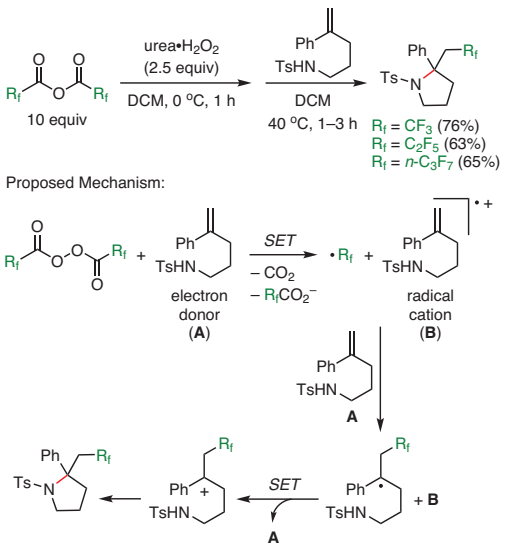


Scheme 10. Scope of the Metal-Free Carbo-fluoroalkylation Reaction with Fluorine-Containing Carboxylic Anhydrides. *(Ref. 25–27)*

of effecting the conversion of the fluoroalkyl-containing alkyl radical intermediate to the desired product in the absence of a catalyst. Nevertheless, we found that the reaction of styrenes bearing a pendant amino group with perfluorocarboxylic anhydrides and urea•H_2O_2 proceeded well, affording perfluoroalkyl-containing pyrrolidine derivatives in good yields. Based on mechanistic investigations, we concluded that the reaction begins with SET between in situ generated diacyl peroxide and styrene to produce the perfluoroalkyl radical and the radical cation of styrene. Then, the perfluoroalkyl radical reacts with another styrene molecule rather than the electron-deficient radical cation, forming a benzyl radical. The benzyl radical is oxidized by the radical cation species as the strongest oxidant in the reaction mixture and generates the key carbocation intermediate. Finally, nucleophilic cyclization gives the desired pyrrolidine product.

4. Conclusion and Outlook

We have briefly summarized the difunctionalization-type fluoroalkylations of alkenes via carbo/heterocycle formation with Togni reagent and fluorine-containing carboxylic anhydrides, focusing mainly on our work. The reactions using Togni reagent provide access to a wide variety of CF_3-containing carbo- and heterocycles by activation with copper or Brønsted acid catalysts or electron-donating additives, including metal iodides. The reactions using fluorine-containing carboxylic anhydrides provide broad access to carbo- and heterocycles containing not only CF_3 but also other fluoroalkyl groups, such as C_2F_5, C_3F_7, and CF_2Cl. In addition to the reactions described here, a great variety of reaction conditions have been reported to date, enabling the synthesis of a diverse array of fluoroalkyl-group-containing molecules. We believe that these fluoroalkylation methods will contribute to the expansion of organofluorine-based compound libraries for pharmaceutical and agrochemical research.



Scheme 11. Metal-Free Amino-perfluoroalkylation Reactions of Styrenes. *(Ref. 28)*

5. Acknowledgments

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About the Authors

Subrata Mukherjee was born in 1991 in Burdwan, India. He completed his B.Sc. (Honors) degree in chemistry at Calcutta University in 2011, and his M.Sc. degree at the Indian Institute of Technology (IIT), Madras, in 2014. He received his Ph.D. degree in 2019 from the National Chemical Laboratory, Pune, under the supervision of Prof. A. T. Biju. Currently, he is working as a postdoctoral researcher in Prof. Sodeoka's research group at RIKEN.

Shintaro Kawamura received his B.Eng. degree in 2007 from Doshisha University. He received his M.Eng. and Ph.D. degrees from Kyoto University. Since 2012, he has worked in Prof. Sodeoka's group at RIKEN. He was promoted from a postdoctoral researcher to a Research Scientist in 2017 and to a Senior Research Scientist in 2021.

Mikiko Sodeoka received her B.S., M.S., and Ph.D. degrees from Chiba University. After working at the Sagami Chemical Research Center, Hokkaido University, Harvard University, and the University of Tokyo, she became a Group Leader at the Sagami Chemical Research Center in 1996. She moved to the University of Tokyo as an Associate Professor and then to Tohoku University as a Full Professor in 2000. Since 2004, she has been a Chief Scientist at RIKEN.

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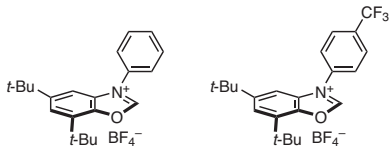
Dong, Z.; MacMillan, D. W. C. *Nature* **2021**, *598*, 451.



deoxazole (**924245**) deoxazole-quat (**924253**)

924245	Deoxazole, ≥95%	1 g, 5 g
924253	Deoxazole-quat, ≥95%	1 g, 5 g

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Global Head of the Chemistry Franchise

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ABOUT OUR COVER

Antoine Lavoisier, the tax collector turned chemist, has been immortalized in this one of the most recognizable paintings of all time. Created in 1788 by Jacques Louis David (1748–1825), one of the best-known French portrait artists of the 18th century, ***Antoine Laurent Lavoisier and Marie Anne Lavoisier*** (oil on canvas, 259.7 x 194.6 cm) languished in obscurity for close to a century on account of the French Revolution that started about a year after the work was completed and led to the tragic death of Mr. Lavoisier.*

What better subject to feature on the cover of this issue of the *Acta*, which celebrates the breadth of chemical research, than Lavoisier who had broad interests in many aspects of chemistry. He is credited with the discovery of oxygen and the composition of water and is one of the founders of modern chemistry, an early developer of chemical nomenclature, and a strong proponent of the metric system.

Detail from ***Antoine Laurent Lavoisier and Marie Anne Lavoisier***. Photo courtesy of The Metropolitan Museum of Art, New York, NY.

Purchase, Mr. and Mrs. Charles Wrightsman Gift, in honor of Everett Fahy, 1977. The Metropolitan Museum of Art, New York, NY.

** To find out more about the tragic fate of Mr. Lavoisier, visit **SigmaAldrich.com/acta***

PRODUCT HIGHLIGHT

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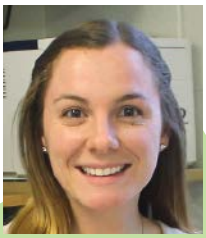
Lewis Acid Catalyzed Carbonyl–Olefin Metathesis (COM): A Tutorial


Ms. A. J. Davis


Dr. J. L. Gomez-Lopez


Prof. C. S. Schindler

Ashlee J. Davis, Jessica L. Gomez-Lopez, and Corinna S. Schindler*

Willard-Henry-Dow Laboratory
Department of Chemistry
University of Michigan
930 North University Avenue
Ann Arbor, MI 48109, USA

Email: corinnas@umich.edu

Keywords. carbonyls; olefins; metathesis; Lewis acid; catalysis; oxetanes; fragmentation; ring-closing; hydrocarbons; ethers.

Abstract. The field of carbonyl–olefin metathesis (COM) has grown significantly in recent years and expanded from stoichiometric reaction protocols to efficient catalytic strategies for ring-closing, ring-opening, and cross(carbonyl–olefin) metathesis. The aim of this review is to classify the types of catalyst currently available for carbonyl–olefin metathesis and highlight substrate–catalyst compatibility across several substrate systems. Although most of the transformations proceed through oxetane formation and subsequent fragmentation, mechanistic insight into unique pathways is also explored. Finally, alternative reactivity of metathesis substrates is discussed.

Outline

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   2.2. Olefins
3. Ring-Closing COM Forming 5-Membered Rings
   3.1. Aryl Ketones
   3.2. Pyrroline Synthesis
   3.3. Aliphatic Ketones
4. Ring-Closing COM Forming 6-Membered Rings
   4.1. Polyaromatic Hydrocarbon Scaffolds
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   5.1. Ring-Opening Systems
   5.2. Cross-Metathesis
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1. Introduction

Over the past decade, Lewis acid catalyzed carbonyl–olefin metathesis (COM) has experienced extensive development.[1] Interest in this reaction stems from its ability to directly form new carbon–carbon double bonds, a fundamentally important functional group relevant to many industrial applications from the production of pharmaceuticals to the manufacture of materials. The carbonyl substrate activation takes place via reversible binding of the Lewis basic carbonyl moiety to the metal center of the Lewis acid catalyst, which allows for catalyst turnover. The first Lewis acid promoted carbonyl–olefin metathesis was reported in 1971 and required stoichiometric amounts of the Lewis acid.[2] Following this initial discovery, a catalytic approach to carbonyl–olefin metathesis remained elusive for more than four decades. In 2016, however, our group reported the first catalytic carbonyl–olefin metathesis reaction of aryl ketones by employing $FeCl_3$ as the Lewis acid catalyst.[3] Subsequent investigations of this transformation have included an in-depth exploration of the mechanism as well as expansion of the reaction paradigm to include a diverse array of starting

materials and products.[4,5] Since that first report, several other research groups have explored other catalyst systems, the substrate scope, and mechanistic details. While ring-closing COM remains to date the most studied and best developed transformation within this reaction group, several new classes of COM have recently been reported, including ring-opening metathesis, cross-metathesis, and transannular variations (**Scheme 1**). This review will focus on the development of new catalysts for each of these systems as well as the expansion of the substrate scope.

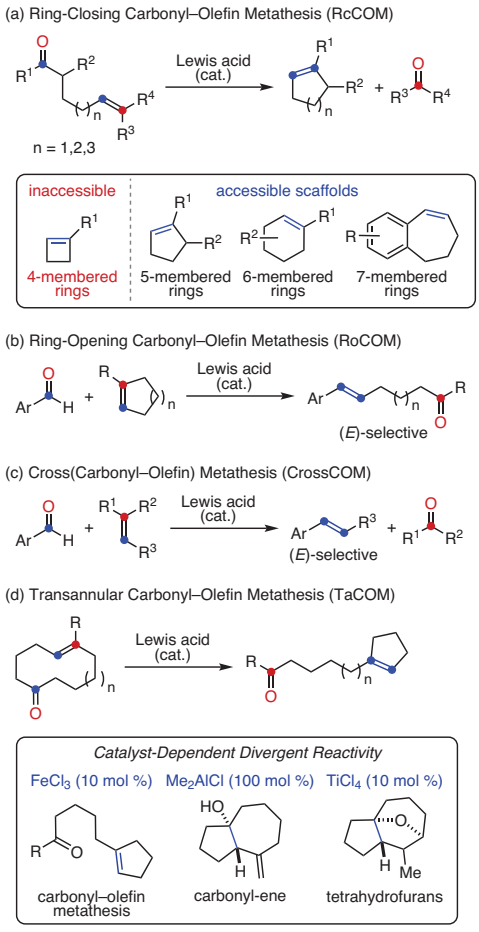
2. Scope of Carbonyls and Olefins Employed

2.1. Carbonyls

As part of our 2017 study on the $FeCl_3$-catalyzed formation of polycyclic aromatic compounds (PACs), our group conducted a thorough investigation of the tolerance of the COM for the carbonyl moiety (**eq 1**).[5] Although the majority of substrates employed in this reaction rely on the use of methyl ketones (R = Me) and aldehydes (R = H), more sterically demanding aliphatic groups, as in isopropyl (R = *i*-Pr, 79% yield) and *tert*-butyl (R = *t*-Bu, 55%) ketones, are also tolerated, providing the metathesis products in moderate-to-good yields. Electron-deficient trifluoromethyl ketones (R = CF_3) and conjugated ketones [R = H_2C=C(Me)] promote the desired transformation in moderate yields, although mild heating is required. β-Ester ketones (e.g., R = EtO_2CCH_2) are selective for reactivity at the ketone moiety, leading to product scaffolds bearing ester functionalities for downstream transformations, while bis-aryl ketones (e.g., R = Ph, 67% yield) are tolerated to provide highly conjugated products.

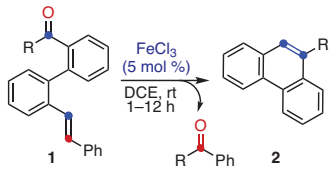
2.2. Olefins

In our similar earlier work, we employed a variety of olefins to determine the scope of alkenes that are suitable for the transformation (**eq 2**).[3] Prenyl-derived olefin (R^1 = R^2 = Me) works best in the reaction leading to nearly quantitative formation of the metathesis product, while the slightly more sterically hindered methyl, phenyl substituted olefin results



Scheme 1. Classes of Carbonyl–Olefin Metathesis (COM).

Carbonyl Scope in the Carbonyl–Olefin Metathesis

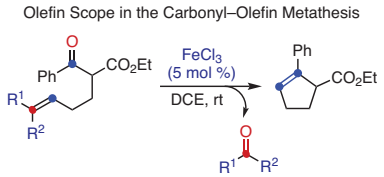


R	Yield of **2**
Me	99%
H	75%
i-Pr	79%
t-Bu[a]	55%
CF_3[a]	52%
H_2C=C(Me)[a]	50%
EtO_2CCH_2	72%
Ph	67%
2-Np	53%

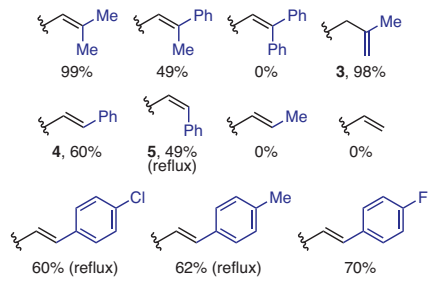
[a] At 50 °C

eq 1 *(Ref. 5)*

Olefin Scope in the Carbonyl–Olefin Metathesis



Starting Alkene Moiety and Yield of the Cyclopentene



eq 2 *(Ref. 3)*

in a diminished yield of 49%. Increasing the sterics further by using the bis-phenyl olefin results in complete shutdown of the reactivity. The chain-extended terminal olefin **3** works exceptionally well, providing the metathesis product in 98% yield through an in situ isomerization to form the prenylated olefin subunit. Both trans- (**4**) and cis-styrenyl (**5**) olefins are compatible with the transformation, providing 60% and 49% yields of the corresponding cyclopentenes, respectively. Crotyl- and allyl-derived olefins do not react, presumably due to the inherent lack of nucleophilic character in each system. Finally, para fluoro-, chloro-, and methyl-substituted styrenyl olefins provide moderate yields (60–70%) of the corresponding cyclopentenes bearing electronically differentiated substituents.

3. Ring-Closing COM Forming 5-Membered Rings

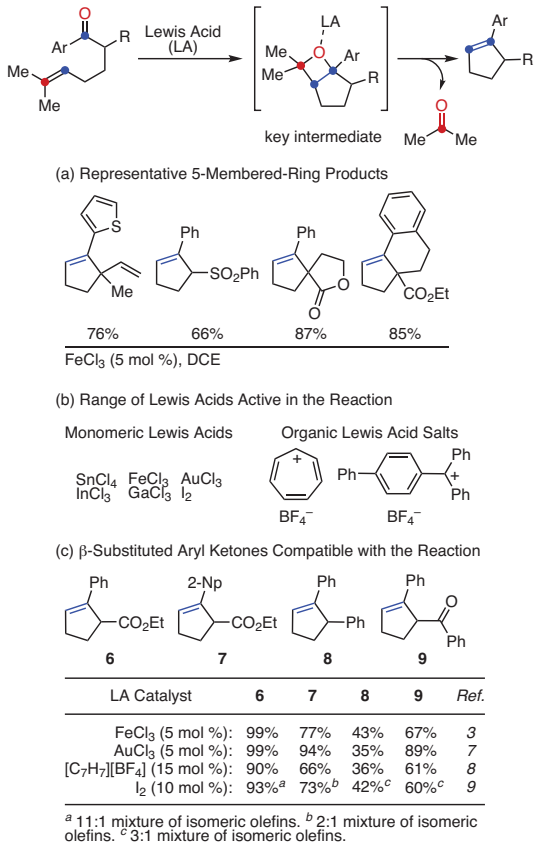
Until recently, strategies for carbonyl–olefin metathesis relied on the use of stoichiometric amounts of Lewis acids.[6,7] These methods often utilized harsh conditions, including the use of $SnCl_4$ and Me_2AlCl, were limited in scope, and proceeded in low-to-moderate yields. In this section, several new catalytic systems developed in our lab as well as by other research groups are described for the Lewis acid catalyzed ring-closing carbonyl–olefin metathesis that forms 5-membered rings. The overall scope of both the catalytic regimes and substrate compatibility are discussed to facilitate the synthetic applications of carbonyl–olefin metathesis.

3.1. Aryl Ketones

In 2016, our group reported the use of catalytic amounts of earth-abundant and environmentally benign $FeCl_3$ to access functionalized cyclopentenes in up to 99% yield through a ring-closing COM strategy.[3] The reaction is proposed to proceed through a [2 + 2] cycloaddition pathway involving an oxetane intermediate (**Scheme 2**).[3,7–9] The overall scope of this $FeCl_3$-catalyzed transformation includes a variety of functional handles such as heteroaromatic groups, quaternary centers, sulfonates, and spirocyclic and polycyclic scaffolds (Scheme 2, Part (a)). Since our seminal finding, other Lewis acids—including $AuCl_3$,[7] tropylium tetrafluoroborate,[8] and molecular iodine[9]—have been found to serve as catalysts for ring-closing COM leading to 5-membered rings (Scheme 2, Part (b)). Specifically, aryl ketones bearing β-carbonyl and β-aryl functionalities can be used as substrates with each of these catalytic systems to access the corresponding metathesis products in up to 99% yield (Scheme 2, Part (c)). Notably, these catalyst systems result in comparable yields across several substrates, although utilizing I_2 as the Lewis acid catalyst often leads to the formation of inseparable diastereomers.

3.2. Pyrroline Synthesis

Pyrrolines have been accessed through Lewis acid catalyzed reactions employing $FeCl_3$, $AuCl_3$, and I_2 (**Scheme 3**).[7,9–11] Li's group has reported the expansion of the $FeCl_3$ catalyzed carbonyl–olefin metathesis to produce *N*-toluenesulfonyl-protected pyrrolines.[10] The method relies on the use of styrenyl-derived olefins, along with superstoichiometric amounts of allyltrimethylsilane to trap the benzaldehyde byproduct and push the reaction to completion. In contrast, our group has reported a strategy for the synthesis of pyrrolines that utilizes prenylated olefins and foregoes the requirement of the silane additive.[11] Specifically, the incorporation of a strongly electron-withdrawing 4-(trifluoromethyl)benzenesulfonyl protecting group (^{F}Ts) on the nitrogen atom lowers its Lewis basic character. This allows for more efficient binding of the Lewis acid to the carbonyl moiety for catalysis, as demonstrated in the synthesis of **10** (50% yield for the Ts group versus 99% yield for the ^{F}Ts group). The starting materials for this transformation can also be rapidly assembled from naturally occurring chiral amino acids, producing in this case enantioenriched pyrrolines under the optimized reaction conditions. We were able to further expand the use of $FeCl_3$ to form pyrrolines stemming from unnatural amino acid derived substrates to produce a wide variety of functionalized molecular scaffolds including heterocycles bearing aryl and allyl groups (**11**, **12**), Lewis basic sites (**13**), and additional heteroaromatic groups (**14**). Molecular iodine has also been employed as a catalyst for the synthesis of pyrrolines, although the scope is limited to glycine-, alanine-, and phenylalanine-derived



Scheme 2. Lewis Acid Catalysts for the Ring-Closing COM of Aryl Ketones. *(Ref. 3,7–9)*

substrates.[9] Additionally, Li demonstrated that glycine-derived compounds undergo this transformation in the presence of catalytic amounts of $AuCl_3$, resulting in pyrroline scaffolds such as **10**.[7]

3.3. Aliphatic Ketones

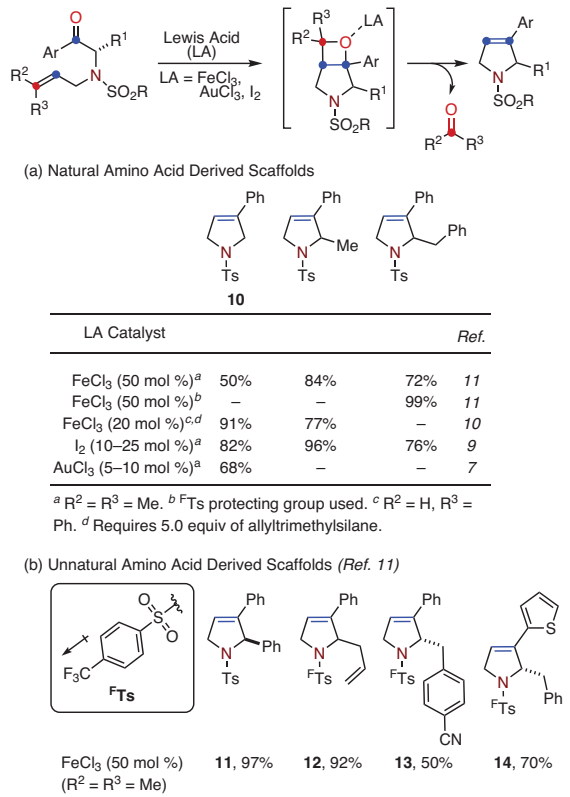
In 2019, our group reported a general protocol for the Lewis acid catalyzed ring-closing COM of aliphatic ketones (**Scheme 4**).[12] To date, this remains the only successful protocol reported that is capable of converting aliphatic substrates to the desired products. The method relies on slightly increased loadings of $FeCl_3$ from 5 mol % to 10 mol %, and proceeds in up to 94% yield. Importantly, kinetic investigations are consistent with a second-order dependence of this transformation on $FeCl_3$;[12] this was subsequently corroborated by EPR, IR, Raman, and DFT studies. The reaction mechanism proceeds via activated intermediate **15** through an in situ complexation requiring a second unit of $FeCl_3$ to form singly bridged homo-dimer **16**, which functions as a Lewis acidic superelectrophile.[13–15] This strategy enables the formation of a variety of trialkyl-substituted cyclopentene scaffolds, including ones with isobutyl and methyl groups at the olefinic carbon. Additionally, the method tolerates electronically differentiated geminal substituents at the α-quaternary center of the cyclopentene ring; including Me,Bn, Me,cinnamyl, and Me,geranyl groups. This strategy also enables the formation of 5,6-fused ring systems in yields of up to 72%. Oxetane **17** was isolated in 52% yield from the corresponding 7-membered-ring precursor, supporting a [2 + 2] cycloaddition as the operative pathway.

4. Ring-Closing COM Forming 6-Membered Rings

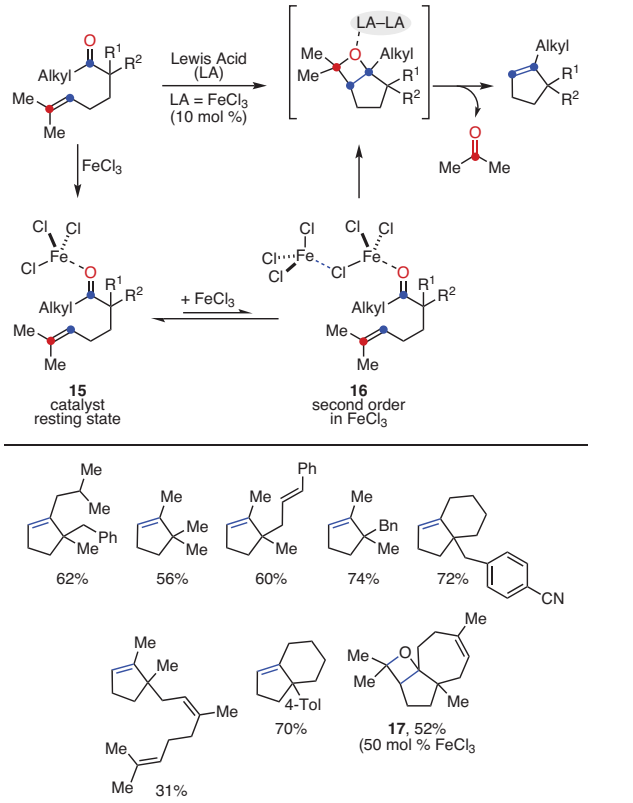
Although several strategies have been reported for the formation of cyclopentenes through ring-closing carbonyl–olefin metathesis, forming the corresponding cyclohexene scaffolds through an analogous approach has remained challenging. This is due in part to the reduced activity of the requisite substrates in the presence of Lewis acids, such as $FeCl_3$. To this end, our group has developed several methods to overcome this limitation, including strategic tuning of substrate electronics and catalyst design. These efforts have enabled access to previously inaccessible cyclic scaffolds.

4.1. Polyaromatic Hydrocarbon Scaffolds

Our group has expanded the use of $FeCl_3$ as a powerful catalyst for COM to include the formation of Polycyclic Aromatic Compounds (PACs).[5] The reaction relies on the use of styrenyl-derived substrates **1** to avoid the competing carbonyl-ene pathways and is driven by the formation of highly conjugated

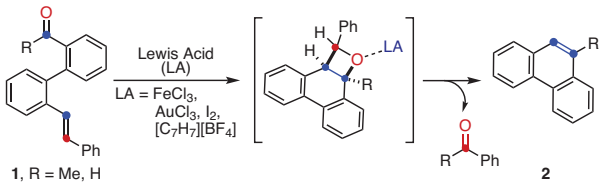


Scheme 3. Ring-Closing COM for Accessing Pyrrolines. *(Ref. 7,9–11)*

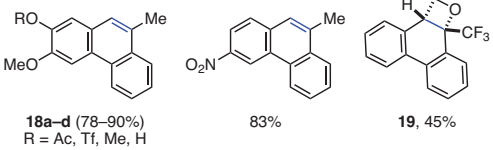


Scheme 4. Fe(III) Homo-dimers Enable COM of Aliphatic Ketones. *(Ref. 12)*

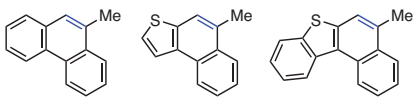
systems (**Scheme 5**).[5,7–9] The PAC products are obtained in up to 99% yield even with relatively low loadings of $FeCl_3$ at just 5 mol %. Furthermore, this strategy enables access to a broad range of metathesis products including scaffolds bearing nitro groups that are generally incompatible with the Lewis acid catalyzed method due to their strong Lewis basicity. Other electronically differentiated phenol-derived structures, such as acetates, triflates, methyl ethers, and hydroxyls were also well tolerated (**18a–d**). Importantly, oxetane **19** (45%) was obtained from the corresponding trifluoromethylated ketone starting material and its isolation provides additional evidence for oxetane formation as the active pathway for this type of metathesis. Our report was followed up by Li group's report of the use of $AuCl_3$ as an alternative Lewis acid for the transformation.[7] Their method can be applied to both crotyl and styrenyl substrates to access compounds **20–22** in good-to-excellent yield (50–95%). Additionally, Nguyen's group reported protocols that employ either tropylium tetrafluoroborate or molecular iodine as catalysts to form product **20** from both crotyl- and prenyl-derived olefins.[8,9] However, both catalysts provide the desired product as an inseparable mixture with the corresponding carbonyl-ene product.



(a) PACs by $FeCl_3$-Catalyzed Carbonyl–Olefin Metathesis *(Ref. 5)*



(b) PACs by Lewis Acid Catalyzed Carbonyl–Olefin Metathesis



LA Catalyst	20	21	22	*Ref.*
$FeCl_3$ (5 mol %):	99%	51%	62%	*5*
$AuCl_3$ (10 mol %):	50%[a]	95%	64%	*7*
I_2 (10 mol %):	94%[b,c]	–	–	*9*
I_2 (10 mol %):	54%[a,d]	–	–	*9*
$[C_7H_7][BF_4]$ (15 mol %):	97%[b,e]	–	–	*8*
$[C_7H_7][BF_4]$ (15 mol %):	49%[a,f]	–	–	*8*

[a] Crotyl-derived substrate used.
[b] Prenyl-derived substrate used.
[c] Isolated as a 2.5:1 mixture with carbonyl-ene product.
[d] Isolated as a 1:1 mixture with carbonyl-ene product.
[e] Isolated as a 2:1 mixture with carbonyl-ene product.
[f] Isolated as a 1.5:1 mixture with carbonyl-ene product.

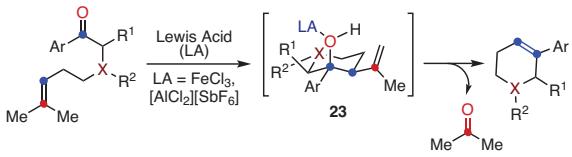
Scheme 5. Polycyclic Aromatic Compounds (PACs) through Ring-Closing COM.

4.2. Cyclohexenes from Aryl Ketones

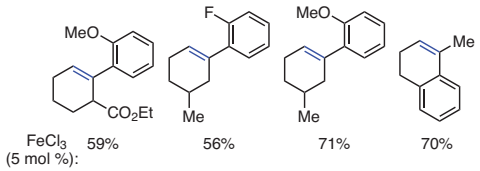
Our group's seminal report on $FeCl_3$ as an active catalyst for ring-closing COM enabled access to cyclohexene scaffolds in moderate-to-good yields (56–71%), although the scope was limited to substrates bearing ortho substituents in the aryl moiety (**Scheme 6**, Part (a)).[3,16] However, general access to these simple scaffolds remained largely unavailable due to the unreactive nature of the starting materials. In 2020, we reported that an Al(III) ion pair catalyst was potent enough to overcome this limitation.[16] Kinetic investigations performed on the system established that this superelectrophilic catalyst promotes metathesis product formation through a novel pathway proceeding via a carbonyl-ene intermediate **23**. This improved protocol enables the facile formation of a broad range of cyclohexene scaffolds including spirocyclic motifs, **24**, structures bearing additional Lewis bases, **25**, and halides, **26**, as well as chroman derivatives such as **27**. Importantly, this system outperforms $FeCl_3$, resulting in significantly improved catalytic activity (Scheme 6, Part (b)).[16]

4.3. Tetrahydropyridines

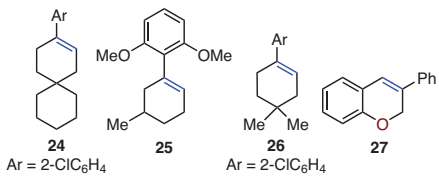
Very recently, we published a general protocol for the synthesis of tetrahydropyridines.[17] Drawing upon insights gained from our reaction protocol for accessing pyrroline scaffolds,[11] this method relies on the use of electron-deficient protecting groups for nitrogen such as ^{F}Ts (**Scheme 7**).[10,17] Amino acids serve as



(a) $FeCl_3$-Catalyzed Formation of Cyclohexenes Limited to Ortho-Substituted Substrates *(Ref. 3)*



(b) Reactivity with Superelectrophile $[AlCl_2][SbF_6]$ *(Ref. 16)*



LA Catalyst	24	25	26	27
$[AlCl_2][SbF_6]$ (10 mol %):	85%	61%	59%	87%

Scheme 6. Carbonyl–ene as an Alternative Active Pathway for Ring-Closing COM.

chiral pool reagents for the rapid assembly of starting materials to form tetrahydropyridines bearing pendant haloaromatic (**29** and **30**) and heteroaromatic (**31**) fragments. Additionally, we demonstrated that the metathesis products can be further functionalized through a one-pot deprotection/reprotection strategy to access diversified products in 92% yield (**32**). As part of their 2017 report, Li's group was also able to access larger N-heterocycles through an $FeCl_3$-promoted strategy.[10] The method, however, relies on stoichiometric quantities of $FeCl_3$ and proceeds with a diminished yield of just 20% of the *N*-^{F}Ts substituted 3-phenyltetrahydropyridine (**28**).

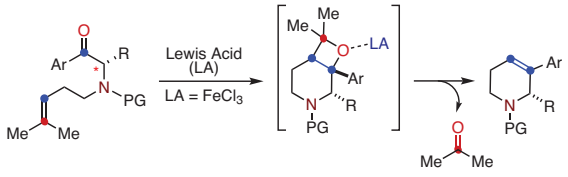
5. Intermolecular COM

5.1. Ring-Opening Systems

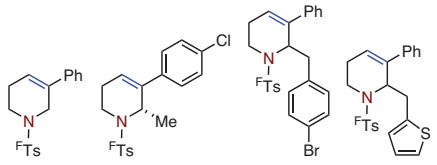
An interesting variant of carbonyl–olefin metathesis is the $GaCl_3$-catalyzed intermolecular ring-opening COM, which we reported in 2018.[18] The method requires four equivalents of aryl aldehyde to proceed and is limited to 5- and 6-membered cyclic olefinic partners (**Scheme 8**).[8,9,18] Mechanistic investigations suggested that the reaction proceeds via a [2 + 2] cycloaddition and subsequent retro-[2 + 2] cycloaddition to form trans olefinic ketones as the exclusive products in up to 47% yield. Mass balance studies revealed that a competing pathway involving a carbonyl–ene reaction and subsequent decomposition is responsible for the diminished yields of the ring-opened COM products. Interestingly, the reaction is not driven by the release of ring-strain, as cycloalkenes with higher ring-strain values do not undergo the desired transformation, whereas 5- and 6-membered rings can be easily converted into the chain-extended aryl ketones. Accessible product scaffolds bearing a pendant group on the benzene ring include aliphatic groups (**34**, **36**, **37**), halides (**36**), and fused rings (**35** and **37**). Additionally, more sterically demanding cycloalkene partners are well tolerated in the reaction paradigm, giving rise to differentially substituted aliphatic ketones such as **38**. Catalytic methods developed by Nguyen also promote intermolecular ring-opening carbonyl metathesis through activation by either molecular iodine or tropylium tetrafluoroborate.[8,9] These two methods are limited to the employment of methyl-substituted cyclic olefins and proceed in yields of up to 59%.

5.2. Cross-Metathesis

A variety of Lewis acidic systems—including $FeCl_3$, molecular iodine, and organic salts—have been employed as catalysts to effect the intermolecular cross(carbonyl–olefin) metathesis (CrossCOM) (**Scheme 9**).[8,9,19,20] Franzén's group reported a general protocol that utilizes trityl tetrafluoroborate as catalyst and aryl aldehydes and trisubstituted olefins as substrates.[19]



(a) Electron-Deficient Nitrogen Protecting Group Facilitates Formation of Tetrahydropyridines via COM

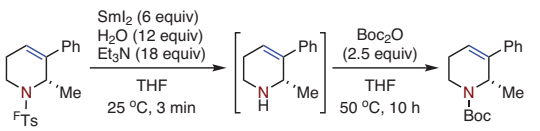


28 **29** **30** **31**

LA Catalyst	28	29	30	31	*Ref.*
$FeCl_3$ (30 mol %):	89%	84%	71%	64%	*17*
$FeCl_3$ (100 mol %)[a]:	20%	---	---	---	*10*

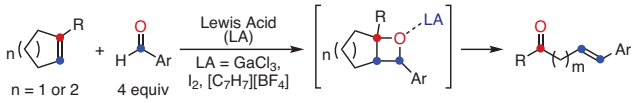
[a] Styrenyl-Derived Substrate and Ts Protecting Group Used.

(b) One-Pot Deprotection–Reprotection Strategy for Accessing Diversified Products *(Ref. 17)*

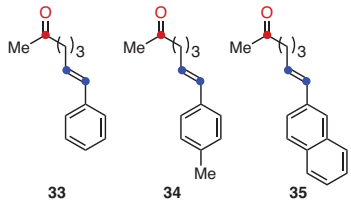


32
92% (2 steps)

Scheme 7. Tetrahydropyridine Scaffolds Accessible through $FeCl_3$-Catalyzed COM.



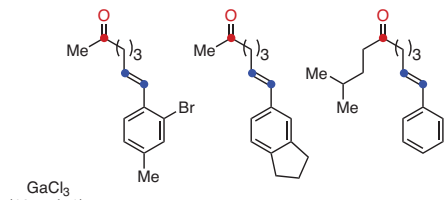
(a) Lewis Acid Effectiveness in the Intermolecular Ring-Opening COM



33 **34** **35**

LA Catalyst	33	34	35	*Ref.*
$GaCl_3$ (10 mol %):	47%	32%	15%	*18*
I_2 (10 mol %):	---	50%	21%	*9*
$[C_7H_7][BF_4]$ (5–15 mol %):	56%	59%	48%	*8*

(b) Sterically Demanding Cycloalkenes Are Tolerated *(Ref. 18)*



$GaCl_3$
(10 mol %): **36**, 39% **37**, 45% **38**, 25%

Scheme 8. Ring-Opening Intermolecular COM.

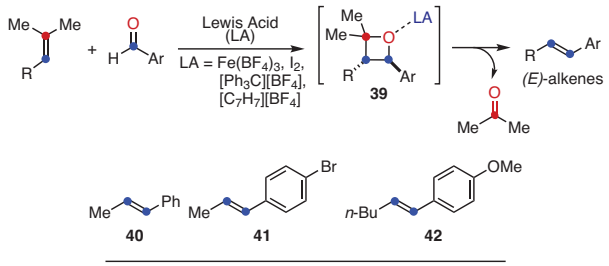
Our group disclosed a method that employs in situ generated $Fe(BF_4)_3$ as an ion pair to generate trans olefins.[20] Similarly, Nguyen's group demonstrated the suitability of molecular iodine[9] and tropylium tetrafluoroborate[8] for CrossCOM. Interestingly, in all cases, the reactions are limited to prenylated olefins and require superstoichiometric amounts of aryl aldehydes (2–5 equivalents) to provide trans-1,2-disubstituted olefins as the exclusive products. Although the starting materials can combine to form four different regioisomeric oxetane intermediates, the reaction proceeds exclusively through trans-oxetane **39** following Lewis acid catalyzed [2 + 2] cycloaddition. Oxetane fragmentation is stereoselective for the (*E*)-olefinic isomer, and independent isomerization studies have revealed that (*Z*) to (*E*) isomerization is unlikely, providing further support for the stereoselectivity.

6. Transannular COM

An interesting, catalytic transannular COM converts steroid-derived scaffolds, e.g. **43** and **44**, into desirable COM cyclopentene products **46** and **47** by utilizing $FeCl_3$ as the Lewis acid catalyst (**Scheme 10**).[21] Mechanistic investigations revealed that the use of other Lewis acids leads to alternative product scaffolds. For example, stoichiometric Me_2AlCl leads to the kinetically favored carbonyl-ene scaffold **45**, while catalytic $SnCl_4$ produces the corresponding tetrahydrofuran **48** through oxetane fragmentation and subsequent intramolecular addition. The $FeCl_3$-catalyzed reaction was demonstrated on a variety of sterically and electronically diverse scaffolds, leading to cyclopentenes bearing pendant acetates (**49**), indenes with both electron-rich (**51**) and electron-poor (**52**) arene substituents, as well as protected ethers (**53**).

7. Heteroatom Tolerance

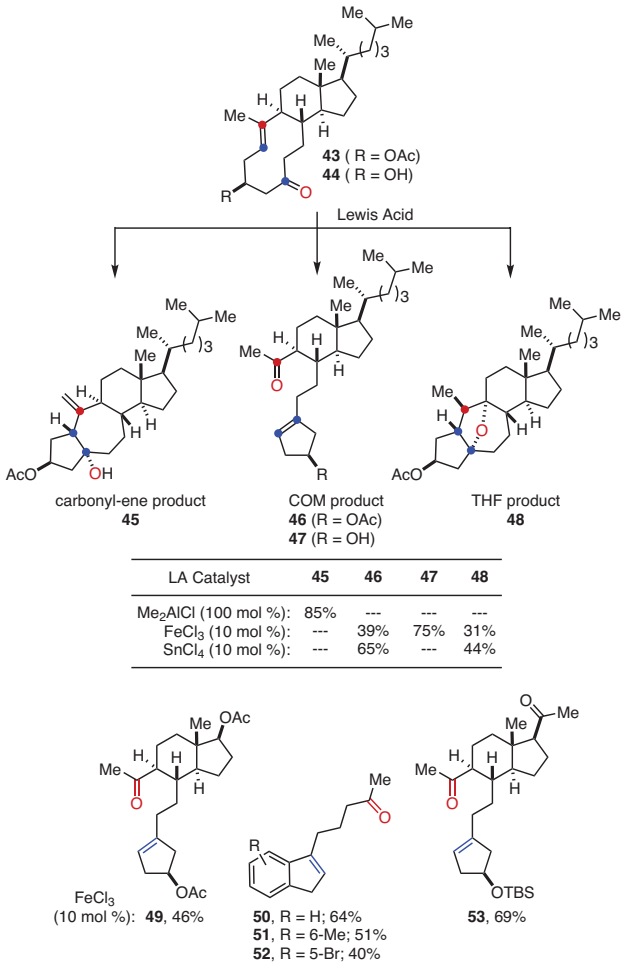
A wide range of heteroatoms, either incorporated, or as pendant substituents, in the cyclopentene/cyclohexene COM product, are tolerated in the $FeCl_3$-catalyzed reaction. These include nitrogen, sulfur, oxygen, and halogen heteroatoms (**Figures 1 and 2**).[3,5,11,17] The incorporation of nitrogen-containing functional groups, which have a strong Lewis basic character and can lead to reaction inhibition in Lewis acid promoted reactions, has seen much improvement over the years. Electron-withdrawing nitro and nitrile groups are well-tolerated, leading to the formation of polycyclic aromatic scaffolds in up to 90% yield (Figure 1, Part (a)).[3,5,11,17] Aryl ketones bearing β-amide groups also result in high yields of the expected, functionalized cyclopentenes (e.g., **54**, 57%).[3] Amines are also tolerated when protected by strong electron-withdrawing groups such as ^{F}Ts (**55**, **56**).[11,17] Similarly well tolerated in a variety of structural systems are halides including fluorine, chlorine, and bromine substituents as well as the trifluoromethyl group (Figure 1, Part (b)).[3,5,11] Oxygenated products, such as pendant lactones, free alcohols, ketones, and esters can be obtained from aryl ketone precursors in up to 87% yield, while oxygen protecting groups



LA Catalyst	40	41	42	Ref.
$Fe(BF_4)_3$ (10 mol %):	51%	51%	---	*20*
$[Ph_3C][BF_4]$ (20 mol %):	68%	55%	---	*19*
I_2 (10 mol %):	50%[a]	---	39%	*9*
$[C_7H_7][BF_4]$ (5–15 mol %):	56%	48%	70%	*8*

[a] 4-Methylbenzaldehyde used as starting aldehyde.

Scheme 9. Intermolecular Cross(Carbonyl–Olefin) Metathesis (CrossCOM).



LA Catalyst	45	46	47	48
Me_2AlCl (100 mol %):	85%	---	---	---
$FeCl_3$ (10 mol %):	---	39%	75%	31%
$SnCl_4$ (10 mol %):	---	65%	---	44%

Scheme 10. Divergent Reactivity of Transannular Systems and Application Scope of the Transannular COM. *(Ref. 21)*

such as triflates, acetates, and ethers are well-tolerated in the formation of highly conjugated polycycles (Figure 2, Part (a)).[3,5] Sulfur-based functional groups such as sulfonyl and sulfonate have been incorporated into COM product scaffolds, while heteroaromatic thiophene and benzothiophene moieties have been successfully incorporated into pyrroline and polyaromatic scaffolds, respectively (Figure 2, Part (b)).[3,5,11]

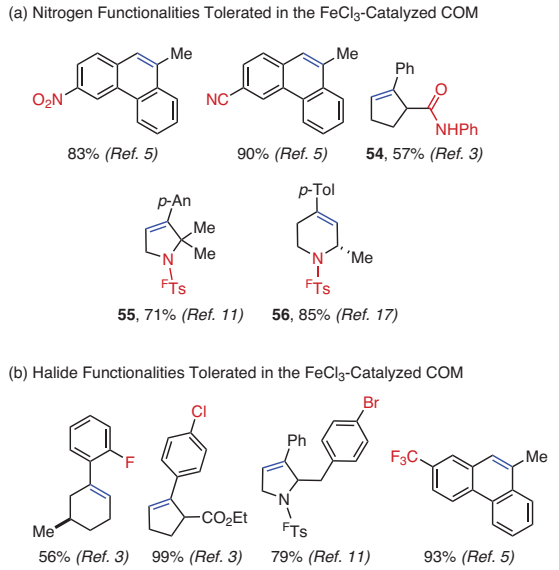


Figure 1. Nitrogen and Halogen Heteroatoms across Different Substrate Classes Tolerated in the $FeCl_3$-Catalyzed COM.

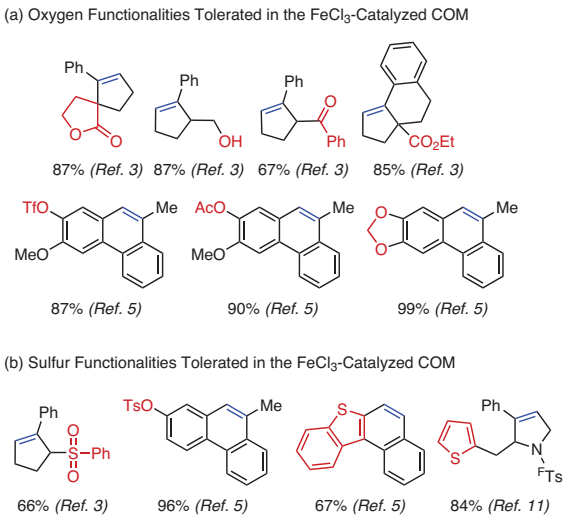
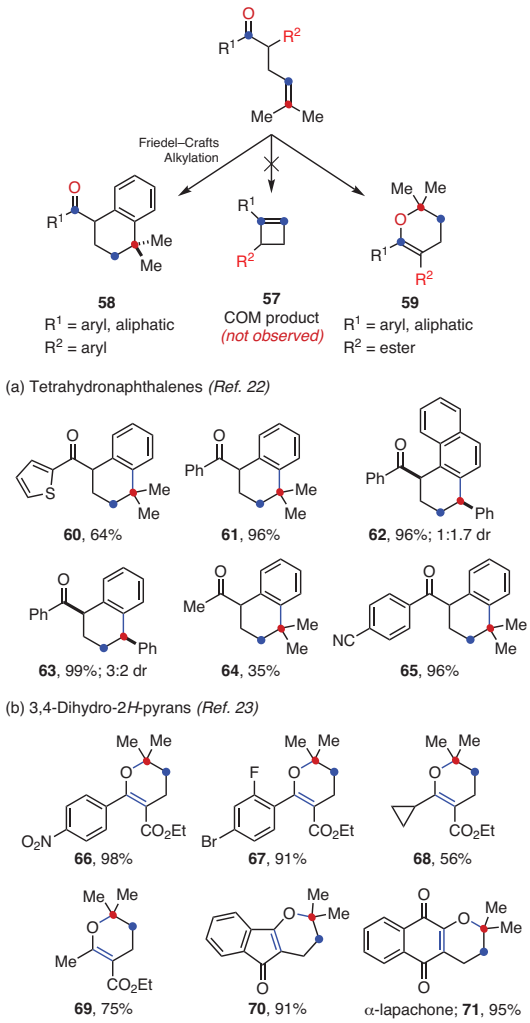


Figure 2. Oxygen and Sulfur Heteroatoms across Different Substrate Classes Tolerated in the $FeCl_3$-Catalyzed COM.

8. Alternative Reactivity for 4,5-Unsaturated Ketone Substrates

Under our standard ring-closing COM conditions, 4,5-hexenones do not yield the expected cyclobutene scaffolds **57**; instead two alternative modes of reactivity are observed (**Scheme 11**).[22,23] When 2-arylhexenones are employed (R^2 = aryl), tetrahydronaphthalene scaffolds **58** can be accessed in up to 99% yield via a Friedel–Crafts alkylation mechanism.[22] Heteroatomic functional groups, such as thiophene **60** and benzonitrile **65** are well-tolerated, resulting in 64% and 96% yield, respectively. Both prenyl (**60**, **61**, **64**, and **65**) and styrenyl (**62**, **63**) olefins are tolerated, although the products arising from styrenes are obtained as mixtures of diastereomers. When alkyl 4,5-hexenones (R^1 = alkyl, R^2 = aryl) are employed as substrates, a significant drop in yield is observed (e.g., **64**, 35%). On the other hand, when R^2 is an ester group—which cannot undergo aromatic substitution—the



Scheme 11. Alternative Product Scaffolds from 4,5-Unsaturated Ketone Substrates under $FeCl_3$ Catalysis (10 mol %).

formation of 3,4-dihydro-2*H*-pyrans **59** is generally observed.[23] Both aryl (**66**, **67**) and aliphatic ketones (**68**, **69**) work exceptionally well in this reaction paradigm, leading to up to 98% yield of the dihydropyran products. 1,3-Diketone systems provide high yields of α,β-unsaturated cyclopentenones, e.g. **70**. Additionally, the method was successfully applied to the synthesis of α-lapachone, **71**, a regioisomer of the clinical anticancer candidate β-lapachone.

9. Mechanistic Considerations

As part of our efforts to expand the understanding of Lewis acid catalyzed carbonyl–olefin metathesis, our group conducted detailed investigations of the underlying mechanisms that form 5- and 6-membered-ring products. A combination of theoretical and experimental studies including synthetic, spectroscopic, kinetic, and computational studies provided data that is consistent with an iron(III)-promoted formation of oxetane intermediate **72** after initial binding of Fe(III) to the carbonyl moiety (**Scheme 12**, Part (a)).[3,24] Oxetane formation was identified as the turnover-limiting step leading to cyclopentene **73**, as determined by an inverse secondary kinetic isotope effect with k_H/k_D = 0.65 ± 0.07. Interestingly, ring-closing strategies for forming 6-membered rings have revealed an alternative pathway (Scheme 12, Part (b)).[16] Kinetic investigations and DFT studies support the reversible formation of carbonyl-ene intermediate **75**, which can either revert back to the activated aryl ketone **74** or proceed forward through hydroalkoxylation to form oxetane **76**.[16] Fragmentation of **76** liberates metathesis product **77** along with the acetone byproduct and regenerates the active catalyst. Interestingly, our studies also support [2 + 2] cycloaddition via **78** as an operative secondary pathway when styrenyl substrates are employed. The substrates lack the β-hydrogens required for carbonyl-ene reactivity, but still furnish the desired metathesis products, albeit in diminished yields. This work is the first demonstration of a carbonyl-ene pathway as a productive route towards metathesis products, rather than a detrimental side reaction.

10. Conclusions

It is evident that carbonyl–olefin metathesis (COM) has developed into an impactful synthetic methodology for accessing a wide variety of cyclic motifs and new carbon–carbon bonds in complex molecular structures from simple and readily available starting materials. The reports highlighted in this review demonstrate the unique ability of Lewis acids to selectively promote carbonyl–olefin metathesis in a catalytic fashion, leading to a broadened substrate scope and excellent yields for both the intra- and intermolecular variants. Although several unique reaction pathways have been reported for the ring-closing COM, they all go through a Lewis acid activated oxetane intermediate which then collapses to yield the metathesis product. We envision that future developments will focus on catalyst design, substrate scope expansion, and mechanistic insight to overcome the current limitations of the methodology. Furthermore, carbonyl–olefin metathesis has the potential to make significant contributions to the area of natural product synthesis and materials chemistry.

11. Acknowledgments

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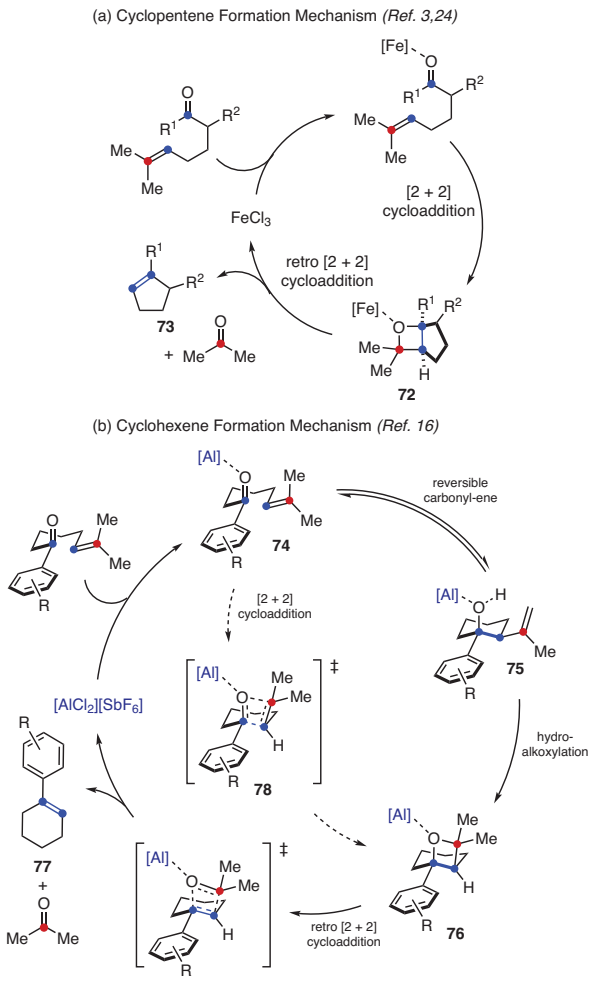

Scheme 12. Alternative Mechanistic Pathways for Carbonyl–Olefin Metathesis.

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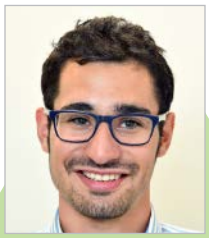
About the Authors

Ashlee J. Davis received her undergraduate degree in 2016 from the University of California, Irvine, under the supervision of Prof. Suzanne A. Blum. She then moved to the University of Michigan in 2017 to pursue a doctoral degree with Prof. Corinna Schindler with a focus on the development and application of Lewis acid catalyzed carbonyl–olefin metathesis transformations. Her research interests include method development, organometallics, and mechanistic investigation.

Jessica L. Gomez-Lopez received her chemistry M.S. degree in 2013 (with Prof. Miguel P. Parra-Hake) and Ph.D. degree in 2016 (with Prof. Valentí n Miranda-Soto) at the Technological Institute of Tijuana. She then joined the Schindler group at the University of Michigan as a postdoctoral fellow. Her research interests include coordination chemistry, organometallics, and method development

Corinna S. Schindler received her undergraduate and M.S. degrees from the Technical University of Munich and her Ph.D. degree in chemistry from ETH Zürich with Prof. Erick M. Carreira (2010). She then completed her postdoctoral studies at Harvard University with Prof. Eric N. Jacobsen in 2013. The development and expansion of Lewis acid catalyzed carbonyl–olefin metathesis reactions have been at the forefront of her research efforts since starting her independent career at the University of Michigan in 2013.

Fluoroalkyl Azides and Triazoles: Unlocking a Novel Chemical Space


Dr. A. Markos


Dr. V. Matoušek


Dr. P. Beier

Athanasios Markos,[a] Václav Matoušek,[b] and Petr Beier*,[a]

[a] Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences
Flemingovo náměstí 2
160 00 Prague, Czech Republic

[b] CF Plus Chemicals s.r.o.
Karásek 1767/1
621 00 Brno, Czech Republic

Email: petr.beier@uochb.cas.cz

Keywords. nitrogen heterocycles; azides; triazoles; cycloaddition; rhodium catalysis; copper catalysis; carbenes; enamides; imidoyl halides.

Abstract. Fluorinated azidoalkanes have emerged recently as novel and surprisingly stable azides. Their easy preparation and commercial availability have opened up new possibilities for their application in organic synthesis. This review is focused on the use of fluorinated azidoalkanes in cycloaddition reactions leading to *N*-fluoroalkyl-1,2,3-triazoles. It also covers the rhodium-catalyzed and acid-mediated transformations of the triazoles to afford a variety of new *N*-fluoroalkyl nitrogen heterocycles and *N*-alkenyl compounds.

Outline

1. Introduction
2. Fluorinated Organic Azides
3. Synthesis of *N*-Fluoroalkyl-1,2,3-triazoles
4. Denitrogenative Reactions of *N*-Fluoroalkyl-1,2,3-triazoles
   4.1. Rhodium-Catalyzed Reactions of *N*-Fluoroalkyl-1,2,3-triazoles
   4.2. Acid-Mediated Transformations of *N*-Fluoroalkyl-1,2,3-triazoles
5. Conclusion
6. References

1. Introduction

Nitrogen-containing compounds; such as amines, amides, azides, and nitrogen heterocycles; are some of the cornerstones of organic chemistry, but their combination with fluorinated groups has not been widely studied. This research area has gained in momentum only recently, driven by the unique physicochemical characteristics of fluorinated molecular structures. For example, in materials chemistry, fluorinated groups allow the tuning of desirable properties.[1,2] In medicinal chemistry and agrochemistry, fluorine atoms and fluorinated groups alter metabolism, bioavailability, and other important drug characteristics, or they open up new reactive pathways.[3–9] The importance of fluorinated groups in medicinal chemistry is illustrated by the 72 drugs containing the CF_3 group approved up to the year 2020 (69 of these drugs contain the CF_3 group bound to carbon and the other three drugs contain the OCF_3 functionality). None of these drugs, however, contain the N–CF_3 functionality (amines or azoles) which may be due to their challenging synthesis.[10] The study of fluorinated nitrogen compounds is expected to bring about new possibilities in the chemistry space. In this article, the recently emerged fluorinated organic azides and especially α-fluorinated ones are reviewed, including their properties and methods of synthesis. This is followed by an overview of their reactivity, focusing on their use in the preparation of *N*-fluoroalkyl-1,2,3-triazoles. These heterocycles serve as excellent starting substrates for the synthesis of novel fluorinated nitrogen heterocycles and *N*-alkenyl compounds such as enamides.

2. Fluorinated Organic Azides

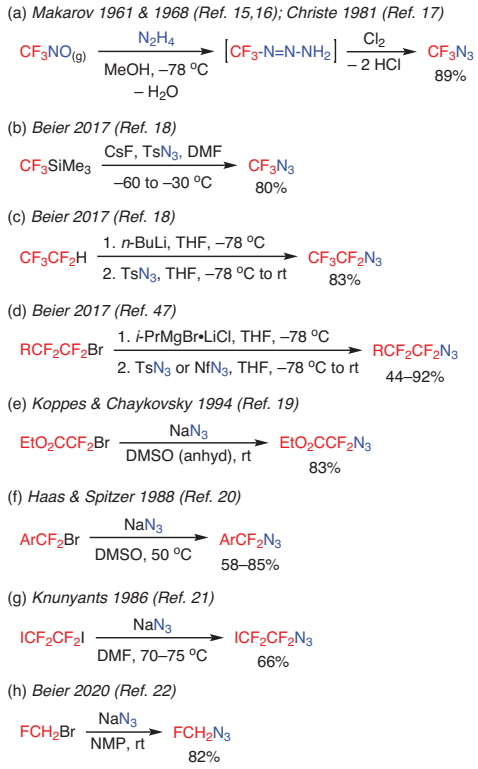
Organic azides are valuable and versatile intermediates in synthesis.[11,12] They serve as amine or nitrene precursors[13] and are indispensable in heterocycle synthesis. More recently, organic azides have found application in materials science, drug discovery, and in biochemistry as ligation reagents or photoaffinity labels.[11] In general, low-molecular-weight azides,

metal azides, and polyazido compounds are unstable and often decompose explosively. In contrast, fluorinated organic azides, in particular α-fluorinated azidoalkanes, have been much less investigated, although their stability is far higher than that of their non-fluorinated counterparts.[14] However, the lack of methods for their synthesis and the concerns over the possible explosive character of unknown fluorinated azidoalkanes have precluded their development and systematic study as reagents in synthesis.

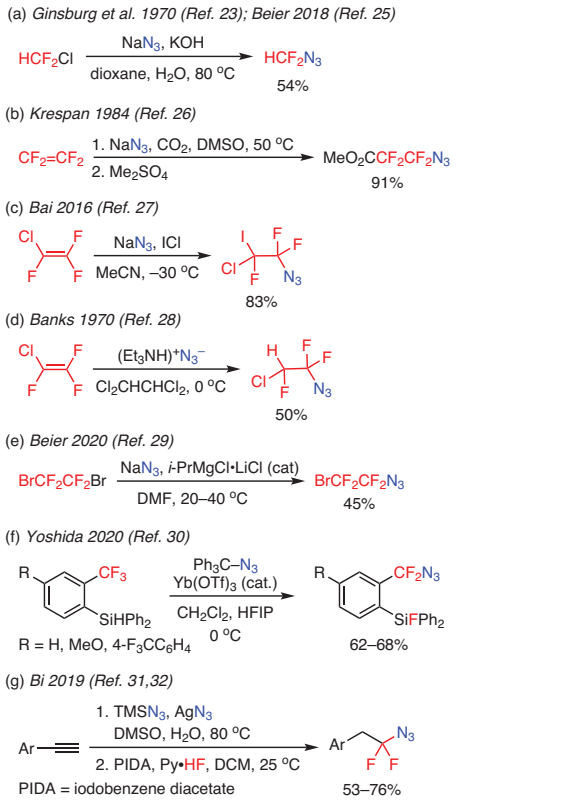
The most important methods for the synthesis of α-fluorinated azidoalkanes are shown in **Schemes 1** and **2**. Azidotrifluoromethane was prepared in two steps from toxic gaseous CF_3NO (Scheme 1, Part (a)).[15–17] A more recent and convenient method starts from the Ruppert–Prakash reagent (CF_3TMS) and its higher analogues, and is based on the nucleophilic fluoroalkylation of electrophilic azide sources, such as tosyl or nonaflyl azides (Scheme 1, Part (b)).[18] Reactions of fluorinated carbanions with electrophilic azides lead to other useful fluorinated azides (Scheme 1, Parts (c) and (d)).[18] In some cases, halide displacement with sodium azide can be performed (Scheme 1, Parts (e)–(h));[19–22] however, this reaction failed with perfluoroalkyl iodides and bromides. Azidodifluoromethane is synthetized via a difluorocarbene as the reactive intermediate (Scheme 2, Part (a)).[23–25] The addition of a nucleophilic azide to fluorinated olefins and quenching of the resulting fluorinated carbanions with suitable electrophiles (CO_2, I^+, H^+) affords the corresponding β-substituted polyfluorinated azidoethanes (Scheme 2, Parts (b)–(d)).[26–28] A variant of the addition of azide to in situ generated tetrafluoroethylene is shown in Scheme 2, Part (e).[29] Here, the starting Halon 2402 ($BrCH_2CH_2Br$) undergoes bromophilic attack with $N_3CF_2CF_2^-$ (generated in situ from reaction of sodium azide with tetrafluoroethylene intermediate) that enables the closing of the catalytic cycle. Lewis acid $Yb(OTf)_3$ catalyzes the unusual defluorination–azidation of 1,2-silyl(trifluoromethyl)arenes with trityl azide (Scheme 2, Part (f)).[30] The reaction shown in Scheme 2, Part (g), is a unique hydroazidation of alkynes, which is followed by *gem*-difluorination with in situ formed $PhIF_2$•HF and then 1,2-aryl migration. The *gem*-difluorination is limited to electron-rich aryl groups, whereas alkyl and electron-poor substituents on the alkyne lead to vicinal difluorination by azide migration to form the regioisomeric 1-azido-2,2-difluoroalkanes.[31,32]

β-Fluorinated organic azides are readily prepared by S_N2 reactions starting from sodium azide and the corresponding triflates or mesylates in aprotic polar solvents[33,34] or by other special methods.[31,35]

One- and two-carbon fluorinated azides are typically very volatile compounds. Their thermal stability has been evaluated by heating their $CDCl_3$ solutions in a sealed NMR tube and observing the possible decomposition by ^1H and ^{19}F NMR



Scheme 1. Synthetic Methods for Forming α-Fluorinated Azidoalkanes.



Scheme 2. Additional Synthetic Methods for Forming α-Fluorinated Azidoalkanes.

spectroscopy. CF_3N_3, HCF_2N_3, and $BrCF_2CF_2N_3$ are stable to at least 150 °C,[18,25,29] while FCH_2N_3 in chloroform solution decomposed at ambient temperature.[22] The decomposition is presumed to take place by nitrogen elimination to form a nitrene, [1,2]-hydrogen shift from carbon to nitrogen, and ultimately fragmentation to form HCN and HF. Some of these azides are now commercially available (**Table 1**). The synthesis and reactivity of α-fluorinated azidoalkanes have been reviewed recently.[14]

3. Synthesis of *N*-Fluoroalkyl-1,2,3-triazoles

The 1,2,3-triazole scaffold occurs in a number of bioactive compounds possessing antitumor, antimicrobial, or antiviral properties.[36] Furthermore, it has been employed in drug design as a bioisostere of amide bonds,[37] in biochemistry as a ligation unit,[38,39] and in materials chemistry, for example, as a promotor of proton conduction in polymer electrolyte membranes.[40] The use of 1,2,3-triazoles in various applications has been greatly accelerated by the development of copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC). Although, CuAAC is limited to terminal alkynes, this variant of the Huisgen [3 + 2] cycloaddition of azides and alkynes is regioselective, insensitive to solvent, and takes place under mild conditions. For its robustness, efficiency, and wide scope CuAAC has become known as the "click reaction".[41–44]

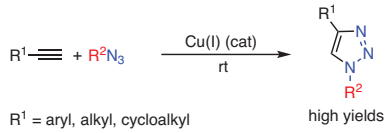
While the thermal [3 + 2] cycloaddition of α,α-difluoroalkyl azides proceeds under harsh conditions with low regioselectivity to give *N*-fluoroalkyl-1,2,3-triazoles,[45,46] CuAAC affords *N*-fluoroalkyl-4-substituted-1,2,3-triazoles in high yields and regioselectivity (**eq 1**).[18,22,25,27,29,47–50]

Functionalized *N*-fluoroalkyl-4,5-disubstituted triazoles are synthesized by reaction of a stoichiometric copper acetylide, an electrophile, and a fluoroalkyl azide (**eq 2**).[18,25,51] In the case of fluoromethyl-, difluoromethyl-, and tetrafluoroethyl-5-iodotriazoles, cross-coupling reactions represent an effective synthetic pathway to *N*-fluoroalkyl-4,5-disubstituted 1,2,3-triazoles,[22,25,47] while with *N*-perfluoroalkyl-5-iodotriazoles the cross-coupling reactions are rather less efficient.[18,51]

Fluorinated azidoalkanes undergo metal-free, enamine-mediated [3 + 2] cycloaddition to form *N*-fluoroalkyl-4,5-disubstituted-1,2,3-triazoles. This reaction works with activated, easily enolizable ketones possessing an electron-withdrawing group at the α carbon (**eq 3**).[22,25,52]

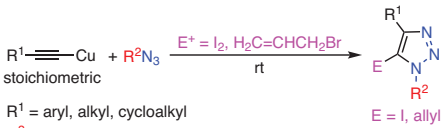
4. Denitrogenative Reactions of *N*-Fluoroalkyl-1,2,3-triazoles

Thanks to the presence of a stabilized aromatic ring, 1,2,3-triazoles are generally thermally and chemically stable compounds. However, certain ring-opening processes of 1,2,3-triazoles do occur. For example, the *N*-phenyl-5-amino-1,2,3-triazole ring system opens when heated and rearranges in a process known as the Dimroth rearrangement (**Scheme 3**, Part (a)).[53] Another group of 1,2,3-triazoles that are amenable



R^1 = aryl, alkyl, cycloalkyl
R^2 = CH_2F, CHF_2, CF_3, C_nF_{2n+1}, CF_2CF_2OAr, CF_2CF_2SAr, CF_2CF_2HetAr, CF_2CF_2Br, $CF_2CF(Cl)I$, $CF_2CF(Cl)SAr$, CF_2CO_2Et, $CF_2CH(OMe)Ar$

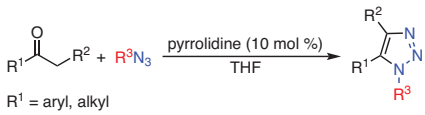
eq 1 *(Ref. 18,22,25,27,29,47–50)*



R^1 = aryl, alkyl, cycloalkyl
R^2 = CH_2F, CHF_2, CF_2CF_3, CF_2CF_2OAr, CF_2CF_2SAr, CF_2CF_2HetAr

E = I, allyl

eq 2 *(Ref. 18,25,51)*



R^1 = aryl, alkyl
R^2 = CN, C(O)R, CO_2R, NO_2, SO_2Ph, $PO(OEt)_2$
R^3 = CH_2F, CHF_2, CF_3, CF_2CF_3

eq 3 *(Ref. 22,25,52)*

Table 1. Boiling Points, Thermal Stability, and Commercial Availability of Selected Fluorinated Azides.

Formula	CAS Registry Number®	Boiling Point	Thermal Stability	Commercial Availability
HCF_2N_3	41796-84-3	8 °C	>80 min at 150 °C	0.5 M in DME
CF_3N_3	3802-95-7	-28 °C	>80 min at 150 °C	0.5 M in THF
$CF_3CF_2N_3$	2055167-74-1			0.15 M in THF
$BrCF_2CF_2N_3$	2476559-31-4	50–52 °C	>80 min at 150 °C	0.5 M in THF
$EtO_2CCF_2N_3$	153755-61-4	117–118 °C		Neat
FCH_2N_3		22 °C	Unstable at rt	
$CF_3CH_2N_3$	846057-92-9	54–55 °C		0.6 M in DME
$HCF_2CH_2N_3$				0.5 M in DME
$HCF_2CF_2CH_2N_3$	846057-93-0	81–82 °C		0.5 M in DME

to ring-opening are the *N*-sulfonyl derivatives, which, in the presence of Rh(II) catalysts, form carbenes that serve as key intermediates in a plethora of transannulations and C–H and X–H insertion reactions (Scheme 5, Part (b)).[54–57]

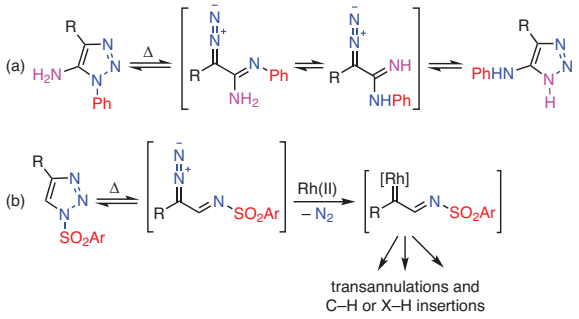
The presence of a fluoroalkyl chain in *N*-fluoroalkyl-1,2,3-triazoles gives rise to unique transformations of these compounds under specific conditions. In this regard, we have recently described rhodium-catalyzed and acid-mediated transformations of *N*-fluoroalkyl-1,2,3-triazoles affording a variety of novel fluorinated heterocycles and useful intermediates for further synthesis.

4.1. Rhodium-Catalyzed Reactions of *N*-Fluoroalkyl-1,2,3-triazoles

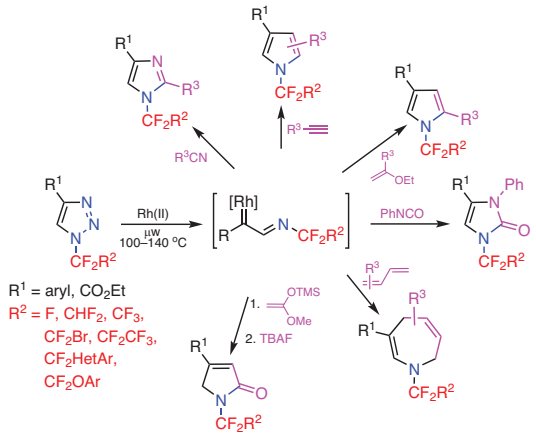
Similarly to *N*-sulfonyl-1,2,3-triazoles, *N*-fluoroalkyl-1,2,3-triazoles[58] undergo various rhodium(II)-catalyzed reactions when heated in a microwave. In this way, previously unknown *N*-fluoroalkyl pyrroles, pyrrolones, imidazoles, imidazolones and azepines can be efficiently prepared (**Scheme 4**).[29,59–61]

Unlike secondary or tertiary *N*-trifluoromethylamines, *N*-trifluoromethyl nitrogen heterocycles, such as pyrazoles or benzimidazoles, are stable towards hydrolysis. These azoles display favorable medicinal chemistry properties, such as increased lipophilicity and Caco-2 permeability, no reactivity with glutathione, and decreased pK_a, which makes them interesting building blocks in drug design.[10] Although *N*-trifluoromethyl azoles are rare compounds,[62,63] this moiety is starting to appear in potential drug candidates. For example, replacing the methyl substituent on nitrogen in the checkpoint kinase 1 (CHK1) inhibitor with the trifluoromethyl group results in suppressed N-dealkylation while maintaining a comparable inhibitory activity.[64]

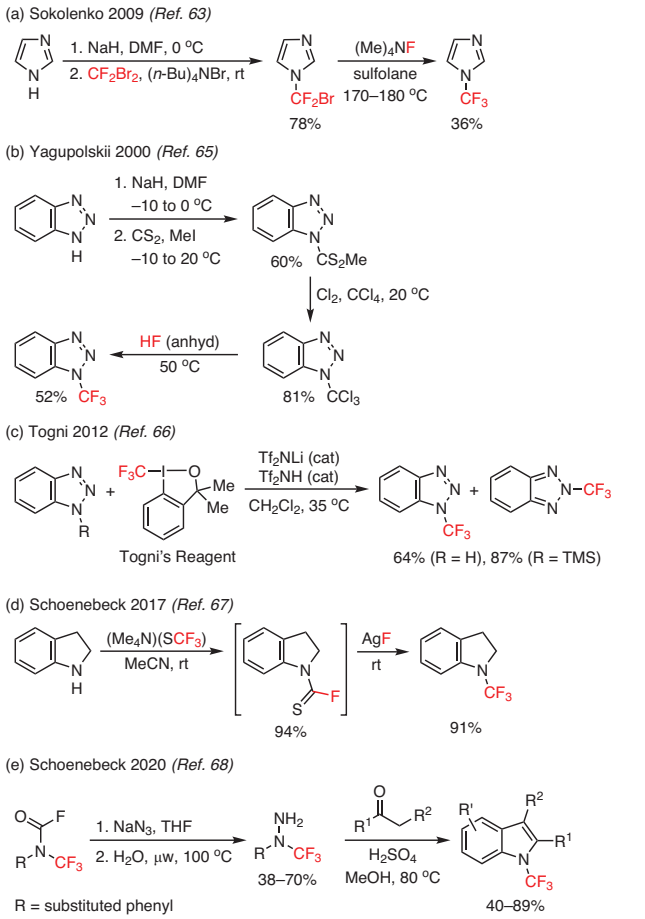
Alternative methods for the synthesis of *N*-trifluoromethyl azoles include: (i) reactions of deprotonated azoles with CF_2Br_2 and subsequent halogen exchange (**Scheme 5**, Part (a)),[63] and (ii) formation of dithiocarbamates, their conversion to *N*-trichloromethyl azoles, and halogen exchange using anhydrous HF (Scheme 5, Part (b)).[65] Direct electrophilic trifluoromethylation of azoles or their *N*-silylated derivatives is not a general reaction and provides a regioisomeric product mixture (Scheme 5, Part (c)).[66] Moreover, secondary amines



Scheme 3. Ring-Opening of 1,2,3-Triazoles. *(Ref. 53–57)*



Scheme 4. Rhodium(II)-Catalyzed Reactions of *N*-Fluoroalkyl Triazoles Leading to the Formation of New Nitrogen Heterocycles. *(Ref. 29,59–61)*



Scheme 5. Alternative Methods for the Synthesis of *N*-Trifluoromethyl Azoles.

have been converted into tertiary *N*-trifluoromethyl amines through the intermediacy of thiocarbamoyl fluorides (Scheme 5, Part (d)).[67] Finally, carbamoyl fluorides react with sodium azide and lead, after Curtius-type rearrangement, to *N*-trifluoromethyl hydrazines. The latter compounds are then used, for example, in the Fisher indole synthesis (Scheme 5, Part (e)).[68]

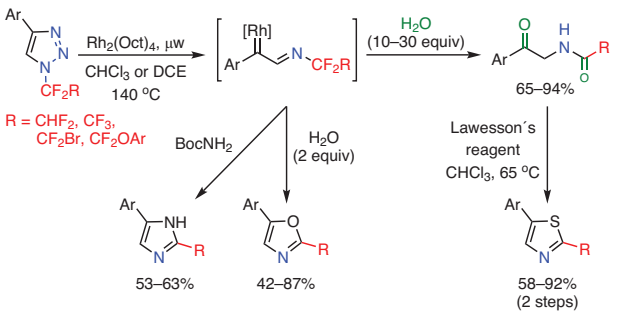
Rhodium(II)-catalyzed tandem ring-opening and defluorinative annulation of *N*-fluoroalkyl-1,2,3-triazoles also represents a general route to 2-fluoroalkyl-1,3-azoles, such as oxazoles, thiazoles, and imidazoles (**Scheme 6**).[29,69] During the process, the nitrogen-bound CF_2 group hydrolyzes and becomes a part of the new five-membered nitrogen heterocycle.

The reactivity of *N*-fluoroalkyl triazoles in rhodium-catalyzed reactions depends on the electronic properties of the fluoroalkyl group as well as on the nature of the substituent at the 4 position in the triazole ring. Their reactivity decreases in the order CF_2CF_3 > CF_3 > CF_2CF_2X > CF_2CF_2H, while *N*-CF_2H triazoles are unreactive. In most of the cases reported, the 4 position of the triazole ring is occupied by aryl groups and, for these, the triazole reactivity increases with increasing electron-donor character of the aryl group, indicating that a cooperative push–pull electronic effect correlates well with the substrate's propensity to undergo a ring opening.

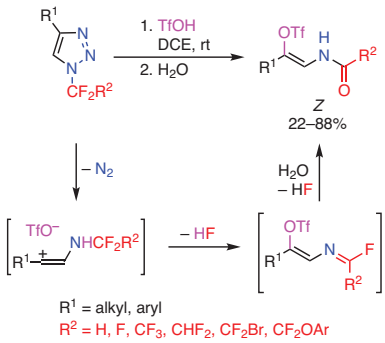
4.2. Acid-Mediated Transformations of *N*-Fluoroalkyl-1,2,3-triazoles

1,2,3-Triazole is both a weak acid (pK_a 9.3) and a weak base (pK_{BH+} 1.2).[70] A similar basicity has been observed for *N*-methyl-1,2,3-triazole, and, upon protonation of the nitrogen at position 3 of the ring with strong acids, *N*-alkyl triazoles form stable salts. However, the behavior of *N*-fluoroalkyl triazoles under strongly acidic conditions is different. Triflic acid mediated denitrogenative transformation led to stable β-enamido sulfonates (**Schemes 7** and **8**).[71] This new triazole ring-opening proceeds at ambient temperature without the need for a metal catalyst and is stereoselective at the C=C bond. Mechanistic investigations and quantum chemical calculations suggested that the reaction proceeds via a vinyl cation.[51] When *N*-trifluoromethyl triazoles were used in the presence of alcohols, (*Z*)-β-enamido carbamates were formed (Scheme 8). Fluorosulfonic acid afforded (*Z*)-β-enamido fluorosulfonates (Scheme 8). In contrast to the rhodium-catalyzed transannulations, these acid-mediated reactions work well even with *N*-difluoromethyl triazoles (but not with *N*-fluoromethyl derivatives).

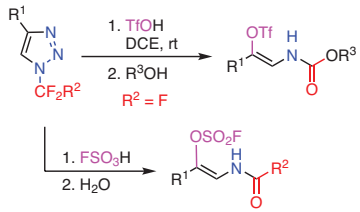
β-Enamido trifates exhibited excellent reactivity in cross-coupling reactions. Suzuki, Sonogashira, and Negishi cross-couplings provided highly functionalized enamides and permitted control of the stereochemistry (**Scheme 9**).[71,72]



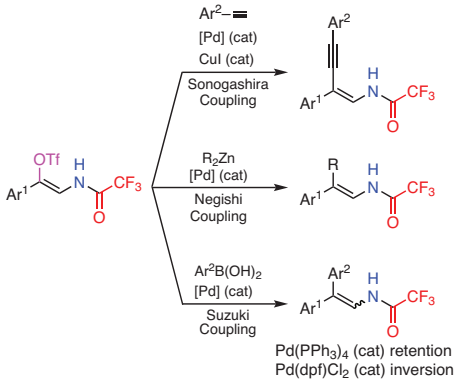
Scheme 6. Tandem Ring-Opening and Defluorinative Annulation of *N*-Fluoroalkyl-1,2,3-triazoles. *(Ref. 29,69)*



Scheme 7. Triflic Acid Mediated Conversion of *N*-Fluoroalkyl-1,2,3-triazoles into (*Z*)-β-Enamido Triflates. *(Ref. 71)*



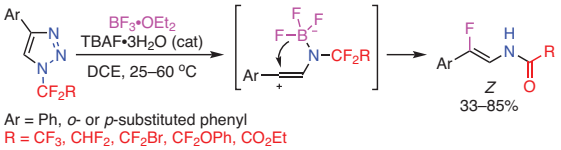
Scheme 8. Triflic and Fluorosulfonic Acid Mediated Transformation of *N*-Fluoroalkyl-1,2,3-Triazoles into Carbamates and (*Z*)-β-Enamido Fluorosulfonates. *(Ref. 71)*



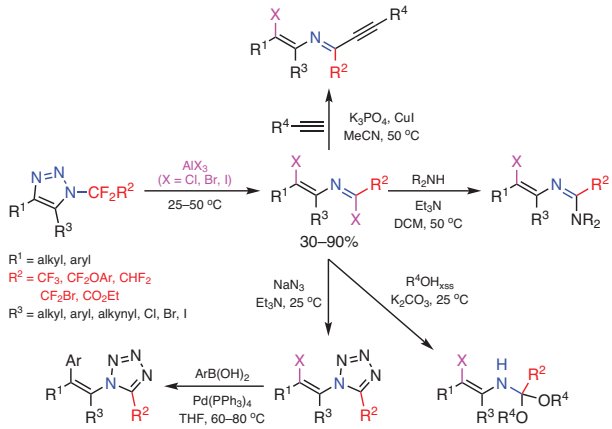
Scheme 9. Cross-Coupling Reactions of (*Z*)-β-Enamido Triflates. *(Ref. 71,72)*

Lewis acids, such as BF_3•OEt_2, promote the opening of the triazole ring in a way similar to that of strong Brønsted acids, again via vinyl cation intermediates. This time, however, vinyl fluorides are formed (**Scheme 10**).[73]

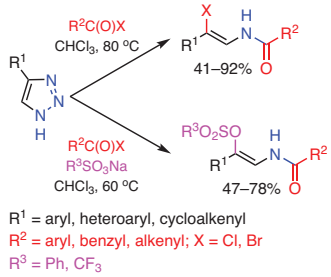
Similarly, aluminum halides (AlX_3, X = Cl, Br, I) mediate the conversion of *N*-fluoroalkyl-1,2,3-triazoles into vinyl halides (**Scheme 11**).[51] In this case, the *N*-difluoromethylene group does not hydrolyze, and imidoyl halides are isolated as stable products. Halide atoms from aluminum are delivered stereoselectively to the carbon atom of the vinyl cation and are exchanged with fluorine atoms of the CF_2 group on nitrogen. The highly functionalized imidoyl halide products are useful building blocks for the synthesis of a variety of stereodefined *N*-alkenyl compounds, can be substituted with nucleophiles, the imidoyl group can undergo cyclization to a tetrazole, or the vinyl halide can engage in cross-coupling reactions. Using this methodology, derivatives of zuclomiphene and enclomiphene drugs containing a halovinyl moiety were prepared.[51]



Scheme 10. BF_3•OEt_2-Mediated Conversion of *N*-Fluoroalkyl-1,2,3-triazoles into (*Z*)-β-Enamido Fluorides. *(Ref. 73)*



Scheme 11. AlX_3-Mediated Conversion of *N*-Fluoroalkyl-1,2,3-triazoles into (*Z*)-Haloalkenyl Imidoyl Halides and Further Transformations of the Latter Compounds. *(Ref. 51)*



Scheme 12. Enamido Halides and Triflates by Acylation of 1,2,3-Triazoles. *(Ref. 74)*

Recently, enamido halides and triflates have been prepared by acylation of 1*H*-1,2,3-triazoles (**Scheme 12**).[74] The reaction of 1,2,3-triazoles with acid halides provided *N*-acyl-1,2,3-triazoles and hydrogen halide, which induced acid-mediated decomposition of the triazoles.

All acid-mediated triazole ring-opening reactions lead to *N*-alkenyl compounds, such as enamides or *N*-alkenyl imidoyl halides, which are versatile synthetic building blocks. The stereoselective transformations of imidoyl halides[75,76] and enamides[77–79] are the key steps in the synthesis of many prominent classes of natural products, pharmaceuticals, and agrochemicals.

5. Conclusion

Fluorinated azidoalkanes are now easily available and mostly stable compounds. They represent a convenient starting point in the synthesis of a wide range of fluorinated nitrogen heterocycles. Cycloaddition leads to *N*-fluoroalkyl-1,2,3-triazoles that can be further transformed using rhodium-catalyzed transannulations, providing access to medicinally interesting heterocycles such as imidazoles, pyrroles, and pyrrolones. Furthermore, the recently reported acid-mediated transformations of *N*-fluoroalkyl-1,2,3-triazoles allow for a novel type of triazole ring-opening and stereoselective formation of *N*-alkenyl compounds such as enamides or *N*-alkenyl imidoyl halides. The synthetic usefulness of these *N*-alkenyl compounds has been demonstrated and goes beyond organofluorine chemistry. Further discoveries in this area; such as reactions of fluorinated azidoalkanes with nucleophilic species, nitrene formation, or strain-promoted cycloadditions; are to be expected.

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About the Authors

Athanasios Markos received his M.Sc. degree in organic chemistry in 2017 from Palacký University of Olomouc, Czechia. In 2021, he obtained his Ph.D. degree in organic chemistry from the Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czechia, under the supervision of Dr. Petr Beier. He is currently a postdoctoral fellow at ETH Zürich, Switzerland, in the group of Prof. Helma Wennemers. His research interests include the development of new synthetic methodology and novel bioorthogonal reactions.

Václav Matoušek received his M.Sc. degree in 2008 from the University of Chemical Technology, Prague, Czechia. He then worked briefly in medicinal chemistry research in the team of Dr. P. Hebeisen at F. Hoffmann-La Roche (Basel, Switzerland) prior to embarking on his Ph.D. studies at ETH Zürich. In 2013, he obtained his Ph.D. degree under the supervision of Prof. Dr. Antonio Togni working in the field of fluoroorganic chemistry. His research at ETH led to the development of novel methodologies for fluoroalkylation as well as the discovery of new substituted hypervalent iodine-fluoroalkyl reagents. In 2014, he founded the ETH spin-off CF Plus Chemicals with the mission to expand the chemical space of fluorinated products for small-molecule drug discovery and to leverage the chemistry of Togni reagents for site-selective protein bioconjugation and for the characterization of protein–protein interactions.

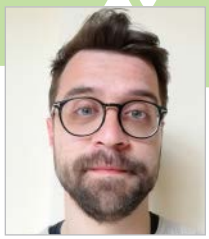
Petr Beier obtained his M.Sc. degree in organic chemistry in 2001 from the University of Pardubice, Czechia, and his Ph.D. degree in organic chemistry in 2004 from St. Andrews University, United Kingdom. After postdoctoral studies at the University of Southern California, Los Angeles, USA (2005–2006), he started his independent career as a research group leader at the Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czechia. His research program focuses on the chemistry of main group elements, methodology development, reactive intermediates, asymmetric synthesis, the investigation of reaction mechanisms, and on organometallic chemistry.

Fluorine Labeling as a Versatile Tool for Probing Nucleic Acid Folding and Interactions by NMR Spectroscopy


Ms. V. Zlínská


Dr. V. Matoušek


Prof. L. Trantírek


Dr. R. Rigo

Vladimíra Zlínská,[a,b] Václav Matoušek,[c] Lukáš Trantírek,[b,*] and Riccardo Rigo[b,*]

[a] Faculty of Science
Masaryk University
Kamenice 5
625 00 Brno, Czech Republic

[b] Central European Institute of Technology
Masaryk University
Kamenice 5
625 00 Brno, Czech Republic

[c] CF Plus Chemicals s.r.o.
Karásek 1767/1
621 00 Brno, Czech Republic

Email: lukas.trantirek@ceitec.muni.cz and riccardo.rigo@ceitec.muni.cz

Abstract. This mini-review provides an overview of approaches to ^{19}F-labeling of nucleic acids. A special attention is paid to applications of ^{19}F-labeled nucleic acids to resolve their polymorphism and characterize their folding and interactions with ligands and proteins in vitro and in living cells by using NMR spectroscopy.

Keywords. ^{19}F NMR spectroscopy; ^{19}F probes; DNA; RNA; ^{19}F-labeling approaches; nucleic acid folding; nucleic acid/small molecule interactions; nucleic acid/protein interactions; in vitro; in vivo.

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3. Applications of ^{19}F NMR Spectroscopy to the Nucleic Acid Field
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1. Introduction

Nucleic acids undergo complex conformational equilibria in the cell. Depending on their primary sequence, they can fold into a variety of structures besides the canonical double helix. The most studied are tetrahelices such as G-quadruplex or i-Motif (occurring at G- and C-rich strands, respectively), triplexes, and hairpins.[1] Each type of DNA secondary structure is further influenced by environmental factors such as pH, molecular crowding, and the presence of cations. Likewise, specific interactions with proteins and small molecules regulate nucleic acid folding, which leads to the fine-tuning of essential biological processes such as replication, DNA repair, and gene expression.[2,3] In this perspective, the characterization of the thermodynamic equilibria of nucleic acids assumes a pivotal role.

Many biophysical methods; such as circular dichroism spectroscopy, calorimetric approaches, and surface plasmon

resonance (SPR); have been exploited for assessing nucleic acid folding and interactions in vitro.[4–6] However, evaluating a complex mixture containing multiple species using these methods can be challenging. In many cases, a further step of data deconvolution is required, and the final output may not provide detailed structural information.[7,8] To overcome this limitation, ^{19}F NMR approaches have taken hold in the nucleic acids field in the last decades. Fluorine has several beneficial physical and chemical properties that can be taken advantage of to analyze the conformations of biomolecules: The ^{19}F nucleus has a nuclear spin of ½ and a natural abundance of 100%. Moreover, it is only 17% less sensitive than the most sensitive NMR active nucleus, ^{1}H .[9] The ^{19}F chemical shift is very sensitive to changes in the local van der Waals environment and electrostatic fields.[10] Thus, even minimal conformational changes could lead to significant chemical shift variation. When compared to the chemical shift range of ^{1}H, the ^{19}F chemical shift range is 100-fold larger. As a result, the ^{19}F NMR spectra are generally well resolved and simple to analyze. These features render the ^{19}F nucleus as an efficient probe for characterizing nucleic acid structures by NMR spectroscopy.

This short review summarizes the recent developments in the ^{19}F labeling of nucleic acids and their ^{19}F NMR applications in the study of nucleic acid folding and interactions.

2. Labeling Strategies for the Preparation of Fluorinated Oligonucleotides

Despite fluorine being one of the most abundant elements in nature, it is underrepresented, if not absent, in biological systems.[11] This feature makes ^{19}F NMR spectra of biomolecules easy to interpret since the background signal is very low. The ^{19}F nucleus, however, must first be inserted into the macromolecule of interest by using exogenous sources and a labeling step. Nowadays, modified nucleotides are incorporated into nucleic acids by utilizing standardized approaches and, in general, fluorinated oligonucleotides are synthesized using either chemical or enzymatic reactions.[12] A commonly employed chemical approach involves the preparation of ^{19}F-modified nucleobases that subsequently serve as building blocks for the solid-phase nucleic acid synthesis. A great variety of ^{19}F modifications can be introduced with comparable efficiencies into the nucleic acid of interest. Moreover, solid-state synthesis allows the site-specific labeling of the oligonucleotide sequence thus enabling the characterization of different regions in the nucleic acid structure. Another chemical approach consists of conjugating the ^{19}F probe with the oligonucleotide after the solid-phase synthesis step. Different reactivities can be exploited in the conjugation process, and click-chemistry methods are worthy of note for their high yields and simple setups.[13]

As an alternative to solid-state chemical synthesis, the enzymatic approach is utilized to synthesize longer oligonucleotides. Nevertheless, this remains the least used method for the preparation of fluorinated oligonucleotides. One of its disadvantages is that the enzymatic approach does not allow the site-specific labeling of sequences. Moreover, only a limited number of ^{19}F-modified nucleobases are substrates of common DNA or RNA polymerases and, thus, can be incorporated into the newly synthesized nucleic acid.[14–16] Only developments in polymerase engineering could overcome this limitation.[17]

Depending on the labeling strategy employed, ^{19}F nuclei can be incorporated into the oligonucleotide at the base, the sugar, or the 3′ or 5′ ends. However, one should keep in mind that the position and type of modification introduced can significantly influence the stability and folding of the ^{19}F-labeled DNA/RNA structure.

2.1. ^{19}F-Labeling at the Nucleobase

The incorporation of ^{19}F nuclei in the nucleobase represents the most exploited approach for ^{19}F NMR applications, and these modifications comprise the most diverse set of ^{19}F-labeled structure types (**Figure 1**). One of the first reported types of base modification incorporates the ^{19}F nucleus at position 5 of the pyrimidine ring (**1**, **2**). When introduced into a canonical DNA duplex, this modification directs the fluorine towards the major groove and results in minimal impact on the thermodynamic stability, binding properties, structure, and function of the DNA.[18,19] The ^{19}F nucleus can also be introduced at position 2 of the purine. For instance, 2-fluoroadenine (2F-Ada, **3**) showed marginal thermodynamic and structural destabilization when incorporated into a short RNA.[20] However, fluorination at position 2 can interfere with C-2 interactions, preventing the assembly of the RNA tertiary structure.[21] Two ^{19}F-labeled derivatives, the 5-fluorobenzofuran **4** and 2-(3-fluorophenyl)benzo[*d*]oxazol-5-amine **5**, were independently developed as dual-probes that are capable of simultaneously detecting variations in the chemical shift of ^{19}F NMR spectra and in the fluorescence intensity.[22,23] Fluorinated base analogue *N*-(deoxyguanosin-8-yl)-7-fluoro-2-aminofluorene **6** and 2,4-difluorotoluene **7** were also successfully incorporated into an oligonucleotide and proved to be useful ^{19}F sensors.[24–27]

The discovery of new applications for ^{19}F NMR spectroscopy has required the development of new probes that can provide increased signal intensity at low oligonucleotide concentration. Some examples of efficient probes containing three or more chemically equivalent ^{19}F nuclei include: 5-trifluoromethylated pyrimidines **8** and **9**,[28] 8-trifluoromethylated purines **10** and **11**,[29,30] 2′-deoxy-*N*^{4}-(6-aminopyridin-2-yl)cytidine derivatives **12** and **13**,[31] 2,2,2-trifluoroacetophenone **14**,[32] 3,5-bis(trifluoromethyl)benzene derivatives **15** and **16**,[33] and 4,4,4-trifluoro-3,3-bis(trifluoromethyl)but-1-yne **17**.[34]

2.2. ^{19}F-Labeling at the Sugar

Fluorination at position 2′ of the ribose is a common method for oligonucleotide labeling (**Figure 2**, probes **18**, **19**, **21**). 2′-F-ribose **18** and 2′-F-arabinose **19** are among the first modifications incorporated into an oligonucleotide. They both increase the stability of the glycosidic bond, but they differ in their influence on the sugar conformation. **19** promotes the 2′-endo sugar conformation and stabilizes the B-form duplex, while **18** favors

the 3′-endo sugar conformation and stabilizes the A-form typical for RNA duplexes.[35,36] Li et al. incorporated a 4′-F-uridine, **20**, into RNA oligonucleotides and observed that this modification predominantly leads to North-type ribose puckering.[37]

Aiming to increase the sensitivity, Himmelstoß et al. introduced a 2′-*O*-trifluoromethyl group (2′-OCF_3, **21**) into an RNA hairpin. However, bulky substituents at 2′ tend to be oriented towards the narrow minor groove, thus causing structure destabilization.[38] Similarly, the incorporation of a 2′-trifluoromethylthio group (2′-SCF_3, **22**) significantly increased the ^{19}F-probe sensitivity and had the same influence on structure stability as the 2′-OCF_3 group.[39–41] Furthermore, novel probes carrying three chemically equivalent ^{19}F nuclei have also been described by Granqvist and Virta.[42] 2′-*O*-(4-CF_3-triazolylmethyl)uridine, **23**, and 4′-*C*-(4-CF_3-triazolylmethyl)-thymidine, **24**, moieties were introduced via click chemistry at position 2′-O and 4′, respectively, and one other moiety, 4′-*C*-(4-trifluoromethylphenyl)uridine, **25**, at position 4′ of the sugar. These modifications did not significantly influence the stability of the nucleic acid structure.

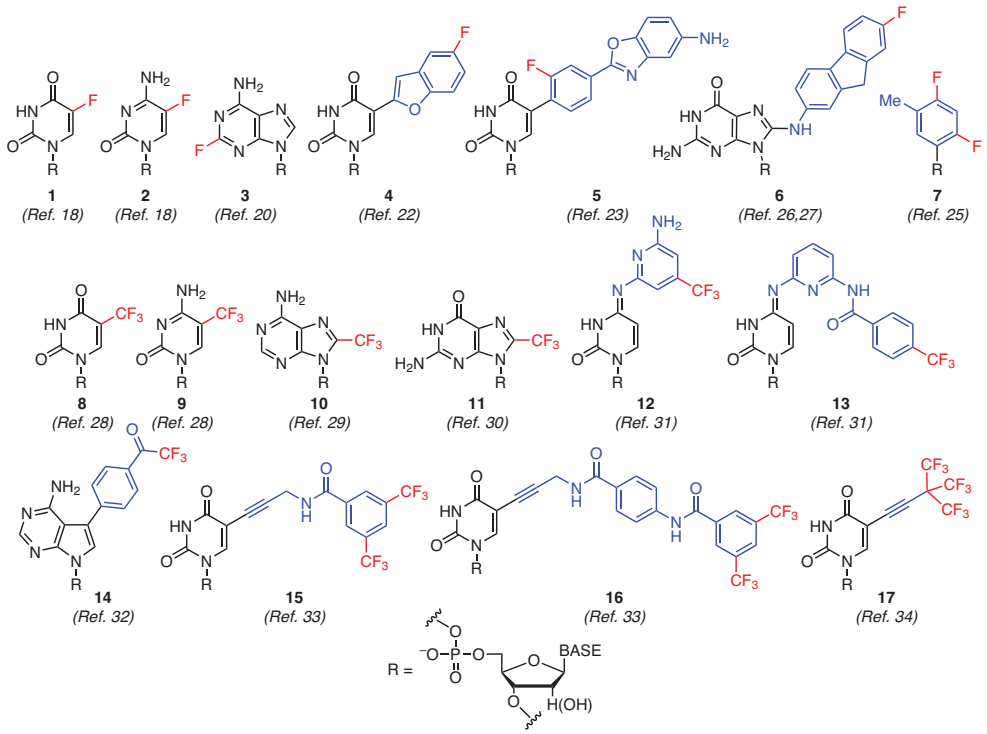

Figure 1. ^{19}F Modifications at the Nucleobase Developed for ^{19}F NMR Applications.

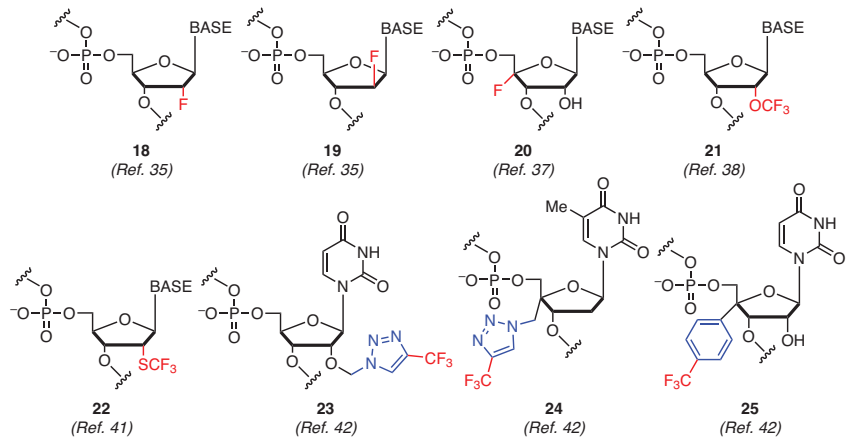

Figure 2. ^{19}F Modifications at the Sugar Moiety Employed in ^{19}F NMR Spectroscopy.

2.3. ^{19}F-Labeling at the 3′ or 5′ End

Labeling an oligonucleotide at the terminal end represents a simple alternative to complicated synthetic methods. Multiple reports on the conjugation of the 3,5-bis(trifluoromethyl)-phenyl moiety via different linkers and chemical reactions have been published (**Figure 3**, probes **26**–**29**). It was found that incorporation of the 3,5-bis(trifluoromethyl)phenyl moiety into the oligonucleotide results in only minor structural changes.[43–47] Bao and Xu applied the same synthetic approach to incorporate a 3-(trifluoromethoxy)phenyl moiety (**30**) at the 5′ end of a DNA oligonucleotide with similar efficiency.[47] It is also worth mentioning that Baranowski et al. developed a novel synthetic method for substituting ^{19}F for the 5′ terminal oxygen, and this fluorophosphate modification proved to be a versatile tool for ^{19}F NMR studies (**31**–**33**).[48,49]

3. Applications of ^{19}F NMR Spectroscopy to the Nucleic Acid Field

^{19}F NMR spectroscopy has been extensively applied in quantitative studies of the interactions between fluorinated small molecules and macromolecules.[50–53] Developments in labeling approaches have permitted the extension of this technique to various aspects of the nucleic acid field. In the last decades, ^{19}F NMR spectroscopy has shown tremendous flexibility in characterizing the structure and function of DNA and RNA macromolecules. In the following paragraphs, we report on new relevant applications of ^{19}F NMR spectroscopy and provide some selected examples.

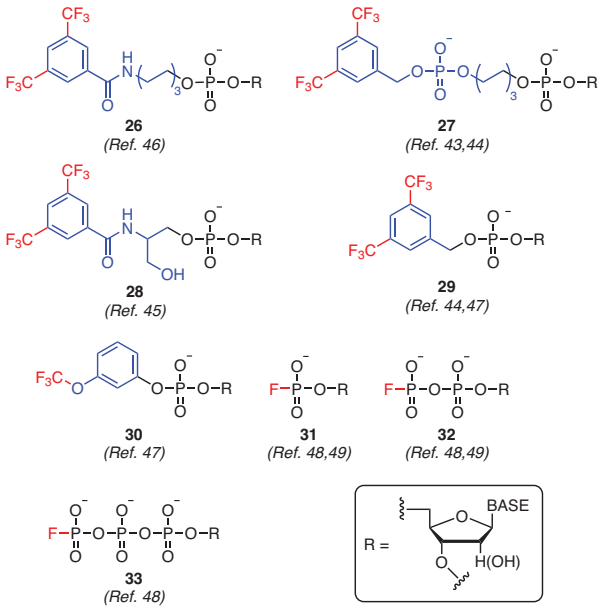

Figure 3. ^{19}F Modifications at the 5′ End for Use in ^{19}F NMR Spectroscopy.

3.1. Characterization of Nucleic Acid Folding Equilibria by ^{19}F NMR Spectroscopy

The simple interpretation of 1D ^{19}F NMR spectra has turned this technique into a powerful tool for studying nucleic acid folding in a complex mixture. As a first application, ^{19}F labels were used for probing hybridization processes and many research groups independently demonstrated that ^{19}F probes could report on changes in the nucleic acid local microenvironment associated with base pairing with the complementary strand. For instance, Sigurdsson and co-workers have reported that the introduction of a nucleoside carrying a perfluorinated *tert*-butyl group (Figure 1, **17**) into a short oligonucleotide allows discrimination between single-stranded and double-stranded DNA.[34] Moreover, they were able to follow the thermal denaturation of the double-strand form by using ^{19}F NMR. Another fluorine-modified nucleobase analogue (**7**) of uridine was successfully used to monitor the complex folding equilibria of a short RNA oligonucleotide where single-strand, double-strand, and hairpin forms were in mutual exchange.[25] A similar system was studied by utilizing an enzymatic approach for incorporating fluorine-modified nucleotides, **5**, in the selected DNA oligonucleotide.[23] Bistable DNA and RNA hairpins, where two hairpin forms convert one into another, were also employed as model systems to demonstrate the ability of ^{19}F probes to detect minor structural differences in nucleic acid folding.[38,40,54–56] Micura and co-workers were even able to distinguish between a stem-loop hairpin and the formation of a compact pseudoknot by using 2′-SCF_3 modified RNA.[41]

Intriguingly, besides studies on systems in which canonical duplexes are in equilibrium with single-stranded forms, fluorine has also been utilized to probe hybridizations involving non-canonical base pairings. For example, Tanabe's group was able to discriminate between a perfect DNA duplex from mismatched and bulged structures by introducing a modified uridine with a 3,5-bis(trifluoromethyl)benzene unit (**15**, **16**) in the oligonucleotide under study.[33] Moreover, many research groups have independently applied ^{19}F probes to characterize the formation and thermal denaturation of DNA triplexes.[31,42,57,58]

In addition, ^{19}F NMR spectroscopy has allowed the elucidation of complex biological processes. In this context, Virta and co-workers have analyzed the steps of RNA strand invasion by utilizing two different ^{19}F probes (**17**, **24**) and using TAR RNA from HIV-1 as a model.[58,59] Moreover, the conversion from B- to Z-DNA has been studied by employing ^{19}F NMR. Bao et al. introduced a trifluoromethyl-guanosine probe (**11**) into an oligonucleotide to observe the B- to Z-form transition in vitro and in mammalian cells.[60] Kowalska and co-workers monitored i-motif formation and stability by incorporating **31** and **32** at the 5′ end of a short C-rich oligonucleotide.[49]

^{19}F NMR has also significantly impacted the characterization of G-quadruplex structures, whose polymorphic nature in many cases prevents any detailed structural analysis with standard techniques. Xu's research group, among others, has characterized the folding features of telomeric RNA and

DNA G-quadruplexes both in vitro and in the cell by using oligonucleotides bearing a 3,5-bis(trifluoromethyl)phenyl group at the 5′ end (**27**).[43,44] This probe proved to be versatile and permitted discrimination between different G-quadruplex conformations. It was even employed to assess the G-quadruplex molecularity, allowing dimers to be distinguished from monomers. Additionally, the same group, but with a shorter linker, was attached at the 5′ end (**29**) and it allowed the monitoring of the denaturation processes as well as the formation of DNA/RNA hybrid G-quadruplex.[47]

3.2. Evaluation of Nucleic Acid–Ligand/Protein Interactions by ^{19}F NMR Spectroscopy

Due to the sensitivity of ^{19}F to structural variations, interactions of ions, small molecules, or proteins with nucleic acids can be detected as chemical shift changes in ^{19}F NMR spectra.

In 2011, Sakamoto et al. developed a thrombin-binding aptamer labeled at the 5′ end with a 3,5-bis(trifluoromethyl)-benzyl group (**27**) and validated this probe as a valuable and selective K^+ ion detector.[46] Introducing two 5-fluorouracil modifications (**1**) into a riboswitch, Lilley and co-workers quantitatively studied its interaction with Mg^{2+} ions.[61]

Fluorination of adenines at position 2 (**3**) in a riboswitch oligonucleotide allowed the characterization of its interaction with the ligand by ^{19}F NMR.[62] It is worth pointing out that this modification did not produce significant perturbation of the ligand-binding activity of the riboswitch. In 2021, Krafčík et al. labeled the telomeric G-quadruplex at the 5′ end using the 3,5-bis(trifluoromethyl)benzyl moiety (**27**) and tested a small library of G-quadruplex binders both in vitro and in the living cells.[63] Mitoxantrone was tested by Kowalska and co-workers as an i-motif binder by introducing **31** at the 5' terminus of the C-rich telomeric strand.[49] Konrat, Micura, and co-workers replaced the 2′-OH group at different positions within a short hairpin RNA with a fluorine atom (**18**) and mapped the interaction of this modified macromolecule with different ligands, namely tobramycin, streptomycin, and flavine mononucleotide.[64] Furthermore, Micura's group tested a novel 2′-SCF_3 modification (**22**) on a riboswitch oligonucleotide sensitive to tobramycin and monitored the rearrangement of its secondary structure after ligand binding.[39]

The 2′-SCF_3 modification (**22**) was further tested for RNA–protein interactions by studying the binding of a domain of the ribonucleoprotein U1A to an ^{19}F-labeled stem–loop RNA.[39] Moreover, the RNA–peptide interaction was further evaluated by solid-state NMR experiments using the ^{19}F label at position 2′ of the ribose (**18**).[65] The incorporation of two 5-fluorocytosines (**2**) into a short DNA duplex allowed characterization of the interaction between HhaI methyltransferase and its DNA substrate.[66] Another ^{19}F label that is utilized for analyzing DNA–protein interactions is 8-trifluoromethyl-2′-deoxyguanosine (**11**). Xu and co-workers incorporated this probe into a Z-DNA and studied its interaction with the Z_α domain of ADAR1 protein.[30] Probe **32** was inserted into a thrombin-binding aptamer forming a G-quadruplex structure to analyze its interaction with thrombin.[49] Plavec, Zhou, and co-workers evaluated the enzymatic activity of RNase H2 by introducing **20** into a short duplex.[37]

3.3. Application of ^{19}F-Labeling to in-Cell NMR Spectroscopy

^{19}F-labeling enables the detection of different nucleic acid conformations within a complex mixture by NMR. In this respect, it represents a valuable and complementary tool to in-cell ^{1}H NMR spectroscopy. In 2017, Xu and co-workers introduced a 3,5-bis(trifluoromethyl)benzyl group (**27**) at the 5′ terminus of telomeric RNA and demonstrated the predominant formation of two subunits stacked G-quadruplexes in living cells of *Xenopus laevis* oocytes.[43] The dual-probe 5-fluorobenzofuran (**4**) was attached at position 5 of 2′-deoxyuridine, and it allowed observation of the telomeric DNA forming a hybrid-type and a parallel topology of G-quadruplex in *Xenopus laevis* oocytes.[22] To study G-quadruplex structural topologies in human cells, Bao et al. attached a 3,5-bis(trifluoromethyl)benzyl moiety (**27**) at the 5′ terminus of telomeric DNA and investigated its structure in HeLa cell line. Their studies revealed the formation of two-hybrid type and two-tetrad antiparallel DNA G-quadruplex structures in the human cell environment.[44] The same research group further investigated the stability of DNA–RNA-hybrid G-quadruplex in human cells by using a similar ^{19}F probe (**27**) attached at the 5′ end of the RNA and the 3-(trifluoromethoxy)phenyl group (**30**) at the 5′ end of the DNA. The outcome proved that DNA–RNA-hybrid G-quadruplex can be formed in the environment of HeLa cells.[47] In 2020, Xu's group incorporated 8-trifluoromethyl-2′-deoxyguanosine (**11**) into the DNA to investigate the stability of Z-DNA in human cells.[30] ^{19}F in-cell NMR spectroscopy was recently applied to the screening of G-quadruplex binders and it showed that in vitro DNA–small–molecule complex stability does not always reflect its stability in the living cells.[63]

4. Conclusion and Future Opportunities

^{19}F NMR spectroscopy is a powerful and versatile tool that can provide detailed information about multiple aspects of nucleic acid folding and interactions both in vitro and in living cells. The interpretation of the simple spectra enables the study of complex folding equilibria without the requirement of complicated data analysis and deconvolution. Despite the promises of this approach, ^{19}F NMR spectroscopy still presents several limitations in this context. For instance, and to the best of our knowledge, no single ^{19}F probe has been shown to be applicable in any system and under any condition. Thus, the optimal experimental settings must be selected on a case-by-case basis. Moreover, most of the ^{19}F probes or precursors are not commercially available. Another limitation has to do with the balance between resolution and sensitivity. For example, probes **18** and **19** show excellent peak dispersion and resolution in ^{19}F NMR spectra that easily distinguish among different nucleic acid conformations; however, their single ^{19}F nucleus does not provide the strong signal intensity necessary for many ^{19}F NMR applications. On the other hand, many probes

containing more ^{19}F nuclei, which boosts their sensitivity, show low signal dispersion. Developing novel ^{19}F probes and new labeling approaches is much needed to overcome these limitations. In this way, the application of ^{19}F NMR spectroscopy in the field of nucleic acids could be fully exploited.

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About the Authors

Vladimíra Zlínská received her bachelor's degree in biochemistry in 2020 from Masaryk University. She is currently completing her master's degree in genomics and proteomics and plans to enroll in a Ph.D. program at Masaryk University. The research project she is presently involved in focuses on the characterization of nucleic acids by using ^{19}F NMR spectroscopy.

Václav Matoušek obtained his M.Sc. degree (2008) from the University of Chemical Technology, Prague, Czechia. He then worked briefly in medicinal chemistry research in the team of Dr. P. Hebeisen at F. Hoffmann-La Roche AG (Basel, Switzerland) prior to embarking on his Ph.D. studies at ETH Zürich. In 2013, he obtained his Ph.D. degree under the supervision of Prof. Dr. Antonio Togni, working in the field of fluoroorganic chemistry. His research at ETH led to the development of novel methodologies for fluoroalkylation as well as the discovery of new substituted hypervalent iodine–fluoroalkyl reagents. In 2014, he founded the ETH spin-off CF Plus Chemicals with the mission to expand the chemical space of fluorinated products for small-molecule drug discovery and to leverage the chemistry of Togni reagents for site-selective protein bioconjugation and for the characterization of protein–protein interactions.

Lukáš Trantírek received his Ph.D. degree in organic chemistry in 2001 from Masaryk University. He then trained as a postdoctoral fellow in the laboratory of Prof. Juli Feigon at the University of California, Los Angeles, and in the laboratory of Prof. Norbert Müller at the Johannes Kepler University in Linz, Austria. Between 2009 and 2015, he worked as a principal investigator at the University of Utrecht in the Netherlands. He joined the Central European Institute of Technology in 2012 and is presently a senior research group leader and associate professor of biomolecular chemistry. Among his awards, he is especially proud of his 2009 NWO VIDI and 2021 Rudolf Lukes Award for his contribution to organic, bioorganic, and medical chemistry. His research focuses on understanding the chemistry of nucleic acids in cells and on in-cell NMR spectroscopy.

Riccardo Rigo received his M.Sc. degree in medicinal chemistry in 2014 from the University of Padova. He obtained his Ph.D. degree in molecular sciences from the same

institution under the supervision of Prof. Claudia Sissi, where he focused on the biophysical characterization of nucleic acid folding in vitro. In 2019, he carried out postdoctoral studies on TET2 dioxygenase and its involvement in mast cell activation with Drs. Erinn Soucie and Patrice Dubreuil at the Research Cancer Centre of Marseille (CRCM). He then joined Professor Trantirek's research group at the Central European Institute of Technology (CEITEC) in Brno, where he studied epigenetic and environmental factors affecting nucleic acid folding in living cells. He is currently establishing his research group at the University of Padova. His main research interest is deciphering the role of nucleic acid structures in biological processes.

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