Perfluoroaryl Azides: Reactivities, Unique Reactions and Their Applications in the Synthesis of Theranostic Agents

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Cheap, functional, reliable things (reactions) unleash the creativity of people who then build stuff (applications) that you could not imagine.

G. M. Whitesides, 2014
Abstract

The work centers around perfluoroaryl azides (PFAAs), and their ability to undergo certain fast and robust transformations. The chemistry was further applied for biomedical applications.

The first section focuses on the azide-aldehyde-amine cycloaddition using PFAAs. Experimental and computational investigations uncovered a fast azide-enamine cycloaddition to form triazolines, which spontaneously rearrange into stable amidine products. In addition, this transformation was explored in the formulation of pure nanodrugs. Because this reaction can introduce a phenyl and a perfluoroaryl moiety enabling supramolecular interactions near the antibiotic drug, the resulting ciprofloxacin derivatives formed nano-sized aggregates by precipitation, which displayed aggregation-induced emission for bacterial imaging as well as enhanced size-dependent antibacterial efficacy.

In the second section, the high electrophilicity of PFAAs was explored to transform azides to aryl amides. The reactivity of PFAAs in the thioacid/azide reaction was studied. In addition, PFAAs were discovered to react with phenylacetaldehyde to form aryl amides via an azide-enol cycloaddition, similar to the perfluoroaryl azide-aldehyde-amine reaction. This strategy of amide synthesis was furthermore generalized through a combination of base-catalyzed azide-enolate cycloaddition reaction and acid- or heat-promoted rearrangement of triazolines.

The last section describes a type of azide fluorogens whose fluorescence can be switched on by a light-initiated intramolecular nitrene insertion into a C-H bond in the neighboring aromatic ring. These fluorogenic structures were efficiently accessed via the direct nucleophilic aromatic substitution of PFAAs.

Keywords: perfluoroaryl azides, click chemistry, dipolar cycloaddition, enamine, triazoline, amidine, aggregation-induced emission, pure nanodrugs, aryl amide, thioacid/azide reaction, nucleophilic aromatic substitution, azide-masked fluorophore, nitrene insertion.
Abbreviations

AIE  Aggregation-induced emission
CuAAC  Copper-catalyzed azide-alkyne cycloaddition
DBU  1,8-Diazabicyclo[5.4.0]undec-7-ene
DEU  N,N-Diethylurea
DG  Directing group
DLS  Dynamic light scattering
DMA  N,N-Dimethylacetamide
DMAP  4-Dimethylaminopyridine
DMEU  1,3-Dimethyl-2-imidazolidinone
DMPU  1,3-Dimethyltetrahydropyrimidin-2(1H)-one
D-A  Donor-acceptor
E. coli  Escherichia coli
ee  Enantiomeric excess
eq.  Equation
equiv.  Equivalents
E_T  Molar transition energy
FMO  Frontier molecular orbital
h  Hour(s)
HOMO  Highest occupied molecular orbital
HPLC  High-performance liquid chromatography
HRMS  High-resolution mass spectrometry
HTS  High-throughput screening
H_Abs  Hydrogen abstraction
H-shift  Hydrogen shift
ISC  Intersystem crossing
LUMO  Lowest unoccupied molecular orbital
MIC  Minimum inhibitory concentration
NHC  N-Heterocyclic carbene
NMP  N-Methyl-2-pyrrolidone
NMR  Nuclear magnetic resonance
NOESY  Nuclear Overhauser effect spectroscopy
<table>
<thead>
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<th>Definition</th>
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<tr>
<td>NPs</td>
<td>Nanoparticles</td>
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<tr>
<td>PAL</td>
<td>Photoaffinity labeling</td>
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<td>PALM</td>
<td>Photoactivated localization microscopy</td>
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<tr>
<td>PBS</td>
<td>Phosphate-buffered saline</td>
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<tr>
<td>PDI</td>
<td>Polydispersity index</td>
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<td>PFAA</td>
<td>Perfluoroaryl azides</td>
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<tr>
<td>PNDs</td>
<td>Pure nanodrugs</td>
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<tr>
<td>r.t.</td>
<td>Room temperature</td>
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<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
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<tr>
<td>S_NAr</td>
<td>Nucleophilic aromatic substitution</td>
</tr>
<tr>
<td>SPAAC</td>
<td>Strain-promoted azide-alkyne cycloaddition</td>
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<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
</tr>
<tr>
<td>TMU</td>
<td>Tetramethylurea</td>
</tr>
<tr>
<td>TS</td>
<td>Transition state</td>
</tr>
<tr>
<td>UV/UV-vis</td>
<td>Ultraviolet/Ultraviolet-visible</td>
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<tr>
<td>vol %</td>
<td>Volume percentage</td>
</tr>
<tr>
<td>$\xi$</td>
<td>Zeta-potential</td>
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List of Publications

This thesis is based on the following papers, referred to in the text by their Roman numerals I-VI:

I. 1,3-Dipolar cycloaddition reactivities of perfluorinated aryl azides with enamines and strained dipolarophiles
   Sheng Xie,# Steven A. Lopez,# Olof Ramström,* Mingdi Yan,* and K. N. Houk*
   J. Am. Chem. Soc. 2015, 137, 2958-66

II. Design and synthesis of theranostic antibiotic nanodrugs that display size-dependent antibacterial activity and luminescence
    Sheng Xie, Sesha Manuguri, Nanjing Hao, Yang Zhang, Juan Zhou, Olof Ramström,* and Mingdi Yan*
    Manuscript

III. Anilide formation from thioacids and perfluoroaryl azides
     Sheng Xie,# Ryo Fukumoto,# Olof Ramström,* and Mingdi Yan*
     J. Org. Chem. 2015, 80, 4392-7

IV. $N,N$-Diethylurea-catalyzed amidation between electron-deficient aryl azides and phenylacetaldehydes
    Sheng Xie, Olof Ramström,* and Mingdi Yan*
    Org. Lett. 2015, 17, 636-9

V. Base-catalysed synthesis of aryl amides from aryl azides and aldehydes
   Sheng Xie, Yang Zhang, Olof Ramström,* and Mingdi Yan*
   Manuscript

VI. Photo-activatable switch-on fluorescence through intramolecular C-H insertion of azide derivatives
    Sheng Xie, Olof Ramström,* and Mingdi Yan*
    Manuscript

# These authors contributed equally
Paper not included in this thesis:

VII. **Perfluoroaryl azide-aldehyde-amine cycloaddition for surface and nanomaterial functionalization**
Sheng Xie, Juan Zhou, Xuan Chen, Na Kong, Gerry Hammer, David G. Castner, Olof Ramström,* and Mingdi Yan*
*Manuscript.*

VIII. **Metal-free carbohydrate immobilization on nanoparticles using perfluoroaryl azide-based azide-aldehyde-amine cycloaddition**
Na Kong, Sheng Xie, Juan Zhou, Margarita Menéndez, JaeHyeung Park, Dolores Solís, Olof Ramström,* and Mingdi Yan*
*Manuscript.*

IX. **Quantitative fluorine NMR (19F qNMR) to determine carbohydrate density on glyconanomaterials synthesized from perfluoroaryl azide-functionalized silica nanoparticles by click reaction**
Na Kong, Juan Zhou, JaeHyeung Park, Sheng Xie, Olof Ramström,* and Mingdi Yan*

X. **Lipase-catalyzed kinetic resolution of 3-phenyloxazolidin-2-one derivatives: cascade O- and N- acylations**
Yang Zhang, Yan Zhang, Sheng Xie, Mingdi Yan,* and Olof Ramström*
*Submitted*

XI. **Enzyme- and ruthenium dynamic kinetic resolution for asymmetric synthesis of 5-substituted N-aryloxazolidinons involving cascade acylations**
Yang Zhang, Sheng Xie, Mingdi Yan,* and Olof Ramström*
*Manuscript*

XII. **Dynamic covalent chemistry of aldehyde enamines: Sc(III) and Bi(III) catalysis of enamine exchange**
Yang Zhang, Sheng Xie, Mingdi Yan,* and Olof Ramström*
*Manuscript*
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Chemistry plays an integral role in bridging fundamental physics with biology, nanoscience, xeno-sciences and applied sciences.\textsuperscript{1-4} With unique capabilities of manipulation of molecules and matter, chemistry has the power to practically solve many global problems and advance our fundamental understanding in science and technology as a whole.\textsuperscript{5,6} These many advances have been seen, for example in genome sequencing, drug discovery/chemotherapy, fuels/food production and environmental protection.\textsuperscript{1-4} In this era, chemistry – a subject studying the composition, structure, properties and change of matter – is expanding its mission from “pure molecules”, to “everything that is composed of molecules”.\textsuperscript{6} This gives new opportunities, yet challenges, in particular to chemists engaging in the development of new molecular transformations.

The unique capability of synthetic chemists – manipulation of molecules/atoms – is performed through chemical reactions. Organic synthesis is a mature discipline where reliable rules and reactivity are well established.\textsuperscript{7} This development has led to an ever expanding range of structures for different applications. In addition, synthetic methodologies have enabled access to some of the most complicated molecules ever discovered in nature, generally prepared by different routes compared to the biosynthetic pathways.\textsuperscript{7}

The use of chemical reactions can furthermore be very different from compound synthesis. An interesting area is to establish reaction-based functional applications, in similar ways as within living systems, so that functions or properties can be established through chemical transformations. This line of thought has led to a well-established field called “chemical biology”, the use of chemical methods to study biological problems. This idea has also rapidly expanded at the scientific interfaces, such as reaction-based devices utilized in areas of computing, medicine and energy production.\textsuperscript{8}

\textit{The most fundamental and lasting objective of synthesis is not production of new compounds, but production of properties.}

G. S. Hammond, 1968

\section*{1.1. From reactions to functions}

Chemistry intersects with other scientific disciplines through molecular events at both the molecular and supramolecular levels. Thus, the “function” of a chemical transformation could be either properties emanating from any starting materials, intermediates or products, or the transformation itself interpreted as an informational event. The uses of a reaction are therefore dependent on its
performance. Chemical approaches have led to a variety of established reaction-based applications, such as conjugation, labelling,9 and sensing.10

Besides the required, specific functions, most of the appreciated chemical reactions that are used to address multidisciplinary problems are also of high reliability. First, a robust reaction contributes to the reliability of the entire system. Secondly, reliability is necessary for practical purposes. These features can be exemplified by “click” chemistry11 and “bioorthogonal chemistry”.12 “Click” chemistry refers to a set of robust, selective reactions (e.g., CuAAC)13 for efficient construction of substances from small modular units.11 The concept of using reliable reactions to make connectivity has also been greatly expanded in conjugation applications.14-20 “Bioorthogonal chemistry” additionally features reliable ligation that can proceed inside of living systems without interfering with native biochemical processes.12 An example of this is strain-promoted azide-alkyne cycloaddition (SPAAC).21,22 In the words of chemistry, these chemical reactions possess one or a few characteristics:

- Fast rates
- High yields
- Chemo-selectivity
- Orthogonality (to physical, biological processes…)

New, reliable transformations are therefore of great interest, in view of the fact that popular chemical approaches are highly limited to a few well-known transformations. However, although perhaps lacking in general reliability, many chemical transformations have practical uses, resulting in new possibilities in manipulation of molecules and matter. In this context, it is encouraging that the selectivity and reactivity of a transformation can be improved by design, such as in the case of SPAAC.

1.2. Organic azides

Organic azides have gained an important role at the interfaces of chemistry, biology, medical and material sciences.23,24 In particular, azide-mediated coupling reactions, which exhibit excellent chemo-selectivity and high reactivity, have been employed in a wide range of applications.15,25-27 In addition, organic azides are easy to handle, stable, and orthogonal to physiological processes.

1.2.1. General reactivities

Azides are a unique class of compounds: energy-rich with high physical lability as seen in explosives, but yet with remarkable chemical inertness.23 Due to the many possible polar resonance structures (Fig. 1a), organic azides have chemical reactivities which can be grouped in several distinctive modes of transformations (Fig. 1b). The azide can undergo reaction by nucleophilic
attack of the inner nitrogen on an electrophile, addition of a nucleophile on the slightly electrophilic outer nitrogen or these processes can occur simultaneously resulting in a formal cycloaddition reaction. Alternatively, extrusion of N₂ generates a nitrene.

\[
R-N_3 \rightleftharpoons [R-N=N=\ddot{N}] \rightleftharpoons [R-N=N=N] \rightleftharpoons [R-N=N\equiv\ddot{N}]
\]

Figure 1. (a) Resonance structures of azides. (b) Reactivity of organic azides.²³

1.2.2. Azide-based coupling reactions

Several known azide-based coupling reactions are shown in Figure 2. The first example is Curtius’ acyl azide-amine reaction discovered in the early 20th century (eq. 1).²⁸ This reaction has been widely used in amide synthesis and peptide coupling. However, the low thermal stability of acyl azides limits the use of this reaction.²⁹

\[
\text{Acyl azide-amine amide coupling} \quad \begin{array}{c}
\text{O}_3
\\ \text{H}_2\text{N}\\ \text{Base}
\end{array} \quad \begin{array}{c}
\text{O}_3
\\ \text{N}
\\ \text{H}
\end{array} \quad (\text{eq. 1})
\]

\[
\text{Cu/RuAAC} \quad \begin{array}{c}
\text{N}_3
\\ \equiv
\\ \equiv
\\ \equiv
\\ \text{Cu/Ru}
\end{array} \quad \begin{array}{c}
\text{N}_3
\\ \equiv
\\ \equiv
\\ \equiv
\\ \equiv
\end{array} \quad (\text{eq. 2})
\]

\[
\text{SPAAC} \quad \begin{array}{c}
\text{N}_3
\\ \equiv
\\ \equiv
\\ \equiv
\end{array} \quad \begin{array}{c}
\text{N}_3
\\ \equiv
\\ \equiv
\\ \equiv
\end{array} \quad (\text{eq. 3})
\]

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\text{Staudinger figation} \quad \begin{array}{c}
\text{N}_3
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\\ \equiv
\\ \equiv
\\ \equiv
\\ \equiv
\\ \equiv
\end{array} \quad \begin{array}{c}
\text{O}
\\ \text{MeO}
\\ \text{Ph}_3\text{P}
\\ \equiv
\\ \equiv
\\ \equiv
\\ \equiv
\\ \equiv
\end{array} \quad (\text{eq. 4})
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\text{“Suffo-click”} \quad \begin{array}{c}
\text{O}_3
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Figure 2. Examples of azide-based coupling reactions.
The use of azides increased extraordinarily since the introduction of click chemistry, in particular the Cu- or Ru-catalyzed azide-alkyne cycloaddition (CuAAC/RuAAC) (eq. 2). This transformation provides a highly reliable and mild way to make covalent conjugates and has evolved as an “omnipotent ligation method”. However, this reaction is not without issues as the metal catalysts can be detrimental to biomolecules. For example, copper species in the CuAAC reaction induced denaturation of proteins, as well as degradation of DNA and polysaccharides. Furthermore, in surface/nanomaterial functionalization (heterogeneous conditions), a significant amount of copper salts (5-20 mol %, or 0.1-1 mM) is required to attain high efficiency, a condition which is troublesome due to the difficulty associated with its removal when the use of polar solvents was not possible. Moreover, the use of azide-functionalized monolayers on surfaces could result in high coupling efficiency, whereas terminal alkyne-monolayers are much less efficient due to the reduced accessibility of the activated alkyne-Cu(I) species on the surface. This also complicates the use of the CuAAC because the installation of an alkyne tag on high molecular-weight molecules is generally much more troublesome than introducing an azide tag.

As a result, several catalyst-free azide ligations have been developed, including SPAAC (eq. 3), Staudinger ligation (eq. 4) and sulfonyl azide/thioacid reaction (eq. 5). The only, yet obvious, limitation in these metal-free coupling reactions is the installation of coupling tags, which include strain-loaded alkynes, thiocarboxylic groups or phosphine moieties. These functional groups may not be readily accessible or stable, thus leaving room for improvement in organic azide-based coupling reactions.

### 1.3. Perfluoroaryl azides

An interesting class of azides is perfluoroaryl azides (PFAAs, Fig. 3), which have proven valuable for functionalization of materials and surfaces and in photoaffinity labeling via reaction of the nitrene intermediates formed by photolysis or thermolysis of the azide. This thesis centers around this class of azides, to explore their new reactivities in azide-based reactions. This section summarizes their properties.

#### 1.3.1. Synthesis

PFAAs (Fig. 1) can be readily synthesized via nucleophilic aromatic substitution (SNAr) of commercially available pentafluoroaromatics by azide salts, providing a straightforward and highly efficient protocol (Fig. 3). Another method proceeds via diazotization of perfluoroaryl aniline (1.3) followed by SNAr, however, this procedure has been primarily used for the synthesis of pentafluorophenyl azide.
1.3.2. Stability

The obtained PFAAs are generally sensitive to <350 nm UV-light under which they decompose to release N\textsubscript{2}. Also, due to the highly electron-poor aromatic ring,\textsuperscript{44} they showed high electron-affinity and suffered from decomposition under electron beams.\textsuperscript{45}

Nevertheless, PFAAs displayed good thermal stability as long as they are kept in the dark (Fig. 4).\textsuperscript{46} Moreover, PFAAs are chemically inert at biological conditions, as exemplified by the wide use of PFAAs in photoaffinity labeling (PAL) experiments. This class of azides is stable and has been widely applied in material and biomedical researches.

1.3.3. Nitrene-mediated reactivities

PFAAs are well known for their nitrene chemistry, as will be discussed below. Figure 5 shows some key transformations when aryl azides are used as the nitrene precursors.\textsuperscript{49-51}

Banks and coworkers firstly reported the highly efficient formation of a C-H insertion product upon the thermolysis of 4-azido-2,3,5,6-tetrafluoropyridine in cyclohexane.\textsuperscript{52} This reactivity is strikingly different from other phenyl
azides where intramolecular ring-expansion (e.g., to form ketenimine, Fig. 5) is the dominant pathway for the phenyl nitrene. Platz and coworkers further found a particularly high yield of insertion products for 2,6-difluorinated aryl azides. The ‘ortho-fluorine’ effect was proposed, stating that the two neighboring fluorines diverted singlet PFAA nitrenes away from the ring-expansion rearrangement with ~5 kcal/mol increased energy barrier. This effect results in a long lifetime of singlet nitrenes, thus promoting the insertion/addition reactions of PFAA nitrenes with a variety of structures including amines, alkenes, alkanes and sulfides (Fig. 5, gray highlight). The presence of fluorines at the meta- or para- positions in PFAAs, in addition to the two ortho-fluorines, furthermore enhanced these nitrene insertion/addition reactions.

Because of the “ortho-fluorine” effect, PFAAs are also very different from other electron-deficient aryl azides, in particular nitro-substituted phenyl azides. Singlet nitrenes from nitro-substituted phenyl azides underwent facile intersystem crossing (ISC) to the triplet state, which then dimerized to azo compounds (Fig. 5).

**Figure 5.** Reactions of aryl nitrenes from azides. (Habs: proton abstraction, ISC: intersystem crossing, promoted under cryogenic temperature or with triplet sensitizers)

**PFAA nitrenes in biological and materials science.** The high insertion/addition reactivities of PFAA nitrenes have been explored in PAL experiments since the 1990s, for example, to study the binding structures of
chloride channels,\textsuperscript{59} yeast RNA polymerase III transcription complexes\textsuperscript{60} and many others.\textsuperscript{61-63} Besides, PFAAs has also been employed to modify biomolecules,\textsuperscript{64} or directly used as radical generators in photodynamic therapy.\textsuperscript{65}

Another application of PFAA nitrenes is when PFAAs are employed as hetero-bifunctional coupling reagents in the surface and nanomaterial functionalization.\textsuperscript{46,66} The strategy consists of pre-immobilization of PFAA on surfaces to form an azide layer, followed by photo-initiated nitrene-mediated coupling. The conjugation can be efficient with various molecules without the requirement of making specific coupling tags. Therefore, this protocol is fairly versatile, efficient and it is even possible to integrate with photolithography technology. The only issue is the lack of control over selective coupling events, which may result in a mixture of molecular conjugates.

**PFAAs Nitrenes in organic synthesis.** Nitrene insertion/addition is a facile reaction pathway to obtain other nitrogen-containing compounds. However, when azides are used as nitrene precursors, the efficiency of synthesis is highly structure-dependent. For example, in the photolysis of azides in toluene (Fig. 6), PFAAs gave the amination product in good yields, while phenyl azides with other substituents gave significantly lower yields.\textsuperscript{57}

![Figure 6. Photochemical benzylic C-H insertion of azides.\textsuperscript{57}](image)

Strategies to control the reactivity of nitrenes from azides have been explored.\textsuperscript{67,68} Stepwise, metal-catalyzed nitrene transfer is a very attractive strategy that has been developed recently. In this regard, among azides, PFAAs showed to be among the most efficient nitrene precursors in the metal-catalyzed amination and aziridination. For example, Che and coworkers reported that ruthenium(II)-porphyrin complexes worked well, in combination with PFAAs, in the amination of non-activated sp\textsuperscript{3} C-H bonds, as well as in stereo-selective aziridination of olefins (Fig. 7a).\textsuperscript{69} In the studies with cobalt(II)-porphyrin complexes, PFAAs again performed well in the amidation of aldehydic C-H bonds,\textsuperscript{70} and aziridination of styrene (Fig. 7b).\textsuperscript{43} It was proposed that the strong electron-withdrawing perfluoroaryl group contributed to the high electrophilicity of the nitrene-metal complex, thereby facilitating
hydrogen abstraction of the aldehyde proton.\textsuperscript{43} Note that neither reaction systems worked for other types of azides.

\textbf{Figure 7.} (a) Ru(II)-porphyrin-catalyzed\textsuperscript{69} and (b) Co(II)-porphyrin-catalyzed nitrene transfer and insertion reactions of PFAAs.\textsuperscript{43,70}

The unique reactivities of PFAAs in these metal-catalyzed nitrene transformations support the “controlled formation” of reactive metal-nitrene intermediates. The reactivities can be explained by the formation of paramagnetic metalloradicals, although a wide range of metal-nitrene complexes can exist (Fig. 8).\textsuperscript{71,72} The understanding of these metal-nitrene complexes is still limited and not conclusive, even though trapping of nitrenes with metal complexes were long observed.\textsuperscript{73} The stability and chemical reactivities of these complexes are generally influenced by a number of factors and depend on the choice of ligand, metal and nitrene precursors (e.g., azides). Of different PFAAs, studies have only been performed on pentafluorophenyl azide in the metal-nitrene complex formation. The pentafluorophenyl nitrene-metal complexes have higher reactivities, compared to the ones from more electron-rich azides, as was observed for metal-nitrene complexes involving Pt, Mo, Rh, Ru or Co as metal cores. In isolable metal-nitrene complexes (with Mo\textsuperscript{74}, Rh\textsuperscript{75} and Pt\textsuperscript{76}), higher reactivities in hydrogenation, cycloaddition and radical reactions were shown, compared to metal-nitrene complexes from other azides. These results suggest that PFAAs are among the most efficient azides due to the higher electron-deficiency.
1.3.4. 1,3-Dipolar cycloadditions

1,3-Dipolar cycloaddition is a reaction between a dipole and a dipolarophile to form a five-membered ring. Primarily due to concerted formation of two bonds, this class of reaction is highly chemo-selective.

For PFAAs, the azide-alkyne cycloaddition has been extensively studied. Azides belong to HOMO-LUMO-controlled dipoles. The highly electronegative fluorine atoms in PFAAs make the azido group strongly electrophilic, which promotes their reaction with nucleophilic dipolarophiles. The regioselectivity of this cycloaddition is also controlled by the HOMO-LUMO interactions between the azide and the alkyne. Specifically, electron-rich dipolarophiles react faster with electron deficient azides in a regiospecific manner, and vice versa. This is exemplified by the higher regioselectivity favoring 1,4-triazoles, using PFAAs in reaction with phenylacetylene, in contrast to phenyl azide that gave almost equal formation of 1,4- and 1,5-triazoles. Interestingly in aqueous conditions, cycloaddition of PFAAs formed 1,4-triazoles regioselectively with many alkynes. PFAAs in Cu/RuAAC and under heat-promoted conditions have furthermore been shown to produce polytriazoles (Fig. 9). The thermal reaction yielded the shortest polymer with mixed regioisomers. Owing to the steric repulsion in 1,5-triazole formation, RuAAC produced lower molecular-weight polymers, while CuAAC yielded the longest polymers with exclusive 1,4-triazoles.
In the solid state, PFAA-based AAC can be performed at r.t. by virtue of the arene-perfluoroarene $\pi-\pi$ interactions (Fig. 10). \(^{83,84}\) This process is pressure-promoted and gives exclusive formation of 1,4-triazoles.\(^{85,86}\)

When changing to highly strained aliphatic cyclooctyne 1.12, PFAAs showed better reactivity than many other azides (Fig. 11).\(^{87}\) The accelerated cycloadditions were attributed to the inverted frontier molecular orbital (FMO) interactions between the LUMO\(_{azide}\) and the HOMO\(_{alkyne}\) when highly electron-deficient azides are used. In contrast, when other alkynes, including benzoannulated cyclooctynes, are used, the FMO interactions are between the
HOMO\textsubscript{azide} and the LUMO\textsubscript{alkyne} and PFAAs displayed slower cycloadditions compared to aliphatic azides.\textsuperscript{88}

![Figure 11. Azides in AAC with aliphatic cyclooctyne 1.12.\textsuperscript{87} k(rel): relative rate constants.][figure11]

1.3.5. Reduction and other transformations

**Reduction.** Azides can be reduced. The reduction potential correlates with the energy of LUMO: the lower the LUMO, the easier it is to add electrons to the compound. As perfluorination of the aromatic ring substantially lowers the azide LUMO by withdrawal of electrons, PFAAs show higher sensitivity than phenyl azides to reducing agents, for example stannous chloride dihydrate.\textsuperscript{89}

Interestingly, azides can be selectively reduced by thiols.\textsuperscript{90,91} Dithiols, such as dithiothreitol, are capable of reducing PFAAs into their respective anilines quickly under physiological conditions. The reduction requires two sequential transformations to complete a reducing cycle, which hints on the high selectivity (Fig. 12).\textsuperscript{92} The selective reduction has been employed for monitoring H\textsubscript{2}S \textit{in vivo} using aryl azide-masked fluorophores.

![Figure 12. Proposed selective reduction of azide by dithiols.][figure12]

When using monothiols, the reduction shows generally > 2-3 orders slower reaction kinetics than for dithiols.\textsuperscript{93} Reduction by thiols can be significantly enhanced under basic conditions, indicating that the addition of the thiolate anion is involved in the rate-determining step (Fig. 12). Also for this type of reduction, electron-withdrawing substituents on the aromatic ring accelerate
the reduction, which supports that PFAAs suffer from relatively fast reduction by thiols.\textsuperscript{94}

**Other transformations.** PFAAs have also been applied to many other transformations.\textsuperscript{95} An important example is the Staudinger reaction where, for example, PFAA 1.14 reacted with triphenylphosphine (1.15) to give ylide 1.16 in 93\% isolated yield at r.t. (Fig. 13).\textsuperscript{95} Ylide 1.16 proved stable during isolation, while many ylides from other azides hydrolyzed easily to amines.

![Figure 13. PFAAs in Staudinger reaction.\textsuperscript{95}](image)

1.4. Aim of this thesis

As described in the above, a number of challenges remain in current azide-mediated coupling reactions. Two of the major challenges are:

- Toxicity and compatibility of metal catalysts
- Requirement of very specific coupling groups (e.g., strained alkynes)

To address these limitations, PFAAs have been studied as pre-activated azide substrates (due to the many F-atoms), which retain the unique properties of organic azides. The aim was to develop simple and reliable PFAA-based reactions, with a focus on cycloaddition reactions, and to explore the use of these reactions in the fields of biomedical research and materials science.

In chapter 2, an azide-aldehyde-amine cycloaddition using PFAAs is presented in detail. This method is explored as a modular modification of drug molecules for pure nanodrug formulation. In chapter 3, transformations of PFAAs to aryl amides are discussed. In chapter 4, the thesis ends with an exploration of nitrenes formed from PFAAs and their application in fluorescent imaging, which is to show the rich potential of PFAAs other than being used in coupling reactions.
Perfluoroaryl Azide-Aldehyde-Amine Reaction

(Paper I-II)

Since the 1960s, azides have been observed to react smoothly with enolizable aldehydes (2.3) and amines to give triazolines (2.4) via the azide-enamine cycloaddition (Fig. 14). However, the resulting triazolines were unstable and could rearrange into triazoles, amidines, imines, amides, pyrroles and so on. The sluggish transformation of triazolines limited the use of this smooth multi-component 1,3-dipolar cycloaddition. We however found that when PFAAs were used, the reaction underwent fast and reliable transformation to yield stable amidines. The reaction has been further established in applications in biomedical and materials science.

\[
\begin{align*}
R - N_3 & \quad + \quad R_3 - N & \quad + \quad H_\text{O} - R_1 & \quad \rightarrow \quad R - N - N & \quad \quad R_3 - N & \quad \quad R_2 - R_1 \\
2.1 & \quad 2.2 & \quad 2.3 & \quad 2.4
\end{align*}
\]

Figure 14. Azide-aldehyde-amine cycloaddition to form triazolines (2.4) and subsequent rearrangements.

2.1. Scope of the reaction

Methyl 4-amino-2,3,5,6-tetrafluorobenzoate (PFAA 1) was used as the model azide to react with an aldehyde and an amine in MeOH (Fig. 15). These reactions were carried out under ambient conditions to give amidine 2.5 in high yields. This is very different from phenyl azide which gave mainly triazolines.

The reaction has a broad substrate scope. Secondary aliphatic amines, either cyclic (2.5a-c, >90%) or acyclic (2.5d, 83%), gave amidines in high isolated yields. Primary aliphatic amine also showed clean transformation (2.5e, 91%). This inspired us to explore the use of unprotected amino acids as amines. Three of the most frequent N-terminal amino acids in natural
proteins\textsuperscript{100}: \textit{L}-alanine, \textit{L}-leucine and \textit{L}-methionine, were tested. After optimization, the best reaction condition was the use of 2:1 \textendash{} 4:1 DMSO/H\textsubscript{2}O as solvent at 60 °C (0.1 M, homogeneous condition), where in general clean transformations (>85\%) were accomplished within 2 h as shown by \textsuperscript{19}F NMR. The lower isolated yields witnessed for certain products (\ref{2.5f}, 79\%; \ref{2.5g}, 74\%; \ref{2.5h}, 74\%) were attributed to the poor isolation efficiency by flash column chromatography on these acidic products. The reaction can be performed without protection of the acid group, which is an advantage in comparison to popular carbodiimide amine coupling reactions. Aniline also displayed good compatibility (\ref{2.5i}, 81\%), albeit with a slower conversion than aliphatic amines. Diphenylamine, a sterically hindered and nucleophilic-deactivated aniline, gave amidine \ref{2.5j} in 74\% isolated yield after 5 days.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure_15}
\caption{Scope of aldehydes and amines. (a) Reaction conditions: PFAA \textsuperscript{1} (0.10 M), \ref{2.2} (0.105 M), \ref{2.3} (0.105 M), 1 mmol scale, MeOH, r.t., isolated after 12 h. (b) DMF, \textsubscript{N}\textsubscript{2}. (c) PFAA \textsuperscript{1} (0.05 M), amino acid (0.075 M), phenylacetaldehyde (0.075 M), DMSO/H\textsubscript{2}O 3:1, 60 °C, 2 h. (d) By \textsuperscript{19}F NMR. (e) Phenylacetaldehyde (2.5 equiv.), aniline (2.5 equiv.), 24 h, 40 °C. (f) Phenylacetaldehyde (2.5 equiv.), aniline (2.5 equiv.), 40 °C, 5 d. (g) 40 °C, 2 h.}
\end{figure}
Carbonyl compounds were furthermore screened against piperidine and PFAA 1. Enolizable aldehydes generally showed neat and fast triazoline formation. However, the triazolines formed from non-activated aldehydes, for example butyraldehyde and 3-phenylpropanal, rearranged into amidines at a much slower rate than those formed from phenylacetaldehyde. In these cases, polar protic solvents such as methanol were found to promote the rearrangement efficiently. At 40 °C in methanol, the amidines were isolated in good yields within 4 h (2.5k, 85%; 2.5m, 71%; 2.5n, 92%). An alternative way to accelerate the rearrangement was to add acids when the cycloaddition was complete. However, this operation could lead to aniline by-products.

The acyclic amidine bond in these derivatives was tested to be near-neutral, due to the highly electron-withdrawing perfluoroaryl group. These amidine derivatives formed from secondary amines showed high stability, being resistant to thermal heating and acid/base treatment (pH 1-10). The amino acid derivatives (2.5k-2.5n) suffered from decomposition of the amidine bond when heated under highly concentrated condition, but were stable in diluted solutions for a long time.

### 2.2. Mechanistic investigations

We studied the reactions of PFAAs (1-6) with phenylacetaldehyde enamines (A-D) and ketone enamines (E-H) experimentally and by computations. The results were compared to those of phenyl azide (2.6), benzyl azide (2.7) and tosyl azide (2.8). The cycloaddition and rearrangement steps are discussed separately in following sections.

![Figure 16. Dipoles (azides) and dipolarophiles (enamines) in this section.](image-url)
2.2.1. Enhanced cycloaddition

We tested the reaction of PFAA 1 with phenylacetaldehyde enamine A in CDCl₃ (Fig. 17). The formation of triazoline was only observed at a very early stage. It was identified by a pair of doublets in the range of 4.7-5.7 ppm in the \(^1\)H NMR spectra.\(^{101}\) Attempts to separate the triazoline failed; the compound decomposed upon column chromatography on silica gel or recrystallization from diisopropyl ether.\(^{98}\) Nevertheless, both \(^1\)H and \(^{19}\)F NMR spectra displayed only one triazoline isomer. This high regioselectivity is consistent with other azide-enamine cycloaddition reactions.\(^{101}\) The regioisomer 2.9 could be proposed, based on the experimental and computational results in the reaction between phenyl azides and phenylacetaldehyde enamines.\(^{101,102}\) Corresponding triazolines were detected for all PFAAs (1-6) and aldehyde enamines (A-D), where the amidine products were isolated in good yields (Paper I).

![Reaction profile of aldehyde enamine A with PFAA 1 monitored by \(^{19}\)F NMR. Squares: amidine 2.5a; Circles: triazoline 2.9; Triangles: amidine + triazoline. Reaction conditions: azide (106.8 mM), enamine (42.7 mM), CDCl₃ (0.8 mL), 20 °C.](image)

Kinetic studies using \(^{19}\)F NMR were conducted, where different components in the reaction mixture could be followed. A typical reaction profile, presented as the concentration vs. the reaction time, is shown in Figure 17. It supported a fast cycloaddition reaction followed by a slow decomposition step. By fitting the kinetic data using the reaction model, the rate constants for both the cycloaddition (\(k_1\)) and the decomposition (\(k_2\)) were obtained (Table 1).
Table 1 shows the rates of different azides against phenylacetaldehyde enamine B. The reaction between PFAA 1 and enamine B displayed close to four orders of magnitude rate enhancement ($k_{\text{rel}}$) compared to that of phenyl azide 2.6. Additionally, an electron withdrawing group para to the azide group further accelerated the reaction (PFAAs 3-6). In the case of $p$-nitro-substituted PFAA 4, the cycloaddition rate constant reached $1.22 \pm 0.03$ M s$^{-1}$, i.e., more than five orders of magnitude higher than that of phenyl azide. In contrast, benzyl azide underwent extremely slow cycloaddition. Tosyl azide (2.8) displayed fast conversions comparable with PFAAs, however yielded a mixture of products.

<table>
<thead>
<tr>
<th>azide</th>
<th>$k_1$ ($10^{-2}$ M$^{-1}$ s$^{-1}$)</th>
<th>$k_{\text{rel}}$</th>
<th>$\Delta G_{\text{exp}}$ (kcal mol$^{-1}$)</th>
<th>$k_2$ ($10^{-5}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PFAA 1</td>
<td>7.22 ± 0.04</td>
<td>9081</td>
<td>18.7 ± 0.1</td>
<td>2.7 ± 0.1</td>
</tr>
<tr>
<td>PFAA 2</td>
<td>1.05 ± 0.03</td>
<td>1321</td>
<td>19.8 ± 0.1</td>
<td>-</td>
</tr>
<tr>
<td>PFAA 3</td>
<td>97.1 ± 2.4</td>
<td>122138</td>
<td>17.2 ± 0.1</td>
<td>33 ± 12</td>
</tr>
<tr>
<td>PFAA 4</td>
<td>121.6 ± 3.2</td>
<td>152956</td>
<td>17.0 ± 0.1</td>
<td>84 ± 9</td>
</tr>
<tr>
<td>PFAA 5</td>
<td>35.9 ± 0.6</td>
<td>45157</td>
<td>7.7 ± 0.1</td>
<td>27 ± 1</td>
</tr>
<tr>
<td>PFAA 6</td>
<td>80.8 ± 0.2</td>
<td>101635</td>
<td>17.3 ± 0.1</td>
<td>27 ± 7</td>
</tr>
<tr>
<td>2.6</td>
<td>$1.85 \pm 0.02 \times 10^{-3}$</td>
<td>1</td>
<td>24.0 ± 0.1</td>
<td>-</td>
</tr>
<tr>
<td>2.7</td>
<td>$&lt; 10^{-5}$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.8</td>
<td>16.0 ± 0.2</td>
<td>20126</td>
<td>18.2 ± 0.1</td>
<td>-</td>
</tr>
</tbody>
</table>

$^a$Conditions: [Azide]:[Enamine] 2:1, $^1$H and $^{19}$F NMR in CDCl$_3$, 20 °C, $r^2 > 0.995$.

$^b$k$_{\text{rel}}$: $k_1(k_{\text{rel}})/k_2$. $^c$Calculated by Eyring equation ($k_B = 1$) from $k_1$. $^d$r$^2 = 0.987.

Table 2. Cycloadditions rates of PFAA 1 or 2 with enamines (A-D)$^a$

<table>
<thead>
<tr>
<th>enamine</th>
<th>$k_1 \times 10^{-2}$ M$^{-1}$ s$^{-1}$</th>
<th>$k_{\text{rel}}$</th>
<th>$k_1(1)/k_1(2)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.639 ± 0.001 (1)</td>
<td>0.098 ± 0.002 (1)</td>
<td>6.5</td>
</tr>
<tr>
<td>B</td>
<td>7.22 ± 0.04 (11)</td>
<td>1.05 ± 0.03 (11)</td>
<td>6.9</td>
</tr>
<tr>
<td>C</td>
<td>17.6 ± 0.2 (27)</td>
<td>2.29 ± 0.06 (23)</td>
<td>7.7</td>
</tr>
<tr>
<td>D</td>
<td>10.1 ± 0.1 (16)</td>
<td>1.35 ± 0.03 (14)</td>
<td>7.4</td>
</tr>
</tbody>
</table>

$^a$By $^1$H and $^{19}$F NMR in CDCl$_3$, [Azide]:[Enamine] 2:1, 20 °C, $r^2 > 0.995$. 

17
Table 2 shows the cycloaddition rates of aldehyde enamine A-D with PFAA 1 and PFAA 2. It has been reported that the reactivity of different enamines towards electrophiles generally decrease in the order of pyrrolidine > piperidine > morpholine enamines, following the order of their nucleophilicity. In our studies, a similar trend was observed for both PFAA 1 and 2. The acyclic diethylamine enamine D was shown to be slightly more reactive than piperidine enamine B.

2.2.2. With ketone enamines

When the PFAAs were treated with acetophenone enamines (E-H), no amidine product was detected; perfluoroanilines 2.11 were instead isolated as the major products (Fig. 18a). Both the 19F and 1H NMR spectra confirmed formation of triazolines 2.10. The decomposition of the triazolines was accompanied by a rapid color change from light yellow to dark red as well as the evolution of N₂. In the contrast, clean conversion to the amidine structure (2.13) was observed when tosyl azide was treated with enamine F (Fig. 18b).¹⁰⁴,¹⁰⁵

![Figure 18. Cycloaddition of PFAAs (a) and tosyl azide (b) with ketone enamines.](image)

The different product formation between tosyl azide and PFAA s in cycloaddition with ketone enamines as well as aldehyde enamines is very interesting. The decomposition of the triazolines is likely influenced by the groups neighbouring the azido group, besides the electron-deficiency in the two types of azides.

2.2.3. Concerted cycloaddition

Two possible reaction pathways in the cycloaddition step are shown in Fig. 19 for PFAAs. Concerted cycloaddition is widely accepted in 1,3-dipolar cycloaddition reactions.¹⁰⁷ In the azide-enamine cycloadditions, Munk and coworkers suggested a deviation from the classic concerted mechanism, stating
“the polar transition state model”, which was supported by a large charge-separated transition state revealed by the Hammett correlation. This was recently studied computationally by Houk and coworkers as a concerted mechanism having an asynchronous transition state. Stepwise cycloaddition via zwitterionic intermediates is an alternative mechanism, which was proposed only in reactions between some highly electron-withdrawing azides (e.g., sulfonyl azide or o-nitrophenyl azides) and cyclic ketene N,X-acetal enamines (X = O, S or NR).

Figure 19. Concerted (a) and stepwise (b) perfluoroaryl azide-enamine cycloaddition.

To verify the two pathways, the effect of solvent on the cycloaddition rate ($k_1$) was evaluated. The correlation of the rate constant to the solvent polarity $Z$-value ($E_T$, molar transition energy) is frequently used to elucidate the properties of transition states in reactions. The poor correlation ($R^2 = 0.73$) in this case agreed with a similar observation with phenyl azides (Fig. 20). In contrast, the decomposition rate ($k_2$) showed a highly linear correlation to the solvent polarity ($R^2 = 0.99$). These results supported a concerted cycloaddition for PFAAs.
Figure 20. Correlation of the solvent polarity ($E_T$, molar transition energy) to cycloaddition rate $k_1$ (●) and decomposition rate $k_2$ (■). Conditions: [PFAA I]:[Enamine B] 2:1, in CDCl$_3$, CD$_3$OD or CD$_3$CN, 20 °C.

The concerted cycloaddition was further supported by computations. The calculated transition state structures (TS) for both the concerted and stepwise modes of the cycloaddition are shown in Figure 21. The activation free energies ($\Delta G^\ddagger$) for the stepwise reaction are higher than the $\Delta G^\ddagger$ of the corresponding concerted cycloaddition by 5–7 kcal mol$^{-1}$. The $\Delta G^\ddagger$ decreased for the transition state structures involving increasingly electron-deficient aryl azides in the order of TS-c (phenyl azide 2.6, $\Delta G^\ddagger = 25.3$), TS-b (PFAA 2, $\Delta G^\ddagger = 21.7$), and TS-a (PFAA 1, $\Delta G^\ddagger = 21.0$).

Figure 21. First row: the concerted transition state structures for the cycloaddition of PFAA 1 (TS-a), PFAA 2 (TS-b) and phenyl azide 2.6 (TS-c) with aldehyde enamine B. Secondary row: the corresponding stepwise transition structures. Computed using M06-2X/6-311+G(d,p)/IEFPCM$^{CHCl_3}$/M06-2X/6-31G(d)/IEFPCM$^{CHCl_3}$. Bond lengths are in Å, and reported energies, are Gibbs free energies in kcal mol$^{-1}$ determined assuming a standard state of 1M at 298.15 K.
Figure 22. The distortion/interaction model in the azide-alkene cycloaddition.

Figure 23. Graph of activation, distortion, and interaction energies for TS-a (enamine B + PFAA 1), TS-b (enamine B + PFAA 2), and TS-c (enamine B + 2.6) in the distortion/interaction model. (black: activation energies, green: distortion energies of dipolarophile, blue: distortion energies of azides, red: interaction energies). Calculated using M06-2X/6-311+G(d,p)/IEFPCM(CHCl3)/M06-2X/6-31G(d)/IEFPCM(CHCl3).

Figure 24. The LUMOs of azides 2.6, PFAA 2, PFAA 1 and the HOMO of enamine B, by calculation using HF/6-31G(d)//M06-2X/6-31G(d)/IEF-PCM(CHCl3).
The distortion/interaction model (Fig. 22) was employed to deduce the origins of the azide reactivity differences by computation. The activation energy ($\Delta E_{\text{act}}$) is dissected into distortion energy ($\Delta E_{\text{dist}}$) and interaction energy ($\Delta E_{\text{int}}$). The distortion energy is further dissected into $\Delta E_{\text{dist}}(\text{azide})$ and $\Delta E_{\text{dist}}(\text{alkene})$ which are required to distort the azide and alkene into their respective transition state geometries, without allowing the fragments to interact. The interaction energy is the energy of the interaction between the distorted cycloaddends, consisting of a net stabilizing quantity that results from charge transfer of occupied-vacant orbital interactions, electrostatic interactions, polarization, and closed-shell (steric) repulsions. Figure 23 shows a graph of $\Delta E_{\text{act}}$ (black), $\Delta E_{\text{dist}}$ (enamine, green), $\Delta E_{\text{dist}}$ (azide, blue), and $\Delta E_{\text{int}}$ (red) for TS-a, TS-b, and TS-c. It showed that TS-c has the highest activation energy because of increased distortion and reduced interaction between the azide and the enamine.

An FMO analysis was conducted to understand the contribution of interaction energies to the activation energies in these cycloadditions. Fig. 24 shows the calculated molecular orbitals of enamine B and PFAA 1, 2 and phenyl azide (2.6). The stabilizing orbital interaction is mainly between the HOMO of enamine B and the LUMO of the azides. Perfluorination of the aryl ring substantially lowers the azide LUMO energies of PFAA 1 and 2, and thus results in smaller HOMO-LUMO gaps and stronger FMO interactions. In addition, the asynchronicity in these cycloadditions is also believed to arise from the strong orbital interaction, and to a greater extent in the case of PFAAs.

2.2.4. Stereo-selective rearrangement

Mechanism studies on the decomposition of triazolines have not proven conclusive. Pocar and coworkers proposed a stepwise decomposition of triazolines formed from phenyl azide and enamines (Fig. 25a). Concerted decomposition was proposed only recently in the decomposition of triazolines formed from sulfonyl azides and enamines (Fig. 25b). These two pathways varied in two key steps: the N$_2$-extrusion and the 1,2 H-shift.
Figure 25. Stepwise (a) and concerted (b) decomposition of triazolines.

For PFAAs, the NMR spectra showed cleanly a direct transformation of the triazolines to amidines. The rearrangement rates of triazolines are summarized in Fig. 26. The rates increased with a more electron-withdrawing $R$-substituent on the aryl azide ring, fitting a positive and linear quasi-Hammett correlation with $\rho [\sigma_p – \log (k_\alpha/k_F)] = 4.6$, ($r^2 = 0.99$). This supported the involvement of charge separation in the rate-determining steps. The result is further supported by a linear solvent polarity correlation to the rates of rearrangement in Fig. 20. In addition, the length of $[\text{CH}_2]_n$ also exerted a significant influence on the rearrangement rates: for $n \geq 1$, the rates were almost 100 times slower than those of $n = 0$ when phenylacetaldehyde was used.

Figure 26. Structural factors on rearrangement rate ($k_2$) of triazolines 2.25.

To evaluate the stepwise or concerted rearrangement, we studied the decomposition of triazolines 2.27 formed from trisubstituted enamine 2.26 (Fig. 27). As illustrated in Figure 25, a stereospecific triazoline (2.27a or 2.27b) has a chance to undergo racemization during H-shift (structure 2.21) in the stepwise rearrangement (dashed line, Fig. 27). In contrast, concerted decomposition should in principle only give stereospecific products, e.g., 2.27a to 2.28a, and 2.27b to 2.28b.
Triazolines 2.27a and 2.27b crystalized out of solution as racemates, after mixing of trisubstituted enamines (2.26, and its minor cis isomer) with PFAA 2 in diethyl ether at -20 °C. Racemate triazolines 2.27 (triazoline isomers m and n) decomposed into amidines 2.28 (amidine isomers M and N) at r.t., as was characterized by HPLC (Fig. 28a). Pure isomers 2.27a and 2.27b were then separated by chiral HPLC before decomposition. In sequential decomposition studies of the pure isomers, stereospecific rearrangement was discovered: triazoline m to amidine M (Fig. 28b), triazoline n to amidine N (Fig. 28c). The stereospecific decomposition was also observed for PFAA 1 in similar studies.

The rearrangement of isomer n was conducted under UV-light or in the presence of trifluoroacetic acid. UV-activation is believed to give di-radical 2.30 after N2-extrusion, which leads to aziridine-mediated transformations (Fig. 29). Upon treatment with a brønsted acid, protonation occurred at the
inner nitrogen, which in many cases leads to the stabilized diazo compound (2.33, Fig. 29). These two treatments can (in theory) open pathways for racemization of amidines during the H-shift. Indeed, a slightly higher degree of racemization was observed under UV-light and acidic conditions than by thermal decomposition. However, the amidine was still detected in high enantiomeric excess (isomer N). These studies supported either a concerted or 'synchronous' stepwise process of N₂-extrusion and H-shift. The 'synchronous' stepwise process depicted that the N₂-extrusion and the 1,2 H-shift were highly coupled, when the two processes could be distinguished on the time-scale.

**Figure 29. Rearrangement of triazolines under UV-light or in the presence of acidic protons.**

### 2.3. Formulation of pure nanodrugs showing aggregation-induced emission

The PFAA-aldehyde-amine reaction could install a perfluoroaryl ring and a phenyl ring linked closely by an amidine bond, for example, in ciprofloxacin derivatives (Fig. 30). The perfluoroaryl ring near the phenyl ring was expected to induce strong interactions between either the perfluoroaryl and phenyl rings, or between two perfluoroaryl rings. These supramolecular interactions have been employed in crystal engineering and gelation. In this section, the perfluoroaryl-mediated π–π interactions were exploited to form nano-sized particles by aggregation. Furthermore, these nanoaggregates were applied as aggregation-induced emission (AIE) dots and pure nanodrugs.

AIE has become established in applications in obtaining solid materials for optoelectronic devices, biomedical imaging and smart materials. AIE-active molecules generally have stiffened conformation and rigid structure in the solid aggregates, resulting in restriction of molecular motions which annihilate the emissive process in solution. Propeller molecules were frequently observed with AIE properties. The ciprofloxacin derivatives in this study also have quasi-propeller structures, with the fluoroquinolone-,
perfluoraryl- and phenyl ring systems as three blades as shown in the example of the crystal structure of compound 2.41 (Fig. 30).

![Chemical structures and labels](https://example.com/chemical_images)

**Figure 30.** Modular synthesis of ciprofloxacin derivatives having nearby perfluoroaryl and aryl moieties. (a) Ciprofloxacin (1.0 mmol), azide (2.37, 1.3-2.0 mmol), aldehyde (2.38, 2.0 mmol), acetone (80-160 mL), r.t., 1-3 days; (b) Pd/C (10 mol %), H₂ balloon, THF/MeOH 2:1; (c) 7 days, purified by recrystallization; (d) Norfloxacin was used instead of ciprofloxacin; (e) Piperidine was used instead of ciprofloxacin.

### 2.3.1. AIE activation

**Emission in solutions.** Table 3 summarizes the emission properties of compounds 2.40-2.42, together with ciprofloxacin. The amidine derivatization showed minimal influence in the ground state (absorption); however it had significant impact in the excited states (emission). In contrast to ciprofloxacin, these derivatives showed drastic high emission efficiency (fluorescent quantum yield Φ fluorescents: 20-30%) in dry THF and DCM. The electron-withdrawing amidine group weakened the electron-donating capability of the outer nitrogen atom in the piperazinyl ring. The photo-induced electron-transfer (PET) process from the outer nitrogen to the fluoroquinolone ring, which is the most efficient energy-dissipation pathway in ciprofloxacin, was thus blocked. This led to the enhanced emission after derivatization. In MeCN and MeOH, these derivatives emitted faintly, in contrast to ciprofloxacin which emitted strongly. The decrease of emission and bathochromic shift with the solvent polarity indicated that a twisted intramolecular charge transfer (TICT) process
upon photoexcitation from the donor moiety to the acceptor\textsuperscript{124} was populated in the highly polar solvent. This process is susceptible to non-radiative dissipation pathways, causing the quench of fluorescence.\textsuperscript{125-127}

<table>
<thead>
<tr>
<th>Table 3. Emission of ciprofloxacin and its derivatives (2.40-2.42) in solutions.</th>
</tr>
</thead>
<tbody>
<tr>
<td>solvent</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Benzene</td>
</tr>
<tr>
<td>Toluene</td>
</tr>
<tr>
<td>THF\textsuperscript{a}</td>
</tr>
<tr>
<td>DCM\textsuperscript{a}</td>
</tr>
<tr>
<td>MeOH</td>
</tr>
<tr>
<td>MeCN</td>
</tr>
<tr>
<td>DMSO</td>
</tr>
</tbody>
</table>

Quantum yields were referenced to quinine sulfate ($\Phi_f$: 0.55) in 0.1 N H\textsubscript{2}SO\textsubscript{4} aq. solution. \textsuperscript{a}dry solvent. \textsuperscript{b}from reference\textsuperscript{128}.

**Emission in aggregation state.** Aggregates were prepared by precipitating fluorophores from a good solvent (organic solvent) into a poor solvent (water),\textsuperscript{129} an operation called ‘reprecipitation’. By this protocol, compounds 2.40-2.42, 2.45-2.47 and 2.49 were demonstrated to be AIE-active. Compound 2.40 was used as an example, and was weakly emissive in pure acetonitrile at 457 nm (Fig. 31a-b, $\Phi_f$ = 1\%). After the water content in the solution reached up to 70 vol %, the emission started to increase together with a blue shift. With further increase to 90 vol % of water, the maximal fluorescence intensity was observed ($\Phi_f$ = 12\%), with a 12-fold enhancement over that of its solution in MeCN (Fig. 31b). As shown in the UV-vis spectra (Fig. 31c), the appearance of broad level-off tails due to Mie scattering indicated the formation of aggregates.\textsuperscript{126} Spherical aggregates were observed under scanning electron microscopy (SEM, Fig. 31d).
2.3.2. Aggregation factors

The ‘reprecipitation’ protocol was optimized for the preparation of nanosized aggregates. The aggregates formed immediately when dropping the organic solution (1-10 mg/mL) into water. Table 4 summarizes the characteristics of these aggregates in suspensions by dynamic light scattering (DLS) and fluorescence microscopy. Fig. 32 shows the morphology under transmission electron microscopy (TEM).

The higher water content (water to the organic solvent in precipitation) resulted in a smaller particle size (entries 1-6). The organic solvents also had significant impact on the particle size (entries 7-10). Aggregates of compound 2.40 having diameter down to 40 ~ 60 nm were obtained when DMSO, DMF, acetone or MeCN were used (entry 8-11), which were much smaller than the 90 nm aggregates when THF was used (entry 5). The TEM diffraction pattern confirmed their amorphous nature (Fig. 32B), in contrast to the needle-
shaped nanocrystals which could occasionally be observed in the 99.9 vol % water/MeCN mixture (Fig. 32D).

Interestingly on the TEM grid, well-defined micelle-like structures (d ~ 1000 nm, inner hole: d ~ 100 nm) were observed in a large amount when the suspension of compound \textbf{2.40} in 80 vol % water content was evaporated (Fig. 32A). In contrast, the DLS measurements (80 vol %) showed that the aggregates were 250 nm in size. These results indicated an alternative assembly of these amphiphilic molecules when they were dried on TEM grid.

**Table 4. Properties of aggregates.**

<table>
<thead>
<tr>
<th>entry</th>
<th>comp.</th>
<th>solvent/H&lt;sub&gt;2&lt;/sub&gt;O (%)</th>
<th>particle size (nm)</th>
<th>PDI</th>
<th>ξ (mv)</th>
<th>Ψ&lt;sub&gt;r&lt;/sub&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>\textbf{2.40}</td>
<td>THF (70%)</td>
<td>n.d.</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>\textbf{2.40}</td>
<td>THF (80%)</td>
<td>243 ± 20</td>
<td>0.17</td>
<td>-15 ± 8</td>
<td>2.3</td>
</tr>
<tr>
<td>3</td>
<td>\textbf{2.40}</td>
<td>THF (90%)</td>
<td>254 ± 30</td>
<td>0.14</td>
<td>-24 ± 6</td>
<td>6.9</td>
</tr>
<tr>
<td>4</td>
<td>\textbf{2.40}</td>
<td>THF (95%)</td>
<td>136 ± 20</td>
<td>0.13</td>
<td>-27 ± 7</td>
<td>6.1</td>
</tr>
<tr>
<td>5</td>
<td>\textbf{2.40}</td>
<td>THF (99%)</td>
<td>90 ± 5</td>
<td>0.21</td>
<td>-29 ± 7</td>
<td>8.8</td>
</tr>
<tr>
<td>6</td>
<td>\textbf{2.40}</td>
<td>THF (99.9%)</td>
<td>40 ± 5</td>
<td>0.23</td>
<td>-37 ± 3</td>
<td>11.1</td>
</tr>
<tr>
<td>7</td>
<td>\textbf{2.40}</td>
<td>MeCN (99%)</td>
<td>60 ± 5</td>
<td>0.24</td>
<td>-30 ± 8</td>
<td>8.1</td>
</tr>
<tr>
<td>8</td>
<td>\textbf{2.40}</td>
<td>DMSO (99%)</td>
<td>54 ± 5</td>
<td>0.23</td>
<td>-26 ± 7</td>
<td>10.8</td>
</tr>
<tr>
<td>9</td>
<td>\textbf{2.40}</td>
<td>Acetone (99%)</td>
<td>48 ± 5</td>
<td>0.21</td>
<td>-35 ± 8</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>\textbf{2.40}</td>
<td>DMF (99%)</td>
<td>40 ± 10</td>
<td>0.45</td>
<td>-30 ± 10</td>
<td>8.2</td>
</tr>
<tr>
<td>11</td>
<td>\textbf{2.40}</td>
<td>MeCN (99%)</td>
<td>145 ± 15</td>
<td>0.12</td>
<td>-34 ± 6</td>
<td>9.7</td>
</tr>
<tr>
<td>12</td>
<td>\textbf{2.40}</td>
<td>MeCN (99%)</td>
<td>70 ± 10</td>
<td>0.21</td>
<td>-33 ± 8</td>
<td>13.9</td>
</tr>
<tr>
<td>13</td>
<td>\textbf{2.40}</td>
<td>MeCN (99%)</td>
<td>n.d.*</td>
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</tr>
<tr>
<td>14</td>
<td>\textbf{2.40}</td>
<td>MeCN (99%)</td>
<td>73 ± 10</td>
<td>0.19</td>
<td>-39 ± 6</td>
<td>&lt; 0.1</td>
</tr>
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<td>15</td>
<td>\textbf{2.40}</td>
<td>MeCN (99%)</td>
<td>125 ± 10</td>
<td>0.11</td>
<td>-38 ± 7</td>
<td>0.3</td>
</tr>
<tr>
<td>16</td>
<td>\textbf{2.40}</td>
<td>MeCN (99%)</td>
<td>119 ± 10</td>
<td>0.08</td>
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</tr>
<tr>
<td>17</td>
<td>\textbf{2.40}</td>
<td>MeCN (99%)</td>
<td>132 ± 10</td>
<td>0.12</td>
<td>-37 ± 6</td>
<td>7.4</td>
</tr>
<tr>
<td>18</td>
<td>\textbf{2.40}</td>
<td>MeCN (99%)</td>
<td>n.d.</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>19</td>
<td>\textbf{2.40}</td>
<td>MeCN (99%)</td>
<td>64 ± 5</td>
<td>0.16</td>
<td>-38 ± 5</td>
<td>-</td>
</tr>
<tr>
<td>20</td>
<td>\textbf{2.40}</td>
<td>MeCN (99%)</td>
<td>232 ± 40</td>
<td>0.11</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Organic solution of compound (1-10 mg/mL) was injected into Millipore water to reach a final concentration of 10 µM. Measurements (n = 3) were conducted after reaching equilibrium (1-3 h). Each data was the average of > 3 independent experiments. *Z-average measured by DLS. *Excitation: 334 nm, emission: 455 nm. *Quantum yields were measured after reprecipitation and were referenced to quinine sulfate in 0.1 N aq. H<sub>2</sub>SO<sub>4</sub> solution (Φ<sub>F</sub>: 0.55). *Soluble in the solvent mixture. PDI: polydispersity index. ξ: zeta potential. n.d.: not determined.

Aggregates formed from 99 vol % water/MeCN of different amidine structures were compared. Compounds with a propeller-shaped structure all formed amorphous spherical aggregates (Fig. 32). In contrast, compound \textbf{2.48} without the phenyl ring did not yield analyzable aggregates. Compound \textbf{2.44} (entry 14) with a hydrophilic -COOH group in the perfluoroaryl ring did not show aggregation by DLS because of its high solubility, but assembled to nanoaggregates on TEM grids (Fig. 32G).
Among these derivatives, compound 2.40 with a pentafluorophenyl moiety gave spherical nanoaggregates with the smallest average size. This aggregation of a size down to 60 nm was also confirmed with the norfloxacin derivative 2.49 (entry 19). Moreover, compound 2.50 without the large flat quinolone ring also formed aggregates (entry 20). These results indicated that the nearby pentafluoro phenyl- and phenyl rings were very efficient in inducing nanosized assembly.

Figure 32. Compound 2.40 aggregates in 80 (A), 99 (B), 99.9 (C, D) vol % water/ acetonitrile under TEM. Compound 2.41 (E), 2.42 (F), 2.43 (G) and 2.45 (H) amorphous nanoparticles in 99 vol% water/ acetonitrile under TEM. Concentration: 10 µM. Inset are diffraction patterns, scale: 21 nm.

Figure 33. Storage stability of nanoaggregates 2.40 at 4 °C. Particle diameter (○) and polydispersity index (PDI, ●) were measured by DLS.

Although the aggregates prepared from <90 vol % water content were stable for several hours (they formed crystals slowly), those from >95 vol % of water
exhibited high storage stability in suspensions. Aggregates 2.40 (d~60 nm) showed no significant change in size over two months when stored at 4 °C (Fig. 33). The zeta potential of these aggregates showed the surface charge to be -25 to -40 mV (Table 4), indicating that the polar carboxylate groups of the quinolone ring would be exposed on the surface to stabilize the particle-solution interface.132

2.3.3. Interactions in crystalline state
The high stability of these aggregates suggests that perfluoroaryl and phenyl moieties may play an important role during molecular assembly in the aggregates. The crystal structure of compound 2.41 showed that the perfluoroaryl and phenyl ring preferred to form rigid intermolecular structures (Fig. 34). The intermolecular fluoroaryl-fluoroaryl inter-ring distance was measured to be 3.4-3.5 Å; the phenyl-phenyl rings face-to-face distance was measured to be 4.2 Å. Both supported strong interactions.114 These interactions could rigidify the configuration of molecules in aggregates such that the rotation of the inner N atom in the piperazinyl ring against the quinolone (D-A) ring could be efficiently blocked. This likely resulted in AIE in a similar fashion as many other D-A type AIE-active fluorophores. The especially good properties of 2.40 aggregates: smaller size, better stability and stronger AIE enhancement, are likely associated with a better match of the supramolecular π-π interactions for planar pentafluorophenyl group compared to other substituted perfluoroaryl groups.

![Figure 34. Crystal packing of compound 2.41 showing intermolecular phenyl-phenyl, perfluoroaryl-perfluoroaryl, and fluoroquinolone-fluoroquinolone stacking.](image-url)
2.3.4. Aggregation-enhanced, size-dependent antibacterial efficacy

Formulation of (pro)drug molecules as nano-sized aggregates or crystals, i.e. pure nanodrugs (PNDs),\textsuperscript{133} has recently become a very attractive strategy in drug formulation. PNDs have demonstrated enhanced antitumor efficacy as a result of the improved drug permeability and retention.\textsuperscript{132,134-136} However, the use of PNDs in antibiotic therapeutics was much less studied.

Ciprofloxacin is a second-generation fluoroquinolone antibiotics and has been used for the treatment of various infections.\textsuperscript{137} The emergence of ciprofloxacin-resistant microorganisms\textsuperscript{138} has long been observed and poses a growing public health threat.\textsuperscript{139-141} Ciprofloxacin is currently formulated with acids or bases for systemic administration. We envisioned that an aggregation-form of antibiotics, instead of the molecular drug, would be efficient to avoid the drug resistance for molecular ciprofloxacin.

The reprecipitation protocol was employed to make aggregates with different sizes. The antibacterial activities were tested against Gram-negative \textit{E. coli} ORN178 and ORN208. In particular, ORN208 is a selection of nalidixic acid-resistant variant of \textit{E. coli} K-12.\textsuperscript{142} We found that ORN208 also displayed high ciprofloxacin-resistance with MIC (the lowest concentration of an antibiotic that inhibits growth of the organism) of 250 ng/mL, as in contrast to the MIC of 8 ng/mL against \textit{E. coli} ORN178.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{mic.png}
\caption{MIC of ciprofloxacin and compound 2.40 in 70 vol % (A, dissolved), 90 vol % (B, d \textasciitilde 155 \pm 33 nm), 99 vol % (C, d \textasciitilde 43 \pm 6 nm), 99.9 vol % (D, d \textasciitilde 24 \pm 5 nm) water/MeCN against ciprofloxacin-resistant \textit{E. coli} ORN208. The size of aggregates was determined by TEM/SEM.}
\end{figure}

We observed size-dependent aggregation-enhanced antibacterial activities for these derivatives, in particular compound 2.40 (Fig. 35). The MIC of compound 2.40, when fed in dissolved form, is about 625 \textmu g/mL against \textit{E. coli} ORN208. The nanoaggregates prepared from 99.9 vol % water with an average size of 24 nm gave an MIC of 125 ng/mL. This value is 5000 times
lower than that of compound 2.40 in its fully dissolved state, and also about 3.7 times (mole concentration) lower than that of ciprofloxacin. Moreover, the nanoaggregates showed enhanced antibacterial efficacy with the decreased sizes. The aggregation-enhanced activity was also observed in other derivatives including 2.45 and 2.49, but to a less extent.

Similar size-dependent efficacy was frequently observed in inorganic particles\textsuperscript{143}, for example AgNPs\textsuperscript{144}, where a smaller size resulted in a faster release of toxic Ag\textsuperscript{+} species. Similar effects could be the case here, where the release of drug molecules from smaller aggregates would be faster for smaller aggregates.

We propose that the efficacy of the nanodrugs could also be associated with the enhanced permeation of the drug to reach its target in the form of organic aggregates. This could be either via a direct translocation into the intracellular matrix\textsuperscript{145,146} or dissolution of the aggregates at the outer membrane of bacterial.\textsuperscript{147} The exact mechanism at play however warrants further investigations.

2.4. Summary

The reaction between perfluoroaryl azides with enamines formed from condensation of aldehydes and amines was studied. PFAAs, being highly electron-withdrawing dipoles, underwent a very fast cycloaddition, followed by a clean rearrangement of triazolines to give amidines. The protocol was highlighted with the ability to install perfluoroaryl and phenyl moieties on ciprofloxacin to yield aggregates, which displayed aggregation-induced emission (AIE). The nanoaggregates, as pure nanodrugs, displayed enhanced antibacterial efficacy against ciprofloxacin-resistant \textit{E. coli} ORN208.
3. Aryl Amide Formation

(Paper III-V)

The amide bond is an essential linkage in nature. Its dual roles as both hydrogen-bond donor and acceptor impact the structure and thus function of natural proteins. Aryl amides share these unique properties, and have established wide applications as pharmaceuticals, high performance materials, auxiliaries of metal catalysts, and supramolecular foldamers. However, efficient synthesis of aryl amides is still in demand. Mild reactions to yield aryl amides, in particular from aryl azides, represent attractive transformations.

3.1. Thioacid/azide reaction using PFAAs

3.1.1. Introduction

The thioacid/azide amidation was reported in 1988 and has attracted wide interests in the past decades (Fig. 36). Many azides are reactive with thiocarboxylic acids to give amides. The reaction pathway in the presence of base was proposed to go through thiotriazoline via a step-wise cycloaddition followed by extrusion of N₂ and elemental sulfur.

![Figure 36. Thioacid/azide amidation in the presence of a base.](image)

In this reaction, electron-deficient azides generally show much faster kinetics than those electron-rich ones do. So far, sulfonyl azides are among the fastest ones, being in the range of 10⁻³~10⁻² M s⁻¹. The sulfonyl azide/thioacid reaction has been widely used for site-specific modification of molecules including peptides/proteins, reaction-based sensing of thioacids, and kinetic target-guided synthesis. To find other fast thioacid/azide reactions, highly electron-deficient PFAAs were explored.
3.1.2. Performance in various solvents

In the optimization, PFAA 1 was chosen to react with thioacetic acid to give aryl amide 3.4 (Table 5). 2,6-Lutidine ($pK_a = 6.6$) was used as the base to activate thioacetic acid. When the base was absent, aniline 3.5 was mainly formed (entry 2). The base-promoted amide formation was slower in aprotic solvents than that in protic solvents (entries 3-7). When water was added into acetone or MeOH, the amide formation was dramatically accelerated meanwhile the aniline byproduct decreased (entries 1, 3 vs. 8, 9). One of the best conditions, 2,6-lutidine in 60:40 v/v % water/MeOH solvents, gave amide 3.4 in 92% isolated yield (entry 8). The improved amide formation is attributed to the formation/stabilization of thioacetate anion in the protic environments.

**Table 5. Optimization of the reaction conditions.**

<table>
<thead>
<tr>
<th>entry</th>
<th>base (equiv.)</th>
<th>solvent</th>
<th>yield (%)</th>
<th>comp. 3.4</th>
<th>comp. 3.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2,6-Lutidine (1.3)</td>
<td>MeOH</td>
<td>74</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>MeOH</td>
<td>12</td>
<td>67</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2,6-Lutidine (1.3)</td>
<td>Acetone</td>
<td>41</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2,6-Lutidine (1.3)</td>
<td>DCM</td>
<td>57</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2,6-Lutidine (1.3)</td>
<td>DMF</td>
<td>31</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2,6-Lutidine (1.3)</td>
<td>THF</td>
<td>48</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>2,6-Lutidine (1.3)</td>
<td>DMSO</td>
<td>31</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>2,6-Lutidine (1.3)</td>
<td>MeOH:H$_2$O (6:4)</td>
<td>95 (92$^b$)</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>2,6-Lutidine (1.3)</td>
<td>Acetone:H$_2$O (7:3)</td>
<td>78</td>
<td>3</td>
<td></td>
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<tr>
<td>10</td>
<td>Pyridine (1.3)</td>
<td>MeOH</td>
<td>43</td>
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<td>11</td>
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<td>MeOH</td>
<td>95</td>
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<tr>
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<tr>
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<td>MeOH</td>
<td>53</td>
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<tr>
<td>14</td>
<td>TBAOH (1.0)</td>
<td>Acetone</td>
<td>96</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

Conditions: PFAA 1 (0.22 mmol), 3.3 (0.28 mmol), solvent (0.5 mL). $^a$Determined by $^{19}$F NMR. $^b$Isolated yield.

To improve the performance of this reaction in aprotic solvents, strong bases were required. For example, the use of tetrabutylammonium hydroxide (TBAOH, 1 equiv.) gave amide 3.4 in 96% yields in acetone (entry 14).
The reaction was next optimized in aqueous phase (Table 6). Sodium 4-azidotetrafluorophenylbenzoate (PFAA 7) was used as the model azide due to its high solubility in aqueous phase. These reactions were accomplished within 2 h in water (entry 1) or in PBS buffer (entries 3-5) to form amide 3.10 in high yields. Unlike in organic solvents that gave aniline side-product, no aniline product was detected in aqueous solutions.

**Table 6. Reactions in aqueous solution.**

<table>
<thead>
<tr>
<th>entry</th>
<th>solvent</th>
<th>base (equiv.)</th>
<th>time (min.)</th>
<th>yield (%)&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>-</td>
<td>100</td>
<td>90</td>
</tr>
<tr>
<td>2</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>NaOH (1.0)</td>
<td>20</td>
<td>95 (88&lt;sup&gt;c&lt;/sup&gt;)</td>
</tr>
<tr>
<td>3</td>
<td>PBS pH 7.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>120</td>
<td>95</td>
</tr>
<tr>
<td>4</td>
<td>PBS pH 8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>40</td>
<td>99</td>
</tr>
<tr>
<td>5</td>
<td>PBS pH 9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>-</td>
<td>20</td>
<td>99</td>
</tr>
</tbody>
</table>

Conditions: PFAA 7 (0.44 mmol), 3.3 (0.6 mmol), solvent (1 mL), r.t.. <sup>a</sup>0.2 M. <sup>b</sup>Determined by <sup>19</sup>F NMR in CDCl<sub>3</sub>. <sup>c</sup>Isolated yield.

**3.1.3. Chemoselectivity and reactivity**

Following the recommended protocol to test chemoselectivity,<sup>170</sup> a series of the model reactions (Table 5, entry 11) were carried out with 1 equiv. of an (different) interfering compound in each reaction (Paper III). The final mixture was analyzed when the reaction was completed. The results supported that the thioacid/PFAA amidation had high chemo-selectivity with a range of functional groups.

However, aliphatic amines and thiols showed interference with the reaction and resulted in aniline 3.5 formation (up to 40%) in this reaction. The side reaction has also been observed in the sulfonyl azide/thioacid reaction.<sup>91</sup> For PFAAs, the formation of aniline by-product decreased markedly when water was used as a part of the solvent. The formation of aniline 3.5 in the presence of aliphatic amines (or thiols) is proposed to be a result of competing nucleophilic attack of amine (or thiols) to intermediate 3.11 (Fig. 37). This is supported by the formation of amide 3.13 in the reaction mixture.
Figure 37. Proposed reactions of PFAA 1 with thioacetic acid in the presence of aliphatic amines.

Table 7. PFAA reactivities and reaction kinetics.

<table>
<thead>
<tr>
<th>entry</th>
<th>azide</th>
<th>product</th>
<th>yield (%)(^a)</th>
<th>(k_{\text{obs}} (10^{-3} \text{ M}^{-1} \text{s}^{-1}))(^a)</th>
<th>(k_{\text{rel}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PFAA 1</td>
<td>3.4</td>
<td>95 (92(^b))</td>
<td>16.6 ± 0.4</td>
<td>2.4</td>
</tr>
<tr>
<td>2</td>
<td>PFAA 3</td>
<td>3.6</td>
<td>99 (88(^b))</td>
<td>44 ± 2</td>
<td>6.5</td>
</tr>
<tr>
<td>3</td>
<td>PFAA 2</td>
<td>3.7</td>
<td>87 (86(^b))</td>
<td>6.8 ± 0.1</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>PFAA 6</td>
<td>3.8</td>
<td>99 (91(^b))</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>2.8</td>
<td>3.9</td>
<td>99 (94(^b))</td>
<td>21 ± 0.1</td>
<td>3</td>
</tr>
</tbody>
</table>

Conditions: azide (0.66–0.85 mmol), 2 (1.3 equiv.), r.t. \(^a\)Determined by \(^{19}\text{F} NMR. \(^b\)Isolated yield.

Table 7 shows the reaction rates \((k_{\text{obs}})\) of PFAAs and tosyl azide with thioacetic acid in CD\(_3\)OD, which all fell into the fast kinetic range (>\(10^{-3} \text{ M}^{-1} \text{s}^{-1}\)). Compared with PFAA 1, an electron-withdrawing substituent (CN) at the para-
position increased the rate (entry 3) whereas a less electron-withdrawing F-atom showed relatively slower kinetics (entry 4). The reactivity of PFAAs in this reaction is comparable to that of tosyl azide (entry 5).

### 3.2. Amidation using aryl azides and aldehydes

Although demonstrated with high efficiency in section 3.1, the thioacid/azide reaction has met with limited practical success, due to insufficient accessibility of the thiocarboxylic acid reagents.

#### 3.2.1. Introduction

The transformation in this section has a theoretical basis from Chapter 2, where the rearrangement of triazolines, formed from azides and enamines, into amidines was described (Fig. 38a). We hypothesized that the use of enolates/enols instead of enamines would in principle lead to the corresponding amide (Fig. 38b). In this transformation, enolate 3.14b would undergo 1,3-dipolar cycloaddition with an azide to form 5-hydroxyltriazoline 3.15b, which then rearranges to aryl amide 3.17 and releases N₂ as the only side-product. Although the azide-carbonyl triazoline formation was reported to be efficient under basic conditions, the control over the rearrangement of 5-hydroxyltriazoline 3.15b to amide 3.17 can be a challenge. Depending on the substituents and reaction conditions, 5-hydroxyltriazoline 3.15b can undergo various rearrangements to triazoles, anilines, amides, vinyl ketones or α-amino-ketones.

![Figure 38](image-url)

*Figure 38. (a) PFAA-aldehyde-amine reaction to form amidines; (b) Proposed PFAA-aldehyde reaction to form amides.*

#### 3.2.2. N,N-Diethylurea-catalyzed enol-azide amidation

When PFAA 1 and phenylacetaldehyde (2.35) were mixed in DMF, aryl amide (3.17a, Fig. 39) was isolated in 50% yield within 24 h. A prolonged reaction
time did not result in higher conversions. The reaction proved very clean. When DMPU (1,3-dimethyltetrahydropyrimidin-2(1H)-one) was used as the reaction solvent, the reaction could be accomplished within 24 h to give the aryl amide in 90% yield. Screening of catalysts as well as reaction solvents was conducted, in which N,N-diethylurea (DEU) in DMSO was found to be most efficient (Fig. 39).

![Chemical reaction equation]

**Figure 39.** Screening of catalysts for the model reaction in DMSO.

**Reaction scope.** The optimized condition was 20 mol % DEU in DMSO, where aryl amides 3.17a-e formed in high yields within 4 h at r.t. (Fig. 40).

Phenyl azides carrying electron-deficient functionalities could also work well (3.17i-k). Less electron-deficient o-bromophenyl azide produced aryl amide (3.17m, 32%) in a lower yield and most of the starting azides could be recovered. Phenyl azide did not show any amide formation. Highly electron-deficient tosyl azide did not give amide product after extensive optimization.

The scope is highly constrained to phenylacetaldehyde derivatives. Other non-activated aldehydes such as 3-phenylpropanal gave amides in much lower yields where, for example, product 3.17h was obtained in 20% yield after 48 h at 80 °C. However, when DMPU was used instead of DMSO at 80 °C, the yield of 3.17h was improved to 70% even in the absence of DEU.
Mechanistic Studies. A typical aldol product (3.18) can be isolated from the mixture of phenylacetaldehyde in DMPU (15%, Fig. 41a), which was not detected if PFAA was added at the beginning. These results supported that the aldehyde instead of the azide was activated by the urea. When the amidation of phenylacetaldehyde was performed in D₂O/DMSO mixture, only one deuterated proton was detected at the benzyl position in product 3.17a (Fig. 41b). This indicated that the benzylic proton in phenylacetaldehyde could exchange with D₂O under the catalysis of DEU. When 2-phenylpropanal was used, the only benzylic proton in amide product 3.17b was not deuterated (Fig. 41c). These studies suggest that one of the benzylic protons in the amide product is the previous aldehydic proton (-CHO), which could be accomplished via a hydride shift process.
Formation of triazole was observed when \( o \)-nitrophenyl azide was used (Fig. 42). When a base was used instead of DEU, the formation of triazole increased remarkably where, for example, triazole 3.17i-t was isolated exclusively (92%) when 10 mol % DBU was used. These results supported the formation of triazoline intermediates in the DEU catalysis.

![Figure 42. Azide-phenylacetaldehyde reaction catalyzed by DEU or a base.](image)

DEU and DMPU are generally very weak Brønsted bases\(^{180,181}\) and thus not able to deprotonate the enol form of phenylacetaldehyde with \( pK_a = 9.5 \sim 9.8 \).\(^{182,183}\) The catalytic effect is also strongly influenced by the urea structure. For example, DMEU (1,3-dimethyl-2-imidazolidinone) displayed much lower catalytic effect than TMU (tetramethylurea) and DMPU (Fig. 43), in spite of their similar properties.

![Figure 43. Promotion effect of TMU, DMEU and DMPU on the formation of 3.17a. Conditions: PFAA I (0.25 mmol), phenylacetaldehyde (0.28 mmol), ureas (2 equiv.), DMSO-\( d_6 \) (0.8 mL), r.t., by \(^{19}F\) NMR.](image)

**Reaction mechanism.** We proposed an activated enol-mediated mechanism (Fig. 44), where DEU or other ureas stabilize and activate phenylacetaldehyde in the enol form through hydrogen bonds. DMSO could work cooperatively with DEU as a hydrogen bond acceptor.\(^{184,185}\)

The activated enol species (3.20) then undergo cycloaddition with the highly electrophilic azide to form the triazoline (3.21). The near-neutral conditions in the DEU catalysis promoted a synchronous decomposition of the unstable triazoline, via \( \text{N}_2 \)-extrusion and 1,5-hydride shift to form the aryl
amide (3.22) as the final product. This is similar to the perfluoroaryl azide-
aldehyde-amine reaction, where the electron-deficient perfluoroaryl group
promoted the rearrangement. When a stronger base was present, the triazoline
mainly underwent dehydration/aromatization to give 1,4-triazole 3.23.179

Figure 44. Proposed DEU-catalyzed amide formation.

3.2.3. Base-catalyzed enolate-azide amidation
In the previous section, we have established a mild enol-azide amidation
catalyzed by N,N-diethylurea. This protocol is, however, efficient only with
activated phenylacetaldehyde derivatives and certain electron-withdrawing
azides. We however believed that the “cycloaddition-rearrangement” via
triazolines could be a general strategy for aryl amide synthesis. To achieve
reactions for non-activated aldehydes and azides, base catalysis is required to
furnish the azide-carbonyl cycloaddition. The challenge is thus to control the
rearrangement of the triazoline to the amide.

α-Monosubstituted aldehydes. For α-unsubstituted aldehydes, the major
pathway is dehydration/aromatization to form triazoles when base catalysis is
employed.179 Therefore, cyclohexanecarbaldehyde (3.24), an α-
monosubstituted aldehyde which could not undergo dehydration to form
triazole after cycloaddition with azides, was firstly studied to explore the
rearrangement of triazolines (Table 8). When the reaction was carried out in
DMSO in the presence of 10 mol % cesium carbonate at 30 °C, full conversion
to triazoline 3.25 was observed within 6 h but no amide was detected (entry 1).
After workup with 1 N HCl, aryl amide 3.26a was isolated in 90% yield.
Alternatively, the reaction proceeded at 100 °C, either by microwave or hot
plate, to give aryl amide 3.26a within 1 h in 96% isolated yield (entry 3). A
positive correlation of conversion with basicity was observed. KOH (0.1
equiv.) in DMSO promoted the reaction to complete within half an hour (entry
4). When weak base such as trimethylamine was used, no amide product could
be isolated (entry 6). Owing to its ability to enhance basicity, DMSO showed to be the most efficient solvent (entries 7-8).\textsuperscript{186,187}

\textit{Table 8. Optimization of cyclohexanecarbaldehyde with phenyl azide.}

<table>
<thead>
<tr>
<th>entry</th>
<th>base (0.1 equiv.)</th>
<th>solvent</th>
<th>temp. /time</th>
<th>yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cs\textsubscript{2}CO\textsubscript{3}</td>
<td>DMSO</td>
<td>30 °C/6 h</td>
<td>90\textsuperscript{a}</td>
</tr>
<tr>
<td>2</td>
<td>Cs\textsubscript{2}CO\textsubscript{3}</td>
<td>DMSO</td>
<td>80 °C/1 h</td>
<td>87</td>
</tr>
<tr>
<td>3</td>
<td>Cs\textsubscript{2}CO\textsubscript{3}</td>
<td>DMSO</td>
<td>100 °C/1 h</td>
<td>96</td>
</tr>
<tr>
<td>4</td>
<td>KOH</td>
<td>DMSO</td>
<td>100 °C/0.5 h</td>
<td>96</td>
</tr>
<tr>
<td>5</td>
<td>K\textsubscript{2}CO\textsubscript{3}</td>
<td>DMSO</td>
<td>100 °C/1 h</td>
<td>82</td>
</tr>
<tr>
<td>6</td>
<td>NEt\textsubscript{3}</td>
<td>DMSO</td>
<td>100 °C/1 h</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>KOH</td>
<td>DMF</td>
<td>100 °C/0.5 h</td>
<td>60</td>
</tr>
<tr>
<td>8</td>
<td>KOH</td>
<td>MeCN</td>
<td>100 °C/0.5 h</td>
<td>55</td>
</tr>
</tbody>
</table>

Conditions: \textbf{2.6} (1 mmol), \textbf{3.24a} (1.15~1.25 mmol), solvent (2 mL), microwave heating with capping, isolated yield.\textsuperscript{a}Workup with aq. HCl (1 N).

The substrate scope involving \(\alpha\)-monosubstituted aldehydes was investigated. Under heat-promoted conditions, phenyl azides were well tolerated with various substituents to give aryl amides \textbf{3.26b-o} in high yields (Fig. 45a). Electron-deficient aryl azides generally displayed a faster and cleaner amidation compared to phenyl azide, due to the enhanced cycloaddition between the electron-deficient azides and nucleophilic enolate anions.\textsuperscript{77} Accordingly, electron-rich phenyl azides were slower and gave slightly poorer but still good isolated yields (\textbf{3.26c}, 73%; \textbf{3.26g}, 87%), whereas aldol side products were also observed.

Steric effects of \textit{ortho}-groups on the cycloaddition reaction were evaluated.\textsuperscript{188} 2.6-Dimethylphenyl azide was not reactive (\textbf{3.26o}), likely due to the high steric hindrance of the two \textit{ortho}-methyl groups. In contrast, 2-methylphenyl azide, with monosubstitution in the \textit{ortho}-position, gave aryl amide \textbf{3.26j} in 91% isolated yield. Moreover, 2,6-dichlorophenyl azide also gave aryl amide \textbf{3.26n} in 71% isolated yield.

Heterocyclic aryl azides worked well, for example pyridinyl azide (\textbf{3.26p}, 98%) and 3-azidothiophene (\textbf{3.26q}, 69%). Benzyl azide produced amide \textbf{3.26s} in 35% yield; tosyl azide was however not active at all.
Figure 45. Substrate scope. Conditions: 2.1 (1 mmol), 3.24 (1.15~1.25 mmol), DMSO (2 mL), 100 °C, 0.5 h, isolated yield. (a) 1 h. (b) 80 °C. (c) 30 °C, 6~24 h, quenched by 0.5 M aq. AcOH (2 mL). (d) 80~160 °C, 2~10 h. (e) 120 °C, 1 h. (f) 140 °C, 2 h. (g) 60 °C, 12 h.

The scope regarding α-monosubstituted aldehydes is shown in Fig. 45b. Cyclopentyl aldehyde, acyclic isobutryraldehyde and 2-phenylacetaldehyde performed excellently. Cyclopropane carbaldehyde could not undergo the amide formation (3.26u) after extensive optimization, probably owing to unfavorable formation of the enolate. Phenyl aldehyde and other non-enolizable aldehydes showed no product formation.

The synthesis could be a room temperature process by applying the protocol in entry 1 of table 9. In these cases, the azide-enolate cycloaddition was accomplished within 6-24 h to give the stable triazoline. After acidic workup, the triazoline rearranged to the amide in good yield (3.26g, 78%; 3.26q, 81%). For highly electron-deficient azides, aniline (reduction of aryl azides) formation was also observed; but the yields of aryl amides (3.26f, 3.26k) were still good.

α-Unsubstituted aldehydes (Table 9). Under basic conditions (e.g., 0.1 equiv. KOH in DMSO), 3-phenylpropanal (3.27a) gave triazole 3.28 in 35% yield (entry 1). Aryl amide 3.26cc was not isolated at all. The incomplete azide conversion (42%) was attributed to a large amount of aldol condensation product. To promote the azide conversion, the reaction was adjusted by pre-
stirring the azide with the base, followed by addition of excess aldehyde dropwise (entry 2). The formation of triazole 3.28 could be further prohibited when THF/t-BuOH mixture was used as co-solvent. The aryl amide 3.26cc was thus obtained after quenching with acidic solution (entries 3-6). The best result is entry 6, where dropwise addition of 4 equiv. 3-phenylpropanal into phenyl azide with 2 equiv. of t-BuOK in THF/t-BuOH co-solvent at 20 °C enabled a 95% conversion of azide within 5 minutes and aryl amide 3.26cc was isolated in 68% yield after acid quenching.

**Table 9. Optimization of α-unsubstituted 3-phenylpropanal with phenyl azide.**

<table>
<thead>
<tr>
<th>entry</th>
<th>base (equiv.)</th>
<th>solvent</th>
<th>3.27a (equiv.)</th>
<th>temp./time</th>
<th>conv. d</th>
<th>3.28e (%)</th>
<th>3.26cc%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>KOH (0.1)</td>
<td>DMSO</td>
<td>1.2</td>
<td>100 °C/0.5 h</td>
<td>42</td>
<td>35e</td>
<td>n.d.</td>
</tr>
<tr>
<td>2a</td>
<td>KOH (0.5)</td>
<td>DMSO</td>
<td>3</td>
<td>30 °C/1 h</td>
<td>52</td>
<td>48f</td>
<td>n.d.</td>
</tr>
<tr>
<td>3b</td>
<td>t-BuOK(0.5)</td>
<td>THF/t-BuOH</td>
<td>3.0</td>
<td>20 °C/10 min</td>
<td>10</td>
<td>n.d.</td>
<td>8</td>
</tr>
<tr>
<td>4b</td>
<td>t-BuOK(1.1)</td>
<td>THF/t-BuOH</td>
<td>3.0</td>
<td>20 °C/6 min</td>
<td>70</td>
<td>5d</td>
<td>48 (66f)</td>
</tr>
<tr>
<td>5b</td>
<td>t-BuOK(1.5)</td>
<td>THF/t-BuOH</td>
<td>4.0</td>
<td>20 °C/5 min</td>
<td>71</td>
<td>10d</td>
<td>57 (86f)</td>
</tr>
<tr>
<td>6b</td>
<td>t-BuOK(2.0)</td>
<td>THF/t-BuOH</td>
<td>4.0</td>
<td>20 °C/5 min</td>
<td>&gt; 95</td>
<td>14d</td>
<td>68 ± 7f</td>
</tr>
</tbody>
</table>

*2.6 (1 mmol), DMSO (2 mL), workup with 1.5 AcOH solution (2 mL). a Protocol: to a solution of 2.6 (1 mmol) and base in THF/t-BuOH (1 mL/0.5 mL) under vigorous stirring, aldehyde 3.27a in THF (0.5 mL) was added dropwise within 0.5 minute. After the azide conversion was completed, the solution was then quenched by 1.5 M aq. AcOH (2 mL). b Determined by 1H NMR. c Isolated yield. d Yield based on recycled azide. e Average of three experiments. n.d.: not detected.

This strategy performed well with many other α-unsubstituted aldehydes and aryl azides (Table 10). For electron-deficient azides (entries 3, 4) whose triazolines suffered from facile dehydration under basic conditions, the protocol regarding reagent concentration, reaction time, base loading and acid quenching required additional optimization to maximize the isolated yield of aryl amides.
Protocol: to a solution of 2.1 (1 mmol) and base in THF/t-BuOH(1 mL/0.5 mL) under vigorous stirring, aldehyde 3.27 (4 mmol) in THF (0.5 mL) was added dropwise within 0.5 minute. After the reaction was completed, the solution was quenched with 1.5 M aq. AcOH (2 mL). 4 Isolated yield. 5 Solvent volume was doubled.

**PFAA-aldehyde amidation.** The reaction could be efficiently applied to PFAAs. Generally, the amidation proceeded within 1-12 h at r.t. and the aryl amide could be isolated in high yields (Fig. 46). Neither heating-promotion nor acid workup was required to force the rearrangement of triazolines (triazolines were not detected in all attempts by NMR). For α-unsubstituted aldehydes, PFAAs directly gave aryl amides in high yields (3.26pp-3.26vv). α-Unsubstituted aldehydes generally led to significant amount of triazoles when reacting with azides other than PFAAs. The preference of decomposition/rearrangement over dehydration/aromatization is attributed to the exceptionally electron-deficient perfluoroaryl group, which facilitates the extrusion of N₂. This observation is consistent with the PFAA-aldehyde-amine reaction in which amidines were formed cleanly from the corresponding 5-amino-triazolines. ⁸⁸
Figure 46. Synthesis of perfluoroaryl amides. Conditions: PFAA (1 mmol), 2,3 (1.15 - 1.25 mmol), DMSO (2 mL), 1-12 h, isolated yield. (a) 60 °C. (b) 50 °C, 4 d. (c) 4-Dimethylaminopyridine was used as base. (d) 40 °C, 16 h.

3.3. Summary

This chapter reports three transformations from aryl azides to aryl amides. The thioacid/PFAA amidation reaction was first studied and demonstrated high efficiency in the various solvent conditions, in particular in aqueous media. Owing to the similarities of the enols/enolates and the enamines as electron-rich nucleophiles, the enolate/enol-azide cycloaddition was explored. In this regards, N,N-diethylurea was discovered as an efficient organic catalyst to promote the amidation between PFAAs and phenylacetaldehyde derivatives at room temperature. The “cycloaddition-rearrangement” strategy via triazolines was then generalized with the combination of base-catalyzed cycloaddition and acid/heat-promoted rearrangement, to provide a facile aryl amide synthesis with especially high efficiency for highly electron-deficient aryl amides.
4. Nitrene-mediated Fluorescence Switch-on

(Paper VI)

PFAAs are well-known substrates for nitrene chemistry giving high yields of insertion products (section 1.3.2). In this chapter, we report the use of the nitrene transformation with PFAAs to design fluorogenic structures, which become fluorescent upon photoactivation.

4.1. Introduction

Fluorescence imaging has emerged as an important tool to study biomedical problems under living conditions. The weakness is, however, the low spatial resolution (~ 250 nm) imposed by light diffraction. As shown by the 2014 Nobel prize in chemistry, this barrier can nevertheless be overcome on the basis of switching events. One of the most appreciated switching is by photo-controllable chemical transformation of molecular fluorophores, for example in photo-activated localization microscopy (PALM). These photo-controllable transformations have an essential impact on the quality of imaging, and new transformations are still in demand.

Figure 47. (a) Azide-to-amine photochemical process (dark to fluorescent switch-on) in PALM. (b) Our strategy.

Azido group has been widely used to mask fluorophores in non-fluorescent dark states. Often, the fluorescent states can be switched on upon transformation of azides, for example, through reduction, photolysis or “click” conjugation. The azido group displays orthogonal properties to other chemical processes, and thus these azide-masked fluorophores are highly stable and offer a good dark background. In particular, the photochemical
azide-to-amine transformation has been developed as a very successful strategy in PALM.\textsuperscript{197-201} The reaction produces aryl nitrene after $N_2$ extrusion, which then gets protonated through, most likely, hydrogen abstraction.\textsuperscript{50,204} In practice, these perfluorinated azide-masked dihydrofuran fluorophores were strikingly efficient and able to achieve a spatial resolution down to 36 nm in PMMA films (Fig. 47a).\textsuperscript{199} The good performance was attributed to a clean photo-controllable reduction and as a consequence a higher signal-to-noise ratio compared to non-fluorinated aryl azide-masked fluorophores.\textsuperscript{201} However, these perfluorinated nitrenes would arbitrarily insert into various molecular structures, as has been shown in PAL experiments.\textsuperscript{46,201} These reactions could lead to a variety of different fluorescent molecules, thus weakening the overall signal intensity and causing potential damages to living samples.\textsuperscript{201}

The intramolecular C-H insertion of perfluorinated aryl nitrenes can be an attracting alternative to the azide-to-amine process in mediating fluorescence switch events. Nitrenes formed by thermolysis or photolysis of azides have been reported to efficiently insert to proximal C-H bonds to form $N$-heterocycles.\textsuperscript{23,205} Here, the use of the highly robust nitrene insertion into neighboring aromatics in an intramolecular fashion (Fig. 47b) has been explored to initiate the switch-on fluorescence.

### 4.2. Nucleophilic aromatic substitution of PFAAs

We employed nucleophilic aromatic substitutions (SNAr) to construct the ortho-functionalized fluorinated aryl azides.\textsuperscript{206,207} When PFAA 1 reacted with carbazole 4.5 in DMSO using Cs$_2$CO$_3$ (2 equiv.) as a base, double substituted aryl azide 4.7 formed exclusively at r.t. and was isolated in 86\% yield (entry 1, Table 11). When other solvents such as DMF, MeCN and THF were used (entries 2-4), the formation of azide 4.7 was much slower. For bases with lower strength under these conditions (e.g., NEt$_3$), full transformation of the azide could not be accomplished even when the reaction time was greatly extended (entries 5-6). The stronger sodium hydride and t-BuOK accelerated the reaction, but the isolated yields were lower (entries 7-9). A significant amount of dark polymeric by-products consisting of carbazole moieties were produced. This protocol was applied to other carbazole derivatives, and the corresponding azide derivatives could be isolated in high yields (Fig. 49, product 4.8, 75\%; 4.9, 82\%).

The reaction likely proceeded through two sequential substitutions of a carbazolenide nitranion with the aromatic C-F bonds. Interestingly, mono-substituted product (4.6) was only detected in trace amounts, even when a large excess of azide (8 equiv.) to the carbazole was used. The regiochemistry of 4.6 was determined though $^1$H NMR analysis of the carboxylate methyl ester group, which showed a through-space NOE signal from the ortho-carbazole moiety.\textsuperscript{208} It is hypothesized that the first substitution activated the aryl C-F
bonds and thus greatly facilitated an even faster second substitution at its para-position. In the mono-substituted product 4.6, the large planar carbazole moiety is expected to be almost perpendicular to the central perfluorinated phenyl ring. The perpendicular configuration would strongly decrease the conjugative electron-donation of nitrogen atom.\textsuperscript{209} Therefore, the carbazole moiety activates the ring carbon (C-F) in the para-position to be highly electrophilic for the second S\textsubscript{N}Ar. Besides, steric effect also played a role in the regioselectivity. The resulting pattern of double para-substitutions has also been observed in many nucleophilic substitutions of polyfluoroaromatics.\textsuperscript{210,211}

**Table 11. Optimization of conditions\textsuperscript{a}**

<table>
<thead>
<tr>
<th>entry</th>
<th>base</th>
<th>solvent</th>
<th>time</th>
<th>temp</th>
<th>4.7 (%)\textsuperscript{b}</th>
<th>4.7 : 4.6</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Cs\textsubscript{2}CO\textsubscript{3}</td>
<td>DMSO</td>
<td>12h</td>
<td>r.t.</td>
<td>&gt;97 (86\textsuperscript{c})</td>
<td>n.d.</td>
</tr>
<tr>
<td>2</td>
<td>Cs\textsubscript{2}CO\textsubscript{3}</td>
<td>DMF</td>
<td>12h</td>
<td>r.t.</td>
<td>76</td>
<td>n.d.</td>
</tr>
<tr>
<td>3</td>
<td>Cs\textsubscript{2}CO\textsubscript{3}</td>
<td>MeCN\textsuperscript{d}</td>
<td>12h</td>
<td>reflux</td>
<td>44</td>
<td>14:1</td>
</tr>
<tr>
<td>4</td>
<td>Cs\textsubscript{2}CO\textsubscript{3}</td>
<td>THF</td>
<td>24h</td>
<td>reflux</td>
<td>37</td>
<td>8:1</td>
</tr>
<tr>
<td>5</td>
<td>NEt\textsubscript{3}</td>
<td>DMSO</td>
<td>72h</td>
<td>80 °C</td>
<td>17</td>
<td>3:1</td>
</tr>
<tr>
<td>6</td>
<td>K\textsubscript{2}CO\textsubscript{3}</td>
<td>DMSO</td>
<td>24h</td>
<td>r.t.</td>
<td>75</td>
<td>n.d.</td>
</tr>
<tr>
<td>7</td>
<td>NaH</td>
<td>DMSO</td>
<td>12h</td>
<td>r.t.</td>
<td>53</td>
<td>\textasciicircum{e}</td>
</tr>
<tr>
<td>8</td>
<td>t-BuOK</td>
<td>DMSO</td>
<td>12h</td>
<td>r.t.</td>
<td>68 (52\textsuperscript{e})</td>
<td>\textasciicircum{e}</td>
</tr>
<tr>
<td>9</td>
<td>t-BuOK</td>
<td>MeCN\textsuperscript{d}</td>
<td>3h</td>
<td>r.t.</td>
<td>16</td>
<td>7:1</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Condition: PFAA 1 (0.4 mmol), carbazole (0.8 mmol), base (1 mmol, except for NaH and t-BuOK 0.4 mmol), solvent (5 mL). \textsuperscript{b}By \textsuperscript{1}H NMR using internal standard. \textsuperscript{c}Isolated yield. \textsuperscript{d}20 mL solvent used. \textsuperscript{e}Not calculated. n.d., compound 4.6 was not detected.

The reaction also worked efficiently with phenols as nucleophiles (Fig. 48). In this case, mono-substituted compound 4.11 was isolated in 78% yield. The regio-selectivity was determined by 1D-NOE NMR experiment. Compound 4.11 could furthermore undergo a clean second substitution to yield regioisomers 4.12 and 4.13 in the ratio of \textasciicircum{sim} 1.8:1. However, these two regioisomers were not separated efficiently by conventional methods. Further substitutions could be also observed with the addition of more phenol, but with very low yields.
Figure 48. $S_N$Ar with phenol. (a) PFAA 1 (0.4 mmol), phenol (0.38 mmol), Cs$_2$CO$_3$ (0.5 mmol), DMSO (5 mL), r.t., 12 h; (b) 4.11 (0.3 mmol), phenol (0.3 mmol), Cs$_2$CO$_3$ (0.4 mmol), DMSO (5 mL), r.t., 12 h.

4.3. Photo-activatable fluorescence

Of the azide derivatives, all the para-disubstituted derivatives (4.7-4.9 and 4.12, Fig. 49) displayed switch-on fluorescence upon UV-irradiation. Compound 4.7 was chosen as a model compound to study the conversion. After irradiation of azide 4.7 with 350 nm UV-light in MeOH, product 4.14 was isolated in 68% yield (Fig. 50). Analysis of the crude mixture by $^1$H and $^{19}$F NMR spectroscopy showed a highly clean formation (>90%) of product 4.14. The structure of compound 4.14 was determined by NMR and HRMS, and could also be distinguished from the reduced product 4.15.

Figure 49. Photo-activatable fluorophores

Figure 50. (a) Photo-initiated intramolecular C-H insertion. (b) Reduction to amine.

The photoactivation was followed by UV-vis and fluorescence microscopy. The increased absorption at 376 nm was a result of product 4.14 formation (Fig. 50).
51a). The process was accompanied by a decrease in absorption at 328 nm, stemming mostly from the intact carbazole moiety. The absorption profile could be well fitted into a standard first-order mathematical model, to give a half-life ($t_{1/2}$) of about 12 seconds. Starting compound 4.7 did not give any fluorescence; while the product showed emission centered at 550 nm (Fig. 51b). The amplification of the fluorescent intensity at 550 nm exceeded a factor of 200, indicating a very high contrast between the off (4.7) and on (4.14) states. Moreover, the Stoke shift of the fluorophore 4.14 is as large as 180 nm. The irradiation wavelength for compound 4.7 and the excitation wavelength for product 4.14 are well separated, due to the expanded conjugated system after the reaction. These properties are all desirable and suggest that this transformation has broad applications as a photo-controllable switch.

**Figure 51.** (a) Absorption at 328 nm and 376 nm vs. irradiation time in the photo-activation of compound 4.7. (b) Fluorescence spectra before and after 200 s irradiation, excitation wavelength: 378 nm. Conditions: $10^{-5}$ M in ethanol, UV lamp (0.46 mW cm$^{-2}$).

**Table 12.** Photophysical and photochemical parameters of various azido fluorogens.

<table>
<thead>
<tr>
<th>Comp.</th>
<th>$\lambda_{\text{abs, azido}}$(nm)$^a$</th>
<th>$\lambda_{\text{abs, p}}$(nm)$^b$</th>
<th>$\lambda_{\text{fl}}$(nm)</th>
<th>Yield$^d$ (%)</th>
<th>$\Phi_F$ (%)</th>
<th>$t_{1/2}$ (s)</th>
<th>$\Phi_p$ $^g${λ$p$ nm}$^h$</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>407 {26,700}</td>
<td>463 {20,000}</td>
<td>578</td>
<td>87</td>
<td>0.62</td>
<td>85$^f$</td>
<td>0.017$^h$ {385}</td>
</tr>
<tr>
<td>4.7</td>
<td>328 {12,400}</td>
<td>376 {22,890}</td>
<td>550</td>
<td>80</td>
<td>0.51</td>
<td>15$^f$</td>
<td>0.18$^h$ {350}</td>
</tr>
<tr>
<td>4.8</td>
<td>345 {13,830}</td>
<td>378 {15,670}</td>
<td>538</td>
<td>95</td>
<td>0.76</td>
<td>10$^f$</td>
<td>0.46$^h$ {350}</td>
</tr>
<tr>
<td>4.9</td>
<td>358 {13,830}</td>
<td>394 {16,200}</td>
<td>581</td>
<td>90</td>
<td>0.11</td>
<td>6$^f$</td>
<td>0.62$^h$ {350}</td>
</tr>
</tbody>
</table>

$^a$Peak absorbance and molar absorption of azido fluorogen. $^b$Peak absorbance and molar absorption of product. $^c$Chemical reaction yield to the fluorescent product, determined by $^{19}$F and $^1$H NMR spectroscopy. $^d$Fluorescence quantum yield. $^e$Half time of photoconversion. $^f$Photoconversion quantum yield of azides. $^g$Wavelength used to photoactivate azido fluorogens. $^i$Light intensity: 1.1 mW cm$^{-2}$. $^j$Light intensity: 0.46 mW cm$^{-2}$. $^k$Reference 201. Measurements for compound 4.7-4.9 were done in ethanol at 10 μM concentration.
In Table 12, these azide-masked fluorophores are compared with the established pentafluorophenyl azide-derivatized dihydrofuran fluorophore 4.1 (Fig. 1a). In general, compound 4.7-4.9 showed comparable properties to 4.1 in terms of absorption wavelength ($\lambda_{abs, azido}$), fluorescence wavelength ($\lambda_{abs, p}$)/quantum yield ($\Phi_F$), and chemical reaction yield. The photoconversion quantum yield ($\Phi_p$) measures the probability of the molecule undergoing the azide transformation for each photon absorbed. In comparison to compound 4.1 using the azide-to-amine transformation, the intramolecular insertion of compound 4.7-4.9 in the current study generally displayed higher $\Phi_p$ values by a factor of 10. Masked fluorophores with higher photo-conversion efficiency enable activation at lower illumination intensity, which lead to less potential cell-damage during exposure to UV light.

### 4.4. Further modification

In addition, the photoactivatable fluorogenic unit could be further modified by the use of the carboxylic moiety and the photoactivatable moiety could thus be readily incorporated into different environments. Figure 52 shows the introduction of a $\alpha$-D-mannopyranoside entity into compound 4.7. It showed high solubility in water and the switch-on fluorescence was also retained.

![Figure 52. Synthesis of mannose-conjugated water-soluble photoactivatable azide-masked fluorophore 4.17.](image)

**Figure 52.** Synthesis of mannose-conjugated water-soluble photoactivatable azide-masked fluorophore 4.17. (a) 1, NaOH, methanol, r.t., 5 h; 2, NHS, EDAC, DCM, r.t., 48 h; (b) 2-aminoethyl $\alpha$-D-mannopyranoside, DMF, at dark, r.t., 8 h.

### 4.5. Summary

This chapter describes a type of photoactivatable azide-masked fluorogens, operating through intramolecular nitrene C-H insertion into neighboring aromatic rings (carbazole and phenol). These azide-masked fluorogens were efficiently accessed via the direct nucleophilic aromatic substitution of PFAAs. The properties of this switch-on fluorescence are comparable with the well-known azide-to-amine process utilized in PALM, but show much higher photoconversion efficiency.
5. Future Prospects

While “click” chemistry brings up interests on aliphatic azides, this work has been focused intensively on aryl azides. Aryl azides, and in particular PFAAs, showed markedly different reactivities from aliphatic azides. In case of cycloadditions, their chemical reactivities are complementary. This could possibly allow these reliable cycloadditions with PFAAs to be used complimentarily to aliphatic azide reactions. This difference is caused by the characteristics of the azides which, as mentioned, as HOMO-LUMO-controlled dipoles. The vast difference in reactivity of azides (alkyl azides, phenyl azides, PFAAs, sulfonyl azides), as exemplified in this thesis, is therefore strongly impacted by the neighboring substituent group. For this reason, other types of azides, such as boron azides and phosphoryl azides, could also be of interest in such chemical systems.

The three-component perfluoroaryl azide-aldehyde-amine reaction, as demonstrated, is a catalyst-free, fast and mild conjugation reaction (Chapter 2). Importantly, this reaction can selectively modify amine groups in the presence of carboxylic acid groups, which provides a straightforward functionalization of peptides/proteins. The use of this reaction is currently undergoing, and has been already applied in functionalization of nanomaterials with glycan structures (Paper VIII) and drug molecules (Paper VII).

This thesis describes the rearrangement of triazolines to yield stable amides or amidines. The rearrangement is influenced by a range of factors. For PFAAs, the highly electron-withdrawing perfluoroaryl ring promoted the rearrangement of triazolines at room temperature; however a detailed mechanism warrants further studies. Conditions to induce rearrangement of triazolines to aryl amides have been tested, which provide a very general synthesis of aryl amides from azides and aldehydes (Chapter 3). Therefore, similar decomposition studies on triazolines formed from azide-ename reaction is general slow for non-activated alkenes. It will be of importance to develop catalytic azide-alkene cycloadditions, like the CuAAC in azide-alkyne cycloadditions.

Although triazolines can be synthesized straightforwardly through the azide-alkene cycloaddition, the reaction is general slow for non-activated alkenes. It will be of importance to develop catalytic azide-alkene cycloadditions, like the CuAAC in azide-alkyne cycloadditions.

The well-known responsive nature of azides is highlighted as it provides a simple way to couple input of information by light with molecular events. A current challenge is to activate azides with longer wavelength or less destructive light (or even visible light). In addition, the use of other physical forces, for example, ion beam and electromagnetic field will also be interesting to activate azide in chemical transformations. Improvements on these aspects could perhaps create a useful non-destructive way to combine azide chemistry (i.e., reactions) with physical factors/signals.
6. Concluding Remarks

This thesis resulted in several robust transformations using perfluoroaryl azides. Furthermore, application of these reactions has been preliminarily demonstrated in making luminescent nanodrugs.

The enhanced reactivity of PFAAs is associated with perfluorination, which significantly lowers the LUMO orbital energy of the azide dipole. Therefore, it was shown that PFAAs could undergo fast reactions with reagents of high nucleophilicity. Those include enamines, enols, enolates and thiocarboxylates in this work. The multicomponent perfluoroaryl azide-aldehyde-amine reaction yielding the amidine proceeded through a 1,3-dipolar azide-enamine cycloaddition, followed by a synchronous rearrangement of the obtained triazoline intermediate. Similar cycloaddition-rearrangement pathways were observed in the PFAA/thioacid reactions proceeding through thiotriazolines, and in the aryl azide/aldehyde amidation through 5-hydroxidetriazolines. These reactions are highly selective due to the nature of the cycloadditions with formation of two bonds at about the same time. Moreover, they also showed fast kinetics under mild reaction conditions.

The perfluoroaryl azide-aldehyde-amine reaction provided a simple approach to install perfluoroaryl and aryl moieties to guide formation of supramolecular aggregates. It has been shown with ciprofloxacin, which after derivatization, forming stable nanoaggregates by precipitation. Moreover, the obtained aggregates showed enhanced antibacterial efficacy and aggregation induced emission.

The last part of the thesis introduced a molecularly fluorogenic structure capable of switching on fluorescence upon excitation with photons, which is mediated by nitrene insertion reactions. The process was studied, and was shown to be comparable with the well-established azide-masked fluorophores for high resolution fluorescent imaging. These photo-activatable fluorogens are straightforwardly synthesized by nucleophilic aromatic substitution of PFAAs.

This work highlights the enhanced performance of old-type sluggish reactions centered around the manipulation of azide substrates. In particular, PFAAs are shown as a unique class of pre-activated electron deficient azides applicable in several fast and selective transformations. This thesis calls attentions to the usefulness of azide chemistry in fields beyond just synthetic chemistry.
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Appendix A

The following is a description of my contribution to Publications I to VI, as requested by KTH.

Paper I: I contributed to the formulation of the research problems, performed the majority of the experimental work (except for the computational studies), and wrote the draft manuscript.

Paper II: I contributed to the formulation of the research problems, performed part of the experimental studies, analyzed the data (including material characterization, biological experiments) and wrote the draft manuscript.

Paper III: I contributed to the formulation of the research problems, performed part of the experimental studies and wrote the draft manuscript.

Paper IV: I contributed to the formulation of the research problems, performed the experimental studies and wrote the draft manuscript.

Paper V: I contributed to the formulation of the research problems, performed part of the experimental studies and wrote the draft manuscript.

Paper VI: I contributed to the formulation of the research problems, performed the experimental studies and wrote the draft manuscript.
Appendix B

This Appendix contains spectroscopic data for compounds discussed in this thesis but not reported in publications I-VI.

**Amidine 2.5e.** To a solution of aldehyde (2.5 mmol) and azide (2.0 mmol) in MeOH (12 mL), amine (2.1 mmol) in MeOH (2 mL) was added dropwise at 40 °C. When NMR indicated full conversion (~4 h), silica gel (1.5 g) was added to the mixture and the solvent was evaporated. The resulting crude was further purified by flash column chromatography using hexanes/EtOAc 5:1 (R_f = 0.29) as eluent to provide the entitled compound as white solid (671 mg, 91%). ^1H NMR (400 MHz, DMSO): δ 3.66 (s, 2H), 2.90 (t, 2H, J_HH = 7.2 Hz), 3.49 (br, 2H), 3.55 (dt, 2H, J_HH = 6.0, 6.8 Hz), 3.86 (s, 3H, OCH₃), 6.91 (m, 2H), 7.15-7.32 (m, 8H, Ar-H), 8.03 (t, 1H, NH, J_HH = 5.1 Hz); ^13C NMR (100 MHz, DMSO): δ 33.76, 38.44, 42.43, 54.96, 102.05 (m), 126.42, 126.13, 128.29, 128.33, 128.21, 128.76, 135.26, 135.47 (m), 139.54, 139.58 (dm, 2C, J_CF = 241 Hz), 145.11 (dm, 2C, J_CF = 254 Hz), 160.25, 160.63; ^19F NMR (376 MHz, DMSO): δ -142.28 (m, 2F), -153.13 (m, 2F); ESI-HRMS: Calcd. for C₂₄H₂₁F₄N₂O₂ [M+H]^+: 444.1534, found 445.1526.

**Amidine 2.5f.** To a solution of phenylacetaldehyde (0.75 mmol) and azide (0.50 mmol) in DMSO/H₂O 3:1 solvent (10 mL), L-alanine (0.75 mmol) was added and the mixture was stirred at 60 °C. When NMR indicated full conversion (~4 h), the mixture was lyophilized. The crude was further purified by flash column chromatography using DCM/MeOH 19:1 (R_f = 0.20) as eluent to provide the entitled compounds as colorless oil (157 mg, 77%). ^1H NMR (400 MHz, CDCl₃): δ 2.02 (d, 3H, J_HH = 7.0 Hz), 3.51 (s, 2H), 3.93 (s, 3H), 4.63 (Quintet, 1H, J_HH = 6.7 Hz), 5.50 (d, 1H, J_HH = 6.0 Hz, NH), 7.12 (d, 2H, J_HH = 6.9 Hz), 7.30 (m, 3H), 7.75 (br, 1H, COOH); ^13C NMR (100 MHz, DMSO): δ 33.76, 38.44, 42.43, 54.96, 101.92-102.22 (m), 126.42, 126.13, 128.29, 128.33, 128.21, 128.76, 135.26, 135.47 (m), 139.54, 139.58 (dm, 2C, J_CF = 240.9 Hz), 145.11 (dm, 2C, J_CF = 254.0 Hz), 160.25, 160.63; ^19F NMR (376 MHz, CDCl₃): δ -140.97 (m, 2F), -152.00 (m, 2F); ESI-HRMS: Calcd. for C₁₉H₁₇F₄N₂O₄ [M+H]^+: 413.1124, found 413.1121.

**Amidine 2.5g.** Following a similar protocol as amidine 2.5f. The crude was purified by flash column chromatography using DCM/MeOH 19:1 (R_f = 0.26) as eluent to provide the entitled compounds as pale yellowish oil (166 mg, 74%). ^1H NMR (400 MHz, CDCl₃): δ 2.02 (d, 6H, J_HH = 7.1 Hz), 2.23 (m, 1H), 2.49 (t, 2H, J_HH = 7.0 Hz), 3.52 (s, 2H), 3.93 (s, 3H), 4.78 (t, 1H, J_HH = 5.7 Hz), 5.74 (br, 1H, NH), 7.13 (d, 2H, J_HH = 6.8 Hz), 7.30 (m, 3H), 9.34 (br, 1H, COOH); ^13C NMR (100 MHz, DMSO): δ 33.76, 38.44, 42.43, 54.96, 102.10 (m), 126.42, 126.13, 128.29, 128.33, 128.21, 128.76, 135.26, 135.47 (m), 139.54, 139.58 (dm, 2C, J_CF = 241 Hz), 145.11 (dm, 2C, J_CF = 254 Hz).
Hz), 160.25, 160.63; $^{19}$F NMR (376 MHz, CDCl$_3$): $\delta$ -140.88 (m, 2F), -152.00 (m, 2F); ESI-HRMS: Calcd. for C$_{22}$H$_{23}$F$_4$N$_2$O$_4$ [M+H]$^+$: 455.1594, found 455.1591.

**Amidine 2.5i.** To a solution of aldehyde (2.5 mmol) and azide (2.0 mmol) in MeOH (6 mL), aniline (2.5 mmol) in MeOH (2 mL) was added dropwise at 40 °C. When NMR indicated full conversion (~12 h), silica gel (1.3 g) was added to the reaction mixture and the solvent was subsequently evaporated. The crude was further purified by flash column chromatography using hexanes/EtOAc 4:1 ($R_f = 0.31$) as eluent to provide the entitled compound as pale yellow solid (671 mg, 91%). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 3.66 (s, 2H), 3.96 (s, 3H, OCH$_3$), 6.50 (br, 1H, Ph-NH), 7.09 (t, 1H, Ar-H, $J_{HH} = 7.7$ Hz), 7.23-7.39 (m, 7H, Ar-H), 7.48 (d, 2H, Ar-H, $J_{HH} = 7.7$ Hz); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 39.65, 52.99, 104.52 (m), 120.91, 124.59, 128.19, 129.01, 129.49, 129.55, 133.67, 138.38, 133.37 (m), 139.55 (dm, 2C, $J_{CF} = 243$ Hz), 145.18 (dm, 2C, $J_{CF} = 254$ Hz), 157.32, 161.10; $^{19}$F NMR (376 MHz, CDCl$_3$): $\delta$ -140.83 (m, 2F), -152.02 (m, 2F); ESI-HRMS: Calcd. for C$_{22}$H$_{17}$F$_4$N$_2$O$_2$ [M+H]$^+$: 417.1221, found 417.1220.

**Amidine 2.5j.** Following a similar protocol as amidine 2.5i. Pale greenish solid. $R_f = 0.17$ (hexanes/EtOAc = 9:1). $^1$H NMR (400 MHz, DMSO): $\delta$ 3.77 (s, 2H), 3.84 (s, 3H, OCH$_3$), 6.95 (d, 2H, Ar-H, $J_{HH} = 7.6$ Hz), 7.17-7.24 (m, 9H, Ar-H), 7.35 (t, 4H, $J_{HH} = 7.6$ Hz), $^{13}$C NMR (100 MHz, DMSO): $\delta$ 38.22, 52.98, 103.00 (m), 116.70, 119.64, 126.69, 126.98, 127.68, 128.08, 128.73, 129.18, 129.65, 134.87, 133.36 (m), 138.44 (dm, 2C, $J_{CF} = 243.6$ Hz), 143.41, 144.83 (dm, 2C, $J_{CF} = 254.8$ Hz), 159.96, 162.23; $^{19}$F NMR (376 MHz, DMSO): $\delta$ -141.81 (m, 2F), -152.05 (m, 2F); ESI-HRMS: Calcd. for C$_{28}$H$_{21}$F$_4$N$_2$O$_2$ [M+H]$^+$: 493.1534, found [M+H]$^+$ 493.1515.

**Amidine 2.5k.** Following a similar protocol as amidine 2.5e. The crude was further purified by flash column chromatography using hexanes/EtOAc 9:1 ($R_f = 0.32$) as eluent to provide the entitled compounds as white solid (310 mg, 85%). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 0.84 (t, 3H, $J_{HH} = 7.3$ Hz), 1.45 (Sextet, 2H, $J_{HH} = 7.7$ Hz), 1.62 (m, 4H), 1.68 (m, 2H), 2.17 (t, 2H, $J_{HH} = 7.7$ Hz), 3.55 (m, 4H), 3.93 (s, 3H, OCH$_3$), $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 13.89, 20.33, 24.68, 26.10, 31.41, 46.55 (br), 52.79, 102.60 (m), 135.82 (m), 139.73 (dm, 2C, $J_{CF} = 240$ Hz), 145.92 (dm, 2C, $J_{CF} = 254$ Hz), 161.50, 162.48; $^{19}$F NMR (376 MHz, CDCl$_3$): $\delta$ -141.77 (m, 2F), -153.76 (m, 2F); ESI-HRMS: Calcd. for C$_{17}$H$_{21}$F$_4$N$_2$O$_2$ [M+H]$^+$: 360.1539, found [M+H]$^+$ 360.1538.

**Amidine 2.5m.** Following a similar protocol as amidine 2.5e. White solid. $R_f = 0.38$ (hexanes/EtOAc = 5:1). $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 1.59 (m, 4H), 1.67 (m, 2H), 2.52 (m, 2H), 2.71 (m, 2H), 3.56 (m, 4H), 3.94 (s, 3H, OCH$_3$), 7.03 (dm, 2H, $J_{HH} = 7.0$ Hz), 7.20 (m, 1H), 7.26 (tm, 2H, $J_{HH} = 6.6$ Hz); $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 24.58, 25.99, 31.12, 32.94, 46.59, 52.81, 102.80 (m), 126.77, 128.17, 128.78, 135.46 (m), 139.68 (dm, 2C, $J_{CF} = 240$ Hz), 146.03 (dm, 2C, $J_{CF} = 256$ Hz), 139.63, 161.41, 161.60; $^{19}$F NMR (376 MHz, CDCl$_3$): $\delta$ -141.45 (m, 2F), -153.37 (m, 2F); ESI-HRMS: Calcd. for C$_{22}$H$_{22}$F$_4$N$_2$O$_2$ [M+H]$^+$: 422.1617, found 422.1617.
**Amidine 2.5n.** Following a similar protocol as amidine 2.5e. White solid. \( R_f = 0.30 \) (hexanes/EtOAc= 10:1). \(^1\)H NMR (500 MHz, CDCl\(_3\)): \( \delta_H \) 1.18 (d, 6H, CH\(_3\), \( J_{HH} = 7.2 \) Hz), 1.62 (m, 4H), 1.68 (m, 2H), 2.80 (Septet, 1H, \( J_{HH} = 7.2 \) Hz), 3.54 (m, 4H), 3.93 (s, 3H, OCH\(_3\)) \(^{13}\)C NMR (125 MHz, CDCl\(_3\)): \( \delta \) 19.36, 24.54, 25.87, 31.86, 47.34, 52.63, 101.90 (m), 135.41 (m), 139.14 (dm, 2C, \( J_{CF} = 239 \) Hz), 145.76 (dm, 2C, \( J_{CF} = 260 \) Hz), 161.42, 165.47; \(^{19}\)F NMR (376 MHz, CDCl\(_3\)) : \( \delta \) -141.90 (m, 2F), -154.08 (m, 2F); ESI-HRMS: Calcd. for C\(_{17}\)H\(_{21}\)F\(_4\)N\(_2\)O\(_2\) [M+H]\(^+\) : 361.1539, found 361.1538.

**Amidine 2.28.** Following a similar protocol as amidine 2.5e. White solid. Yield 80%. \( R_f = 0.27 \) (hexanes/EtOAc= 5:1). \(^1\)H NMR (400 MHz, CDCl\(_3\)): \( \delta_H \) 1.60 (d, 3H, \( J = 7.40 \) Hz), 3.32 (m, 2H), 3.43 (m, 2H), 3.53 (m, 4H), 4.06 (q, 1H \( J = 7.40 \) Hz), 7.26 (d, 2H, \( J_{HH} = 7.8 \) Hz), 7.24 (t, 1H, \( J_{HH} = 7.6 \) Hz), 7.35 (t, 2H, \( J_{HH} = 7.8 \) Hz); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \( \delta_H \) 15.12, 39.57, 46.27, 66.32, 125.94, 126.30 (tm, \( J_{CF} = 15 \) Hz), 127.03, 129.09, 136.0 (dm, 1C, \( J_{CF} = 247 \) Hz), 138.12 (dm, 2C, \( J_{CF} = 253 \) Hz), 139.39, 140.03 (dm, 2C, \( J_{CF} = 241 \) Hz), 164.93; \(^{19}\)F NMR (376 MHz, CDCl\(_3\)) : \( \delta \) -152.97 (dm, 2F, \( J_{FF} = 23.0 \) Hz), -164.43 (tm, 2F, \( J_{FF} = 24.0 \) Hz), 166.53 (t, 1F, \( J_{FF} = 22.7 \) Hz); ESI-HRMS: Calcd. for C\(_{10}\)H\(_{18}\)F\(_5\)N\(_2\)O [M+H]\(^+\) : 385.1339, found 385.1338.
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