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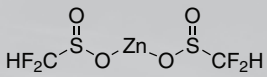

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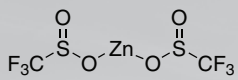

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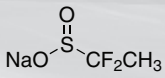

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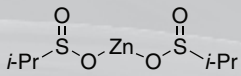

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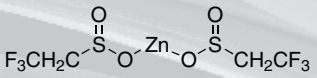

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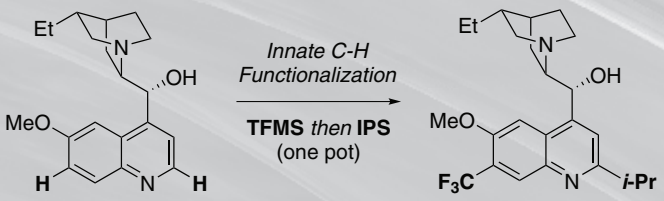



Reference:
Fujiwara, Y.; Dixon, J. A.; O'Hara, F.; Funder, E. D.; Dixon, D. D.; Rodriguez, R. A.; Baxter, R. D.; Herle, B.; Sach, N.; Collins, M. R.; Ishihara, Y.; Baran, P. S. *Nature* **2012**, *492*, 95.


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## ABOUT OUR COVER

Detail from ***Farmhouse in Provence***. Photo Courtesy National Gallery of Art, Washington, DC.

As a young man, van Gogh worked in various jobs in Holland, England, France, and Belgium. Only at age 27, in 1880, did he start his formal art training in Brussels, and continued his training in Holland, where he discovered the work of Rubens, before moving permanently to France in 1886. In Paris, van Gogh studied with Cormon; became acquainted with Pissarro, Monet, and Bernard; and befriended Gauguin. Between 1880 and 1890, van Gogh created close to 900 paintings, before his career was cut short by his suicide at the age of 37. He lived a difficult life: unhappy romances, lack of commercial success, relying on family for financial support, and severe mental illness most of his adult life. It is ironic that an individual who would influence the art world so greatly after his death thought of his life as a terrible waste and of himself as an utter failure.

Van Gogh's early "Dutch period" painting style, with somber tones and dark colors dominating, reflected the artistic influences he came under while in Holland and his strong interest in drawing and painting figures. However, he quickly abandoned the dark colors in favor of brighter, more vibrant colors and bold brushstrokes after moving to Paris and getting introduced to the impressionist and post-impressionist styles. ***Farmhouse in Provence*** (oil on canvas, 46.1 × 60.9 cm), which was completed in June 1888 after van Gogh relocated to the South of France, is a good example of this later style and of his belief that color ought to be the primary medium of expression in a painting. His love of the various shades of yellow* and his strive for realism are evident in the rich golden tones that dominate this painting. Also evident are his quick and thick brushstrokes and the swirling cloud lines that foreshadow the more dramatic swirling-cloud pattern in his masterpiece of about a year later, *The Starry Night*. His use of juxtaposed complementary colors mirrors the technique used by impressionists to enhance the vividness of their works.

**This painting is part of the Ailsa Mellon Bruce Collection at the National Gallery of Art, Washington, DC.**

* *What could be behind van Gogh's affinity for the color yellow? To find out, visit* ***Aldrich.com/acta461***

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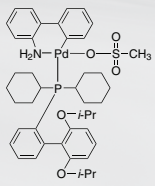

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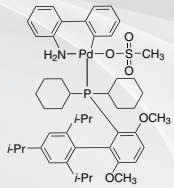

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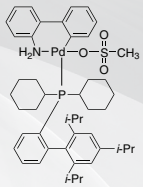

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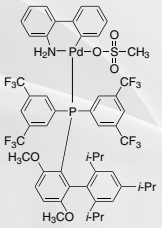

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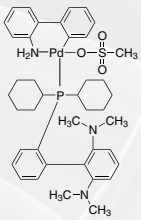

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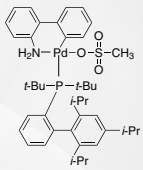

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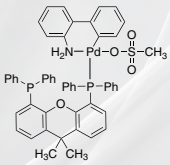

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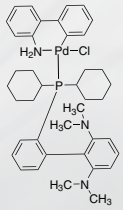

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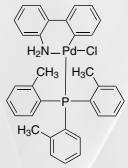

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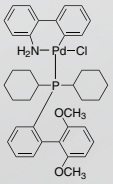

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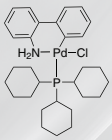

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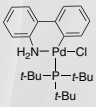

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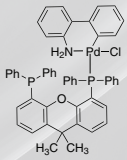

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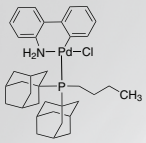

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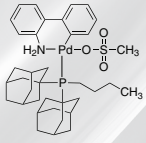

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# Polymer-Incarcerated Metals: Highly Reactive, Recoverable, and Multifunctional Nanocluster Catalysts for Organic Synthesis

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**Keywords.** immobilized catalyst; nanocluster; microencapsulated catalyst; polymer-incarcerated catalyst; green chemistry.

**Abstract.** Various metal(0) and bimetallic nanocluster catalysts have been efficiently immobilized on polystyrene-based polymers using a polymer-incarceration (PI) technique that is based on two procedures: microencapsulation and cross-linking. PI metal nanocluster catalysts have been successfully used for hydrogenation; some coupling reactions; aerobic oxidation of alcohols, hydroquinones, and amines; and direct oxidative esterifications and amidations. One-pot multistep reactions that are catalyzed by PI metal nanoclusters and that combine aerobic oxidation with carbon–carbon or carbon–heteroatom bond formation have been developed. Finally, PI metal nanocluster catalysts have also been applied to more efficient processes such as those taking place in microreactors and flow systems by slightly modifying conventional batch systems.

## Outline



## 1. Introduction

Immobilized catalysts have traditionally been of great interest to synthetic chemists because of several advantages that they have over their non-immobilized counterparts, such as the simplification of product workup and the separation, isolation, and reuse of the catalysts.[1] However, their use in organic synthesis has been relatively limited because, in many cases, immobilized catalysts are less active than the corresponding original catalysts. We have recently been reinvestigating immobilized catalysts from several new perspectives. First, chemical processes with little waste can be expected when immobilized catalysts are utilized, because they can be recovered and reused. This leads to more environmentally benign chemical processes, and immobilized catalysts can thus play a central role in more sustainable chemistry.[1a–j,m,n] Second, the use of immobilized catalysts is expected to be key to high-throughput organic synthesis. Recent advances in combinatorial chemistry require the efficient synthesis of large numbers of structurally distinct compounds.[1a,c,h,k,l,2] Moreover, immobilized catalysts can be applied to reaction integration such as occurs in flow-system and one-pot multistep reactions.[3] For example, immobilized catalysts can be packed in columns, and pure products can be obtained by just passing reactants through the columns, thus

leading to ideal flow systems.[4] We can also expect to immobilize two or more catalysts on the same solid support for use in more efficient one-pot multistep reactions, since nature excels at constructing complex organic molecules through integration of multiple catalytic reactions with high efficiencies in confined enzymatic reaction environments and multienzyme complexes.[5]

The key strategies, reuse of catalysts, and integration of reactions fully fit into the concepts of Green Chemistry proposed by P. Anastas, which is one of the main streams of current and future synthetic organic chemistry since global warming caused by $CO_2$ emissions and the shortage of natural resources are worsening.[1e,i,m,n] While there already exist various synthetic methods that utilize only one of these key concepts, construction of not only recyclable but also multifunctional—defined as the ability of one catalytic system to act on a series of transformations—catalytic systems is still rare and a very challenging task. However, accomplishing this task would bring about the next-generation chemistry in which complex molecules can be synthesized with high resource and energy efficiency.

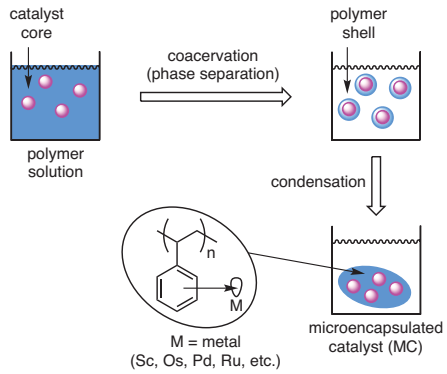
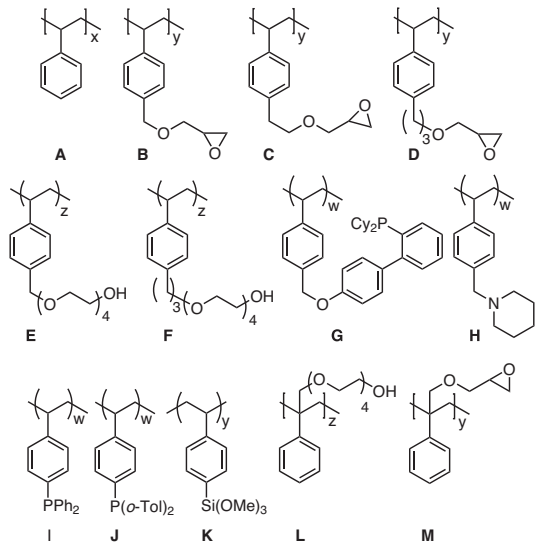



**Figure 1.** Traditional Microencapsulation Technique. *(Ref. 6)*




| Copolymer | Copolymer |
|---|---|
| P1 = A + B + L | PP1 = A + B + I + L |
| P2 = A + C + L | PP2 = A + J + L + M |
| P3 = A + L + M | PP3 = A + G + L + M |
| P4 = A + B + E | NP = A + B + E + H |
| P5 = A + D + F | HP = A + K |

**Figure 2.** Polymers Incorporating Cross-Linking Parts That Are Employed in the Polymer-Incarceration (PI) Method.

### *1.1. Microencapsulated Catalyst*

Back in the early 2000s, we recognized the importance of developing truly efficient polymer-supported catalysts, and settled on the idea of microencapsulated (MC) catalysts.[6] Microcapsules have been employed for coating and isolating substances until such time as their activity is needed, and their applications in medicine and pharmacy have been extensively studied.[7] Recently, much progress has been made in this field; for example, the achievable size of microcapsules has been reduced from a few micrometers to nanometers. Our idea was to apply this microencapsulation technique to the immobilization of catalysts inside polymers. That is, catalysts would be physically enveloped by the polymer backbone, and, at the same time, be immobilized by the interaction between the π electrons of the benzene rings of the polystyrene support and the vacant orbitals of the metal-based catalyst (**Figure 1**).[6,8]

### *1.2. Polymer-Incarcerated (PI) Catalyst*

The polymer-incarceration method is based on two procedures: microencapsulation (MC) and cross-linking. MC is effective for keeping the metal-based catalyst stable, with the interaction between the metal catalyst and the benzene rings in the polymer being strong enough to prevent leaching but not too strong to deactivate the catalyst.[8f] Since MC catalysts are usually employed dissolved in some solvent, recovery and reuse of the catalyst can be difficult. In order to address this issue, cross-linking parts were introduced into the polymer (**Figure 2**), and a new type of catalyst, the polymer-incarcerated (PI) catalyst, was developed.[1j] The PI catalyst can be utilized in most solvents without leaching of the metal.[6,8i,9] In addition to their favorable solvent tolerance, the metal species are "locked up", weakly but with multiple interactions, in the polymer network and cannot aggregate and leach out, resulting in high catalytic activity in the liquid phase (**Figure 3**).

On the other hand, metal nanocluster catalysis has been of great interest in both academia and industry, and has expanded rapidly because of the unique reactivities and selectivities of such catalysts,[10] which can be controlled by the design and preparation of multimetallic nanoclusters.[11] In addition, several recent reports have shown that homogeneous transition-metal-catalyzed reactions affording complex organic molecules can be carried out by employing metal nanocluster catalytic systems.[12]

In this article, we focus on the recent progress achieved with metal nanocluster catalysts immobilized by the polymer-incarceration method, in particular featuring bimetallic effects in immobilized metal nanocluster catalysts. We also survey their applications in reaction integration and multifunctional catalytic systems, such as one-pot multistep transformations and flow-system reactions.

## 2. Polymer-Incarcerated Palladium (PI Pd)

### *2.1. Hydrogenation Reactions*

The first polymer-incarcerated catalyst (PI Pd **1**) was developed by microencapsulating Pd from $Pd(PPh_3)_4$ with copolymer **P1** and then heating the microencapsulated Pd to effect the cross-linking (**Scheme**

**1**, Part (a)).[1j] PI Pd **1** did not dissolve in any organic solvent, four equivalents of $Ph_3P{=}O$ were recovered from the filtrates, and PI Pd **1** was phosphine-free. PI Pd **1** thus prepared showed high activity for the hydrogenation of C–C double bonds (Scheme 1, Part (b)), and could be recycled by a simple procedure without loss of activity or leaching of Pd.

Although PI Pd **1** showed excellent reactivity in hydrogenation reactions, it was assumed that the benzyl ether moieties of PI Pd **1** would be cleaved in the hydrogenation under harsh conditions, such as high hydrogen pressure and high temperature. To address this concern, we designed a new copolymer, **P2**, which has no benzylic ether moiety, in order to develop the more robust PI Pd **2** (**Scheme 2**, Part (a)).[13] A remarkable result was obtained in the hydrogenation of benzothiophene using PI Pd **2** (Scheme 2, Part (b)), whereby the reaction proceeded in excellent yield under conditions milder than those employed in typical procedures known as hydrodesulfurizations (HDS).[14] The excellent activity of PI Pd **2** is possibly due to the highly dispersed palladium in the polymer and the surrounding benzene rings of the polymer which presumably protect the palladium from poisoning by sulfur.

Partial hydrogenation of alkynes to (*Z*)-alkenes is an important process not only in the laboratory but also in industry;[15] however, preventing full hydrogenation is difficult. The Lindlar catalysts [Pd on $CaCO_3$ poisoned by $Pb(OAc)_2$, $PbSO_4$, or $BaSO_4$] are probably the most widely applied heterogeneous catalysts for this transformation,[16] although other heterogeneous[17] and homogeneous catalysts[18] have also been employed. We considered the possibility that phosphine groups in the polymer backbone might partially act as poisoning agents to the metal, thus achieving both chemoselective hydrogenation and suppression of metal leaching.

Copolymer **PP1** was designed and used for the immobilization of Pd to prepare PI Pd **3** (**Scheme 3**, Part (a)).[19] Since, in the process, some phosphine groups were oxidized to the corresponding phosphine oxides, the catalysts were treated with $HSiCl_3$–$Et_3N$ after cross-linking to give phosphinated PI Pd **3**. $^{31}P$ SR-MAS NMR analysis confirmed that the phosphine oxides on the copolymer were completely reduced to the corresponding phosphines. Phosphinated PI Pd **3** was then applied to the hydrogenation of diphenylacetylene and gave partially hydrogenated products selectively without the need to control $H_2$ gas consumption or the addition of external poisoning agents (Scheme 3, Part (b)).[19]

Leaching of the palladium from PI Pd **3** was examined by fluorescence X-ray (XRF) analysis after removal of the catalyst, and no leaching was detected. These results indicate that phosphine moieties can act as moderate poisoning agents of Pd, playing the same role as $PbSO_4$ or $BaSO_4$ in the Lindlar catalyst systems. In general, poisoning of palladium by phosphines is very strong because the phosphorus atoms of phosphines coordinate strongly to palladium atoms, and adsorption of hydrogen molecules and substrates is suppressed by the steric bulk and electronic effects of phosphines. Therefore, hydrogenation does not proceed in the presence of phosphines in homogeneous catalytic systems. In the case of phosphinated PI Pd **3** catalytic systems, however, diphenylphosphino groups in the polymer support might act as "weak" poisoning agents, because coordination of the phosphines to the palladium atoms is restricted by the steric bulk of the polymer support.

We also examined the scope of this catalytic system by employing alkynes having aromatic, aliphatic, hydroxyl, and/or alkoxycarbonyl groups: the corresponding partially hydrogenated products were obtained in good yields without any leaching of palladium. In these

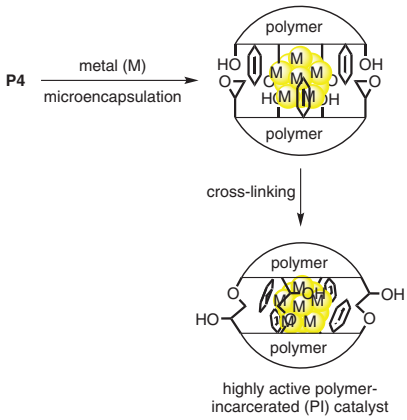
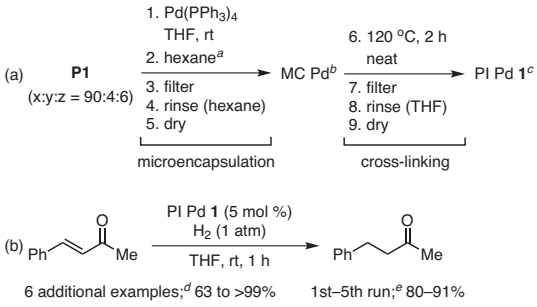
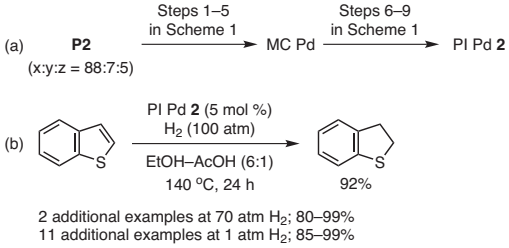



**Figure 3.** The Polymer-Incarceration Approach.




[a] The addition of hexane leads to coacervation (or clustering). [b] MC = microencapsulated. [c] PI = polymer-incarcerated. [d] Of C=C hydrogenation. [e] The catalyst was recovered and reused after simple filtration and drying.

**Scheme 1.** Preparation of Polymer-Incarcerated (PI) Pd(0) and Its Application in Hydrogenation Reactions. *(Ref. 1j)*



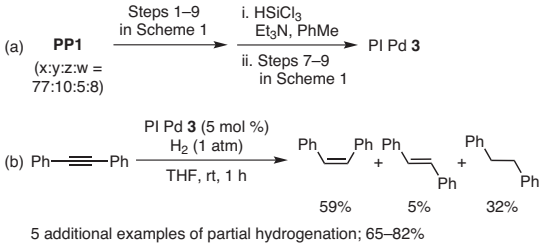

**Scheme 2.** Preparation of Sulfur-Tolerant PI Pd and Its Application in Hydrogenation Reactions under Harsh Conditions. *(Ref. 13)*

hydrogenations, small amounts of the trans products were formed by the Pd-catalyzed isomerization of the cis products.

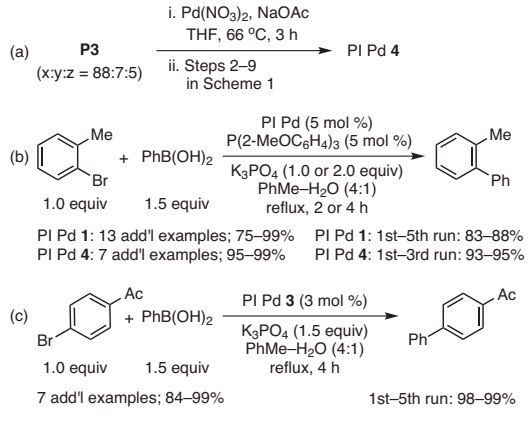
### 2.2. Suzuki–Miyaura Cross-Coupling Reactions

The palladium-catalyzed Suzuki–Miyaura cross-coupling has become one of the most important, powerful, and common methods for forming carbon–carbon bonds, and has been widely used, not only in academia, but also in industry.[20] In general, soluble palladium complexes with ligands such as phosphines, amines, or carbenes are utilized as catalysts, and the choice of ligand often permits tuning of the catalytic activity and selectivity.[21] Despite many attempts to employ palladium metal immobilized on supports, most of the resulting catalysts have had lower catalytic activity than homogeneous catalysts, and the recovery and reuse of such supported catalysts has often not been satisfactory.[22] Although immobilized palladium catalysts based on polymer-supported phosphines or amines have been developed,[23] only immobilized ligands can be used since additional external ligands often induce leaching of the metals.

As palladium in PI Pd **1** is immobilized by weak π interactions with the benzene rings of the copolymer and by the physical envelopment by the polymer backbone, addition of external ligands to tune its reactivity in the Suzuki–Miyaura cross-coupling should be possible without leaching of palladium. After optimization of ligands, bases, and solvents, the best result was obtained when P(2-$MeOC_6H_4$)$_3$ was used as ligand in PhMe–$H_2O$ (4:1) with $K_3PO_4$ as base.[24] No leaching




**Scheme 3.** Preparation of Phosphine-Containing PI Pd and Its Application in Partial Hydrogenation Reactions. *(Ref. 19)*




**Scheme 4.** PI Pd from Inexpensive Pd(II) Salts and Its Application in the Suzuki–Miyaura Cross-Coupling. *(Ref. 24–26a)*

of palladium was detected, and PI Pd **1** could be recycled several times without loss of activity.

Although Pd from Pd($PPh_3$)$_4$ could be successfully immobilized by the PI method to afford PI Pd **1**, the palladium source was relatively expensive. We envisaged that PI Pd **4** could be alternatively prepared from inexpensive Pd(II) salts under suitable reduction conditions. Thus, a solution of Pd($NO_3$)$_2$, NaOAc (1 or 2 equiv), and copolymer **P3** in THF was heated at 66 °C for 3 h. A gradual color change (orange to dark brown) was observed, and palladium was successfully immobilized as PI Pd **4**, and then applied to the Suzuki–Miyaura cross-coupling in the presence of P(2-$MeOC_6H_4$)$_3$, giving the desired product in higher yield than when PI Pd **1** was employed (**Scheme 4**, Part (a) and (b)).[25] A survey of the substrate scope of the PI Pd **4** catalyzed Suzuki–Miyaura coupling revealed that both electron-rich and electron-deficient aryl halides are reactive, and the desired coupling products are obtained in high yields without leaching of palladium. The reaction proceeded well with an aryl chloride in the presence of 2-(dicyclohexylphosphino)biphenyl as ligand, even though the Suzuki–Miyaura cross-coupling of aryl chlorides is generally thought to be difficult.

Since in the preceding cases, the phosphine ligand was lost during filtration of the catalyst, addition of the phosphine ligand was necessary in every recycling use. Employing PI Pd **3**, which was prepared from a phosphinated polymer, in the Suzuki–Miyaura cross-coupling obviated the need to add external phosphine ligands.[26] The structure of the polymer support was found to influence the catalytic activity, and the best results were obtained when the ratio of the diphenylphosphino groups in the polymer to Pd atoms was about 2:1 (Scheme 4, Part (c)).[26] No leaching of Pd was detected in all cases, as confirmed by XRF analysis and the hot-leaching method, indicating that small palladium clusters could be strongly coordinated by both the π electrons of the benzene rings and the phosphines of the polymer support. The role of the phosphines in the polymer support seems to be very important in this reaction: suppressing the leaching of palladium as well as increasing the catalytic activity by acting as ligands.

### 2.3. Amination Reactions

The palladium-catalyzed amination of aryl halides has recently attracted much attention as a direct and efficient C–N bond-forming reaction for the synthesis of a variety of aryl amines, which are commonly found in biologically important compounds.[27] Although many homogeneous palladium-catalyzed aminations have been reported,[28] there are only a few reports of successful heterogeneous catalyst systems for the same transformation.[23d,29] In general, because harsh conditions using strong bases or polar solvents at high temperatures are necessary for the palladium-catalyzed direct amination, the palladium is at a great risk of poisoning, leaching, or aggregation. Therefore, the development of highly effective heterogeneous catalysts without any leaching of palladium has been a central issue for the amination of aryl halides.

With this in mind, we turned our attention to the use of our PI Pd catalyst systems in this reaction. Non-phosphinated PI Pd **1** was not effective for the amination in the absence of external phosphine ligands, and when P(*o*-Tol)$_3$ was added as an external ligand to PI Pd **1**, the catalytic activity increased; however, some leaching of Pd was detected by XRF analysis. Though phosphinated PI Pd **3** showed high catalytic activity even in the absence of external phosphine ligands, the problem of palladium leaching remained. Upon changing the substituent on the phosphorus atom from phenyl to *ortho*-tolyl, PI Pd **5**, prepared from copolymer **PP2**, showed higher catalytic activity and

suppressed the leaching of palladium (**Scheme 5**, Part (a) and (c)).[26b] It is assumed that the reductive-elimination step of the reactions mediated by these two catalysts proceeds more quickly than with PI Pd **3**, because they have bulkier phosphine moieties on the polymer supports. As a result of this faster reductive elimination, polar $Pd^{II}$ species such as Ar–$Pd^{II}$–X would be relatively short-lived and would therefore be less likely to leach out of the polymer supports into polar solvents, especially in the presence of a strong base such as NaO*t*-Bu. Several examples of the direct amination of aryl halides with phosphinated PI Pd **5** catalyst were investigated. Both aryl iodides and bromides with electron-donating groups afforded the desired aminated products in good yields, and, in all cases, no leaching of palladium was observed.

In the first-generation Pd catalysis of aromatic amination reactions, triarylphosphine-based ligands were often employed for their easy availability and stability toward oxidation. However, the reactions are normally conducted at high temperatures and aryl chlorides are unreactive as substrates, mainly because of retardation in a slow oxidative addition step. Accordingly, phosphinated PI Pd **5** on diphenylphosphine basis displayed only low activity with aryl chloride substrates; hence a more active phosphine had to be incorporated. In general, bulky electron-rich phosphines have been shown to enable the direct amination of aryl chlorides under mild conditions,[21a,28c,30] with dialkylbiphenylphosphines being among the most versatile due to their air stability and the high catalytic activity of their Pd complexes.[31] Buchwald's group has extensively studied these ligands and showed that the direct amination of aryl chlorides proceeds in high yield by using these ligands under mild homogeneous conditions.[21a,28c,30a–c,g]

We prepared PI Pd **6** from **PP3** and $Pd(OAc)_2$, and used it to effect the coupling of morpholine with 4-chlorotoluene in good yield and low Pd leaching (Scheme 5, Part (b) and (c)).[32] A survey of the substrate scope of the PI Pd **6** catalyzed direct amination revealed that high yields are obtained with electron-rich and electron-deficient aryl chlorides, and that aromatic and aliphatic primary and secondary amines are all coupled efficiently and in good yields. In addition, the reactions proceeded effectively using a lower catalyst loading (0.4 mol % Pd).

### *2.4. Mizoroki–Heck Reactions and Formation of Subnanometer-Size Pd Clusters*

While PI Pd **1** showed high catalytic activity in hydrogenation, allylation, and Suzuki–Miyaura couplings, the structure of the Pd clusters was not well controlled. As a result, it was thought that, if stable polymer micelles could be formed, smaller-size, well-organized Pd(0) clusters might be generated.

Although copolymer **P1** is amphiphilic, it is difficult for it to form clear micelles because the hydrophilic epoxy branches are attached to the hydrophobic benzene rings. On the other hand, copolymer **P3**, would be expected to form clearer and more stable micelles by phase separation across the polymer backbone between the benzene rings and the epoxy or tetraethylene glycol (TEG) moieties. **P3** would also be expected to adopt several micelle morphologies upon cross-linking between the epoxy and the alcohol moieties of the polymer side chains.

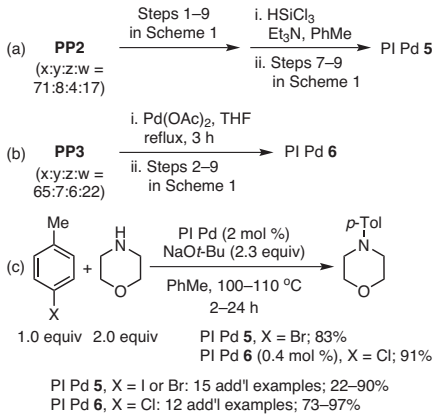
Upon stirring **P3** and $Pd(PPh_3)_4$ in DCM and *t*-AmOH at room temperature for 8 hours, very clear spherical micelles of 200–500 nm diameters (by TEM) were formed. Subsequent heating under solvent-free conditions and addition of MeOH afforded Polymer-Micelle-Incarcerated (PMI) Pd **7** (**Scheme 6**, Part (a)).[33] In contrast, micelles prepared from **P1** and $Pd(PPh_3)_4$ using an identical procedure are not spherical. The different behavior of these two polymers is thought to be due to the structural differences along their polymer backbones.

Since the TEM image of PMI Pd **7** prepared in MeOH–DCM did not reveal recognizable Pd clusters (presumably too small, TEM detection limit 1 nm), PMI Pd 7 was examined by X-ray absorption near-edge structure (XANES) and extended X-ray absorption fine structure (EXAFS) techniques, which revealed the formation of stable, subnanometer Pd(0) clusters contained within micelles. To the best of our knowledge, the Pd clusters produced from random copolymers using this novel method are the smallest reported to date (average cluster diameter ≈ 0.7 nm).

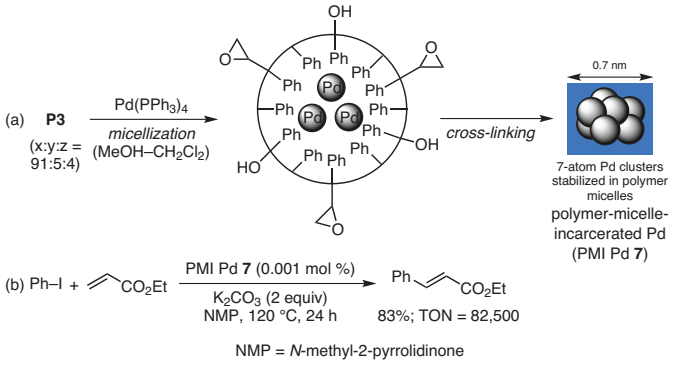
PMI Pd **7** effectively catalyzed the Mizoroki–Heck reaction of iodobenzene with ethyl acrylate, exhibiting a turnover number (TON) of >80,000 (Scheme 6, Part (b)).[33] The possibility that a very small amount of leached palladium could have catalyzed the reaction was discounted by a hot-leaching test and a three-phase test.

## 3. Discovery of Polystyrene-Stabilized Au(0) Nanoclusters

In 1987, Haruta and co-workers reported a low-temperature CO oxidation by molecular oxygen using gold nanoclusters,[34] in which a crucial charge transfer from gold nanoclusters to molecular oxygen results in the concomitant activation of the O–O bond to form a superoxo-like state.[35] After this landmark discovery, numerous



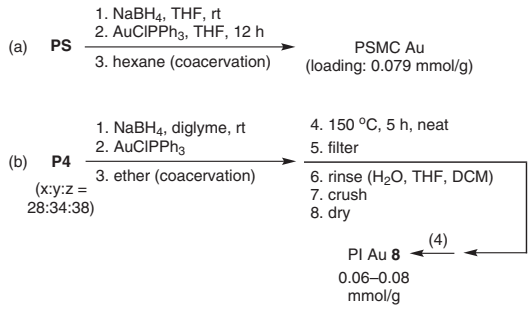

**Scheme 5.** PI Pd with Active Phosphines and Its Application in the Direct Amination of Aryl Halides. *(Ref. 26b,32)*



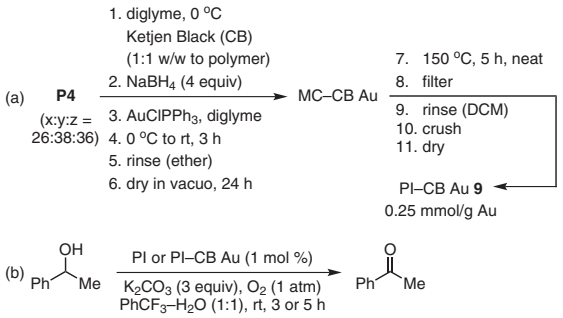

**Scheme 6.** Polymer-Micelle-Incarcerated Palladium (PMI Pd) Catalyst and Its Remarkably High Activity in the Mizoroki–Heck Reaction. *(Ref. 33)*

heterogeneous Au nanocluster catalysts immobilized on inorganic supports were developed, and these catalysts were applied especially to redox transformations.[34c,36] While most of these reactions were conducted under harsh conditions in the gas phase, in the aqueous phase, or under solvent-less conditions, heterogeneous gold nanocluster catalysts, which can be utilized effectively *for organic synthesis under mild conditions*, were few[37] when we started our investigation of the immobilization of Au nanoclusters on organic polymers. We hypothesized that gold nanoclusters prepared in organic solvents and stabilized by organic supports could be truly efficient catalysts for organic synthesis, since most of the gold nanocluster catalysts already reported have been prepared in aqueous media with inorganic materials and, as a result, would have less affinity for hydrophobic organic molecules. Conversely, it is known that aromatic molecules such as benzene can interact weakly with a gold surface or gold nanoclusters via its π electrons.[38] Therefore, we reasoned that, if gold nanoclusters could be stabilized by weak interactions with the aromatic parts of polymers, these gold nanoclusters would become versatile catalysts that could be used for liquid-phase organic synthesis with a wide range of substrates. After some experimentation, we successfully prepared microencapsulated gold nanoclusters (PSMC Au) by reducing $AuClPPh_3$ with $NaBH_4$ in THF in the presence of polystyrene (PS), followed by slow addition of hexane (**Scheme 7**, Part (a)).[39] TEM analysis showed that PSMC Au contains stabilized gold nanoclusters (1 nm) that did not aggregate even after 12 h of stirring.

Having established that gold nanoclusters could be stabilized by the benzene rings of polystyrene, we next prepared polymer-incarcerated gold nanocluster catalyst (PI Au **8**) as a deep purple solid by employing copolymer **P4** (Scheme 7, Part (b)).[39] Small gold nanoclusters (1–3 nm) were observed by TEM analysis, and elemental analysis showed no phosphorus contamination. To the best of our knowledge, this is the first example of stable Au nanoclusters immobilized on a polystyrene-based polymer.




**Scheme 7.** Preparation of Polystyrene-Microencapsulated (PSMC) Au Nanocluster and Polymer-Incarcerated (PI) Au Nanocluster Catalysts. *(Ref. 39)*




| | Reuse Runs | | Add'l Examples | |
|---|---|---|---|---|
| Catalyst | No. | Yield | No. | Yield |
| PI Au **8** | 10 | 99 to >99% | 7 (2° alcohols) | 91 to >99% |
| PI Au **8** | ---- | ---- | 3 (1° alcohols) | 64–79% |
| PI–CB Au **9** | 5 | >99% | 13 (2° alcohols) | 57 to >99% |

**Scheme 8.** Preparation of Au Nanoclusters Incarcerated in Carbon Black–Polymer Nanocomposites and Au Nanocluster Catalyzed Aerobic Oxidation of Alcohols. *(Ref. 39,42)*

## 4. Aerobic Oxidations Catalyzed by PI Metal Nanoclusters

### *4.1. Oxidation of Alcohols to Aldehydes and Ketones*

The selective oxidation of alcohols is one of the most important transformations in organic synthesis.[40] While several metal-based oxidizing reagents have been developed, these reagents usually require stoichiometric amounts of metal oxidants, and thus a large amount of waste is formed. In this respect, the oxidation of alcohols using molecular oxygen catalyzed by reusable heterogeneous catalysts would be ideal from an environmental and atom-economy points of view.[41] Although several excellent heterogeneous catalysts, including gold nanoclusters, for the aerobic oxidation of alcohols to the corresponding aldehydes or ketones have been disclosed,[36a–c] most of them require heating and addition of base, which is undesirable from the viewpoints of energy efficiency, minimization of waste, and selective formation of products.

The PI Au **8** catalyzed aerobic oxidation of 1-phenylethanol proceeded smoothly with no metal leaching in the presence of a weak base ($K_2CO_3$) in benzotrifluoride (BTF, $PhCF_3$)–water at rt under an atmosphere of oxygen (**Scheme 8**).[39] Interestingly, a lower loading of the catalyst (<0.1 mmol/g) exhibited higher activity, presumably because it contained smaller clusters (mainly 1–3 nm), whereas aggregation of nanoclusters in close proximity led to larger clusters (mainly >5 nm) at higher catalyst loadings (>0.1 mmol/g). The catalyst was recovered by simple filtration and reused without significant loss of activity, and the recovered catalyst could be heated at 150 °C for 5 h to facilitate its reuse.

In the case of PI Au **8**, an increase of gold loading amounts was hampered by aggregation of the nanoclusters during the cross-linking step. In order to circumvent this problem, we incorporated carbon black, whose partially graphitic structure was expected to have good affinity for polystyrene-based polymers with π–π interaction. We chose Ketjen Black EC300J (CB), because of its spherical and hollow-shell structure (diameter ~ 45 nm) and its high specific surface area which might allow a high dispersion of the polymer on its surface to form spherical nanocomposites (~55 nm). Au nanoclusters immobilized on Ketjen Black (CB)–polymer nanocomposites (PI–CB Au **9**) were prepared by in situ reduction of $AuClPPh_3$ by $NaBH_4$ in the presence of both copolymer **P4** and CB (Scheme 8, Part (a)).[42] PI–CB Au **9** was highly efficient in the oxidation of 1-phenylethanol, even at a relatively high loading of Au (0.25 mmol/g), because small nanoclusters can be maintained in the highly spread polymer on the surface of CB (Scheme 8, Part (b)).[42]

While PI Au **8** and PI–CB Au **9** showed excellent activity in the aerobic oxidation of secondary alcohols to ketones under mild conditions, the basic conditions employed made the selective oxidation of primary alcohols to aldehydes difficult. In light of recent

reports that doping a secondary metal component into metal catalysts is advantageous for the activity and selectivity of various reactions, we decided to combine gold with a second metal.

New bimetallic catalysts were prepared in the usual way by adding a solution of $AuClPPh_3$ and a secondary metal salt dropwise to a diglyme solution of copolymer **P4** and $NaBH_4$ (**Scheme 9**, Part (a)).[43] STEM and EDS analyses confirmed the size and composition of the clusters. PI Au–Pd and PI Au–Pt contained clusters of 1.5–5 nm in size, and each cluster consisted of both metals. This may suggest that the alloyed clusters are formed by the simultaneous reduction of the metal salts and that they are the active catalysts in this system. The PI bimetallic clusters were then tested for catalytic activity in the aerobic oxidation of alcohols under neutral conditions, and it was revealed that a 1:1 mixture of gold to platinum (PI Au–Pt) provided the most active catalyst. Aromatic secondary alcohols were oxidized smoothly to afford the corresponding ketones in excellent yields. Although aliphatic secondary alcohols had relatively low reactivity, the desired ketones were also obtained in high yields by prolonging the reaction time (Scheme 9, Part (b)). Aromatic and allylic primary alcohols possessing both electron-donating and electron-withdrawing groups were oxidized smoothly under the optimized conditions to afford the corresponding aldehydes in excellent yields without formation of carboxylic acids (Scheme 9, Part (b)). Even more interesting is the observation that the aerobic oxidations also proceeded smoothly in pure water without organic solvents under very mild conditions (Scheme 9, Part (c)).[43]

When PI Au–Pt is used as catalyst for the oxidation of 1-phenylethanol, the α-hydrogen abstraction from the alcohol may be involved in the rate-determining step, as indicated by a kinetic isotope effect study using an α-hydrogen-labeled alcohol ($k_H/k_D$ = 3.0). In addition, the detailed reaction mechanism of the aerobic oxidation of alcohols catalyzed by PI Au **8** was described by Chechik and co-workers, who proved through the use of spin traps and other techniques that the detected H spin adduct is formed by abstracting hydrogen from a Au–H intermediate, which is formed by the gold catalyst abstracting a H atom from the alcohol.[44] Therefore, the alcohol oxidation process takes place through abstraction of hydride or a hydrogen atom from the alcohol by the gold surface, with atmospheric oxygen closing the catalytic cycle by removing the hydrogen from the gold surface, rather than oxidizing the alcohol directly.

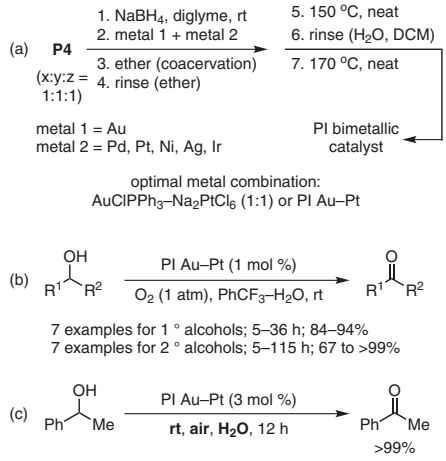
### 4.2. Oxidation of Hydroquinones to Quinones

The quinone structural motif is often found in naturally occurring compounds and in synthetic, biologically active molecules,[45] and quinones are useful in the construction of polycyclic molecules by the Diels–Alder reaction.[45,46] The most important property of quinones is the ease with which they undergo reduction to hydroquinones and vice versa.[47] The oxidation of hydroquinones is one of the most direct ways to prepare the corresponding quinones, and, while various oxidizing agents have been employed for this purpose, reduced oxidants inevitably remain as byproducts in stoichiometric reactions. Several catalysts for the oxidation of hydroquinones with molecular oxygen have been previously reported; however, the substrate scope still requires improvement.[48] In particular, the oxidation of hydroquinones bearing electron-withdrawing groups (EWGs), such as chlorohydroquinone, has either been reported to be difficult or has not been described, presumably because of its high redox potential.

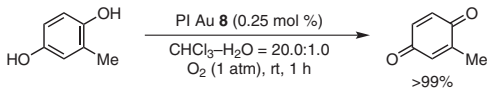
We have successfully utilized PI Au **8** for the oxidation of several hydroquinone derivatives and catechols under atmospheric oxygen or air (**eq 1**).[49] While oxidation of hydroquinones with electron-donating groups (EDGs) occurred smoothly, the oxidation of hydroquinones with electron-withdrawing groups and catechol derivatives was slow; however, the reaction was accelerated by addition of base, affording the corresponding quinones in moderate-to-good yields. In most cases, analytically pure products were obtained by filtration, simple phase separation and concentration without further purification. Better results were obtained with nanocluster PI Pt **10** in the oxidation of hydroquinones with EWGs such as 1,4-tetrachlorohydroquinone. When the oxidation of 1,4-tetrachlorohydroquinone was carried out in the biphasic solvent system chloroform–water (27:1), quantitative formation of the desired product was observed without addition of base. Hydroquinones with both EDGs and EWGs, as well as catechols, were oxidized readily in good-to-excellent yields under additive-free conditions at rt (**Scheme 10**).[50] Catalyst loading could be decreased to as low as 0.05 mol %, and a maximum turnover frequency (TOF) of at least 1000 $h^{-1}$ was observed. The reaction was very clean and selective for all substrates; no significant byproduct was detected, and complete consumption of the starting materials was observed. When the filtered catalyst was washed with $CHCl_3$–NaOH (0.5 M in $H_2O$) after each run, PI Pt **10** could be reused at least 13 times to catalyze the oxidation reaction in almost quantitative yield each time.

### 4.3. Oxidative Benzoxazole Formation

Benzoxazoles and benzothiazoles are of great interest due to their occurrence in a wide range of biologically active natural products and pharmaceutical agents.[51] A popular strategy for the preparation of 2-substituted benzoxazoles and benzothiazoles is the catalytic aerobic




**Scheme 9.** Preparation of PI Bimetallic Nanocluster Catalysts and Their Use in the Oxidation of Alcohols under Mild, Base-Free Conditions. *(Ref. 43)*

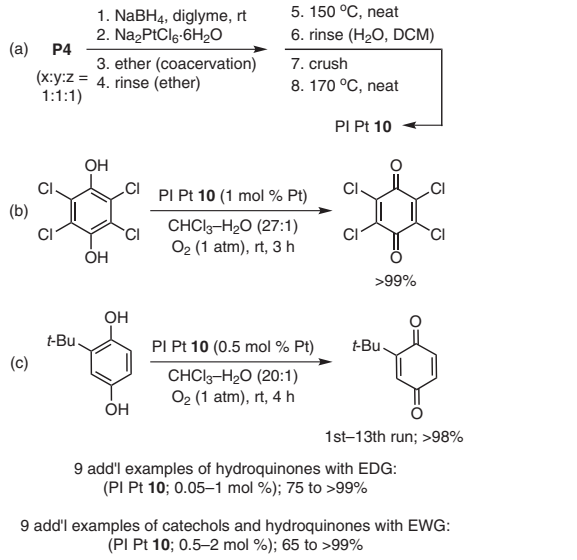



7 additional examples for hydroquinones with EDG (PI Au **8**; 0.25–1 mol %); 86 to >99%
3 additional examples for catechols and hydroquinones with EWG under basic conditions (PI Au **8**; 1 mol %); 55–96%

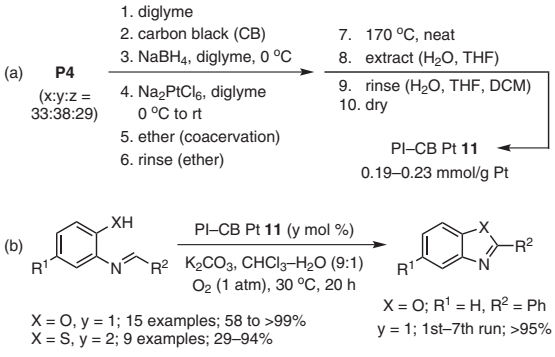
**eq 1** *(Ref. 49)*

oxidative cyclization of phenolic and thiophenolic Schiff bases derived from the condensation of 2-aminophenols or 2-aminothiophenols with aldehydes.[52]

We investigated this oxidative cyclization reaction using various PI and PI–CB metal nanocluster catalysts prepared by following the preparation method of PI Au **8** and PI–CB Au **9**. We found that catalysts containing Pd and Pt nanoclusters provided good results in a chlorinated solvent with a small amount of water, and PI–CB Pt **11** gave the best yield of a benzoxazole under mild conditions (**Scheme 11**).[53] We also found that phenolic imines derived from the condensation of 2-aminophenols with various aromatic aldehydes are easy-to-handle solids that readily convert into the desired 2-substituted benzoxazole in excellent yields under the optimized conditions. On the other hand, Schiff bases derived from non-aromatic aldehydes were not easily isolable and were prepared in situ. In these cases, a slightly modified system gave better results, and 2-substituted benzoxazoles with a non-tertiary aliphatic group could be prepared by our catalytic system. In general, we found that the oxidative cyclization of the thiophenolic Schiff bases to be more sluggish and often required a slightly higher loading of PI–CB Pt **11**. While imines with electron-poor aromatic groups could be converted into the desired benzothiazoles, imines with electron-rich substituents were found to be inferior substrates. In addition, thiophenolic imines derived from aliphatic aldehydes are oxidized extremely well under the optimized reaction conditions. Moreover, PI–CB Pt **11** was reused for seven oxidative cyclization cycles without noticeable loss of activity.




**Scheme 10.** Preparation of PI Pt Nanoclusters and Their Application to the Oxidation of Hydroquinones and Catechols. *(Ref. 50)*




**Scheme 11.** Preparation of Pt Nanoclusters Incarcerated in Carbon Black (CB)–Polymer Nanocomposites, and Their Application in the Oxidative Cyclization of Imines to Benzoxazoles. *(Ref. 53)*

### 4.4. Oxidation of Amines to Imines

Recently, the aerobic oxidation of amines to imines catalyzed by various sizes of Au; such as bulk, microsize, nanosize, and homogeneous Au; has been widely reported.[54] Interestingly, Au clusters 10–50 nm in size sometimes show very high activity,[54d,f,g] although Au clusters over 10 nm in size hardly catalyze other aerobic oxidation reactions. In some cases in this amine oxidation, it was concluded that smaller Au clusters have higher activity;[54e,f] There is, however, no clear and direct evidence that smaller clusters have higher activity for amine oxidation, because there are no reports on the systematic comparison of the activity of different sizes of Au clusters using the same support.

On the basis of studies of the PI Au **8** catalyzed aerobic oxidation of dibenzylamine, a model amine, we formed a working hypothesis that PI Au clusters with a size over 5 nm possess higher activity in the aerobic oxidation of amines than those with a 1–3 nm size, which are generally highly active in the oxidation of alcohols.[55] To obtain PI Au catalysts with larger clusters, PI Au **12** and PI Au **13**, polymer microcapsules—prepared as described in Scheme 7, Part (b)—were heated at 150 °C in mesitylene and decane to effect the cross-linking. Both PI Au **12** (average cluster size = 6.6 nm) and PI Au **13** (average cluster size = 8.5 nm) showed high activity in the amine oxidation. However, when these catalysts were employed in the aerobic oxidation of alcohols, the activity was inversely proportional to their activity in the amine oxidation. These observations made it clear that an important factor in the oxidation of amines is catalyst cluster size, not Au loading, with the optimum cluster size for the aerobic oxidation of amines being larger than that for the oxidation of alcohols.[55]

In order to improve the reactivity further, copolymer **NP** (x:y:z:w = 6:6:6:1), containing tertiary amine moieties, was utilized to prepare PI Au **14** similarly to PI Au **12**. The catalytic activities of PI Au **8**, **12**, and **14** were compared in the aerobic oxidation of dibenzylamine at various temperatures. While PI Au **8** required at least 140 °C to obtain satisfactory results (~90% yield), PI Au **12** and **14** showed good activity even at 100 °C, with PI Au **14** being the best (88% yield at 100 °C). In these studies, secondary amines were oxidized to the corresponding imines, primary benzyl amines to benzylbenzylidene amines, and indoline, tetrahydroquinoline, and tetrahydroisoquinoline were aromatized via a two- or a four-electron oxidation. PI-Au **14** was recovered by simple filtration and drying, and reused at least seven times without loss of activity (**eq 2**).[55]

## 5. One-Pot Multistep Reactions Catalyzed by PI Metal Nanoclusters

One-pot sequential reactions save resources, energy, and time, since they obviate the need for isolating and purifying reaction intermediates. They also allow for the utilization of highly reactive or unstable compounds and/or thermodynamically unfavorable or practically nonexistent intermediates that cannot generally be isolated.[56] While most sequential reactions are performed with homogeneous catalysts, the use of heterogeneous catalysts, especially metal nanoparticle

catalysts, would improve the synthetic utility of cascade reactions by facilitating the recovery and subsequent reuse of the catalysts,[12a] and highly fit the criteria for "green", sustainable chemistry.[1e,i,5]

In addition to the ease of separation from the reaction mixture, heterogeneous support systems for multi-catalytic sequential reactions would allow for the use of mutually incompatible catalysts due to the site isolation of the catalytic species.[57] Moreover, smooth transfer of intermediates from one catalytic system to another within the confined reaction environments of the heterogeneous supports leads to rate acceleration of the overall reactions and results in enhanced selectivities of certain reaction pathways.[5]

### 5.1. Oxidative Methyl Ester Formation

Esterification is traditionally a two-step procedure that includes synthesis of carboxylic acids or activated carboxylic acid derivatives such as anhydrides or acid chlorides.[58] While several facile and economical esterification reactions have been developed, these protocols usually require stoichiometric amounts of reagents and generate a large amount of chemical waste. In this respect, the direct oxidative esterification of alcohols using molecular oxygen and catalyzed by reusable heterogeneous catalysts under mild conditions would be an attractive challenge for both organic synthesis and "green", sustainable chemistry.

Several gold nanocluster catalysts for the aerobic oxidation of alcohols or aldehydes to esters have been reported. Although oxidation of aldehydes to esters proceeds under mild conditions,[59] examples starting from alcohols under mild conditions (atmospheric pressure and ambient temperature) are very limited.[60] The aerobic oxidation of alcohols to esters can be divided into three steps: oxidation of alcohols to aldehydes, hemiacetal formation, and oxidation of hemiacetals to esters. Christensen and co-workers have conducted significant mechanistic studies of the gold-nanoclusters-catalyzed esterification of aldehydes, and showed that the oxidation of aldehydes to esters is much faster than that of alcohols to aldehydes.[59a–c,61]

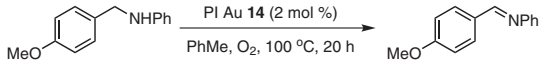
The catalytic activity of PI Au **8** was examined in the oxidation of *para*-methylbenzyl alcohol to methyl *para*-methylbenzoate in methanol in the presence of base under atmospheric oxygen conditions at rt (**eq 3**).[62] It was found that the amount of water in the solvent was very important for reactivity and selectivity; and MeOH–water (500:1) was the most suitable solvent for this reaction. The catalyst could be reused several times without significant loss of activity, and batches of the catalyst with decreased activity after repeated use could also be revived by heating at 170 °C for 4 h. Aromatic alcohols containing both EDGs and EWGs were converted into the corresponding methyl esters in good-to-high yields. Unfortunately, 4-phenyl-1-butanol (an aliphatic alcohol) was not oxidized smoothly (10% yield). Kinetic studies performed with *para*-methylbenzyl alcohol and *para*-methylbenzaldehyde under the optimized conditions indicated that each step is a first-order reaction and that the second step is 30 times faster than the first.[62]

### 5.2. Oxidative Esterification of 1,2- and 1,3-Diol Derivatives

The PI Au **8** catalyzed oxidative esterification of *para*-methylbenzaldehyde using with several alcohols proceeded more smoothly with ethylene glycol than with simple alcohols such as ethanol (**eq 4**).[63] These results are remarkable because polyols, such as ethylene glycol and glycerol itself, are oxidized in the presence of gold nanoclusters.[64] The PI Au **8** catalyzed oxidative esterification of various aldehydes with diols and their derivatives revealed that the yield decreased as the alkyl chain between the two hydroxyl groups increased. These results can be explained by the neighboring-group-participation effect, in which the hemiacetal intermediates are stabilized by the two hydroxyl groups on the surface of the gold nanoclusters, leading to acceleration of the pathway to glycol esterification. In the case of long alkyl chains, it is entropically unfavorable for the two hydroxyl groups to coordinate simultaneously to the gold surface over the long alkyl chain, as confirmed by the results obtained with 1,2- and 1,6-hexanediols. The neighboring-group effect was observed not only with hydroxyl groups, but also with ethereal oxygen atoms. It is worth noting that aliphatic aldehydes could be converted into ethylene glycol monoesters using this catalytic system.

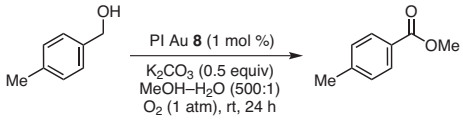
### 5.3. Powerful Method for Amide Synthesis from Alcohols and Amines under Aerobic Conditions

As one of the most important functional groups in chemistry,[65,66] the amide group has traditionally been formed by reactions of carboxylic acids with amines using coupling reagents or by prior conversion of carboxylic acids to derivatives such as acid chlorides or anhydrides.[67] In 2007, however, an important discovery was made by Milstein and co-workers,[68] who described the first coupling between an alcohol and an amine leading to an amide. This clean and simple reaction (molecular hydrogen being the only byproduct) is catalyzed by a




19 add'l examples using PI Au **8** or **14**; 45 to >99% 1st–7th run; >99%

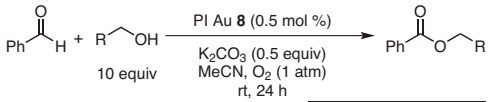
**eq 2** *(Ref. 55)*



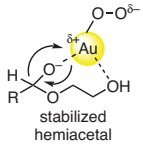

1st–11th run; 86 to >99%
(in runs 8 and 11, the catalyst was heated at 170 °C before use)

8 add'l examples of benzylic and allylic alcohols; 60 to >99%

**eq 3** *(Ref. 62)*



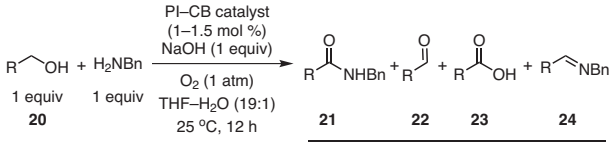

10 add'l examples using ethylene glycol and aldehydes; 70–83%




stabilized hemiacetal

| R | Yield |
|---|---|
| $HOCH_2$ | 83% |
| $HO(CH_2)_2OCH_2$ | 65% |
| $HO(CH_2)_2$ | 64% |
| $MeOCH_2$ | 48% |
| *n*-BuCH(OH) | 32% |
| $HOCH_2CH(OH)$ | 25% |
| $HO(CH_2)_3$ | 20% |
| $HO(CH_2)_4$ | 4% |
| $HO(CH_2)_5$ | 9% |
| Me | 1% |
| MeS | 0% |

**eq 4** *(Ref. 63)*

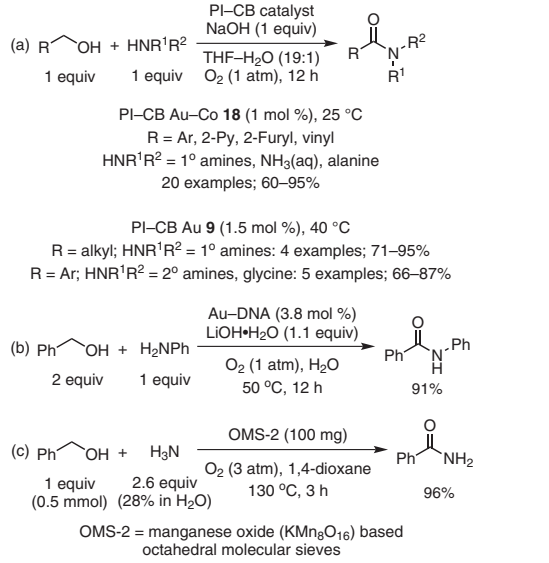



| 20 | R | PI–CB Cat. | GC Yield[a,b] 21 | 24 |
|---|---|---|---|---|
| **a** | $BnCH_2$ [c] | Au (**9**) | 85% | 1% |
| **b** | 4-$MeC_6H_4$ | Au–Pd (**15**) | 2% | 73% |
| **b** | 4-$MeC_6H_4$ | Au–Pt (**16**) | 5% | 75% |
| **b** | 4-$MeC_6H_4$ | Au–Ni (**17**) | 87% | 13% |
| **b** | 4-$MeC_6H_4$ | Au–Co (**18**) | 96% | 4% |
| **b** | 4-$MeC_6H_4$ | Au–Fe (**19**) | 94% | 6% |

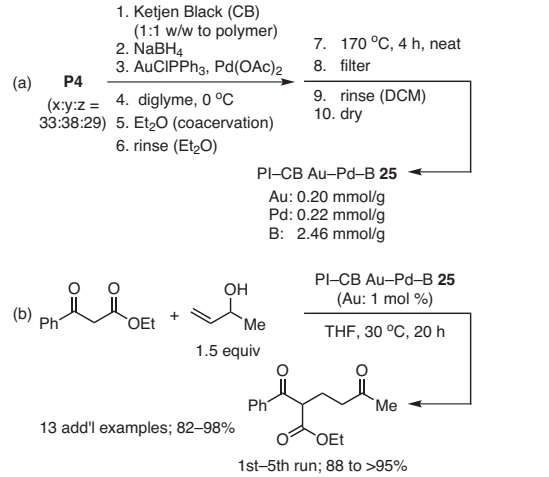
**15**–**19** were prepared similarly to the way the bimetallic catalysts were prepared in Scheme 9, except that, here, Ketjen Black (CB) was added first to copolymer **P4**.

[a] Using dodecane as internal standard. [b] In the cases shown here, **22** and **23** were not detected [c] At 40 °C.

**eq 5** *(Ref. 75)*




**Scheme 12.** Amidation Reactions through a Tandem Oxidation Process (TOP). *(Ref. 75–77)*




**Scheme 13.** Boron-Containing Bimetallic PI Catalysts in the Sequential Aerobic Oxidation–Michael Addition. *(Ref. 80)*

ruthenium pincer complex, and is highly desirable because of its high atom efficiency. While this report inspired several other research groups to further develop the reaction,[69] the latter was conducted under reductive conditions (hydrogen is formed), unsaturated bonds such as C=C were not tolerated, and a relatively high temperature was needed.[69a–c]

In contrast, we chose to explore a tandem oxidative process (TOP) as an alternative pathway for amide formation from an alcohol and an amine. TOP[70] is clearly different from the dehydrogenative pathway in that the reaction proceeds under oxidative conditions and the only byproduct is water. However, this oxidation process is very challenging, because of the many side reactions and byproducts that are possible.[39,54c,f–h,55,60a,64a,71–73] Sakurai's and Haruta's groups studied this process using Au nanocluster catalysts, and reported that the reaction was limited to the formylation of amines with MeOH or formaldehyde.[74]

We chose the catalytic amidation of aliphatic (**20a**) and benzylic (**20b**) primary alcohols as a test of our approach. For this purpose, we prepared various new, bimetallic catalysts, **15**–**19**, incarcerated in carbon black–polymer nanocomposites (**eq 5**).[75] Following extensive experimentation with catalysts, solvents, additives, and temperature, we found that, for **20b**, PI–CB Au–Co **18** is the best catalyst (the desired amide, **21b**, was obtained in 94% isolated yield), while PI–CB Au **9** gives the best results for **20a** (see eq 5).[75] Since our results strongly suggested that imine, benzoic acid, and ester are not intermediates in this reaction, and that the reaction proceeds by a hemiaminal, we thought that cobalt might stabilize the hemiaminal and inhibit the dehydration process, and that, if cobalt and gold nanoclusters were in close proximity, the reaction could then proceed with better selectivity.

To demonstrate the general applicability of the PI–CB Au–X system, various alcohols and amines were tested in amidation reactions. In general, PI–CB Au–Co **18** was used for the combination of benzylic or allylic alcohols and *primary* amines at 25 °C. On the other hand, PI–CB Au **9** was employed for the combination of benzylic alcohols and *secondary* amines or aliphatic alcohols and *primary* amines at 40 °C (**Scheme 12**, Part (a)).[75] Aqueous ammonia and free amino acids could also be utilized directly to afford the corresponding amides in high yields.

Wang and co-workers have developed homogeneous gold nanoclusters stabilized by natural fish sperm DNA for the oxidative amidation from alcohols and amines. In this reaction system, especially excellent results were obtained when combinations of alcohols and aniline derivatives were used (Scheme 12, Part (b)).[76] Mizuno and co-workers have developed a heterogeneous-manganese-catalyzed oxidative primary-amide formation from alcohols and aqueous ammonia under relatively harsh conditions (Scheme 12, Part (c)).[77] Here, a different reaction mechanism was proposed in which a nitrile is formed and is followed by catalytic hydration to afford the amide.

### 5.4. PI–CB Au–Pd–B for Tandem Oxidation–Michael Addition Reactions

In nature, complex organic molecules are constructed elegantly in confined environments through multistep cascade reactions. In order to mimic the catalytic efficiencies of natural chemical processes, synthetic chemists have shown great interest in the preparation and application of self-assembled nanoreactors for catalysis. In addition, the development of one-pot, multistep homogeneous catalysis has been growing due to the inherent efficiency resulting from the elimination of isolation and purification steps of intermediates generated from

traditional iterative synthetic methods.[78] We considered the polymer-incarceration method for the immobilization of two different metal catalysts that can perform two distinct organic transformations, specifically the sequential aerobic oxidation–Michael reaction of 1,3-dicarbonyl compounds with allylic alcohols.

We decided to utilize as catalysts bimetallic Au–M nanoclusters with tetraalkoxyborates derived from $NaBH_4$[79] on a carbon black–polymer nanocomposite (PI–CB Au–M–B), since we anticipated that these heterogeneous catalysts would facilitate the desired sequential reaction under base-free conditions in relatively high concentration. After extensive optimization, we found that the reaction conditions consisting of PI–CB Au–Pd–B **25** in THF gave excellent results; and recovery and reuse of the catalyst was possible without significant loss of activity (**Scheme 13**).[80] A variety of allylic alcohols were good Michael acceptors in this reaction system without any side reaction, such as polymerization or isomerization, being observed. As for 1,3-dicarbonyl compounds, β-keto esters and 1,3-diketones reacted smoothly at 30 °C, but malonate derivatives required elevated temperatures (60 °C).

### 5.5. Remarkable Effect of Bimetallic Nanocluster Catalysts on the Selection of Reaction Pathways in Sequential Reactions

We have shown in the preceding sections striking and remarkable effects of bimetallic nanocluster catalysts. For instance, doping second metals to gold during formation of nanoclusters dramatically changes the reactivity of the aerobic oxidation of alcohols under neutral conditions. We believe that Au plays the major role in aerobic oxidation, while the second metal changes the reactivity of Au electronically by forming an alloy. In the case of the oxidative direct amidation reaction, addition of the iron-group metals to Au nanoclusters dramatically changes the selectivity from imines to amines; in other words, the combination of metals in the nanoclusters changes the reaction pathways. In these catalysts, Au and iron-group metals exist separately in the same polymer support and they may play different roles but act cooperatively in the catalytic system. That is, gold nanoclusters oxidize an alcohol to an aldehyde and a hemiaminal to an amide; on the other hand, iron-group metals facilitate hemiaminal formation and stabilize it. Smooth transfer of the key intermediate, the hemiaminal, from iron group metals to Au nanoclusters within the confined reaction environments of polymer media results in high selectivities and yields of amides.

In the case of the sequential aerobic oxidation–Michael addition reactions, there are two different bimetallic effects. Firstly, Au and Pd form alloyed bimetallic nanoclusters, and this doping changes the reactivity in the oxidation of allylic alcohols under base and water-free conditions. In other words, the second metal tunes the reactivity of Au nanoclusters, and this kind of phenomenon is known as the ligand effect.[11a] Secondly, boron for the Michael reactions and bimetallic nanoclusters for the aerobic oxidation coexist within self-assembled carbon black–polymer nanocomposites to facilitate the sequential reactions efficiently without any side reactions.

There is another example of the remarkable effect of bimetallic nanocluster catalysts in the aerobic oxidation of alcohols. Unlike PI Au **8**, PI–CB Au **9**, or PI Au–Pt; bimetallic nanoclusters immobilized on carbon black–polymer nanocomposites, such as PI–CB Au–Pd **15** and PI–CB Au–Pt **16**, are effective for the oxidation of aliphatic alcohols under mild conditions.[81]

Both catalysts contain bimetal-alloyed nanoclusters; however, the composition ratio in each nanocluster is different. While Au–Pt nanoclusters consist of an almost 1:1 mixture of each component, Au–Pd nanoclusters consist of a 4:1 to 3:1 ratio of Au and Pd, in spite of the almost same loading level in total. The existence of cationic character on the surface of bimetallic nanoclusters, which results from the more electronically positive feature of Pd than of Au, might facilitate the formation of the hemiacetal from the aldehyde and methanol, while it is suggested that there is electron transfer from Au to Pt in Pt–Au alloy nanocluster catalysts.[82]

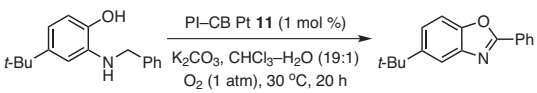
### 5.6. Oxidative Tandem Process for Converting N-Substituted 2-Aminophenols into 2-Substituted Benzoxazoles

We previously established that Pt nanocluster catalysts PI Pt **10** and PI–CB Pt **11** show excellent activity in the oxidation of hydroquinones and in the oxidative benzoxazole formation. We, therefore, hypothesized that, in the presence of the same Pt nanocluster catalysts, a sequential one-pot oxidation of N-substituted 2-aminophenols to *ortho*-quinone imines—which enolize to phenolic imines as a result of the rearomatization of the benzene ring—followed by another oxidation of the phenolic imines would result in benzoxazoles. The reaction proceeded smoothly with N-substituted 2-aminophenols to afford the desired benzoxazoles in high yields in the presence of PI–CB Pt **11** (**eq 6**).[83] In contrast, and while a 3-substituted aminophenol also gave a good result, unsubstituted 2-aminophenols gave poor results, presumably because of the instability of the reactive *ortho*-quinone imine intermediates. Benzoxazoles with alkenyl, alkynyl, and primary aliphatic substituents at the 2 position of the benzoxazole were prepared in good-to-high yields under the optimized reaction conditions.

## 6. Aerobic Oxidation of Highly Functionalized Systems Catalyzed by PI Metal Nanoclusters

### 6.1. In Situ Coupled Oxidation Reactions Catalyzed by PI Pt and ortho-Chloranil

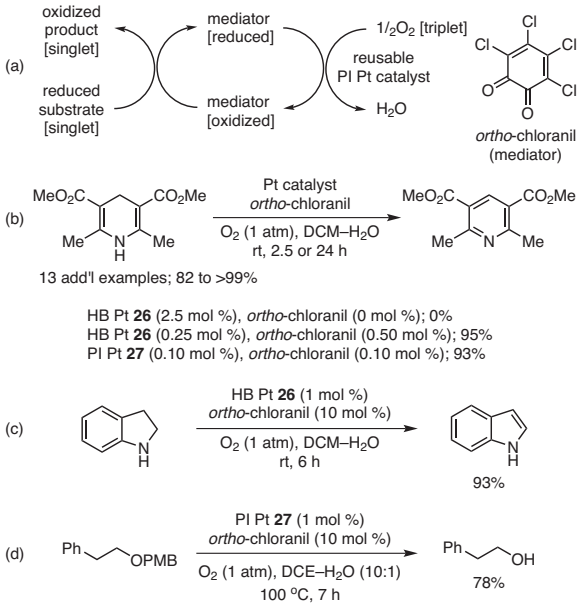
Quinones with a high oxidation potential, such as 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and chloranils, are versatile and important oxidizers in organic synthesis.[47a] However, the stoichiometric use of these quinones often leads to difficulties in removing the concomitant hydroquinone byproducts. The high cost of these expensive oxidizers is another problem. Such drawbacks may be overcome by employing a catalytic amount of the quinone and a stoichiometric amount of a co-oxidant. Of all co-oxidants, molecular oxygen is an ideal stoichiometric oxidizer from environmental and economic points of view, because it is abundant in air, and because water is the sole byproduct of the oxidation. However, because molecular oxygen is a triplet, highly selective direct reactions between molecular oxygen and common singlet organic compounds are difficult under mild conditions in spite of the high oxidation potential of molecular oxygen. An alternative approach is to transfer the oxidation potential of molecular oxygen, in the presence of a reusable catalyst, to singlet organic compounds that can easily react with common organic substrates, leading to a more abundant and environmentally benign oxidation system (**Scheme 14**, Part (a)).[84]



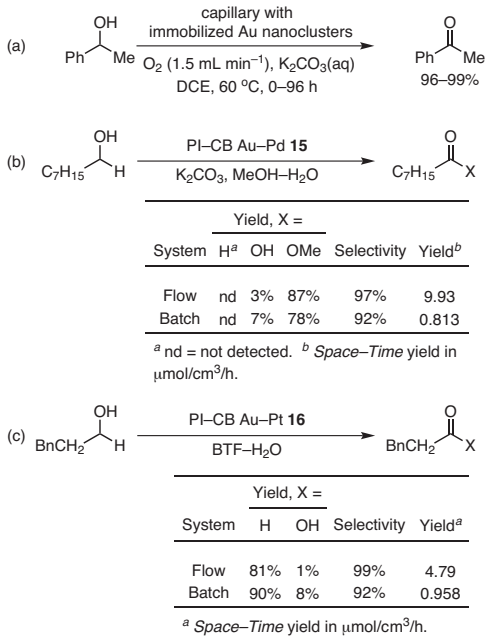

10 additional examples; 65 to >99% 1st–5th run; >95%

**eq 6** *(Ref. 83)*

While Pt nanocluster catalyst PI Pt **10** is effective for the aerobic oxidation of hydroquinones to quinones, it was not suitable for the new protocol since **P4** contains benzyloxy moieties, which may be oxidized by the mediator, *ortho*-chloranil, leading to partial decomposition of the catalyst. For this reason, oxidation-resistant PI platinum catalysts HB Pt **26** and PI Pt **27** were prepared from copolymers **HP** and **P5**, respectively.[85] To demonstrate the viability of the designed in situ coupled oxidation cycle, we investigated the




**Scheme 14.** In Situ Coupled Oxidation Cycles Catalyzed by Reusable Pt Nanocluster Catalysts. *(Ref. 85)*




| System | Yield, X = H[a] | OH | OMe | Selectivity | Yield[b] |
|---|---|---|---|---|---|
| Flow | nd | 3% | 87% | 97% | 9.93 |
| Batch | nd | 7% | 78% | 92% | 0.813 |

[a] nd = not detected. [b] *Space–Time* yield in μmol/cm$^3$/h.

| System | Yield, X = H | OH | Selectivity | Yield[a] |
|---|---|---|---|---|
| Flow | 81% | 1% | 99% | 4.79 |
| Batch | 90% | 8% | 92% | 0.958 |

[a] *Space–Time* yield in μmol/cm$^3$/h.

**Scheme 15.** Multiphase Flow Reactions for the Aerobic Oxidation of Alcohols. *(Ref. 89,90)*

oxidation of Hantzsch's dihydropyridine to pyridine using catalytic amounts of *ortho*-chloranil and Pt catalysts under an oxygen atmosphere (Scheme 14, Part (b)). While HB Pt **26** could not by itself catalyze this transformation, the reaction proceeded smoothly to give the desired product quantitatively in the presence of catalytic amounts of HB Pt **26** or PI Pt **27** and *ortho*-chloranil under atmospheric oxygen. This oxidation system was applicable to other reactions such as the oxidation of indoline to indole and the oxidative deprotection of *para*-methoxybenzyl (PMB) group (Scheme 14, Part (c) and (d)). HB Pt **26** was recovered by simple filtration and reused several times without significant loss of activity.[85]

### 6.2. Application to Microreactors and Flow Systems

To meet the increasing demand for faster, more efficient, and more productive syntheses, extensive efforts have recently been devoted to the integration of chemical reactions in order to create more powerful and speedy methodologies. Yoshida has proposed and classified the integration of chemical reactions into three types: (a) *time and space integration*, whereby all reaction components are mixed at once to perform reactions in one pot; (b) *time integration*, whereby reactions proceed with sequential addition of each reaction component; and (c) *space integration*, whereby reactions are performed on the stream of flow by adding components at different places.[3a,e] *Space integration* (i.e., flow synthesis), in particular, enables a more flexible design of multistep reactions, because reaction time, temperature, method of energy supply (conventional heating, microwave, light, electricity, etc.), reaction phases, and mixing efficiency between each reaction step can all be finely controlled by innovative architectures of flow reactors.[3a,b,d,e] In addition, flow systems can be applied to large-scale production, and high-throughput organic synthesis related to combinatorial library generation and automation.[86] Moreover, flow systems show remarkable compatibility with biphasic reaction systems; such as liquid–liquid, gas–liquid, gas–solid, and solid–liquid; because of their specific interfacial surface area especially in microsize reactors.[4,87]

Gas–liquid–solid multiphase conditions are very rare in flow systems because of insufficient reactivity, and, although several catalytic reactions that use flow systems are known, only a few utilize the metal-catalyzed aerobic oxidation of alcohols.[88] To fill this gap, we developed microsize reactors with high interface area, whose polysiloxane-coated internal surface was modified by immobilizing on it **P4**-derived, microencapsulated Au nanoclusters. The multiphase oxidation reactions—involving two liquid phases (organic and aqueous phases), atmospheric oxygen, and immobilized metal catalysts—were successfully carried out to afford the oxidized product in high yield during 96 h of a continuous flow reaction (**Scheme 15**, Part (a)).[89] While secondary alcohols were oxidized smoothly by this system, the oxidation of primary alcohols to aldehydes required the use of a microsize capillary modified with an Au–Pd bimetallic nanocluster.

While microreactors can be applied to large-scale production by multiplying the number of reactors, flow systems using conventional packed columns are more ubiquitous, because the heterogeneous catalysts developed for batch systems can be utilized directly in them and the total cost to construct the flow systems can be reduced. We investigated the PI–CB bimetallic nanocluster catalyzed aerobic oxidation of alcohols to aldehydes and the oxidative esterification using aliphatic primary alcohols as substrates, and compared the results from the flow systems (using conventional packed columns) with those from the batch systems. In the case of oxidative esterification, the flow system gave both a higher yield and selectivity than the

batch system (Scheme 15, Part (b)).[90] In case of alcohol oxidation, the batch system gave a slightly better yield of the aldehyde, but the flow system gave a higher aldehyde selectivity (Scheme 15, Part (c)).[90] Presumably, controlling the residence time in the column can prevent overoxidation to a carboxylic acid. In addition, the space–time yield of flow systems was about 5–10 times higher than that of batch systems, which demonstrates the high productivity of the flow systems.

## 7. Conclusion

Various metal(0) and bimetallic nanocluster catalysts were efficiently immobilized on polystyrene-based copolymers using a polymer incarceration (PI) technique that was based on two procedures: microencapsulation and cross-linking. Such PI nanocluster catalysts possess several desirable features such as the high stabilization ability of nanoclusters to prevent leaching and aggregation, smooth adsorption and desorption of reactants and products from the nanocluster surface to facilitate reactions under mild conditions, and easy access of substrates to the catalytic center located inside the polymer support. In addition, PI metal nanocluster catalysts are suitable for hydrogenation, some coupling reactions, and aerobic oxidation reactions using atmospheric oxygen or air under very mild conditions. Additional merits of the PI method are the ease of formation of various polymetallic nanoclusters in a systematic fashion and their compatibility with multifunctional catalytic systems. The activities and selectivities we observed with these systems have convinced us of the possibility and importance of polymetallic effects in the development of multifunctional catalytic systems. PI metal nanocluster catalysts can also be applied to more efficient processes such as microreactors and flow systems with only slight modifications of conventional batch systems. These characteristic features of PI nanocluster catalysts—recycling of precious metal catalysts, availability of molecular oxygen or air in oxidation reactions, fabrication of multifunctional catalytic systems, and applicability to integration of chemical reactions—fully meet the criteria of "green", sustainable chemistry and will permit the construction of "Nano Factories", where multiple catalysts located in nanoscale reaction environments will make possible the construction of more complex molecules.

## 8. Acknowledgment

We wish to thank cordially all of our co-workers for their significant contributions to the success of these projects. This work was partially supported by CREST, SORST, ERATO (JST), a Grant-in-Aid for Scientific Research from JSPS, the Global COE Program, the University of Tokyo, MEXT (Japan), and NEDO.

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**Shū Kobayashi** studied at the University of Tokyo and received his Ph.D. degree in 1988 working under the direction of Professor T. Mukaiyama. Following an initial period as assistant professor, he was promoted to lecturer then associate professor at the Science University of Tokyo (SUT). In 1998, he moved to the Graduate School of Pharmaceutical Sciences, the University of Tokyo, as full professor. In 2007, he was appointed to his current position as professor of organic chemistry in the Department of Chemistry, Faculty of Science, The University of Tokyo. He has held various visiting professorships, including at the Université Louis Pasteur, Strasbourg (1993), Kyoto University (1995), Nijmegen University (1996), Philipps University of Marburg (1997), and Paris-Sud (2010). Professor Kobayashi has wide-ranging research interests that include the development of new

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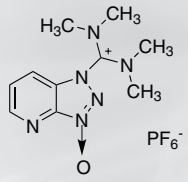

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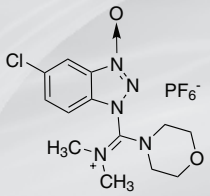

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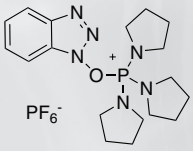

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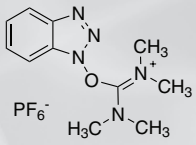

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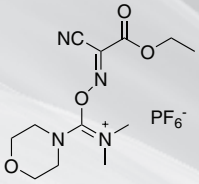

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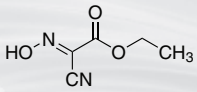

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# Advances in Acylation Methodologies Enabled by Oxyma-Based Reagents


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**Keywords.** oximes; Oxyma; COMU®; acylation; coupling reagents; additives; peptide synthesis; amide; ester.

**Abstract.** The aim of this review is to cover recent advances in acylation chemistry (peptide, amide, and ester bond formation) in which ethyl 2-cyano-2-(hydroxyimino)acetate (Oxyma)-derived reagents are involved. The distinct applications of each class of Oxyma derivatives will be discussed and compared to the behavior of reputed standards in the field.

## Outline



## 1. Introduction

Oximes are some of the most versatile building blocks in organic and organometallic chemistry. The substituents at the α position account for the specific properties of each oxime, such as dissociation constant, solubility, and chelating ability. Oximes are highly polar compounds of moderate acidity, and have a broad range of applications in chemistry and biology. For example, oxime-containing molecules are responsible for the prevention of biofouling in marine submerged materials, and display growth-regulating and fungicide activities in plants.[1,2] Due to their high physiological stability, oximes are also present in prodrugs and natural antibiotics.[3] In synthesis, oximes are involved in alkyl-transfer reactions, in the construction of palladium precatalysts for

carbon–carbon cross-coupling, and in electrocatalysis.[4] In addition, chemoselective protein ligation has recently been envisaged by connecting peptide fragments through an oxime bridge.[5]

Since the 1970s, the beneficial uses of cyanooximes—lacking the typical instability of oximes possessing an α hydrogen—have been extensively investigated in diverse fields of research.[6–12] Their high acidity translates into remarkable aqueous solubility and into the bright yellow color of the resulting anion—a consequence of a UV transition similar to that of the nitro group.[6] Some of the most prominent cyanooximes, bearing distinct electron-withdrawing moieties are depicted in **Figure 1**. The acidic nature of the cyanooximes with electron-withdrawing substituents (e.g.; AmOx (**8**), p$K_a$ = 5.2; Oxyma (**6**), p$K_a$ = 4.60) has led researchers to develop activated derivatives for acylation reactions on the basis of their potential as leaving groups.[13–18] Of all the oxime derivatives examined as acylation promoters, Oxyma (**6**) offers the best balance between reactivity and stability, in addition to its high solubility in a broad spectrum of solvents.[14] These desirable properties and its commercial availability on a large scale have made it a good candidate for investigating its acylation behavior.[19] In spite of its early use as an epimerization-suppressing additive,[14] Oxyma (**6**) remained unnoticed as a coupling reagent in the ensuing decades. Following the reevaluation of Oxyma (**6**) and other acidic oximes by DeGrado's group[20a] and ours,[17,20b,c,21,22] Oxyma (**6**) and its derived coupling reagents (**Figure 2**) have emerged as worthy alternatives to benzotriazoles,[13,14] which had hitherto dominated the field of acyl-transfer reactions.[20–22] In a short period of time, this oxime scaffold has rapidly been adopted in research laboratories to effect a broad range of acylations.[23,24] In the past few years, although some authors have surveyed the available acylation strategies for forming peptide and amide bonds, cyanooximes such as Oxyma (**6**) were either not included, or were described in a very limited number of applications.[25,26] This review offers a unique perspective on Oxyma-based coupling reagents, by focusing on their recently discovered acylation possibilities and by including some of their applications outside of peptide chemistry.

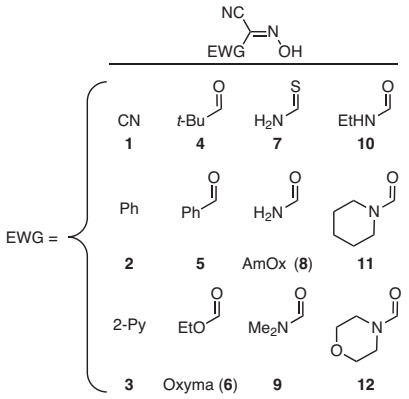



**Figure 1.** Structures of the Better Known Cyanooximes.

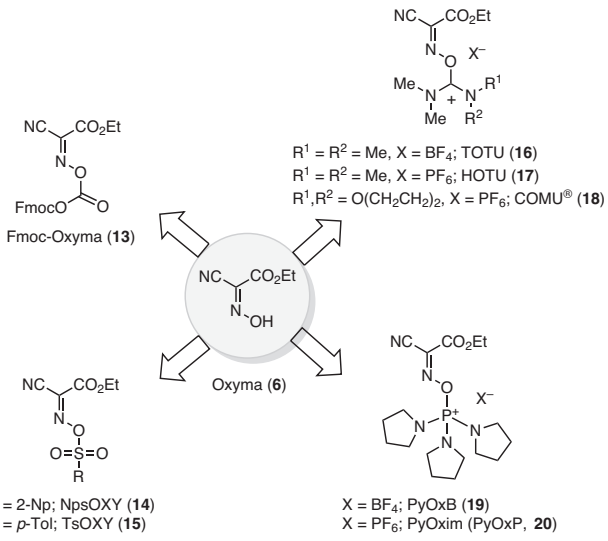



**Figure 2.** Oxyma (**6**)-Based Family of Peptide Coupling Reagents. *(Ref. 16,17,19–22)*

## 2. Oxyma-Based Coupling Reagents

The set of Oxyma-derived coupling reagents includes motifs with varying degrees of electrophilic character, resulting in distinct acylation abilities and diverse applications.[25,26] Thus, uronium salts (e.g., **16**–**18**) contain a markedly reactive carbocation core, and stand out as the preferred choice when powerful activation is required.[25,26] Although the tetramethylamino ones, TOTU (**16**) and HOTU (**17**), were described as peptide coupling reagents in the early 1990s[16]—mimicking the structure of benzotriazolium oxide based uronium salts—they did not gain acceptance in assisting peptide bond formation, and suffered a similar fate to that of the parent Oxyma (**6**) at that time. More recently, the inclusion of morpholine as the proton acceptor moiety in the electron-deficient uronium fragment resulted in COMU® (**18**), with enhanced solubility and acylation potency.[21,27] Oxyma-based phosphonium salts have also been investigated, and found to be especially suited for slow couplings and cyclizations. The tetrafluoroborate (PyOxB, **19**) and hexafluorophosphate (PyOxP, **20**, commercially sold as PyOxim) versions have been designed to stabilize the positively charged phosphorus center.[22] Whereas PyOxB (**19**) has only recently been introduced, the synthesis and characterization of PyOxim (**20**) had already been reported by Hoffmann et al. in 2003, although it was inexplicably not tested at that time.[28] The influence of the counteranion is far from being trivial, since this feature has direct impact on the physical properties (solubility and hydrolytic stability) and, therefore, on the appropriateness of the salt as a cyclization-promoting reagent.[22] Further derivatization of the Oxyma building block with the arylsulfonyl group was accomplished in 2010 by Khattab, resulting in milder activating reagents.[29] The sulfonate esters containing the 2-naphthalene- or *para*-toluenesulfonyl moiety, NpsOXY (**14**) and TsOXY (**15**), exhibit their strongest performance at short preactivation times, thereby ensuring retention of configuration of nearby chiral centers.[29] A completely different aim is achieved with the development of Oxyma carbonates, which contain in their structures the acyl group that is to be transferred to the potential nucleophile.[17] Together with analogues featuring other relevant acidic oximes, Fmoc-Oxyma (**13**) stands out as a reliable reagent for introducing the Fmoc protecting group with minimal impact on oligomers.[17]

Oxyma (**6**) is simply, rapidly, and almost quantitatively accessed in one step from the active-methylene compound ethyl cyanoacetate (**21**) (**Scheme 1**).[16,17,21a,22,29] In this modified Meyer nitrosation, nitrous acid is generated in situ from the reaction of sodium nitrite with an organic acid (acetic or phosphoric acid) at low temperature.[10e,19,30] Oxyma (**6**), isolated as a crystalline white solid, exists in the oxime form as shown

by IR spectroscopic analysis.[10e,19,31] However, in the anionic state (salt), or in solution if the pH is strongly basic, the tautomeric nitroso form is prevalent.[10e,19] Standard solutions of Oxyma (**6**) in DMF or acetonitrile do not shift the equilibrium to the nitroso species, even after prolonged storage or under moderate heating.[32] Subsequent O-functionalization of Oxyma (**6**) with electron-withdrawing groups takes place in the presence of a mild base, and yields the corresponding derivatives in excellent yields and purities (see Scheme 1).[16,17,21a,29] It is worth noting that TOTU (**16**), HOTU (**17**), and COMU® (**18**) are obtained as the O-form isomer (uronium salts), which is more reactive than the typical benzotriazolic N-form (aminium salts).[21a,33] In the case of phosphonium salts PyOxB (**19**) and PyOxP (**20**), an innovative one-pot procedure was implemented, in which the potassium salt of Oxyma (**6**) is formed in situ and then the bromophosphonium salt is added, resulting in enhanced yields in comparison to the original Hoffmann approach.[22,28]

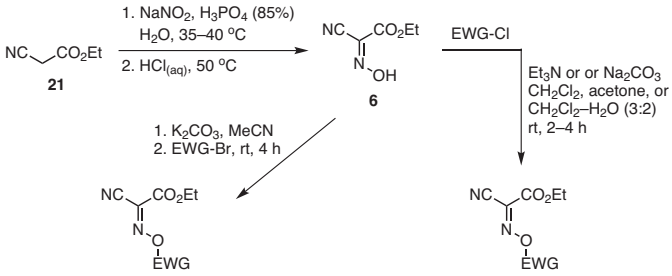
A remarkable feature of Oxyma (**6**)-based reagents is their approximately three-fold enhanced solubility in DMF when compared to their benzotriazole counterparts. This allows the preparation of more concentrated solutions to achieve greater acylation rates.[21,22,34,35] In this regard, not only does the oxime leaving group play an important role, but so does the nature of the electrophilic core.[27] Thus, the morpholine moiety within COMU® (**18**) is responsible for the 50% increased solubility in DMF in comparison to HOTU (**17**).[21,27] Phosphonium salts derived from Oxyma (**6**) also show a high solubility in DCM.[22] Moreover, the highly polar nature of Oxyma (**6**) influences the water solubility of its derivatives, which is essential to removing the coupling byproducts in solution-based acylations.[20b,21c] A risk assessment on Oxyma (**6**) and COMU® (**18**) by means of DSC and ARC calorimetric experiments proved that the likeliness of thermal runaways with these compounds was considerably lower than those with benzotriazole-based reagents, because they decompose in a controlled manner with a relatively low pressure release in contrast to the behavior of benzotriazoles.[20a,21a,36]

## 3. Assisting Peptide Bond Formation

The field of peptide synthesis is continually integrating the latest advances in process technology.[37] One of the areas that have garnered considerable attention is the optimization of the chemical tools promoting amino acid assembly through the formation of an amide bond (referred to as peptide bond in this context).[25] Given the propensity of amino acids to undergo epimerization at the $C_\alpha$ position under strong acid activation, and the subsequent impact of the epimerization on the final purity of the target peptide, acid halides are commonly dismissed for this purpose—in contrast to their role in conventional amide-bond-formation methods.[25,38] Another drawback besets acid azides, the other class of possible acylation reagents, which have been associated with explosive incidents when dried.[25]

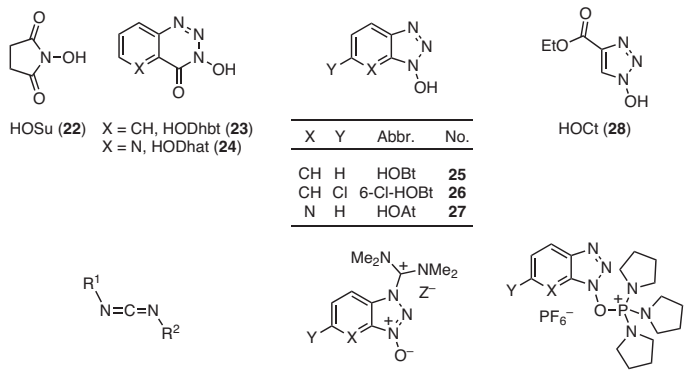
In the search for more balanced acylation strategies, several amino acid active esters have been introduced; these are mainly generated in situ by using a combination of carbodiimide and additive, or by utilizing standalone coupling reagents (**Figure 3**).[19,25] With the exception of electron-withdrawing phenols and triazines, the majority of active esters are based on *N*-hydroxylamines as leaving groups, such as triazoles, benzotriazines, succinimides, and especially benzotriazoles.[19,39] Although carbodiimides [DCC (**29**) and DIC (**30**) in the solid phase; and typically EDC (**31**) in solution] were originally proposed as sole acylation reagents in the coupling medium, the high levels of *N*-acylisourea and epimerized stereoisomers prompted the use of the abovementioned *N*-hydroxylamines as additives, with the aim of favoring the presence of *N*-hydroxylamine active esters in situ.[40]

In this scenario, benzotriazoles have prevailed due to the versatility of their scaffold and their generally accessible prices. The pioneering work of König and Geiger in the early 1970s with the introduction of HOBt (**25**) as racemization-suppressing additive continued for a few years later with the implementation of HOAt (**27**, the most potent and expensive analogue) and 6-Cl-HOBt (**26**, analogue of medium reactivity and more accessible potency-to-cost ratio).[41–43] Although its reactivity is somewhat lower than that of HOBt (**25**), succinimide [HOSu (**22**)] has also attracted much interest because its water solubility allows the acylation to be performed in aqueous media.[44] Benzotriazines HODhbt (**23**) and HODhat (**24**) were initially well received because their performance approached that of HOAt (**27**); however, ring-opening side reactions compromised their continued use.[41a,45] Other templates, such as triazole HOCt (**28**), which was introduced by Jiang et al., have also shown promising performance.[46] Although they were part of a mild and reliable coupling methodology, carbodiimides have




| Abbr. | No. | Yield | Ref. |
|---|---|---|---|
| Fmoc-Oxyma | **13** | 91% | *17* |
| NpsOXY | **14** | 92% | *29* |
| TsOXY | **15** | 86% | *29* |
| TOTU | **16** | 44% | *16* |
| HOTU | **17** | 82% | *21a* |
| COMU® | **18** | 89% | *21a* |

**Scheme 1.** Synthesis of Oxyma (**6**) and Derived Reagents.




| X | Y | Abbr. | No. |
|---|---|---|---|
| CH | H | HOBt | **25** |
| CH | Cl | 6-Cl-HOBt | **26** |
| N | H | HOAt | **27** |

| $R^1$ | $R^2$ | Abbr. | No. |
|---|---|---|---|
| Cy | Cy | DCC | **29** |
| *i*-Pr | *i*-Pr | DIC | **30** |
| Et | $(H_2C)_3NHMe_2^+$ $Cl^-$ | EDC | **31** |

| X | Y | Z | Abbr. | No. |
|---|---|---|---|---|
| CH | H | $PF_6$ | HBTU | **32** |
| CH | H | $BF_4$ | TBTU | **33** |
| CH | Cl | $PF_6$ | HCTU | **34** |
| CH | Cl | $BF_4$ | TCTU | **35** |
| N | H | $PF_6$ | HATU | **36** |

| X | Y | Abbr. | No. |
|---|---|---|---|
| CH | H | PyBOP® | **37** |
| N | H | PyAOP | **38** |
| CH | Cl | PyClock® | **39** |

**Figure 3.** Most Relevant *N*-Hydroxylamines, Carbodiimides, and Onium Salts Employed in Peptide-Bond Formation.

slowly been replaced by the more powerful onium (aminium/uronium and phosphonium) salts.[25] Those standalone coupling reagents contain an *N*-hydroxylamine moiety in their structure and, like the corresponding additives, the predominant ones have a benzotriazole core. Aminium salts HBTU/TBTU (**32**/**33**), HCTU/TCTU (**34**/**35**), and HATU (**36**) have been associated with demanding couplings; whereas phosphonium salts PyBOP® (**37**), PyAOP (**38**), and PyClock® (**39**) are preferred in slow-rate acylations.[42,47,48]

In spite of their reliability in routine peptide couplings, some of these highly reactive species still give rise to incomplete acylations in difficult sequences, detrimental side reactions, and/or safety concerns.[25,36] Particularly troublesome has been the consideration of benzotriazole-based reagents as Class 1 explosives, which has severely restricted their overseas transport.[36] Alternative, groundbreaking strategies for the assembly of demanding amino acids have been recently devised, such as isonitrile-mediated peptide bond formation (Danishefsky's group)[49] or umpolung stereoselective peptide synthesis (Johnston's lab).[50] However, the limited availability of the corresponding building blocks may hinder their wide adoption in the field.[49,50] The need for chemical tools that rapidly accomplish the elongation of the peptide chain in a safe and efficient manner has led our group to reevaluate Oxyma (**6**) and its derivatives for this purpose, and has led to the development of excellent alternatives to classical methods.

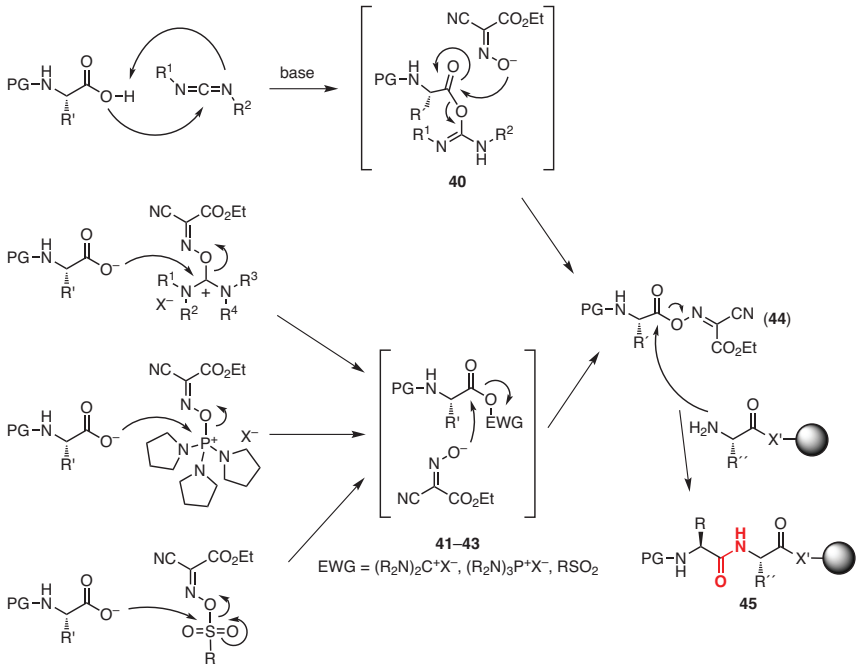
### *3.1. Manual Synthesis*

#### 3.1.1. Linear Couplings

Most of the coupling steps performed in a peptide synthesis laboratory are carried out in a linear fashion, anchoring the growing peptide chain onto a polymeric solid support.[51a] Therefore, the majority of experiments employing Oxyma (**6**), either as additive to carbodiimides or contained in standalone coupling reagents, have been performed as linear syntheses. The proposed mechanism of amino acid activation with Oxyma-based reagents and subsequent aminolysis is detailed in **Scheme 2**.[51b] It must be stressed that, regardless of the chosen coupling strategy, the active species that is ultimately generated in situ corresponds to the Oxyma amino acid ester **44** (if aminolysis does not occur in the precursor to **44**). In the case of carbodiimides, a strongly activated *O*-acylisourea intermediate (**40**) is primarily formed after deprotonation of the carboxylic acid by the carbodiimide and its attachment to the electron-deficient carbon center of the carbodiimide.[19,25] Reaction of Oxyma (**6**) with **40** converts it into the oxime active ester **44**.

A slightly different mechanism takes place with standalone coupling reagents, which, unlike carbodiimides, require the presence of base to initiate the acylation process.[25] Nucleophilic attack of the carboxylate anion onto the electrophilic center (carbon, phosphorus, or sulfur) generates a highly reactive species (**41**–**43**) which is not isolated. This reactive species undergoes acyl transfer with Oxyma (**6**), which is previously released as the leaving group in the first step of the process. In the last step, aminolysis of the Oxyma active ester (**44**) leads to the desired peptide **45**. In all cases, the driving force for the acylation is the highly stable byproduct (urea, phosphoramide, or sulfonate) released after reaction of the Oxyma anion with the first intermediate.[25] Although all strategies form the oxime active ester **44**, different types of reagent are associated with varying acylation rates and potencies, which consequently leads to the assumption that the formation of the Oxyma active ester **44** (i.e., the attachment of carboxylate to the reactive core of the Oxyma reagent) is rate-determining.

Epimerization is the most troublesome side reaction faced by peptide chemists, which can take place through oxazolone formation or via enolization of the linear peptide.[14b,25d,52] Given that separation of epimerized byproducts from the target peptide by chromatographic methods is extremely challenging, the loss of chiral integrity has




**Scheme 2.** Proposed Mechanism of Peptide-Bond Formation Using Various Oxyma (**6**)-Based Coupling Reagents. *(Ref. 51b)*

a direct impact on the final purity of the compound, which raises major concerns in the manufacture of bioactive peptides.[53] Indeed, the capacity of a coupling reagent to reduce the proportion of stereomutated analogues is a decisive factor in the activator selection process.[52,54] Usually, strong activation methods (such as those utilizing acyl halides) are avoided, and carbodiimide-based approaches are employed in combination with additives to balance the reactivity of the active species.[19,38,41a,b] The outstanding potential of Oxyma (**6**) to retain the optical purity of the activated amino acid had already been envisaged a few decades ago separately by Itoh and Izdebski.[13,14] First, Itoh reported full suppression of epimerization in the coupling of **Z-Gly-Phe-OH** to H-Gly-OEt by utilizing DCC–Oxyma, and a greater reduction of stereomutation in the assembly of **Ac-Ile-Gly-OEt**, when this coupling cocktail is employed than when HOBt (**25**) or HOSu (**22**) is used.[13] Shortly thereafter, Izdebski obtained moderate epimerization control with Oxyma when the Young peptide model was utilized.[14] However, the extraordinary ability of this oxime to minimize loss of chirality was not unambiguously proved until 2009, when our group invested efforts in examining in depth this remarkable feature of Oxyma and its derived coupling reagents, either in stepwise or segment couplings.[20b,21a,22,29]

### 3.1.1.1. [1 + 1] Couplings

Selection of an adequate peptide model is central to highlighting the relative ability of various reagents to promote retention of chirality. To that end, the activation of epimerization-prone α-phenylglycine (Phg) provides a suitable scenario for evaluating the performance of a coupling activator in stepwise peptide synthesis.[55] Thus, Oxyma-based activators have been evaluated in the coupling of Z-Phg-OH with H-Pro-$NH_2$ in solution to form **Z-Phg-Pro-$NH_2$**.[56] Remarkably, the extent of **Z-D-Phg-Pro-$NH_2$** formed using DIC (**30**)–Oxyma (**6**), COMU® (**18**), HOTU (**17**), PyOxB (**19**), or PyOxim (**20**) was maintained in all cases below 1%.[20a,21a,22]

Hence, the performance of each subfamily of Oxyma-derived reagents exceeds that of the corresponding benzotriazoles, even HOAt-based ones, which represents a hallmark in the field. Particularly impressive is the almost negligible stereomutation level obtained with Oxyma-containing uronium salts HOTU (**17**) and COMU® (**18**) (0.17% and 0.12%, respectively), which thus allow a substantially higher conservation of chirality than HBTU (**32**) and HATU (**36**) (8.2% and 3.1%, respectively).[21a] Moreover, uronium salt COMU® (**18**) affords **Z-Phg-Pro-$NH_2$** in 93% yield also when only 1 equivalent of base is employed, owing to its morpholine-based moiety.[21a,27] Although Oxyma-based phosphonium salts gave rise to slightly less optically pure crudes than HOTU (**17**) and COMU® (**18**), PyOxim (**20**) induced a two-fold higher retention of configuration than the tetrafluoroborate analogue PyOxB (**19**) (0.3% and 0.6% stereomutation, respectively)—still, both surpassed the performance of HOBt (**25**), 6-Cl-HOBt (**26**), and HOAt (**27**) (5.8%, 1.6%, and 2.2% stereomutation, respectively).[22] In a solid-phase approach to the same dipeptide, the notorious difference in epimerization reduction between PyOxim (**20**) or PyOxB (**19**) and benzotriazole-based phosphonium salts was even more pronounced.[22] Interestingly, both base-mediated coupling strategies (utilizing uronium and phosphonium salts) afforded less epimerized dipeptide than DIC (**30**)–Oxyma (**6**), which required a short preactivation to ensure full acid activation.[20a] Nevertheless, the content of DL stereoisomer when using Oxyma (**6**) as additive was much lower than in analogous syntheses using HOAt (**27**) and HOBt (**25**) (1.0% vs 3.3% and 9.3% stereomutation, respectively).[20a] In addition, the presence of Oxyma (**6**) in the coupling medium not only favors preservation of chirality in carbodiimide-based peptide bond formation, but also when combined to coupling reagents, such as in fluoroformamidinium salt TFFH, because the additive speeds up the transition to the less reactive active ester.[57] Thus, addition of Oxyma (**6**) to TFFH coupling mixtures promotes a huge reduction of epimerization (from 7.4% to 0.5%).[57]

Oxyma-based reagents continued to prove their great capacity to preserve optical configuration during the stepwise assembly of dipeptide **Z-Phe-Val-OMe**. Although less epimerization-prone than Z-Phg-OH, this model system allowed a clear visualization of the effectiveness of Oxyma-sulfonates NpsOXY (**14**) and TsOXY (**15**), and the tetramethyluronium salt HOTU (**17**).[29,58] NpsOXY (**14**) not only led to more effective control of epimerization than benzotriazole counterparts NpsOBt and NpsOAt (4.8% vs 12.9% and 6.7% stereomutation, respectively), it also afforded the dipeptide in considerably higher yields (92% vs 69% and 54%, respectively).[29] Moreover, the overall process was much faster with the Oxyma-derived reagent, because long preactivation times were avoided.[29] A similar scenario was followed with the tosyl analogue TsOXY (**15**), which slightly underperformed the HOBt analogue in the percentage of DL isomer present (2.0% vs 1.4%), but gave a better yield of the desired dipeptide (91% vs 52%).[29] An even more impressive performance was provided by HOTU (**17**) in the same reaction (using Z-Phe-OH), affording less than 1% of the dipeptide epimer.[58] The extraordinary retention of configuration observed with Oxyma-based reagents is evident when compared to that afforded by HOBt-based coupling systems [DCC (**29**)–HOBt (**25**), BOP, and TBTU (**33**)], which produced 39–44% of the epimer.[47c] NpsOXY (**14**) and TsOXY (**15**) also showed a great capacity to prevent loss of chirality and to give higher isolated yields than benzotriazole reagents in the stepwise assembly of **Z-Phe-Ala-OMe**, **Z-Val-Val-OMe**, and **Z-Val-Ala-OMe**.[29] Oxyma (**6**) has also been employed to reduce the extent of Cys isomerization in the on-resin elongation of **H-Gly-Cys-Phe-$NH_2$**, achieving epimerization suppression comparable to that of HOAt (**27**) (0.1% epimer with 5 min preactivation) and higher yields.[20a]

### 3.1.1.2. [2 + 1] Couplings

Loss of chiral integrity is particularly severe during activation of peptide fragments (even at the dipeptide stage), since the concentration of the epimerization-prone oxazolone intermediate is higher here than in the coupling of urethane-protected amino acids.[25] Therefore, the ability of Oxyma-based reagents to enhance the optical purity in segment couplings has also been examined. The activation of dipeptide **Z-Phe-Val-OH**, and its subsequent coupling with H-Pro-$NH_2$ to give **Z-Phe-Val-Pro-$NH_2$**, was employed as a model system, which gave rise to higher epimerization than the stepwise coupling.[59] In this type of coupling, the nature of the base plays an important role in the preservation of chirality of the activated dipeptide starting material (**Z-Phe-Val-OH**). For example, peptide crudes of higher optical purity were obtained with TMP (2,4,6-trimethylpyridine) than with DIEA:[21a,57] Employing 2 equiv of DIEA, COMU® (**18**) and HOTU (**17**) each led to a higher retention of configuration than HBTU (**32**), but both were less efficient than HATU (**36**) (19.3% and 23.6% vs 27.7% and 13.9% stereomutation, respectively).[21a] In contrast, when only 1 equiv of TMP was used, the level of stereomutation was uniformly decreased, with COMU® (**18**) producing the smallest percentage of LDL epimer (3.5%) while maintaining yields over 90%.[21a] A similar level of control of optical purity was observed using PyOxim (**20**) and PyOxB (**19**) (5.7% and 5.3% stereomutation, respectively), which improved upon the epimerization degree obtained with PyBOP® (**37**) and PyClock® (**39**) (12.5% and 8.6% stereomutation, respectively).[22] Furthermore, the

addition of Oxyma (**6**) to PyOxim (**20**) resulted in further reduction of the impact of epimerization to a level (3.4%) similar to that obtained with COMU® (**18**) and 1 equiv of TMP.[21a,22] A comparable performance was observed vis-à-vis the corresponding *N*-hydroxylamines, with Oxyma (**6**) performing at a level (3.8% stereomutation) close to that (2.1% stereomutation) of HOAt (**27**).[20a] In the assembly of **Z-Phg-Pro-NH$_2$**, the use of Oxyma (**6**) as additive to fluoroformamidinium salts reduces further the impact of the LDL epimer from 23% to 2.8%, thereby standing out as a promising low-epimerization approach.[57] Other fragment systems were investigated, such as the [2 + 1] assembly of tripeptides **Z-Gly-Phe-Ala-OMe**, **Z-Gly-Phe-Val-OMe**, and **Z-Gly-Val-Val-OMe**.[58] Using these peptide platforms, HOTU (**17**) achieved an extraordinarily low degree of DL epimer (<1%), in contrast to the poor retention of configuration induced by cyano-2-pyridyloxime- and triazine-based reagents (6–50% stereomutation).[58] In addition, PyOxim (**20**) showed a considerably greater conservation of chirality than PyBOP® (**37**), PyAOP (**38**), and PyClock® (**39**) in the [3 + 3] synthesis of **Z-Gly-Gly-Val-Pro-Gly-Gly-NH$_2$**.[22]

Coupling reagents based on Oxyma (**6**) stand out as the preferred acylating species for assembling sterically demanding sequences.[20,21a,22] Thus, Oxyma (**6**), COMU® (**18**), HOTU (**17**), and PyOxim (**20**) displayed an impressive performance in the coupling of *N*Me-amino acids and Aib residues.[20,21a,22] Remarkably, the acylation capacity of Oxyma-derived reagents often exceeds that of HOAt-based analogues, and the gap in the performance of both classes of activator increases as the steric hindrance of the amino acid increases.[20,21a,22] Steric interactions can account for this behavior given the rigidity of the benzotriazole core. An excellent platform to test the coupling efficiency of a given reagent is the Leu-enkephalin pentapeptide, an endogeneous hormone, modified at the two central Gly residues (**H-Tyr-AA-AA-Phe-Leu-NH$_2$**).[47c] In the on-resin elongation of the *N*Me-Gly analogue, Oxyma (**6**) afforded 91% of the pentapeptide with short, 5-min coupling times—a performance superior to that of HOBt (**25**) in the same reaction model.[20a] Oxyma (**6**) even surpassed the acylation abiliy of HOAt (**27**) in the synthesis of the *N*Me-Ala-enkephalin pentapeptide (79% vs 74% using 30-min coupling times),[20a] whereas in the assembly of the *N*Me-Leu derivative, Oxyma-based COMU® (**18**) performed at an intermediate level between HOAt- and HOBt-containing aminium salts.[21a] Taking advantage of their extraordinary capacity to assemble N-methylated residues, COMU® (**18**) and Oxyma (**6**) were recently combined and proved more efficient than the HATU (**36**)–HOAt (**27**) system in a linear sequence leading to an *N*Me-rich cyclic antitumor depsipeptide.[35] Furthermore, the COMU® (**18**)–Oxyma (**6**) system was compatible with the activation of Alloc-based residues.[35]

In light of the steric and conformational restrictions that an α,α-disubstituted amino acid residue such as Aib would introduce in peptide sequences, several Aib-containing peptide models have been employed to further investigate the capacity of Oxyma-based reagents to assist in the coupling of bulky junctions.[60,61] In particular, the elongation of the Aib-enkephalin pentapeptide has been broadly used to amplify differences in the performance of coupling reagents.[47a,62] Oxyma (**6**), COMU® (**18**), HOTU (**17**), and PyOxim (**20**) displayed an outstanding efficiency in the manual SPPS elongation of Aib-Aib-containing peptide **H-Tyr-Aib-Aib-Phe-Leu-NH$_2$**, often reaching percentages of the target product close to completion.[20,21a,22,57,63] In all cases, a clear superiority of Oxyma-based reagents over HOAt-, and HOBt-derived ones was observed. Using a carbodiimide approach, Oxyma (**6**) as additive rendered a much higher content of the target pentapeptide than HOBt (**25**) and HOAt (**27**), regardless of the coupling time applied (69% vs 19% and 55% of pentapeptide after 1-h double couplings).[20] The same trend was observed with the corresponding uronium salts, with HOTU (**17**) and COMU® (**18**) yielding an impressive 99.0% and 99.7% of the Aib-enkephalin pentapeptide [vs 83.0% and 47.0% for HATU (**36**) and HBTU (**32**), respectively].[21a] In addition, both Oxyma-based uronium salts accomplished conversions higher than 87% with substantially reduced protocols.[21a] Similarly, PyOxim (**20**) showed greater acylation capacity than PyBOP® (**37**), PyAOP (**38**), and PyClock® (**39**) in this Aib-Aib linear system (98% vs 49%, 85%, and 77% of **H-Tyr-Aib-Aib-Phe-Leu-NH$_2$** after 30-min double couplings).[22] Moreover, the addition of Oxyma (**6**) to other standalone coupling reagents enhanced their efficiency to a greater extent than HOBt (**25**) and HOAt (**27**) did.[57,63] Thus, the combination of TFFH and Oxyma (**6**) raised the percentage of the target Aib-peptide in comparison to TFFH alone (98% vs 95%).[57] A more dramatic increase in the yield of **H-Tyr-Aib-Aib-Phe-Leu-NH$_2$** was observed with triazine-based DFET by the inclusion of Oxyma (**6**) in the reaction mixture (94% vs 55%).[63] Recently, several cyanoacetamidooximes (**8–12**) showed promising performance in this peptide system, as replacements for HOSu (**22**).[64] Dimeric versions of this scaffold, 3,4- and 8,9-enkephalin decapeptides, have been employed to compare the performance of various phosphonium salt coupling reagents: PyOxim (**20**) produced the highest percentage (95–96%) of the desired peptide.[22]

Oxyma-based reagents have also been tested in the solid-phase elongation of ACP (65–74) decapeptide, a commonly established model platform, either in its naturally ocurring sequence (**46**) or as the Aib$^{67}$-Aib$^{68}$ analogue (**47**) (**eq 1**).[21a,22,59,65] HOTU (**17**) and COMU® (**18**) were compared in the synthesis of the unmodified decapeptide, **46**, under a fast acylation protocol consisting of 2-min couplings.[21a] Under these conditions, COMU® (**18**) produced ACP (**46**) to a higher extent than HOTU (**17**) (79% vs 66%), demonstrating the enhanced reactivity of the dimethylmorpholino skeleton.[21a] In comparison to a previous synthesis carried out under identical coupling conditions, COMU® (**18**) led to a percentage of ACP (**46**) similar to that by HATU (**36**), but much higher than that by HBTU (**32**) (80% and 46% of **46**, respectively).[66] The low acylation extent obtained with phosphate DEPBT (6%) highlights the difficulty of this synthesis.[45b] The preparation of the Aib$^{67}$-Aib$^{68}$ derivative (**47**) proved even more demanding, requiring longer coupling times to obtain a similar percentage of the desired peptide.[22] However, the Oxyma-based phosphonium salt PyOxim (**20**) outperformed the benzotriazole analogues PyBOP® (**37**) and PyAOP (**38**) (81% vs 48 and 64%), giving rise to minimal amounts of des-Aib.[22]

The benefits of using Oxyma-based reagents also extend to solution-phase approaches, as has been proven in the previous discussion of epimerization systems.[20,21a,22,29] In 1991, Breipohl and König showed the suitability of Oxyma-based TOTU (**16**) and HOTU (**17**) for the preparation of various peptides in solution, such as **Fmoc-D-Hyp-Gly-O*t*Bu** and **Fmoc-Leu-Arg-Pro-azaGly-NH$_2$**, the latter in a [2 + 2] fashion.[16] Additionally, TOTU (**16**) was employed in the activation of aromatic acids, like benzophenone-4-carboxylic acid, and subsequent coupling to the ε-amino group of Lys.[16] One of the most remarkable advantages over benzotriazole-based reagents is the enhanced solubility of Oxyma-derived byproducts, which results in the preparation of more concentrated coupling mixtures and easier byproduct removal during workup.[21,22,34,35,67] The outstanding acylation potential of COMU® (**18**) and HOTU (**17**) vis-à-vis HATU (**36**) was highlighted in dipeptide models containing Val and Aib residues.[21a] Generally, Oxyma-based uronium salts showed faster acylation rates, better yields, and required less of the base during assembly of **Fmoc-Val-Val-NH$_2$** and **Z-Aib-Val-OMe**.[21]

The scope of applications of Oxyma-based reagents extends

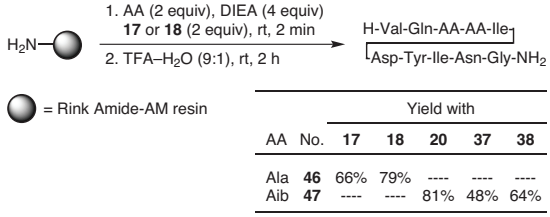
to Boc-based peptide synthesis.[57,68] An initial report by Khattab in 2010 confirmed the compatibility of Oxyma (**6**) with this protecting group, in a solution-phase approach to Leu-enkephalin pentapeptide using Boc-amino acids (except for the *N*-terminal Tyr residue).[57] Thus, TFFH–Oxyma (**6**) mediated couplings and TFA–DCM (1:1) deprotection cycles afforded the target peptide in high purity (98.3% by HPLC) and 69% yield.[57] Additionally, in a recently published work, Alloc- and Boc-protected γ-aminoproline residues were stepwise and alternately assembled in the solid phase by means of DIC (**30**)–Oxyma (**6**) couplings.[68c] However, in comparison with HOBt (**25**) and HOAt (**27**) based coupling reagents, the superiority of the Oxyma (**6**) template is not as evident here as it is in Fmoc-based peptide synthesis. Hence, Alewood and collaborators compared the performance of HBTU (**32**), HCTU (**34**), and COMU® (**18**) in Boc SPPS using different solid supports and fast coupling protocols.[68a] Although COMU® (**18**) performed better in PEG-based resins than in polystyrene ones, its coupling efficiency did not exceed that of HBTU (**32**) or HCTU (**34**) in any of the cases investigated.[68a] Bearing in mind that Boc-amino acids are much less bulky than the Fmoc analogues, the steric bulk of the coupling reagent (hypothetically favoring Oxyma) should not play a crucial role in the activation step. Finally, a groundbreaking ligation methodology for chemical protein synthesis, based on 5-oxaproline, has been described very recently.[68b] In the key coupling of the precious (*S*)-*N*-Boc-5-oxaproline (**48**) onto the solid-phase-attached peptide chain (**49**), COMU® (**18**) was preferred over HCTU (**34**) (**Scheme 3**).[68b] After cleavage from the resin, the oxaproline-peptide (**50**) was chemoselectively ligated with an α-keto acid fragment (**51**) to yield the target sequence (**52**). COMU® (**18**) was also employed in the Fmoc-SPSS of both peptide fragments, when difficult junctions required stronger activation than possible with HCTU (**34**).[68b]

Additional examples of the benefits of using Oxyma-based reagents include: (i) Sawada and Gellman's solid-phase elongation of a γ-amino acid containing 14-mer peptide designed to resemble α-helix motifs.[69] (ii) The use of Oxyma (**6**) by Royo's and Feliu's groups to acylate constrained γ-aminoPro foldamers en route to a battery of dual antimicrobial peptide–cell-penetrating peptide antitumor compounds.[68c] (iii) The use of COMU® (**18**) to synthesize two bioactive cyclopeptides on Barlos's 2-chlorotrityl solid support,[70] which illustrates the full compatibility of Oxyma-based reagents with acid-sensitive resins.[35,71] (iv) COMU® (**18**) has been employed in an optimized protocol for the synthesis of a complement 5a antagonist cyclopeptide, active against Alzheimer's disease and sclerosis.[71] (v) The great solubility of Oxyma (**6**) in organic solvents has prompted its use in the evaluation of green alternatives to DMF.[32] Thus, employing DIC (**30**)–Oxyma (**6**) assisted couplings, the suitability of acetonitrile as solvent, in combination with PEG-based resins, was surveyed in the assembly of several peptides, including ACP (65–74) (**46**) and Leu-enkephalin.[72] The extent of epimerization during assembly of Fmoc-Phe-OH onto H-Leu-Rink-resin was kept at minimal levels using Oxyma (**6**) in this solvent.[72] A few decades ago, DIC (**30**)–Oxyma (**6**) assisted peptide couplings in THF were also reported.[13b]

### 3.1.2. Cyclizations

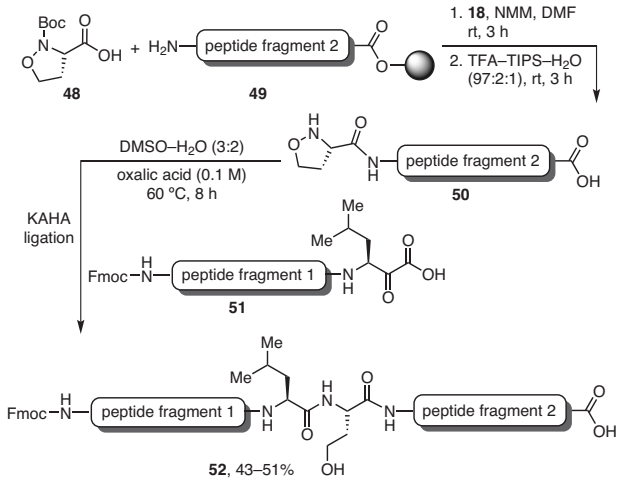
The markedly slow acylation rate in couplings leading to cyclic peptides determines their particular methodological requirements. Thus, the use of uronium salts to activate the carboxylic acid is dismissed in these cases, since guanidylation of the amino group occurs to a great extent as result of the notorious electrophilicity of the reagent's skeleton.[73] In contrast to linear couplings, peptide cyclizations are generally carried out in high dilution in order to avoid the formation of unwanted linear or cyclic dimers. To this end, phosphates (e.g., DEBPT), phosphoryl azides (e.g., DPPA), phosphonic acid anhydrides (e.g., T3P), carbodiimides, and phosphonium salts are the most convenient reagents.[47,74] Acylating agents based on the Oxyma scaffold (**6**)—among which DIC (**30**)–Oxyma (**6**) and the corresponding phosphonium salts **19** and **20** are worth noting—have also been sporadically used in peptide cyclization steps. As would be expected, uronium salt COMU® (**18**) is not the best suited for this type of coupling, since the *N*-terminal guanidylated peptide is obtained as the major if not only product.[22,75]

The Oxyma (**6**)-derived phosphonium salts PyOxB (**19**) and PyOxim (**20**) have been developed as safer alternatives to benzotriazole-based PyBOP® (**37**), PyAOP (**38**), and PyClock® (**39**).[22] PyOxB (**19**) and PyOxim (**20**) exhibit improved solubility, enhanced capacity to retain optical purity, and increased acylation potency in linear couplings over benzotriazole-containing reagents.[22] In cyclizations, however, hydrolytic stability of the coupling reagent is of utmost importance given that the acylation rate is very slow and couplings usually take several hours to complete. Therefore, in view of the poor stability of the tetrafluoroborate salt PyOxB (**19**) (25% reagent left in DMF after 5 hours), the performance of this analogue in slow couplings is compromised.[22] On the other hand, PyOxim (**20**) shows higher stability in acetone and DMF than all benzotriazole counterparts, consequently standing out as a promising choice for cyclizations. To practically test the performance of PyOxB (**19**) and PyOxim (**20**) in cyclic couplings, the linear peptide (**53**) was mixed with the reagent and excess DIEA in DMF and, after 24 hours, the proportion of cyclic




| AA | No. | Yield with 17 | 18 | 20 | 37 | 38 |
|---|---|---|---|---|---|---|
| Ala | **46** | 66% | 79% | ---- | ---- | ---- |
| Aib | **47** | ---- | ---- | 81% | 48% | 64% |

**eq 1** *(Ref. 21a,22)*



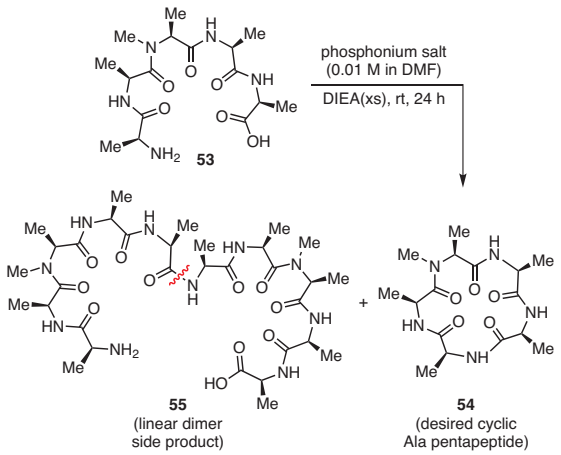

**Scheme 3.** COMU® (**18**) in the Implementation of Boc-protected KAHA Ligation. *(Ref. 68b)*

material (**54**) and linear dimer (**55**) was determined by HPLC (**eq 2**).[22,76] As envisaged, the level of cyclic peptide (**54**) in the PyOxB (**19**)-promoted reaction was one of the lowest, comparable to its level from the PyBOP® (**37**)-assisted synthesis (47% and 43% of **54**, respectively). In contrast, PyOxim (**20**) afforded the highest purity of **54** (70% vs 54% with PyAOP) among the phosphonium salts tested.[22]

In a recent publication, Hinou et al. considered the use of DIC (**30**)–Oxyma (**6**) in cyclizations performed in fluorinated solvents such as 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) and 2,2,2-trifluoroethanol (TFE).[75] As a result of the hydrogen-bond acceptor nature of these solvents, the twin effects of enhanced reagent solubility and boosted cyclization rate were observed.[75,77] Thus, HFIP–DCM and TFE–DCM solvent systems were chiefly investigated during cyclization to the hexapeptide core (**57**) of an antifreeze glycopeptide at 10 mM concentration (**eq 3**).[78] For example, in TFE–DCM (1:1) as opposed to HFIP–DCM, Oxyma (**6**) was the most effective additive (lowest yield of intermolecular coupling product **59**), providing 84% of cyclic material **57** in 1 h, thereby surpassing the cyclization abilities of HOBt (**25**) and HOAt (**27**). The authors attributed these results to the favorable steric factors in the case of DIC (**30**)–Oxyma (**6**).[20a,21a,75]

### 3.2. Automated Conventional Synthesis

One of the advantages of the introduction of solid-phase synthesis in peptide research is the possibility of applying automated technologies, as result of the iterative coupling–deprotection cycles for peptide elongation. Nowadays, peptide synthesizers offer advanced protocols for allowing fast assembly of long sequences, which would be tedious in a manual approach.[54] Most of them are compatible with Boc- and Fmoc-SPPS, and some are designed to enable parallel synthesis or assistance by microwave or infrared heating. Taking into account that solutions of coupling reagents in DMF or NMP need to be functional for several hours during the automated assembly, hydrolytic stability of the reagents is pivotal to the success of the process. Therefore, the hydrolytic stability of Oxyma-based reagents will be discussed.




| No. | Phos. Salt | 54 | 55 |
|---|---|---|---|
| **20** | PyOxim | 70% | 20% |
| **39** | PyClock® | 61% | 24% |
| **38** | PyAOP | 54% | 36% |
| **19** | PyOxB | 47% | 38% |
| **37** | PyBOP® | 43% | 29% |

**eq 2** *(Ref. 22)*

In 2006, an exhaustive study by Hachmann and Lebl of the performance of various coupling reagents in the automated preparation of difficult sequences concluded that carbodiimides were the most suitable reagents for use in peptide synthesizers, given their prolonged stability and the high purity of the final peptide.[54] With regard to Oxyma (**6**), our group has shown that its solutions in DMF, MeCN, and NMP are stable even at 40 ºC for at least 30 days.[32] Our group investigated the compatibility of the DIC (**30**)–Oxyma (**6**) coupling system with the ABI 433A peptide synthesizer, using a Fmoc/*t*-Bu strategy on a 0.1 mmol scale.[20a] ACP (65–74) decapeptide **46** was selected as peptide model, in view of its demanding sequence.[47a,60] For comparison purposes, 0.2 M DMF solutions of Oxyma (**6**), HOBt (**25**), and HOAt (**27**) were prepared, which allowed a clear visualization of their distinct acylation abilities.[20a] Oxyma (**6**) gave rise to almost 70% of the target decapeptide **46** and one of the lowest contents of des-Val deletion peptide (2.1%), thereby performing at an intermediate level between HOBt (**25**) and HOAt (**27**) (62% and 72% of **46**, respectively). It is worth noting that the efficiency of DIC (**30**)–Oxyma (**6**) is thus similar to that obtained a few years ago with more powerful onium salts in the same peptidic target, also conducted in an automated approach with tilted plate centrifugator.[20,54]

With respect to the use of onium salts with peptide robots, the stability of the reagent to hydrolysis in organic solution is an essential factor to consider. Unfortunately, questions have been raised[34,79] as to whether commercial samples of Oxyma-based uronium salts are as stable in solution in an open vial as the stability studies of the lab-synthesized versions had indicated.[21a] Recently, Behrentd and co-workers investigated the stability of various reagents in DMF-$d_7$ by means of $^1$H NMR in open and closed vials.[34] These authors reported that, in an open vial, approximately 50% of COMU® (**18**) remained active after 24 hours and that there was only 14% of reagent left after 2 days.[34] With regard to TOTU (**16**), stability was improved in comparison to COMU® (**18**, 72% remaining after 1 day), but hydrolysis was still much faster than those of benzotriazole salts, which remained almost unaltered after 2 days. A similar trend could be observed in closed-vial experiments, with COMU® (**18**) being less stable than the rest of the uronium salts.[34] Under these conditions that mimick those of automated synthesizers, there was still 67% of COMU® (**18**) left after 2 days, which would result in efficient peptide assembly of most sequences.[34] Even more dramatic results were obtained in a similar HPLC-based study conducted by Jensen's group,[79a] who found that, whereas 95% of HBTU (**32**) remains in DMF solution after 2 days in an open vial, COMU® (**18**) is completely hydrolyzed after 5 hours. In other words, the half-life of COMU® (**18**) in DMF is 3 hours, whereas those of HBTU (**32**) and HATU (**36**) are a few days each.[79b] However, in closed containers, the percentage of COMU® (**18**) after 23 hours rises to 85%.[79a] These authors also showed that the stability of Boc-Ala-OH active esters generated with COMU® (**18**) is much lower than the one formed with HBTU (**32**).[79] In order to evaluate the effect of solvent purity on the large discrepancy between the results obtained in our group and those obtained by others, a commercial sample of COMU® (**18**) was dissolved in DMF batches of varied purity and its content was checked by HPLC in a closed vial.[32] Although residual water in the solvent enhances the breakdown rate of the reagent, the most relevant factor is the presence of free amines in DMF, which can be removed by aspiration.[21c,32,79b] Nonetheless, the stability values are still far from those of our freshly synthesized material.[21] Traces of chloroformamidinium salt, morpholine, or impurities from the potassium salt of Oxyma (**6**) in the multikilogram-scale synthesis of COMU® are likely to be responsible for such distinct behavior

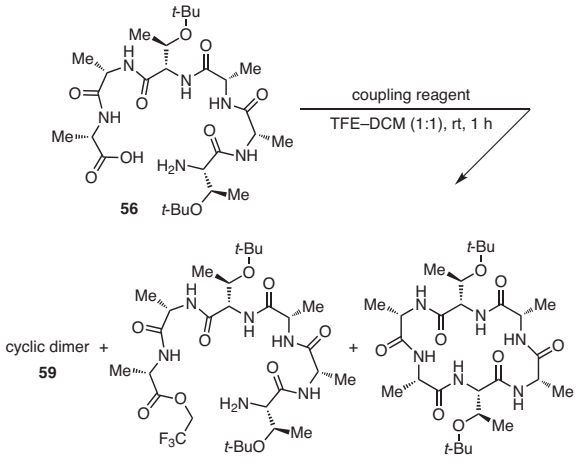
from our samples. In contrast to COMU® (**18**), the stability of the Oxyma-based *phosphonium* salts (**19**, **20**) produced in our group was confirmed in the Behrentd evaluation.[22,34] Hence, PyOxim (**20**) was hydrolyzed in DMF to a similar extent as PyBOP® (**37**) in open vial after 24 hours.[34] Comparison in a closed vial showed that PyOxim (**20**) is slightly more stable than PyBOP® (**37**) (86% vs 81% remaining after 24 h).[34]

The reduced stability of some Oxyma-based onium salts has not decreased their efficiency in the assembly of demanding sequences in conventional peptide synthesizers (without heating or microwave irradiation).[34,80] TOTU (**16**), COMU® (**18**), and PyOxim (**20**) were tested in the manual assembly of the MeLeu-analogue of Leu-enkephalin pentapeptide (**H-Tyr-MeLeu-MeLeu-Phe-Leu-NH$_2$**) in an ABI 433A automated robot.[21a,34] Surprisingly, in spite of the high hydrolysis rates of COMU® (**18**) and TOTU (**16**) reported in the same communication, both were considerably more efficient than HOBt (**25**) and 6-Cl-HOBt (**26**), and were only surpassed by HATU (**36**).[34] PyOxim (**20**) afforded the target peptide in a yield (40%) comparable to those obtained with COMU® (**18**) and TOTU (**16**), and much higher than that achieved with PyBOP® (4%).[34] A recent publication by Chantell and colleagues supports the compatibility of Oxyma-based reagents with peptide synthesizers applying fast protocols.[80,81] COMU® (**18**) and PyOxim (**20**) were evaluated in the automated assembly of several demanding sequences; including ACP decapeptide (**46**), G-LHRH, GHRP-6, 9Pbw0, and linear oxytocin; in a SYMPHONY® robot with short, 2- and 20-min couplings.[80] COMU® (**18**) was particularly suited to perform fast peptide synthesis and afforded the highest purities, regardless of the peptide model tested.[80] On the other hand, PyOxim (**20**) had an acylation potency comparable to that of PyClock® (**39**) and PyBOP® (**37**) in 20-min couplings, although its activation rate was slower than those of other reagents.[80] For example, while PyOxim (**20**) gave rise to purities of 54% and 90% of hexapeptide GHRP-6 using 2- and 20-min couplings, respectively, COMU® (**18**) rendered 92% and 94% of GHRP-6, surpassing the efficiency of HATU (**36**) and HCTU (**34**).

### 3.3. Microwave-Assisted Synthesis

The implementation of microwave irradiation in peptide synthesis is a powerful tool to accelerate coupling and deprotection steps.[82] Since its introduction in the field by Wang and colleagues in 1991, microwave-assisted SPPS has evolved steadily, and, nowadays, the technique is commonly combined with automated synthesizers to rapidly achieve difficult syntheses in high yields.[82a,83] One of the most remarkable advantages offered by microwave irradiation is the precise control of the temperature during coupling and temporary-group removal. Furthermore, during the elongation of long or hydrophobic sequences, chain aggregation is avoided as a result of the nonthermal dipolar polarization effect,[84] even though thermal and nonthermal effects are still not easily distinguished.[85] Our group was interested in combining Oxyma-containing reagents, mainly Oxyma (**6**) and COMU® (**18**), and microwave-assisted peptide synthesizers in order to develop an ultimate synthetic methodology.[21b] In the original communication, we reported the low thermal stability of Oxyma (**6**) and COMU® (**18**), which is connected to their low decomposition onset,[20,21] and calorimetric measurements recommend keeping the coupling temperatures at values close to room temperature (≤74 °C for Oxyma and ≤41 °C for COMU®).[20,21] However, no safety incidents have been reported by our group or others when Oxyma (**6**) and COMU® (**18**) have been heated at 50–80 °C in the process of assisting peptide coupling steps, consistent with the nonhazardous decomposition profile of both Oxyma-based reagents.[20,21,79,82a,86] In addition, the stability of Oxyma (**6**) to amine nucleophiles was tested under microwave irradiation at 80 °C, and it was found that addition byproducts only arose when extreme conditions were employed.[20a] Based on these findings, we assessed the efficiency of COMU® (**18**) in a CEM® Liberty peptide synthesizer.[21b] In 6-min couplings at 80 °C, COMU® (**18**) afforded an impressive amount (92%) of the demanding Aib-pentapeptide (**H-Tyr-Aib-Aib-Phe-Leu-NH$_2$**), showing greater efficiency in this system than HATU (**36**) and HBTU (**32**), which produced only 79% and 23% of the target peptide, respectively.[21b] After our initial report, other groups have reported exciting results from combining COMU® (**18**) with microwave irradiation.[79a,86a,b,e]

Jensen's group has extensively revised the compatibility of COMU® (**18**) with microwave-assisted automated synthesizers, in particular using a Syro *Wave*™ robot, in the assembly of sterically encumbered sequences,[79a,86a–d] such as the elongation of the Jung–Redemann sequence (**H-Trp-Phe-Thr-Thr-Leu-Ile-Ser-Thr-Ile-Met-NH$_2$**), a decapeptide based on the MuLV CTL epitope.[79a,86c,d,87] In a recent communication, the capacity of stock solutions of COMU® (**18**), HBTU (**32**), and HATU (**36**) to assist the assembly of the abovementioned decapeptide was analyzed.[79] Unfortunately, a solution of COMU® (**18**) in standard DMF stored 4 hours was unable to produce the target peptide, although the use of anhydrous solvents raised the percentage to 39%.[79b] Benzotriazole-based reagents showed a consistent performance over time, although HATU (**36**), similarly to COMU® (**18**), rendered none of the decapeptide after 48 h of storage.[79b] However, employing fresh solutions, COMU® (**18**) was clearly superior to HBTU (**32**) and comparable to HATU (**36**) (56% vs 46% and 60% decapeptide, respectively), while DIC (**30**)–Oxyma (**6**) was the preferred coupling system (54–58% of decapeptide, regardless of reagent storage time).[79] According to the authors, the low hydrolytic stability of COMU® (**18**) can be solved in automated



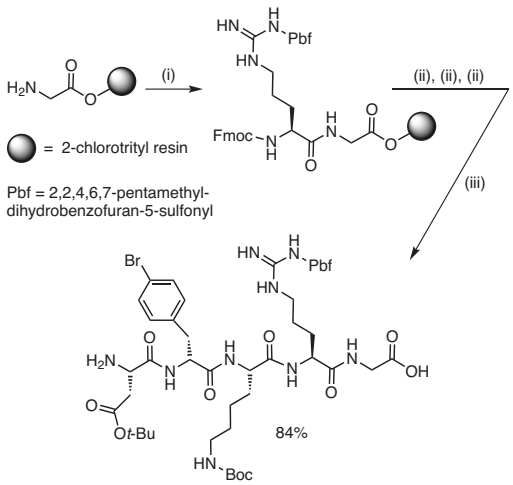

| No. | Reagent | 57 | 58 | 59 |
|---|---|---|---|---|
| **30** | DIC | 5% | 89% | 1% |
| **30,27** | DIC–HOAt | 82% | 3% | 11% |
| **30,25** | DIC–HOBt | 78% | 10% | 11% |
| **30,6** | DIC–Oxyma | 85% | 3% | 6% |

Yields of **57**–**59** were estimated from HPLC peak areas at 220 nm.

**eq 3** *(Ref. 78)*

synthesizers by placing anhydrous solutions in closed vials.[79b] For example, after 24 hours of storage in closed vials, stock solutions of COMU® (**18**) afforded Leu-enkephalin pentapeptide **H-Tyr-Gly-Gly-Phe-Leu-NH$_2$** in good yields.[79b] The Jung–Redemann model decapeptide was further utilized to study the performance of several activators in microwave synthesizers, using HOBt (**25**)–HOAt (**27**) (4:1) to solubilize various onium salts.[86c,d] Once again, the acylation ability of COMU® (**18**) surpassed that of HOBt (**25**)-, and HOAt (**27**)-derived aminium and phosphonium salts, affording the Jung–Redemann decapeptide in 70% purity.[86c,d] The same group next checked the capacity of COMU® (**18**) to assist the assembly of *N*Me residues in a Syro *Wave*™ microwave synthesizer.[86a,b] Here, COMU® (**18**) was slightly less efficient than HATU (**36**)–HOAt (**27**) and DIC (**30**)–HOAt (**27**) systems (59% vs 75% and 76%, respectively) in the solid-phase elongation of **H-MeAla-MeIle-MeGly-NH$_2$** tripeptide by means of 20-min couplings at 75 ºC.[86b] Nonetheless, in a separate communication, the authors reported that the coupling of Fmoc-Ala-OH onto the highly demanding **H-MeIle-Gly-Tyr-Gly-Gly-Phe-Leu-peptidyl** resin is preferrably conducted using COMU® (**18**) than any of the HOAt (**27**)-based systems (86% vs 62% and 76%, respectively).[86a] Following the same trend, COMU® (**18**) and DIC (**30**)–Oxyma (**6**) exhibited a higher acylation potency than HATU (**36**) in the challenging coupling of Fmoc-Aib-OH with resin-bound **H-Aib-Ile-Asp(O*t*-Bu)-Tyr(O*t*-Bu)-Ile-Asn(Trt)-Gly** (87% and 90% conversion vs 72% for HATU (**36**) after 20-min couplings at 75 ºC in the Syro *Wave*™).[79b]

Recently, COMU® (**18**) performed better than HBTU (**32**) in a microwave-mediated manual approach using a 2-chlorotrityl resin to prepare a linear RGD-based pentapeptide as a precursor to a cyclic $\alpha_v\beta_3$ integrin-specific targeting ligand that has potential applications in tumor imaging.[86e,88] Hence, Yamada, Shimizu, and co-workers obtained the target linear peptide in high purity and 84% isolated yield by means of 10-min couplings under controlled microwave heating at 50 ºC (**Scheme 4**), thus improving on the HBTU (**32**)-mediated original process (39% yield), which rendered incomplete couplings.[86e] Moreover, the authors applied the same COMU® (**18**)-based microwave methodology to assemble the N-methylated RGD-based cilengitide peptide.[89] In contrast to HATU (**36**), which required triple coupling reactions, COMU® (**18**) successfully produced the desired peptide with only double couplings.[86a]




(i) Fmoc-Arg(Pbf)-OH (5 equiv), **18** (5 equiv), DIEA (10 equiv), DMF, μw, 50 ºC, 10 min. (ii) (a) piperidine (20% in DMF), μw, 50 ºC, 3 min; (b) Fmoc-Lys(Boc)-OH (5 equiv), **18** (5 equiv), DIEA (10 equiv), DMF, μw, 50 ºC, 10 min. (iii) (a) piperidine (20% in DMF), μw, 50 ºC, 3 min; (b) HOAc–TFE–DCM (1:1:4), rt, 1.5 h.

**Scheme 4.** COMU® (**18**)-Mediated Solid-Phase Synthesis of a Linear Integrin Ligand. *(Ref. 86e)*

## 4. Assisting Amide Bond Formation

Besides its important role in biologically relevant molecules, such as proteins or heterocyclic natural products,[90] the amide bond is also of utmost importance in industry and, particularly, in the development of active pharmaceutical ingredients (APIs).[91] The most utilized nonenzymatic synthesis of amide bonds is the reaction between an acyl chloride and an amine; however, the strong reaction conditions employed are often not compatible with sensitive protecting groups.[92] Thus, alternative methods to form an amide bond under mild conditions have been pursued.[25b,d,93] Recently, new approaches have been reported that employ $AlMe_3$ to catalyze the reaction between an unactivated acid and an amine or ones that utilize fluorous Mukaiyama reagents at room temperature.[94]

In addition, acyl and aminyl radicals have recently been reported to generate amide bonds under oxidizing conditions, although aromatic or conjugated aldehyde precursors are required.[95] Alternative acylating strategies, recently described in the literature, consist of using carbonyldiimidazole (CDI) and pyridinium salts as starting materials, or utilizing acyltrifluoroborates and hydroxylamines in water to effect chemoselective amidation at room temperature.[96] Oxygen-containing precursors other than carboxylic acids, such as aldehydes or alcohols, have also been surveyed.[95,97] Transition-metal-mediated catalysis, achieving accurate chemoselectivity and regioselectivity, has also been described.[97b,c,98]

In a "green chemistry" approach, Nageswar and co-workers employed a bioglycerol-based recyclable carbon catalyst to obtain amides from aldehydes and hydroxylamine hydrochloride in good yields.[97a] To avoid the use of hazardous organic solvents, additional "green chemistry" methods have been developed for the acylation of the amino group: One example is a solvent-free CDI amidation that shortens reaction times and is suitable for Boc protection.[99] Another example is an elegant conversion of aldehydes into amides by using a copper catalyst in aqueous media.[97b]

While the preceding amidation strategies are valid and useful, coupling reagents that are traditionally employed in peptide synthesis have nevertheless been adopted in organic synthesis to effect the acylation of amino groups, given the mild reaction conditions and strong activation offered by these reagents. In recent years, Oxyma-based reagents, mainly Oxyma (**6**) and COMU® (**18**), have enjoyed great popularity in the organic chemistry community, which has applied their outstanding acylation capacity to establish new amide bonds in diverse chemical environments.

### 4.1. Nucleoside-5'-carboxamide Synthesis

In a joint publication, Jacobson's and Katritch's groups disclosed that COMU® (**18**) is useful in the synthesis of several bioactive adenosine-5'-carboxamido analogues (**eq 4**),[100] which act as agonists to various subfamilies of Adenosine Receptors (ARs) in the submicromolar range. AR targeting is of utmost biological relevance since this receptor is regarded as a therapeutic target for cancer, cardiovascular, and neurodegenerative diseases among others, and some of its agonists are used in myocardial imaging.[101] Based on a rationally designed

approach, the affinities of various 5'-carboxamido ligands (differing in the nature of the N-alkyl substitution) for adenosine receptors $A_1$, $A_{2A}$, and $A_3$ were calculated and the most promising ligands were chemically prepared.[100] Remarkably, the sensitive adenosine 5'-carboxylic acid starting material (containing a chiral $C_\alpha$ center) maintained its chiral integrity in this COMU® (**18**)-assisted acylation. Furthermore, COMU® (**18**) was compatible with halogenated, constrained, cyclic, and chiral amines; and promoted selective acylation in the presence of primary amides and free carboxylic acids.[100]

### 4.2. Weinreb Amide Synthesis

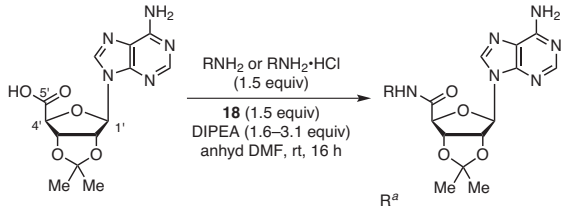
*N*-Methyl-*N*-methoxycarboxamides (commonly known as Weinreb amides) are a unique class of amides, which are synthetically appreciated for enabling the reduction of carboxyl groups to aldehydes or ketones.[102] Tyrrell's group has reported the application of COMU® (**18**) in the safe and efficient transformation of Boc-amino acids into the corresponding *C*-terminal Weinreb amides.[67] The amides were obtained in 63–97% yields by adding COMU® (**18**) and the Boc-amino acid to a DMF solution of the free *N*-methoxy-*N*-methylamine, generated in situ from the hydrochloride salt using excess DIPEA at low temperature (**eq 5**).[67]

COMU® (**18**)-mediated Weinreb amide synthesis offers several benefits. Firstly, the progress of the coupling reaction can be visually monitored by a color change from yellow to orange (pink to colorless using TMP).[21,67] Secondly, COMU® (**18**) effects fast acylation rates (complete conversion in less than 3 hours), exceeding the performance of triazine-based CDMT, which required longer coupling times in the synthesis of the Boc-alanine analogue.[67] Thirdly, the enhanced water solubility of COMU® byproducts allows the isolation of crudes that do not require further purification by column chromatography, as verified by NMR. Fourthly, COMU® (**18**) allowed the optical purity of the amino acid substrate to be preserved: only <1% of the epimeric product was detected by chiral HPLC, reaffirming the results obtained previously in our group.[21a,27,67]

### 4.3. Oligobenzamide Synthesis

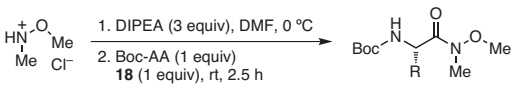
Oligomeric N-alkylated aminomethyl benzamides (extended peptoids or arylopeptoids) form one of the least studied peptidomimetic templates, which, nevertheless, have great potential as foldamers.[103] Hjelmgaard et al. optimized the synthetic route towards *para*- and *meta*-arylopeptoids in solution and in the solid phase using Oxyma-based COMU® (**18**).[104] In the preliminary solution-phase studies, the authors introduced a novel submonomer approach, consisting in iterative acylation–substitution cycles employing isopropyl as model nitrogen side chain to assemble trimeric *para*- and *meta*-arylopeptoids starting from 4-(bromomethyl)benzoyl bromide.[104a] However, in order to form longer oligomers, a trimeric-fragment approach was envisaged, in analogy to peptide segment coupling (**Scheme 5**).[104a] COMU® (**18**) and HOTU (**17**) effected a faster acylation rate than HATU (**36**) and especially faster than PyBOP® (**37**), PyBroP®, DPPA, and DIC (**30**), with COMU® (**18**) forming the active species in less than 2 minutes.[104a] The nature of the solvent was highly relevant, with DCM–DMF and DCM–NMP mixtures being preferred over DCM alone.[104a] Using the optimized combination of solvents, COMU® (**18**) achieved 88–90% conversion in 24 hours, whereas HATU (**36**) rendered only 65–75% conversion.[104a] Thus, hexa- (**62**) and nonameric (**63**) *para*-arylopeptoids were assembled in 55–82% yields by employing COMU® (**18**)-mediated acylations in DCM–DMF (4:1).[104a] NMR experiments showed that the hexa- and nonameric arylopeptoids were predominantly in the cis form, especially as the steric hindrance of the side chain increased. Unfortunately, the difficult separation of dimethylmorpholinourea from the meta analogues led the authors to employ HATU (**36**) for the elongation of these meta analogues.[104a]

The preceding solution-phase approach was extended to the solid phase by utilizing 2-chlorotrityl or Rink amide-polystyrene resins.[104b] Following optimization of the previous solution-phase studies by building chloromethyl submonomer analogues, heterooligomeric *meta*- and *para*-arylopeptoids were elongated stepwise on-resin by means of COMU® (**18**) activation (**Scheme 6**).[104b] Once again, acylation was fastest with COMU® (**18**), which led to a two-fold, four-fold, and six-fold higher rate than with HOTU (**17**), HATU (**36**), and PyBroP®, respectively—the most prominent non-Oxyma reagents tested. Remarkably, meta isomers were successfully produced with COMU®



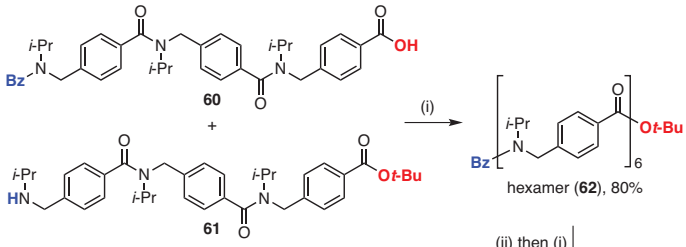

[a] The acetonide group was cleaved with HCl (1 N) in dioxane at rt to give the products with the two free hydroxyl groups in 18–79% yields.

**eq 4** *(Ref. 100)*




Boc-AA (AA = Ala, Val, Leu, Ile, Phe, Trp, Tyr, Met, Pro, or Thia-Pro)

**eq 5** *(Ref. 67)*




(i) **18** (1.2 equiv), $Et_3N$ (4 equiv), DCM–DMF (4:1), 0 °C to rt, 72 h. (ii) TFA, DCM, 0 °C, 3 h.

**Scheme 5.** COMU® (**18**)-Mediated Submonomer Approach to Oligoaryloamides in Solution. *(Ref. 104a)*

(**18**) in the solid-phase technique, in contrast to the results observed in solution.[104] In the subsequent steps, monomeric units bearing isopropyl and 2-morpholinoethyl side chains were introduced to afford the corresponding dimer (**67**) and trimer (**68**). Iterative COMU® (**18**)-assisted acylation–substitution steps allowed the isolation of a dodecameric heteroarylopeptoid (**69**).[104b]

### 4.4. Amino Group Conjugation

One of the most recently surveyed applications of Oxyma (**6**) as acylating reagent is the functionalization of amino-bearing species such as particles with linkers, fluorophores, and biologically active entities, with implications in imaging, sensing, chemical biology, and drug delivery, to name a few.[68c,105–107] In particular, Bradley's group has taken advantage of the acylation potential of Oxyma (**6**) to modify amino-grafted micro- and nanoparticles, together with intrinsically fluorescent particles.[105] In their first communication, the authors disclosed a novel technique (zeta potential analysis) to monitor the extent of chemical modifications directly on-particle.[105a] With the aim of demonstrating the effectiveness of this technique that is based on the electric field generated by charged particles, aminomethyl submicron polystyrene beads were modified with compounds bearing anionic, cationic, or neutral moieties.[105a] Thus, aryl, alkyl, aminoacyl, and aminopoly(ethylene glycol) chains were attached to the amino group of microparticles using DIC (**30**)–Oxyma (**6**), thereby showing the great versatility of substrate acid activation (**Scheme 7**).[105a] Noteworthy in the assembly of the 6-hydroxycaprioic acid derivative is the selectivity between hydroxy and amino acylation that was achieved. As further proof of the compatibility of Oxyma (**6**) with microwave heating, a 10-min preactivated solution of the carboxylic acid was added to a suspension of the particles in DMF, followed by microwave irradiation at 60 ºC.[105a] The potency of this acylation methodology was demonstrated in the application of short, 20-min coupling times to efficiently obtain the altered particles. In addition, PEG-containing particles were subsequently modified with NTA-adipic acid linkers using Oxyma (**6**)-mediated couplings, in order to improve zeta potential measurements.[105a] Following this study, Bradley and colleagues developed fluorescent particles by copolymerization of polystyrene with diverse fluorescein-based units, a typical fluorescence imaging label.[105b,108] Once particles of different sizes were obtained, the aforementioned zeta potential technique was applied to characterize the extent of introduction of PEG units by means of DIC (**30**)–Oxyma (**6**)-mediated couplings at 60 ºC with microwave irradiation.[105b] In the same report, Oxyma (**6**) was responsible for the successful conjugation of aminopolystyrene beads with 5- or 6-fluorescein as a control experiment.[105b]

Recently, an additional example of the use of Oxyma (**6**) to assist in the labelling of amino groups with PEG linkers has been provided by Rosés et al.[68c] The aim of their research was to develop a repurposing approach to the CECMEL-11 antimicrobial undecapeptide, by testing the anticancer activity of a series of conjugates with a cell-penetrating peptide to enhance targeted cell delivery.[68c,109] The synthetic strategy consisted, first, of the solid-phase assembly of a hexapeptide γ-aminoproline foldamer featuring alternate $N_\alpha$-alkyl chains and functioning as a cell-penetrating peptide (**70**). After removal of the $N_\gamma$-terminal Fmoc protecting group, Oxyma (**6**) was employed to introduce 8-Fmoc-amino-3,6-dioxaoctanoic acid (Fmoc-NH-PEG-$CO_2$H, **71**), proving its capacity to activate flexible carboxylic acids (**Scheme 8**).[68c] Finally, several antimicrobial peptides, chiefly differing in the nature of two residues, were stepwise assembled on the PEG-CPP-resin (**72**).[68c] The various antimicrobial-PEG-CPP conjugates (**73**) showed significant activity against MDA-MB-231 human breast tumor cells and specific delivery, in addition to low cytotoxicity, in normal human cells.[68c] In a similar labelling context, Della Ciana suggested the use of Oxyma-based COMU® (**18**) in the application of a novel approach to conjugate amino-containing bioactive compounds with biomarkers.[107]

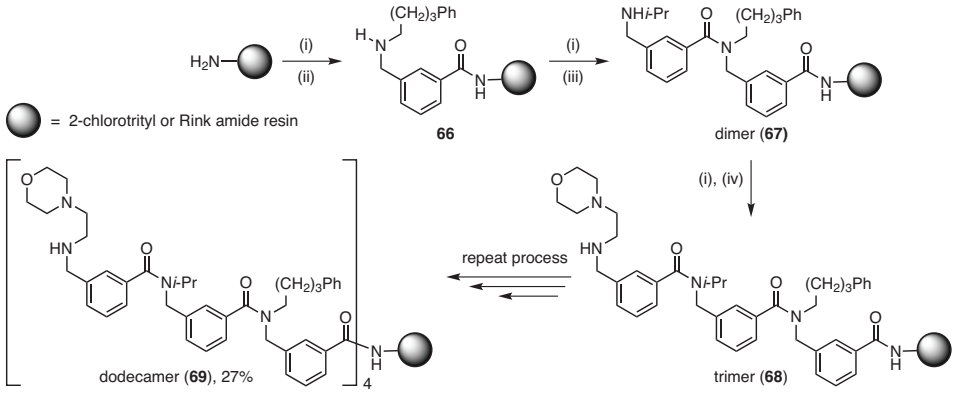
### 4.5. Other Amide Scaffolds

In contrast to aliphatic amines, anilines are strongly deactivated towards nucleophilic attack and, therefore, are challenging substrates for testing the acylation potency of a coupling reagent. In 2010, we examined COMU® (**18**), together with an analogue featuring isonitroso Meldrum's acid, in the acylation of *para*-chloroaniline with Z-Aib-OH.[110a] In spite of this highly demanding junction, combining a poorly reactive amine and a sterically encumbered acid, COMU® (**18**) rendered 89% of the anilide in only 3 hours and an impressive 98% after 1 day.[110a] The acylation rate and yield were much higher than those with the HOBt analogue and slightly superior to those with the HOAt derivative, although the isonitroso Meldrum's acid counterpart was the most reactive of all.[110a] Another example of aniline acylation was reported by Brandt and Blagg, who designed monoenomycins as simpler versions of the antitumor macrocycle trienomycin A that still retain its potent anticancer activity.[110b,111] The authors employed COMU® (**18**) to efficiently form the anilide intermediate selectively over the ester in 93% yield, showcasing another useful application COMU® (**18**) (**Scheme 9**).[110b]

Similarly to RGD (Arg-Gly-Asp) peptides, LDV (Leu-Asp-Val)-containing sequences specifically bind integrin receptors, in this case $\alpha_4\beta_1$, which is overexpressed in leukemia cells.[112] Recently, Oxyma-based activators were involved in the functionalization of LDV peptidomimetics with oligo(ethylene glycol) (OEG) linkers,[113] whereby carboxylic moieties were labelled with OEG by means of COMU® (**18**)-mediated acylations in moderate-to-high yields (50–80%).[113] Finally, COMU® (**18**) has been applied in the acylation of aromatic amines and anilines for *C*-terminal peptide modification.[114] *C*-Terminal amides are of biological interest, prompting several strategies to be devised for their attachment to the peptide chain.[115] The approach of Kwiatkowska et al. consists of the incorporation of Fmoc-Lys-OAllyl into 2-chlorotrityl through the side chain of lysine, followed by Boc protection.[114] After allyl removal, COMU® (**18**) is employed to attach the diverse amines to the *C*-terminal peptide chain.[114]

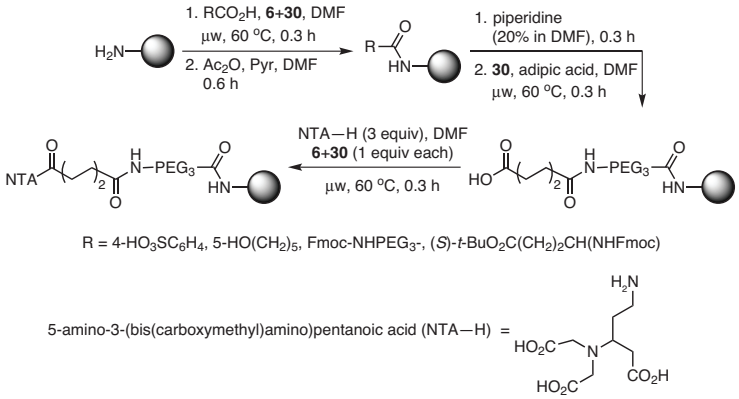
## 5. Assisting Ester Bond Formation

In contrast to the more nucleophilic amines, alcohols require an activating species of enhanced reactivity in order to undergo acylation, as demonstrated earlier by the fact that COMU® (**18**) preferably reacts with poorly reactive anilines rather than with primary alcohols, when both are present in the same structure.[110b] Additionally, alcohol acylation must compete with water or other protic solvents and, consequently, a completely inert atmosphere needs to be set prior to esterification. In spite of the growing interest in aldehydes as substrates for esterification under oxidative conditions—either catalyzed by transition metals or by proline-derived organocatalysts—the best strategies for ester bond formation have focused on acyl transfer from an activated carboxylic acid.[116] A large number of traditional methods are available and generally employ harsh reaction conditions and/or lead to low regioselectivities, such as what happens in the Fischer esterification with acyl chlorides and anhydrides, or in the Bayer–Villiger oxidation.[117] However, when the alcohol to be acylated also contains an acid- or base-sensitive functionality, or is highly




(i) 3-ClCH$_2$C$_6$H$_4$CO$_2$H (**64**) (3 equiv), **18** (3.5 equiv), DIPEA (7 equiv), NMP, rt, 0.3 h. (ii) Ph(CH$_2$)$_4$NH$_2$ (**65**, 20 equiv), DMSO, 50 °C, 1 h. (iii) *i*-PrNH$_2$ (20 equiv), DMSO, 50 °C, 1 h. (iv) O(CH$_2$CH$_2$)$_2$N(CH$_2$)$_2$NH$_2$ (20 equiv), DMSO, 50 °C, 1 h.

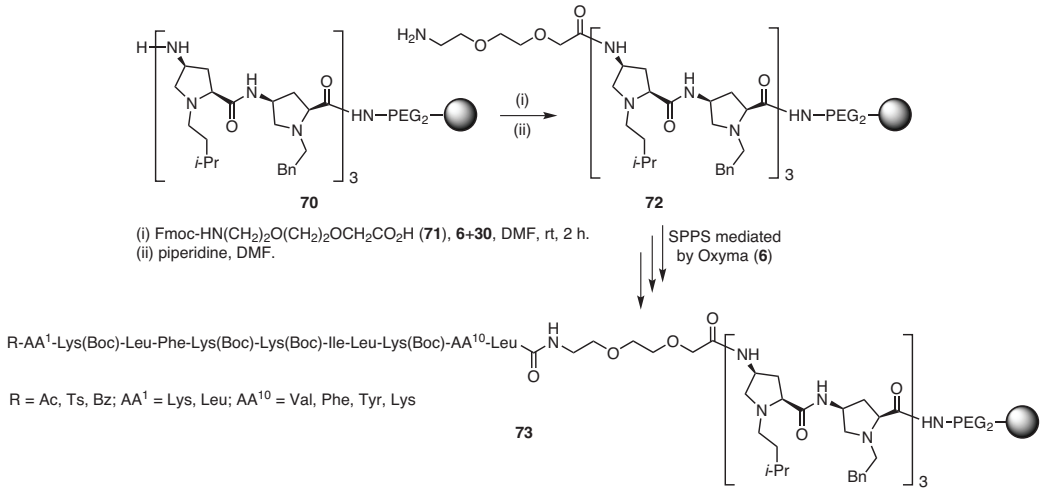
**Scheme 6.** COMU® (**18**)-Mediated Approach to Heteroaryloamides on Solid Phase. *(Ref. 104b)*




R = 4-HO$_3$SC$_6$H$_4$, 5-HO(CH$_2$)$_5$, Fmoc-NHPEG$_3$-, (*S*)-*t*-BuO$_2$C(CH$_2$)$_2$CH(NHFmoc)

5-amino-3-(bis(carboxymethyl)amino)pentanoic acid (NTA—H) =

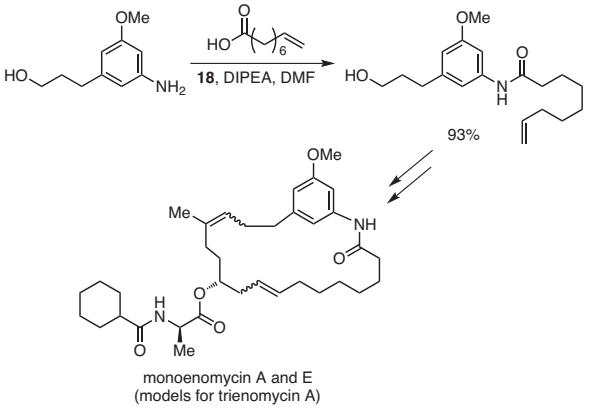
**Scheme 7.** Oxyma-Mediated Functionalization of Amino-Grafted Microparticles. *(Ref. 105a)*



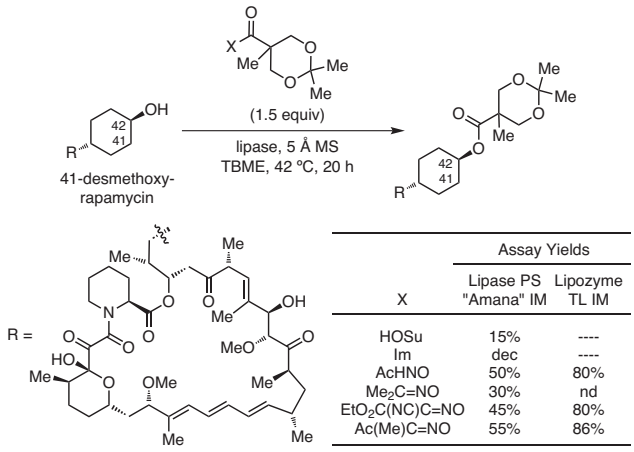

**Scheme 8.** Oxyma-Based Solid-Phase Synthesis of Various Antimicrobial-CPP Conjugates. *(Ref. 68c)*

sterically hindered, the arsenal of suitable methodologies is reduced. Such substrates are more conveniently acylated by using Mitsunobu's conditions, Mukaiyama's 2-halopyridinium salts, CDI esters with Brønsted acid or pyridine catalysis, phosphate–carboxylate mixed anhydrides, or, more recently, hypervalent iodine(III) iodosodilactones in conjuction with DMAP and $PPh_3$.[118]

In recent years, coupling reagents commonly employed in peptide synthesis have been proposed as milder alternatives for ester bond formation, since most of them can be stored for several weeks, and acylations can usually be carried out at room temperature. In the past decade, aminium salts TBTU (**33**) and HBTU (**32**), together with HOBt (**25**), in combination with DCC (**29**)–DMAP, were investigated and found to perform satisfactorily.[119] In light of the previously described acylation results with Oxyma-based reagents in peptide bond formation, the recycling of these efficient activators in alcohol acylations stands out as a promising approach.[20–22] Indeed, in the short period of time since we first disclosed the implementation of this approach, other groups have also reported its use for efficient ester bond formation.[120] The esterification pathway with Oxyma-based reagents is very similar to the one depicted in Scheme 2 for the acylation of amides. As in the corresponding peptide bond formation, the carboxylate anion reacts with the electrophilic portion of the uronium salt, as in HOTU (**17**) or COMU® (**18**), resulting in an *O*-acylisourea intermediate which immediately undergoes nucleophilic attack by Oxyma (**6**) that is present in the medium. The Oxyma-actived ester is then subjected to alcoholysis by the substrate alcohol, giving rise to the desired ester bond and Oxyma (and urea) as byproducts.



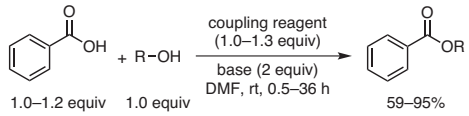

**Scheme 9.** Application of COMU® (**18**) in Anilide Formation en Route to Monoenomycin A and E. *(Ref. 110b)*




| X | Assay Yields: Lipase PS "Amana" IM | Assay Yields: Lipozyme TL IM |
|---|---|---|
| HOSu | 15% | ---- |
| Im | dec | ---- |
| AcHNO | 50% | 80% |
| $Me_2C$=NO | 30% | nd |
| $EtO_2C(NC)C$=NO | 45% | 80% |
| Ac(Me)C=NO | 55% | 86% |

dec = decomposed; nd = not determined; Im = imidazole; TBME = *tert*-butyl methyl ether.

**eq 6** *(Ref. 120a)*




R = *i*-Pr, *t*-Bu, *c*-Pent, 4-$IC_6H_4O(CH_2)_2$
Base = DBU, DIPEA, MTBD
Coupling reagent = TATU, TBTU (**33**), COMU® (**18**)

**eq 7** *(Ref. 120b)*

## 5.1. Lypase-Catalyzed Acylation of Secondary Alcohols

A remarkable acylation strategy combining biocatalysts and *N*-hydroxylamine esters was reported by Storz and colleagues[120a] in 2010 as an alternative to the hazardous vinyl ester approach[121] for the selective acylation of alcohols in stereochemically complex macrocycles. The authors proved that certain lipases are able to promote the esterification of secondary alcohols regioselectively in the presence of other secondary or tertiary alcohols,[120a] as in the extraordinarily difficult C42-esterificaction of 41-desmethoxy-rapamycin (a structural feature present in the members of the rapamycin family of polyketide macrocycles with the highest antitumor activity[122]) with a quaternary carboxylic acid (**eq 6**).[120a] Thus, in conjuction with *Burkholderia cepacia*, the Oxyma ester achieved a more efficient acylation of the C42 alcohol than the corresponding HOSu and imidazole esters. In comparison to the other oxime ester (55%) and hydroxamate (50%), the Oxyma ester slightly underperformed (45%) in combination with *Burkholderia cepacia*, although an impressive increase in yield (80%) was achieved by switching the biocatalyst to *Thermomyces lanuginosus*.[120a]

## 5.2. Selective Acylation of Tertiary Alcohols

Recently, a thorough study of the esterification ability of onium salts typically employed in peptide bond formation has been conducted by Twibanire and Grindley.[120b] The acylation of primary, secondary, and tertiary alcohols with various carboxylic acids at room temperature was screened with COMU® (**18**), TATU, and TBTU (**33**) (**eq 7**).[21a,120b] Using primary alcohols, benzotriazole derivatives displayed higher acylation efficiencies than COMU® (**18**), which required much longer esterification times.[120b] However, COMU® (**18**) achieved higher conversions than HOBt-based TBTU (**33**) when secondary alcohols, such as isopropanol and cyclopentanol, were employed. The nature of the organic base exerted a great impact on the performance of the reagents, with the majority of the acylations being carried out in the presence of DBU, since esterification with DIEA did not occur using TATU and TBTU (**33**), in contrast to COMU® (**18**). However, only MTBD (7-methyl-1,5,7-triazabicyclo[4.4.0]dec-5-ene)[123] was capable of promoting the acylation of tertiary alcohols with COMU® (**18**), which did not react in the presence of DBU,[120b] while the highly reactive TATU showed no conversion even when employing MTBD. Furthermore, COMU® (**18**) could be used without preactivation of the carboxylic acid, in contrast to the 30-min preincubation time

required by the benzotriazole derivatives. According to the authors, the outstanding potency of COMU® (**18**) in the acylation of tertiary alcohols is proof that its rate-determining step depends on the substrate, switching to the final alcoholysis of the Oxyma-active ester with the stronger base MTBD.[120b]

## 6. Introduction of Urethane-type Protecting Groups

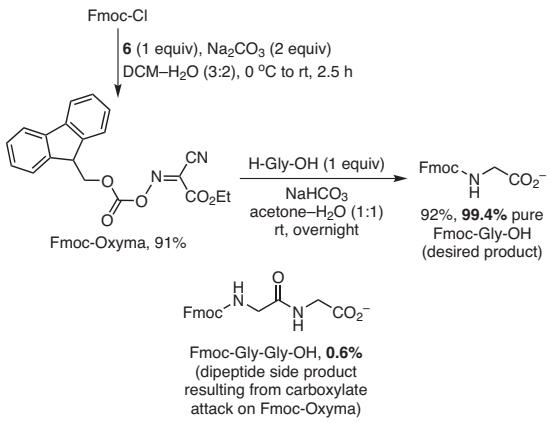
Oxime moieties have recently addressed fundamental issues in the introduction of amino acid blocking groups.[17,18] Orthogonal masking is essential in peptide chemistry and, therefore, several N-protecting groups have been developed such as Boc, Fmoc, Alloc, Z, or pNZ, which present a broad range of deprotection conditions.[124] Among these, Fmoc-based SPPS is slowly but firmly replacing Boc chemistry in virtue of its milder deprotection and peptide-cleavage conditions.[125] However, its introduction into amino acid building blocks is not without troublesome side reactions.[124] Although the use of the chloroformate (Fmoc-Cl) represents the most potent strategy, it often contaminates the Fmoc-amino acid with substantial amounts of oligomers as a result of its strong activation.[126] Moreover, many milder approaches, such as the use of the azidoformate (Fmoc-$N_3$) and *N*-hydroxysuccinimidyl carbonate (Fmoc-OSu) are scattered in the literature.[125,127] Although these Fmoc-introducing reagents succeed in controlling oligomer formation, they are explosion-prone or give rise to other side reactions (Lossen rearrangement).[128] Other approximations include Fmoc introduction with 2-MBT, 5-norbornene-2,3-dicarboximidyl, pentafluorophenyl, and HOBt carbonates; with symmetrical pyrocarbonates, or via in situ bis(trimethylsilylation) steps.[129]

In 1977, Itoh opened the way for the implementation of oximes as leaving groups in active carbonates for urethane-type protection.[15] In that early work, α-phenylcyanooxime (**2**) was proposed for Boc protection (Boc-ON), which still stands today as an alternative to Boc-anhydride and azidoformate.[15,130] Among several oxime templates tested, Boc-ON showed enough stability to be isolated in a crystalline form.[15] The oxime-based reagent was used to promote $N_\alpha$-Boc protection of various amino acids including Arg, Cys, Ile, Met, Phg, Pro, and Trp in just 5 hours and 80–99% yields. Moreover, the corresponding oxime could be easily removed from the medium. In contrast to the azidoformate, Boc-ON allowed complete protection of glycine in 2 hours at room temperature, whereas the former required 20 hours at 40 ºC.[15]

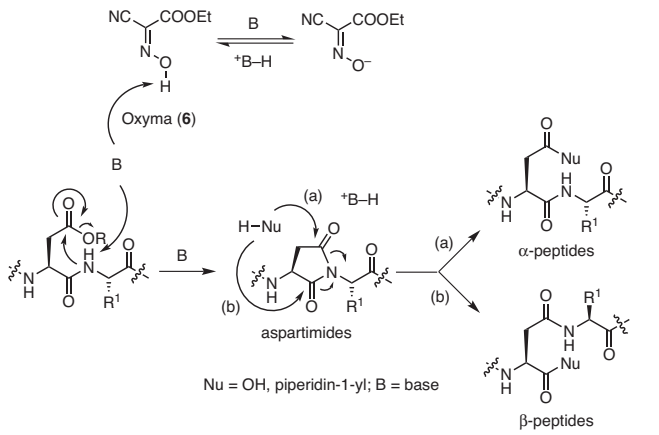
More than 30 years later, oximes were again included in carbonates for $N_\alpha$-protection.[17] Various oxime templates (**1**, **2**, **6**) were considered for the construction of Fmoc-carbonates. In view of the extraordinary performance of Oxyma (**6**) in diverse acylation steps, its carbonate analogue was included in the designed set of reagents (**Scheme 10**).[17] Thus, Fmoc-Oxyma was successfully obtained in 91% yield after recrystallization, by reaction with Fmoc-chloroformate under Schotten–Bauman basic conditions.[17] Its performance was subsequently tested in the protection of H-Gly-OH, which stands out as the most challenging residue for Fmoc introduction, since its low steric hindrance promotes the presence of Fmoc-dipeptides.[127b,129a] Thus, Fmoc-Oxyma rendered Fmoc-Gly-OH in high yield (92.1%) and purity (99.4%) in the presence of $NaHCO_3$ at controlled pH,[17] and the level of Fmoc-dipeptide (**Fmoc-Gly-Gly-OH**) side product was minimal. An even lower impact of oligomerization was found in the crude obtained with cyano-2-pyridyloxime (**3**) (0.01%), as a result of its moderate acidity, which was also utilized to build an efficient Alloc-oxime carbonate.[17] An additional advantage of Fmoc-Oxyma was its facile removal during workup. Very recently, cyanoacetamido scaffolds (featuring unsubstituted or *N*-piperidinyl-, *N*-morpholinyl-, or *N*-ethyl-substituted amides) were surveyed for the introduction of Fmoc, given their simpler synthetic accessibility in comparison to the 2-pyridyl analogue.[18] The moderate activation of these oximes translated into minimal dipeptide formation (<0.17%).

## 7. Other Applications in Peptide Chemistry

The acidity of *N*-hydroxylamines, including oximes, is rarely high in the context of organic molecules.[19] In particular, the dissociation constant of Oxyma (**6**) ($pK_a = 4.60$) is comparable to that of acetic acid ($pK_a = 4.75$).[13a] Thus, apart from serving as an excellent leaving group in acylations, Oxyma (**6**) can be advantageous in other branches of peptide chemistry.[20b,131] Its acidic nature helps prevent the occurrence of base-catalyzed side reactions such as aspartimide formation and proline-derived overcoupling.[131] The undesired intramolecular cyclization of Asp residues leading to an aminosuccinyl-modified backbone (commonly referred to as aspartimides) is one of the most troublesome side reactions in peptide synthesis.[132] Although acid-catalyzed mechanisms are reported in the Boc-approach, aspartimide formation can be dramatic in Fmoc SPPS, since this side reaction takes place in every deprotection cycle once Asp is incorporated into the sequence.[133] Hence, the presence of base accelerates nucleophilic attack of the backbone amide of the Asp residue onto the β-carboxy ester side chain, resulting in an intermediate containing an aminosuccinyl moiety.[131,134] Attack of nucleophiles such as piperidine or water onto this intermediate aspartimide peptide results in the corresponding modified α- and β-peptides.[133c,135] In order to reduce the extent of this unwanted side reaction, efforts have been dedicated to enhancing the steric hindrance of the base or β-side-chain protecting group by introducing pseudoprolines or attaching amide backbone protectants.[132] Although some of these approaches completely suppress aspartimide and piperidide formation, they are difficult to implement in routine SPPS. A simpler and effective alternative consists in the addition of acidic *N*-hydroxylamines, such as HOBt (**25**), and polyhalogenated phenols to the Fmoc deprotection cocktail.[133b,135,136] Considering the successful implementation of Oxyma (**6**) in the plethora of coupling activators, we envisaged that this oxime could be valuable in reducing the impact of aspartimide formation. Indeed, we compared the behavior of Oxyma (**6**), HOBt (**25**), and HOAt (**27**) in a piperidine cocktail employed to remove Fmoc from resin-bound **Fmoc-Ala-Orn-Asp-Gly-Tyr-Ile-$NH_2$**.[131] This sequence




**Scheme 10.** Synthesis and Performance of Fmoc-Oxyma in the Protection of H-Gly-OH. *(Ref. 17)*




**Scheme 11.** Proposed Oxyma (**6**)-Mediated Mechanism of Inhibition of Undesirable Aspartimides and Derived Products. *(Ref. 131,134)*

is well suited to checking the performance of *N*-hydroxylamines, since it combines a strongly aspartimide-prone Asp-Gly junction and Asp-Gly-Tyr-Ile domain.[133a] After a double 6 + 6 hour treatment and cleavage from the resin, Oxyma (**6**) yielded the highest percentage of the desired α-peptide, independently of the concentration of additive in DMF.[131] The mechanim of action remains unknown, although the most accepted hypothesis is competition with the backbone amide for the base in the medium when Oxyma (**6**) is added.[133b]

In addition, Oxyma (**6**) has also been investigated in the prevention of amino acid overattachment caused by the basicity of proline ($pK_a$ = 10.6), an unprecedented side reaction in peptide chemistry.[20b,131] As a result, we undertook the evaluation of proline-based overcoupling during solid-phase elongation of Pro-enkephalin (**H-Tyr-Pro-Pro-Phe-Leu-NH$_2$**), by comparing the effect induced by Oxyma (**6**) with those of HOBt (**25**) and HOAt (**27**).[131] The experimental approach consisted in mixing peptides presenting Pro at the *N*-terminus with the corresponding Fmoc-amino acid (Pro and Tyr) for 2 hours, followed by addition of carbodiimide and a 0.1 M solution of the *N*-hydroxylamine in DMF. All additives slightly increased the purity of the target Pro-enkephalin, with Oxyma (**6**) reducing almost completely the content of +Pro hexapeptide (0.39%).[131]

## 8. Conclusions

This review highlighted the increasing use of Oxyma (**6**) in peptide, amide, and ester chemistry, owing to its outstanding acylation potential vis-à-vis other activators in the coupling reaction. Although there is still room for improvement in certain of their aspects (i.e., uronium salt stability), Oxyma-based reagents are versatile acylating agents and stand out as cost-saving and efficient alternatives to benzotriazole-based reagents. In view of the great acceptance that Oxyma (**6**) has received in such a short period of time, further exciting applications will no doubt follow in the near future.

## 9. Acknowledgments

Research in the laboratory of the authors was partially funded by the Secretaría de Estado de Cooperación Internacional (AECI), the CICYT (CTQ2009-07758), the Generalitat de Catalunya (2009SGR 1024), the Institute for Research in Biomedicine Barcelona (IRB Barcelona), and the Barcelona Science Park. We thank Mr. Yoav Luxembourg for his encouragement in this field.

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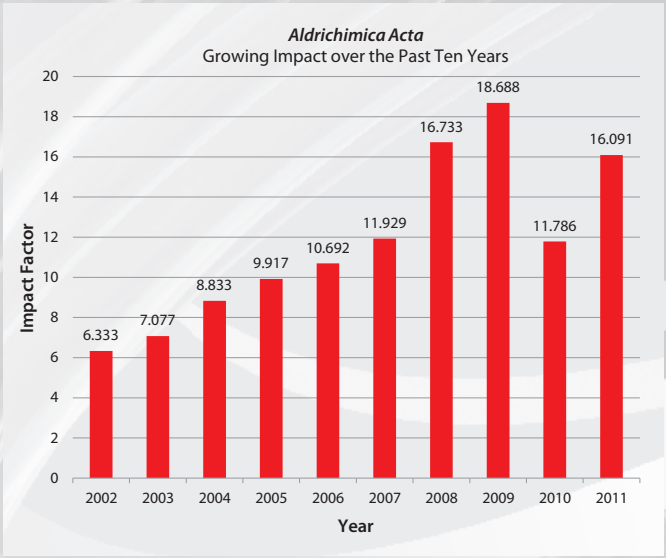


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VOL. 46, NO. 2 • 2013

ALDRICH Chemistry

# Aldrichimica ACTA

Aminoacyl Benzotriazolides: Versatile Reagents for the Preparation of Peptides and Their Mimetics and Conjugates

Recent Advances in the Prins Cyclization

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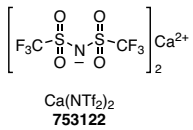
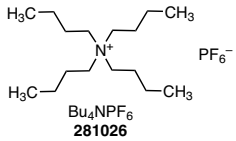


$Ca(NTf_2)_2$
**753122**



$Bu_4NPF_6$
**281026**

| | | |
|---|---|---|
| 753122 | Calcium(II) bis(trifluoromethanesulfonimide), 95% | 1 g |
| 281026 | Tetrabutylammonium hexafluorophosphate, 98% | 5 g<br>25 g<br>100 g |

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Detail from ***Breezing Up (A Fair Wind)***. Photo courtesy National Gallery of Art, Washington, DC.

Winslow Homer completed ***Breezing Up (A Fair Wind)*** (oil on canvas, 61.5 x 97 cm) in 1876, the U.S. centennial year. One of the most celebrated American artists of the nineteenth and early part of the twentieth centuries, Homer had little formal training as a painter, and seems to have developed his plein-air style of objective realism on his own. He was a prolific artist who excelled in several media and travelled widely, depicting a variety of themes, from battlegrounds of the Civil War to leisurely scenes of recreation.

An iconic American painting and one of Homer's most celebrated works, ***A Fair Wind*** received wide acclaim when it was first exhibited in 1876, and was chosen by the U.S. Postal Service in 1962 for a commemorative postage stamp honoring the artist. This seascape touches upon a favorite theme of his, the struggles of man against powerful natural forces. Here the light sail boat is returning home with the day's catch and a seemingly relaxed crew, unbothered or perhaps accustomed to the choppy waters. Unlike some of his later seascapes, this has a warm feel and more optimistic message,* perhaps meant to make a statement about the future of the young country following the uncertain and dangerous years of the Civil War.

**This painting is a gift of the W. L. and May T. Mellon Foundation to the National Gallery of Art, Washington, DC.**

** Homer included a traditional symbol of hope in this painting. Can you guess what it is? To find out, visit* ***Aldrich.com/acta462***

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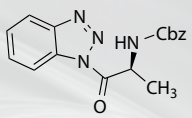

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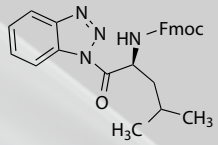

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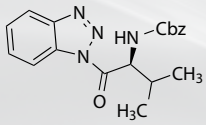

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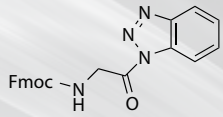

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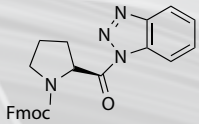

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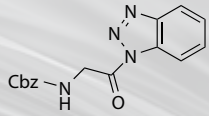

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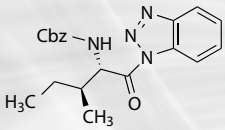

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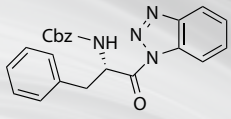

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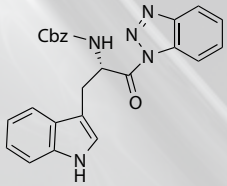

**737704**
**Z-Trp-Bt**


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# Aminoacyl Benzotriazolides: Versatile Reagents for the Preparation of Peptides and Their Mimetics and Conjugates


Dr. S. S. Panda


Dr. C. D. Hall


Dr. E. Scriven


Prof. A. R. Katritzky

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**Keywords.** benzotriazole; peptide synthesis; peptidomimetics; conjugates; methodology; coupling reagent.

**Abstract.** *N*-(Protected α-aminoacyl)benzotriazoles are efficient acylating reagents that offer many advantages in the preparation of peptides and their mimetics and conjugates. Advances in methodology, made possible by these novel reagents, have given rise to solution- and solid-phase preparative techniques for generating complex peptides and peptide conjugates, which are useful in the construction of diverse libraries of building blocks for medicinal chemistry.

## Outline



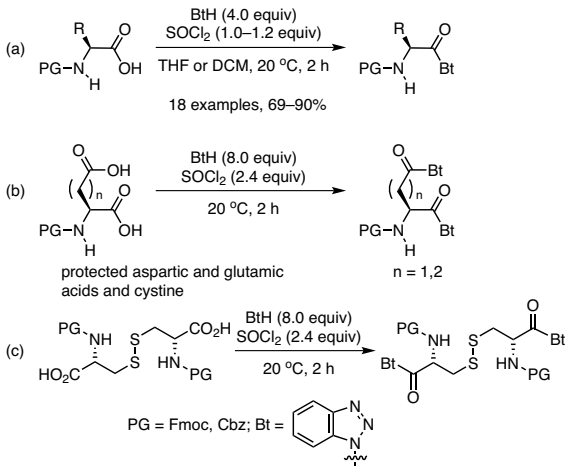
## 1. Introduction

Azolides are compounds in which an azole residue is attached to an acyl group. It has long been known that the azole nucleus in azolides can behave as a leaving group, and this property was explored widely by Staab as early as 1960.[1,2] Katritzky and co-workers have reported that, of the azoles, benzotriazole is a particularly versatile synthetic auxiliary with attractive properties, easily inserted into or removed from molecules and endowing them with a range of useful reactivities.[3] Much of this work has been summarized[4] and, from 1985 to 2012,

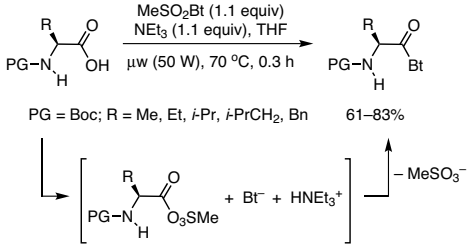
some 1,000 publications have appeared dealing with benzotriazole. We have since found that benzotriazolides (*N*-acylbenzotriazoles) have many advantageous properties relative to acid chlorides. More recently, the utility of benzotriazolides in peptide chemistry has achieved prominence, and reports of numerous applications have appeared as highlighted in this review.

## 2. Synthesis of Aminoacylbenzotriazoles

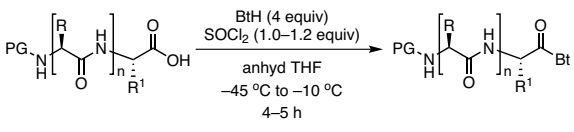
Acylbenzotriazoles are versatile reagents in which the benzotriazol-1-yl (Bt) group serves as a surrogate for halogen and is easily displaced by nitrogen, sulfur, oxygen, or carbon nucleophiles.[4] Acylbenzotriazoles, however, offer numerous advantages over their halogen analogues since they are isolated in high yields, in crystalline form, are usually stable in air (and even to water for short periods at 20 °C), and are more reactive than the corresponding *N*-acylimidazoles. Thus, they can effect peptide coupling in $H_2O$–THF or $H_2O$–MeCN by using unprotected amino acids with the distinct advantage that, if the temperature is controlled, chirality within the component amino acids is preserved. Although synthesis of aminoacylbenzotriazoles requires protection of the α-amino function with either Boc, Fmoc, or Cbz (or by protonation); other common functional groups, especially OH but also SH, $CONH_2$, and $CO_2H$ can be left unprotected.




**Scheme 1.** Preparation of Fmoc- or Cbz-Protected α-Aminoacylbenzotriazoles. *(Ref. 5,9)*




**Scheme 2.** Preparation of Boc-Protected α-Aminoacylbenzotriazoles from Acid-Sensitive or Boc-Protected Amino Acids. *(Ref. 6,7)*




**eq 1** *(Ref. 11)*

### 2.1. From Fmoc- or Cbz-Protected Amino Groups

The first general method for the preparation of aminoacylbenzotriazoles involves the condensation of a protected amino acid with benzotriazole and thionyl chloride (1.0–1.2 equiv) in THF or DCM (**Scheme 1**, Part (a)).[5–9] Excess benzotriazole is required to neutralize the two equivalents of HCl formed, and the method is not applicable to benzotriazolides containing the acid-sensitive Boc group. A wide range of amino acids may be used affording high yields. In addition, di-Bt derivatives are generated when aspartic acid, glutamic acid, or the S–S dimer of cysteine comprise the starting amino acids (Scheme 1, Parts (b) and (c)). The method is extremely versatile, and excess benzotriazole is easily removed by washing with either acid or base. Most significantly, chirality within the starting amino acids is retained in the majority of cases and the protected aminoacylbenzotriazoles may be stored at room temperature for many weeks.

### 2.2. From Boc-Protected Amino Groups

In cases where the amino acid is Boc-protected or sensitive to thionyl chloride, the sodium or trialkylammonium salt of the amino acid may be converted into the benzotriazolide by treatment with 1-(methanesulfonyl)benzotriazole ($BtO_2SMe$ or BtMs) (**Scheme 2**).[6,7] This clean, preparative method is enhanced in some cases by crystallization of the triazolide from water with concurrent removal of the water-soluble methanesulfonate byproduct.

The thionyl chloride method can often be employed to prepare N-protected α-aminoacylbenzotriazoles (61–99%) without protection of potentially reactive side chains such as aliphatic OH (Ser, Thr), aromatic OH (Tyr), thiol SH (Cys), indole NH (Trp), and amide NH (Asn or Gln).[7–9] There are, however, amino acids (e.g., His, Glu, Lys, and Asp) with side chains that do require protection (e.g., with Ts, Bn, or Cbz groups) in order to generate good yields of the benzotriazolides by reaction with either BtH–$SOCl_2$ or BtMs.[10]

## 3. Synthesis of Oligopeptidoyl Benzotriazolides

All of the methods employed to prepare Fmoc-, Cbz-, and Boc-protected aminoacylbenzotriazoles may also be used to prepare N-protected benzotriazolides of dipeptides. Likewise, N-protected tri- through hexapeptides may be converted into the corresponding benzotriazolides (**eq 1**).[11] Each benzotriazolide may then couple with amino acids or peptides to form N-protected tetra- through heptapeptides (vide infra).

## 4. Application to the Synthesis of Peptides

### 4.1. Natural Peptides and Isopeptides

A wide range of amino acids including many of those with additional, unprotected functional groups (Ser, Tyr, Cys, Trp, Pro, Asp, Glu, Lys, and Arg) couple with N-protected aminoacylbenzotriazoles in aqueous acetonitrile (MeCN–$H_2O$, 7:3) at 20 °C to produce dipeptides in 47–98% yields (**eq 2**).[9,12–17] Enantiopure dipeptides (LL and LD)

are obtained in high purity (>99% in most cases) without the use of chromatography, thus highlighting the significant utility of the method. However, protection of a carboxylic acid function as its benzyl ester is advantageous in some instances.

The synthesis of tripeptides is achieved by either a fragment-coupling procedure[9,12,16] or by stepwise coupling.[12,15] In the former, N-protected dipeptides are converted into their benzotriazole derivatives at –10 °C (in order to avoid racemization), which are then coupled with amino acids to form tripeptides (**Scheme 3**).[15,18,19] Tri- and tetrapeptides are similarly prepared in good yields (74–94%) by a stepwise procedure, but usually at a lower temperature (–10 °C) again to prevent racemization.[9]

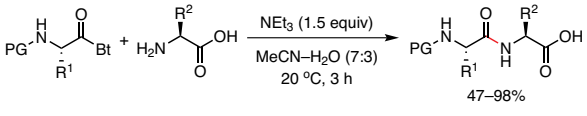
The first examples of penta-, hexa-, and heptapeptides prepared by the Bt technology were generated using microwave-assisted, solid-phase peptide synthesis (SPPS). In this technique, *N*-Fmoc-α-aminoacyl benzotriazolides were employed to attach Fmoc-protected α-aminoacyl groups to a Rink resin, which was then utilized for the synthesis of di- to heptapeptides in 20–68% yields.[18,20,21] In contrast, and although useful, SPPS and enzymatic techniques often afford low yields,[22,23] and are unsuitable for larger-scale preparations.

Recently, N-protected α-tri-, tetra-, and pentapeptidoylbenzotriazoles were coupled with free amino acids, dipeptides, and tripeptides in aqueous acetonitrile at 0 °C to afford N-terminal-protected polypeptides in isolated yields of 61–92%.[11] This reaction has been applied to amino acids (or small peptides) containing free OH, SH, or indole NH groups, and proceeds with no detectable racemization (**eq 3**);[11] it applies equally well to the synthesis of isopeptides.[24]

### 4.2. Difficult-to-Prepare Peptide Sequences

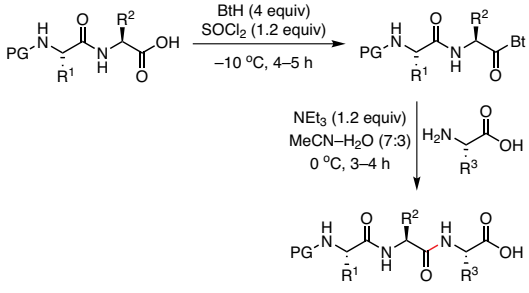
Peptaibols, a group of antibiotics isolated from soil fungi, contain hindered amino acids such as 2-methylalanine (Aib), 2-ethylalanine, and 2,2-diethylglycine and, hence, α-substituted and α,α-disubstituted amino acids are important in peptide and medicinal chemistry.[25] Likewise, N-substituted peptides are important since they are constituents of cyclosporins[26] and exhibit antibiotic,[27] anticancer,[28] and antiviral[29] activities. Benzotriazole technology has proved valuable in the synthesis of hindered dipeptides from either N-protected Aib benzotriazolides and amino acids (**Scheme 4**, Part (a)) or C-terminus Aib dipeptides from N-protected aminoacylbenzotriazoles (Scheme 4, Part (b)) in isolated yields of 67–92%.[17]

It is well known that certain peptide sequences, particularly those containing valine (e.g., H-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala-$NH_2$), are difficult to prepare, and the classical approaches are often characterized by low yields, aggregation, and β-sheet formation leading to racemization. The stepwise SPPS synthesis of difficult peptides utilizing N-protected aminoacylbenzotriazoles and microwave acceleration has been shown to facilitate amide-bond formation (22–37% yields, 89 to >99% purities) and to reduce aggregation.[21]



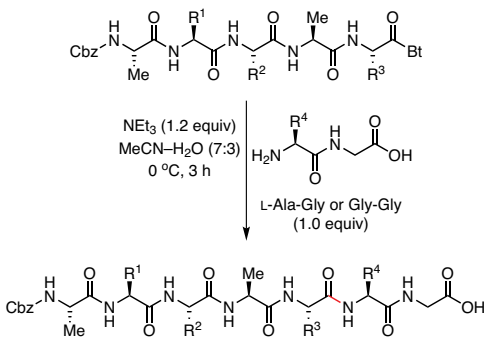

PG = Boc, Cbz, Fmoc
$R^1$ = Me, *i*-Pr, Bn, $MeS(CH_2)_2$, $H_2N(O)C(CH_2)_2$, (indol-3-yl)$CH_2$
$R^2$ = Me, *i*-Pr, Bn, $HOCH_2$, $HSCH_2$, $MeS(CH_2)_2$, $H_2N(O)C(CH_2)_2$, (indol-3-yl)$CH_2$

**eq 2** *(Ref. 9,12–17)*




PG = Cbz, Fmoc; $R^1$ = Me, Bn, $MeS(CH_2)_2$, (indol-3-yl)$CH_2$
$R^2$ = Me, Bn, $MeS(CH_2)_2$, $H_2N(O)C(CH_2)_2$, (indol-3-yl)$CH_2$
$R^3$ = Me, *s*-Bu, Bn, $HOCH_2$, $HSCH_2$, $MeS(CH_2)_2$, (indol-3-yl)$CH_2$

**Scheme 3**. Preparation of Tripeptides by Fragment Coupling. *(Ref. 15,18,19)*

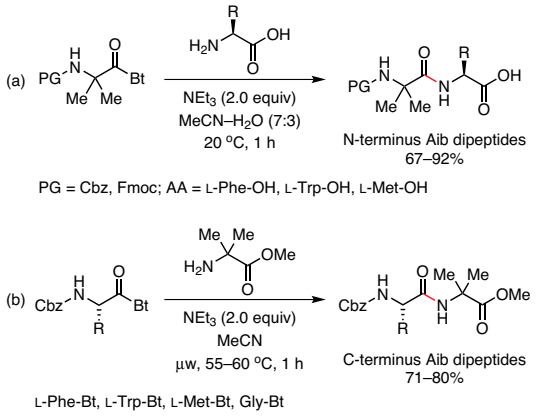



*N*-Cbz-α-heptapeptide
no racemization observed

| $R^1$ | $R^2$ | $R^3$ | $R^4$ | Yield |
|---|---|---|---|---|
| Bn | H | Bn | Me | 82% |
| *i*-Bu | Me | Me | H | 79% |

Example of the synthesis of N-terminal-protected polypeptides

**eq 3** *(Ref. 11)*



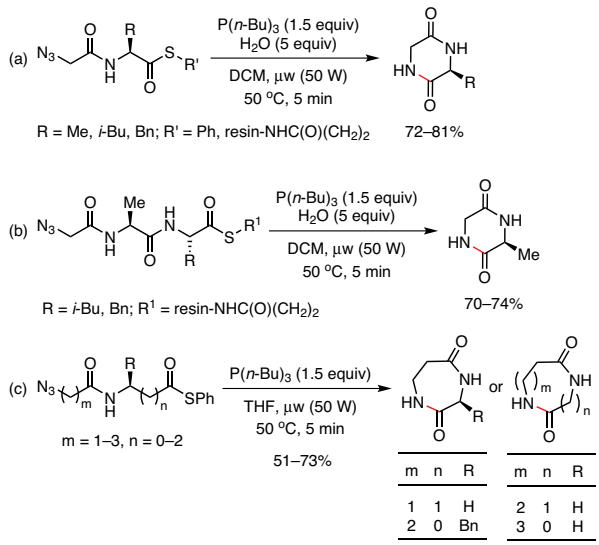

PG = Cbz, Fmoc; AA = L-Phe-OH, L-Trp-OH, L-Met-OH

L-Phe-Bt, L-Trp-Bt, L-Met-Bt, Gly-Bt

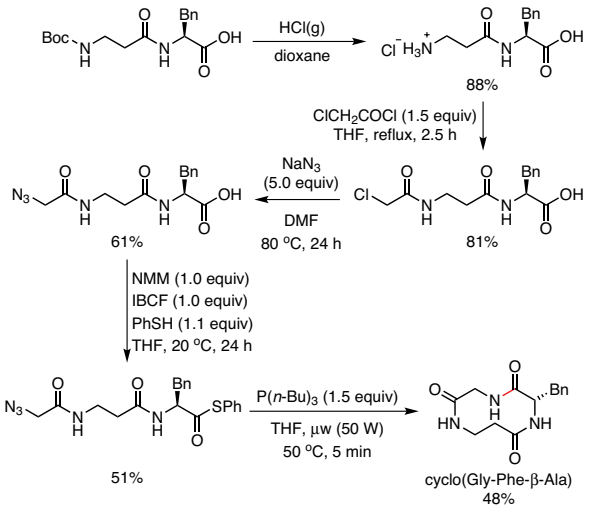
**Scheme 4.** Peptide Sequences with Sterically Hindered Amino Acid Residues from *N*-(Cbz-α-aminoacyl)benzotriazoles. *(Ref. 17)*

### 4.3. Cyclic Dipeptides (2,5-Diketopiperazines) and Tripeptides

Solution- and solid-phase (**Scheme 5**, Part (a)) Staudinger-type cyclizations afford efficient routes to hetero-2,5-diketopiperazines from protected azido dipeptide thioesters under microwave irradiation.[30] However, attempts to synthesize cyclic *tripeptides* by this method resulted in the unprecedented cleavage of an amide group rather than a thioester to form 2,5-diketopiperazines again (Scheme 5, Part (b)),[30] an observation that highlights the stability of a six-membered ring over its nine-membered analogue. Seven- and eight-membered-ring cyclic dipeptides can, however, be prepared in moderate-to-good yields by a Staudinger-type ring closure of a series of azido dipeptide thioesters (Scheme 5, Part (c)).[31] The work was extended to the solution-phase synthesis of a ten-membered-ring cyclic tripeptide in 48% yield (**Scheme 6**).[31]




**Scheme 5.** Attempted Syntheses of Cyclic Tripeptides, Leading Instead to Cyclic Dipeptides (2,5-Diketopiperazines). *(Ref. 30,31)*




**Scheme 6.** Solution-Phase Synthesis of a Ten-Membered-Ring Cyclic Tripeptide. *(Ref. 31)*

## 5. Synthesis of Peptidomimetics

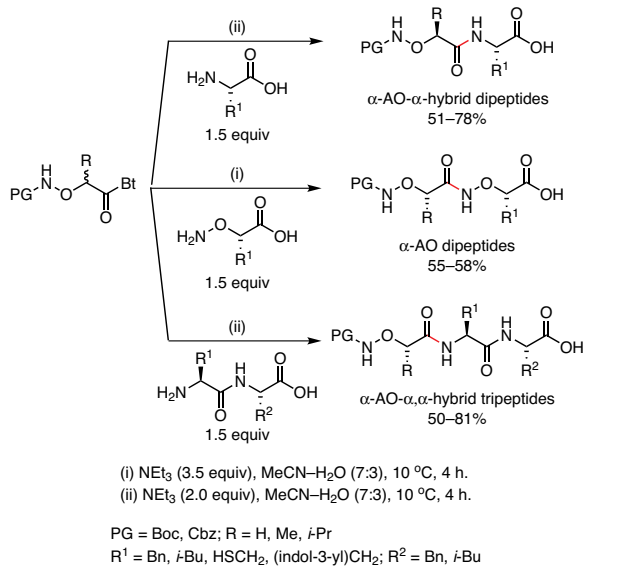
Peptidomimetics are small, protein-like molecules designed to mimic natural peptides by replacement of an amide bond or other element of the natural peptide. Clinical applications of bioactive natural peptides, for instance as hormones or enzyme inhibitors, have been limited by their susceptibility to rapid hydrolysis by peptidases. The corresponding peptidomimetics are not subject to this limitation, and, consequently, have been designed to exhibit high affinity for specific receptors, good metabolic stability toward endogenous proteases, greater oral bioavailability, and longer duration of action. To meet the need for good synthetic approaches to these peptidomimetics, flexible, high-yield, enantiospecific benzotriazole-mediated synthetic routes to six different structural types of peptidomimetics have been developed. These syntheses employ microwave-assisted benzotriazole acylation as the key step.[32–34]

### 5.1. Aminoxypeptides

α-Aminoxy acids [$RCH(ONH_2)CO_2H$] are peptidomimetics that resist enzymatic degradation. Peptidomimetics as a class are of interest as bioisosteric α-amino acids and as analogues of β-amino acids.[35–38] They have been used to prepare aminoxypeptides, α-aminoxy-α-hybrid dipeptides, and α-aminoxy-α,α-hybrid tripeptides from unprotected amino acids. This methodology has been extended to other aminoxy-hybrid dipeptides and tripeptides starting from protected aminoxyacyl benzotriazolides (**Scheme 7**).[38]

### 5.2. Depsipeptides

Depsipeptides contain both amino acid and hydroxy acid units with amide and/or ester bonds. Natural depsipeptides exhibit antifungal, antimicrobial, and anti-inflammatory activities, and certain depsipeptides have been used in cancer treatment.[39] A comparative study of standard coupling agents used to produce depsipeptides revealed variable yields and often long coupling times.[40] *N*-Cbz(depsidipeptidoyl)benzotriazoles were found to be useful for coupling with amino acids (N-acylation)




**Scheme 7.** Preparation of Aminoxy and Other Peptides from α-Aminoxy Benzotriazolides. *(Ref. 38)*

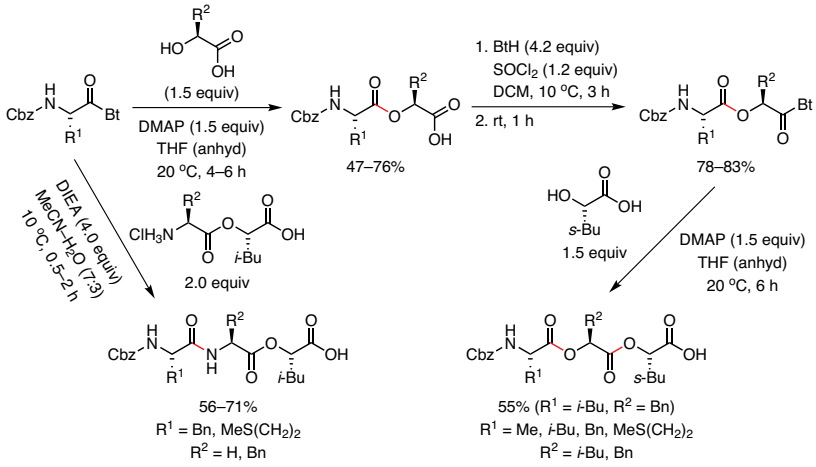
and α-hydroxy acids (O-acylation) to give depsidi- and depsitripeptides in good yields under mild conditions (**Scheme 8**).[41] This approach was applied to the preparation of unprotected depsidipeptides, which were converted into unprotected depsitripeptides.

### 5.3. Azapeptides

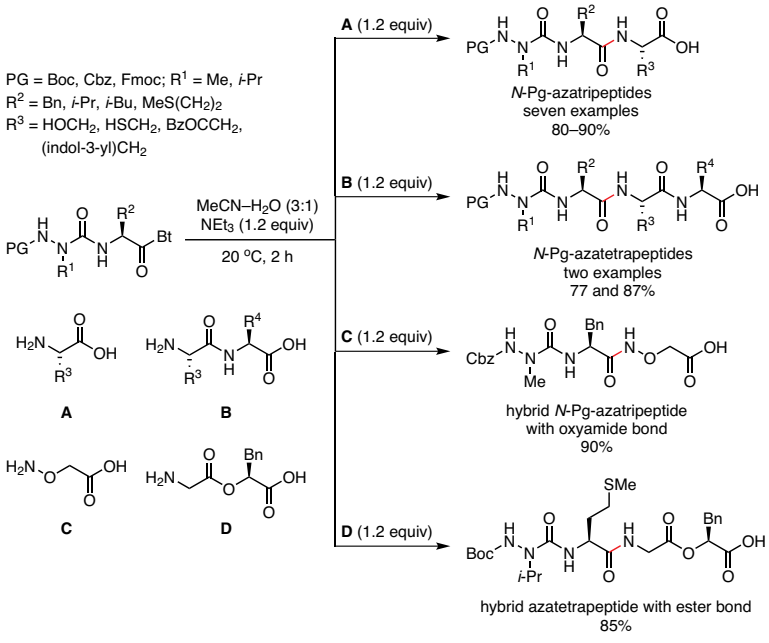
Azapeptides are peptidomimetics in which the α-CH group of one or more amino acid residues is replaced by a nitrogen atom. They are of interest for the generation of receptor ligands, enzyme inhibitors, and clinically approved drugs,[42] and those with electrophilic moieties act as inhibitors of cysteine proteases.[43,44] *N*-(*N*-Pg-α-Azadipeptidoyl)-benzotriazoles have been prepared from amino acid esters in four steps and 48–77% overall yields and utilized for the synthesis of *N*-Pg-azatripeptides, *N*-Pg-azatetrapeptides, and hybrid azapeptides (**Scheme 9**).[45] This methodology proved valuable for the insertion of an aza-amino acid unit into an azatripeptide chain for the synthesis of the previously unknown aza-analogues of the endogenous opioid peptide neurotransmitter Leu-enkephalin, found in animals and humans.[45]

### 5.4. Hydrazinopeptides

The replacement of an α-amino acid unit by a β-amino acid unit is a well-known strategy in the search for pharmacologically active peptides,[46]



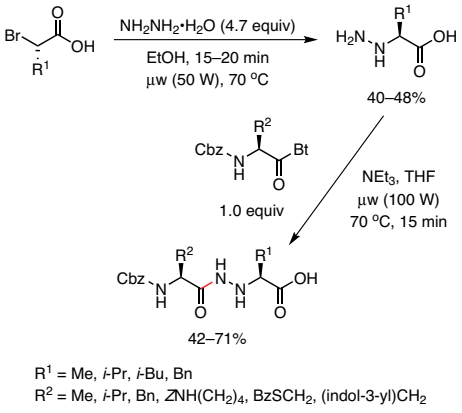

**Scheme 8.** Depsitripeptides from Cbz(depsidipeptidoyl)benzotriazoles. *(Ref. 41)*



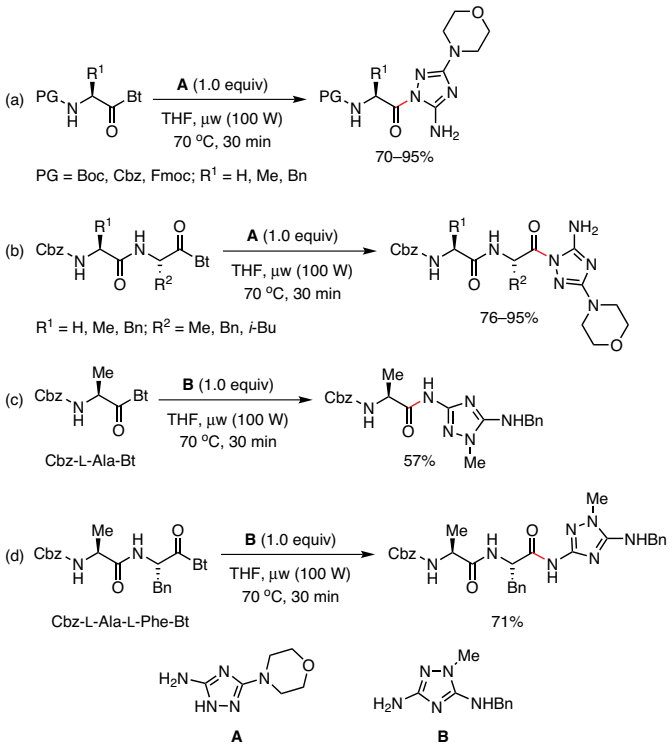

**Scheme 9.** Preparation of Azapeptides from *N*-(*N*-Pg-α-Azadipeptidoyl)benzotriazoles. *(Ref. 45)*

and replacement of the $C^{\alpha}$ and/or the $C^{\beta}$ atom in such β-amino acid building blocks by a hetero atom offers an attractive extension of the β-peptide concept.[47]

An alternate pathway to chirally pure α-hydrazino acids[48] is based on microwave irradiation during the conversion of α-bromo acids into α-hydrazino acids by hydrazine hydrate. The corresponding benzotriazolides afford hydrazine hybrid peptides (**Scheme 10**).[49]




**Scheme 10.** Preparation of Hydrazinodipeptides. *(Ref. 49)*




**Scheme 11.** Microwave-Assisted Acylation of (a) 3,5-Diamino-1,2,4-triazole, and (b) the Exocyclic Amino Group of 3,5-Diamino-1,2,4-triazole. *(Ref. 52)*

### 5.5. Heterocyclic Peptidomimetics

1,2,4-Triazoles have been employed for the bioisosteric replacement of the amide bond,[50] since 1,2,4-triazoles exhibit a wide range of antifungal and antibacterial activities.[51] 3,5-Diamino-1,2,4-triazole has been coupled to di- and tripeptides at either ring or exocyclic nitrogens, using the benzotriazole methodology, to give potential building blocks for the preparation of peptidomimetics (**Scheme 11**).[52]

### 5.6. Cyclic Peptidomimetics

In 2009, the benzotriazole methodology was extended to achieve cysteine S-acylation under mild conditions.[53] This has now been employed to couple an *N*-acylbis(benzotriazole) with cysteine to give a bis(*S*-acylcysteine), which, upon treatment with another equivalent of *N*-acylbis(benzotriazole), affords cyclic peptide mimetics in 64–72% yields (**Scheme 12**).[54]

## 6. Synthesis of Tagged Peptides and Peptidomimetics

### 6.1. Fluorescent Labels

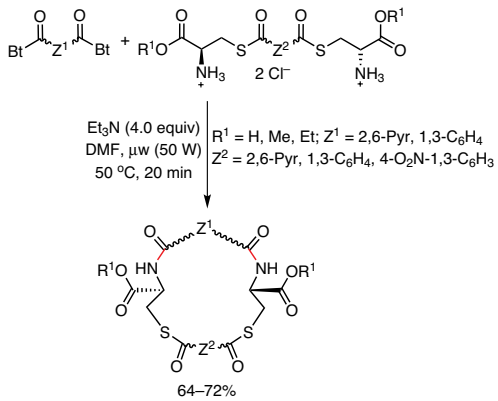
Fluorescent labeling of biological systems is of great importance. There is also increasing interest in establishing methods for the incorporation of non-natural amino acids into proteins without suppression of binding ability.[55]

#### 6.1.1. Coumarin-Labeled Peptides and Peptidomimetics

Coumarins are constituents of many commercially important fluorescent dyes since they offer high-emission quantum yields, photostability, and good solubility in most solvents. Synthetic methods for the incorporation of coumarin into amino acids and peptides are now available. (Coumarin-3-ylcarbonyl)benzotriazoles react readily with a variety of aminoxy acids in aqueous acetonitrile at room temperature to form coumarin-labeled aminoxy acids.[56] Coumarin-labeled amino acids have been prepared in a similar way[57] and coumarin-labeled aminoxy hybrid peptides have been obtained in two steps from the respective benzotriazoles (**Scheme 13**).[56] The 7-methoxycoumarin derivatives have quantum yields of 0.35–0.71 and may therefore be useful in peptide assays.

#### 6.1.2. 6-Chloro-2,3-naphthalimides and Water-Soluble Fluorescent Tags

Organic fluorophores that contain a naphthalene nucleus are of interest since, on binding with a substrate, they often exhibit significant changes




**Scheme 12.** Preparation of Cyclic Peptidomimetics. *(Ref. 54b)*

in their fluorescence spectra, quantum yields, and lifetimes in different solvents. The benzotriazole methodology offers access to new 6-chloro-2,3-naphthalimide derivatives (**Scheme 14**).[58]

### 6.2. Azo-Dye-Labeled Peptides

Azo-arene carboxylic acids are widely used as molecular switches in life sciences.[59–61] The coupling of an azo-dye carboxylic acid to a biological moiety has, in many cases, required harsh conditions and given poor yields.[62,63] In contrast, *N*-(4-arylazobenzoyl)benzotriazole and glycine in DMF–water at 20 °C give the coupled product in 99% yield. Similarly, other amino acids undergo this facile coupling (**Scheme 15**).[64]

## 7. Peptide Conjugates

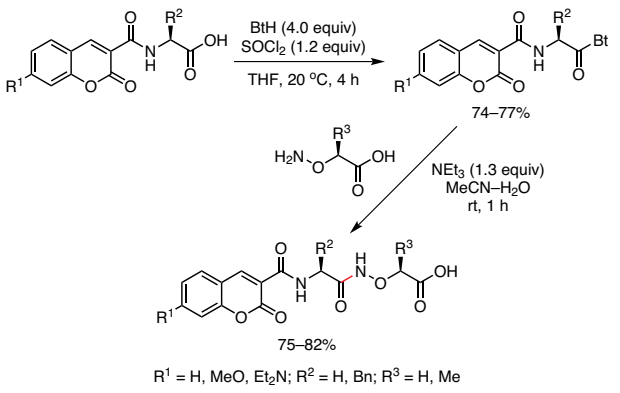
Conjugates comprise peptides attached to another molecular skeleton, usually through either the carboxyl group (C-terminus) or the amino group (N-terminus) of an amino acid.

### 7.1. Conjugates of Sugars

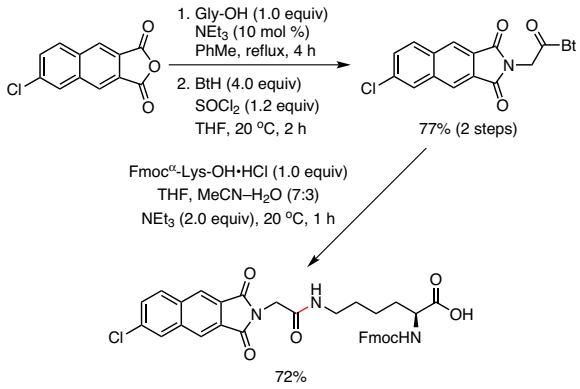
Numerous naturally occurring carbohydrate conjugates link a sugar glycosidically to an α-amino acid unit of a peptide or protein. Considerable effort has been devoted to the synthesis of N- and O-linked glycopeptide conjugates utilizing both solution- and solid-phase methodologies.[65–69] The benzotriazole methodology is advantageous for the solution-phase synthesis of chirally pure *O*-(α-aminoacyl)-[70] and *N*-(α-aminoacyl)sugar conjugates.[71] A typical procedure utilizes DMAP catalysis and microwave irradiation to give 82–92% yields of O- or N-coupled products (**Scheme 16**, Part (a)).[71] The same benzotriazole-based method also provides a convenient and efficient route to Cbz-protected tri- and tetrapeptide conjugates with sugars (Scheme 16, Part (b)).[72]

### 7.2. Conjugates of Heterocycles

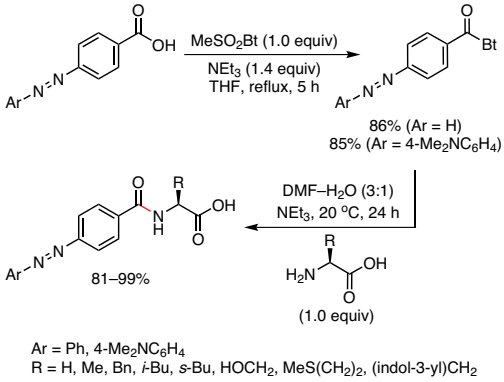
(α-Aminoacyl)amino-substituted heterocycles are of considerable importance as synthetic intermediates (e.g., for endomorphin-2 (EM-2) analogues),[73] and because of their diverse biological activity (e.g., as inhibitors of bacterial RND efflux pump[74–76] and of tumor necrosis factor-α converting enzyme (TACE) GW 3333[77]). Until recently, only a few reports had described the preparation of α-aminoacyl conjugates



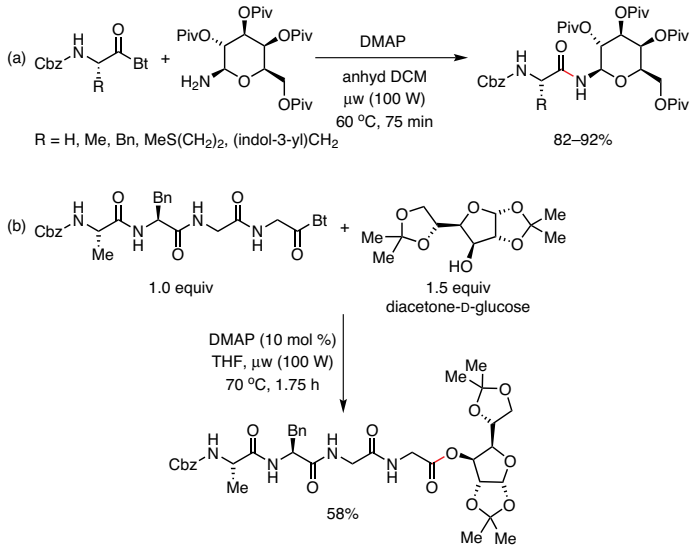

**Scheme 13.** Preparation of Coumarin-Labeled Aminoxy Hybrid Peptides. *(Ref. 56)*




**Scheme 14.** Preparation of 6-Chloro-2,3-naphthalimide and Water-Soluble Fluorescent Tags. *(Ref. 58)*




**Scheme 15.** Preparation of Azo-Dye-Labeled Amino Acids. *(Ref. 64)*




**Scheme 16.** Examples of (a) (α-Aminoacyl)sugar Conjugate and (b) Tetrapeptide Sugar Conjugate Synthesis. *(Ref. 71,72)*

of heterocycles by C- or N-acylation of heterocycles with amino acids. Kraus and co-workers[78] reported that "N-acylation of weakly nucleophilic heterocyclic amines by protected amino acid is not a straightforward reaction which could be achieved under any standard coupling conditions".

*N*-Aminoacyl benzotriazolides now enable the synthesis of chirally pure α-aminoacyl conjugates of heterocycles even from weakly nucleophilic heterocyclic amines by N-acylation in DMF under microwave irradiation.[79] The analogous C-acylation of lithiated methylpyridine or methylquinolone in THF at –20 °C for 1–3 h with *N*-aminoacyl benzotriazolides gave the corresponding α-aminoacyl C-linked conjugates (**Scheme 17**).[80] The convenient and efficient formation of Cbz-protected tri- (e.g., Z-L-Val-L-Phe-Gly-NH-(2-Pyr)) and tetrapeptide (e.g., Z-L-Phe-Gly-L-Leu-Gly-NH-(*N*-methylpiperazine)) conjugates with heterocyclic nuclei of biological importance succeeds under a variety of reaction conditions.[72,80]

### 7.3. Conjugates of Vitamins

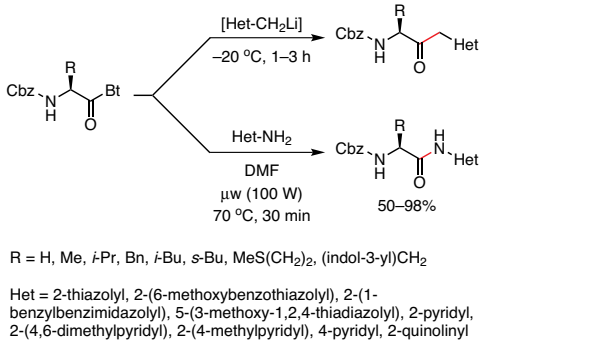
Recent approaches to enhance vitamin uptake include covalently bonding the vitamins to peptides. Water-soluble vitamins are usually transported into cells by potocytosis.[81] Zhang and McCormick[82] have proposed the delivery of vitamin B6 by receptor-mediated transport in eukaryotic cells with the amine of a peptide–vitamin conjugate, which facilitates the cell uptake of peptide and transport into the cytosol.[82,83] The benzotriazole methodology enables the efficient coupling of amino acids and peptides to vitamins, again utilizing microwave irradiation to shorten reaction times and minimize epimerization.[84]

Niacin and biotin benzotriazolides couple with free amino acids, dipeptides, and tripeptides ($NEt_3$, MeCN–$H_2O$, μw, 70 °C) to give the corresponding bioconjugates **1** and **2** in yields of 43–81% and 35–82%, respectively (**Figure 1**).[84] Amino acid and peptide conjugates of vitamin D3 (Figure 1, **3**) are obtained by O-acylation of cholecalciferol with Cbz-protected acylbenzotriazoles in the presence of DMAP in THF and under microwave irradiation (50 W, 70 °C) for 1–2 h.[84] Amino acid and peptide conjugates of α-tocopherol (Figure 1, **4**) are formed by O-acylation of α-tocopherol with Cbz-protected acylbenzotriazoles under microwave irradiation (20 W, 50 °C, 0.3 h) in anhydrous DMF in the presence of potassium carbonate.[84]

### 7.4. Conjugates of Pharmaceuticals

Improving the efficacy of therapeutics, particularly through enhanced local delivery to a diseased cell, is an important topic in pharmaceutical R&D. These techniques are helping to solve traditional drug delivery challenges, such as poor cellular uptake and/or non-specific toxicity.

The utilization of prodrugs that temporarily mask the acidic groups of NSAIDs may increase uptake and reduce irritation caused by direct contact.[85,86] Indomethacin, diclofenac,[87] ibuprofen, and naproxen,[87–91] are among well-known NSAIDs that have been modified by linking to natural amino acids, as reported by numerous investigators. We have recently shown that ibuprofen and naproxen bioconjugates are readily




**Scheme 17.** α-Aminoacyl Conjugates of Amino-Substituted Heterocycles. *(Ref. 79,80)*

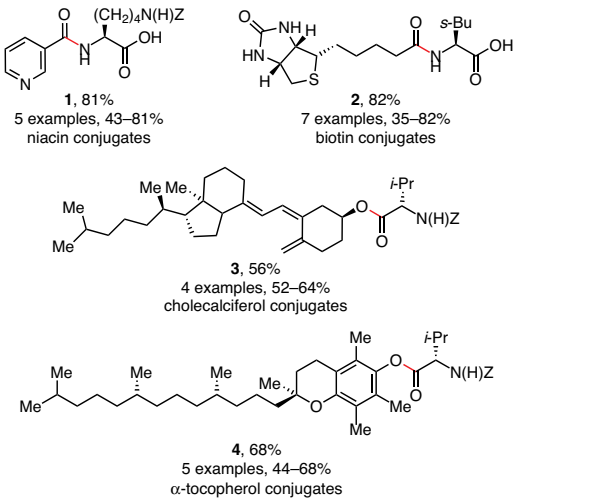
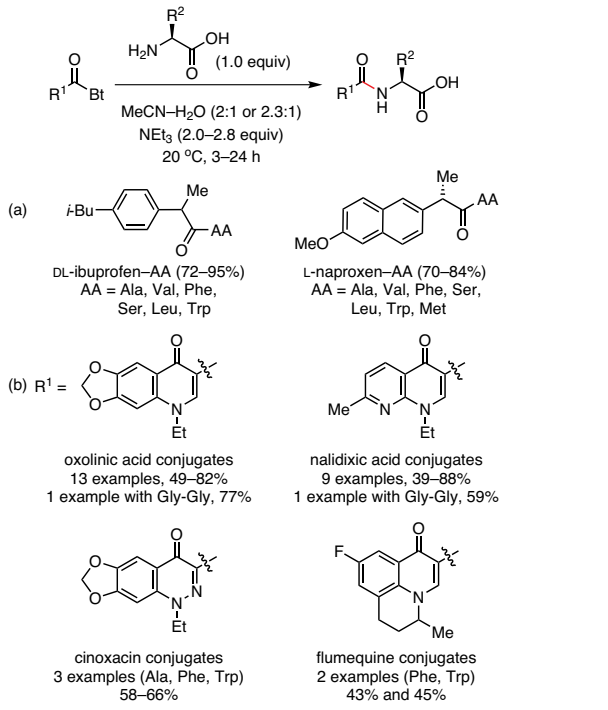



**Figure 1**. Peptide Conjugates of Vitamins Prepared by the Benzotriazole Methodology. *(Ref. 84)*




**Scheme 18.** Preparation of Peptide Conjugates of Pharmaceuticals. *(Ref. 92,100)*

prepared by reacting NSAID benzotriazolides with amino acids and dipeptides in aqueous acetonitrile–triethylamine at 20 °C (**Scheme 18**, Part (a)).[92]

Prodrugs formed by linking quinolone acids with amino acid esters are more lipophilic than the parent drugs,[93,94] and show enhanced in vivo antibacterial properties[95,96] with pronounced therapeutic effects against *Pseudomonas aeruginosa*,[97] *Escherichia coli*,[98] *Staphylococcus aureus*,[98] and *Salmonella typhi*.[96] Additional wide-ranging biological activities include anti-allergic,[99] antihypertensive,[99] bronchodilation,[99] and binding to bovine serum albumin.[92] Amino acid conjugates of quinolone antibiotics were prepared in 39–88% yields by coupling free amino acids with benzotriazole-activated oxolinic acid, nalidixic acid, cinoxacin, or flumequine (Scheme 18, Part (b)).[100,101]

### 7.5. Conjugates of Plant Hormones

Indole-3-acetic acid (IAA), an indispensable plant hormone (auxin), also occurs naturally as "conjugates" linked to amino acids.[102] Gene expression and cell division, elongation, and differentiation in plant tissue are all regulated by these indole-3-acetic acid auxins. Another endogenous plant hormone, indole-3-propionic acid (IPA), and its amino acid conjugates interact with serum albumin.[103] Plant hormone benzotriazolides—prepared in 86–90% yields by standard treatment of indole-3-acetic acid and indole-3-propionic acid—coupled with diverse amino acids to give conjugates in 40–70% yields (**Scheme 19**).[104]

### 7.6. Aminoxy Acid Conjugates of Peptidomimetics and Hydrazino Acid Conjugates

The benzotriazole methodology enables convenient and efficient synthesis of novel aminoxy acid containing conjugates even at hindered nucleophilic centers in steroids, terpenes, sugars, and nucleosides.[105] The benzotriazolides of α-hydrazino acids were used to generate hydrazine acid conjugates through N-, O-, S-, and C-acylations in good yields (49–88%).[49]

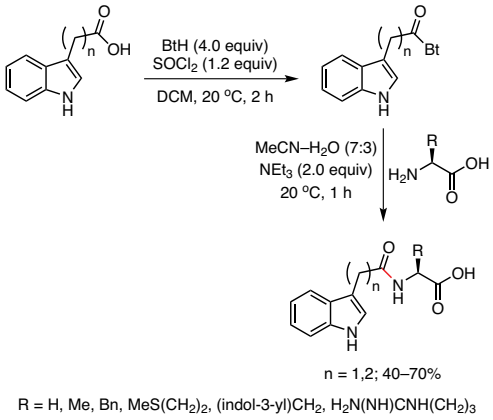
## 8. Differential N-, O-, and S-Acylations

Isopeptides are used for the detection and capture of ubiquitinating and de-ubiquitinating enzymes using activity-based protein profiling (ABPP).[106] The presence of an additional amino group in *N*-, *O*-, or *S*-acyl isopeptides generally increases their hydrophilicity, which is advantageous in effecting their purification by HPLC. The native peptides can then be generated from the corresponding *N*-, *O*-, or *S*-acyl isopeptides via an N to N,[107] O to N,[108] or S to N[6,109,110] intramolecular acyl migration reaction. These findings have led to the synthesis of peptides containing difficult sequences.[111]

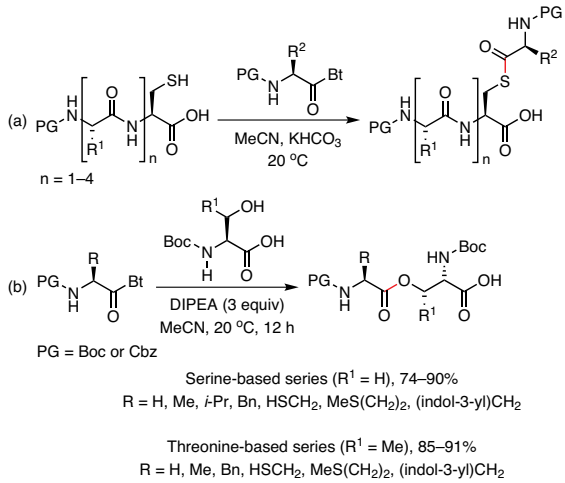
### 8.1. S- and O-Acyl Isopeptides

*S*-Acyl isopeptides are usually less likely to aggregate in solution and therefore are easier to synthesize and purify relative to the corresponding native peptides. S-Acylation of protected cysteine-containing peptides was carried out in the presence of $KHCO_3$ at 20 °C in acetonitrile (**Schemes 20**, Part (a)).[6,109,110] Selective S-acylation of cysteine was also carried out in acetonitrile–water mixture in the absence of base.

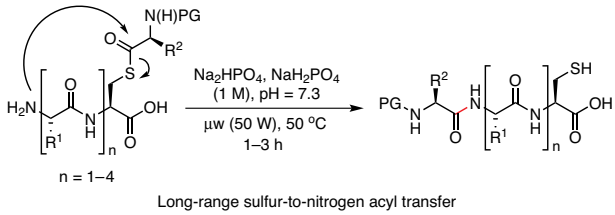
*O*-Acylation of protected serine and protected threonine with various *N*-Pg-(α-aminoacyl)benzotriazoles in the presence of diisopropylethylamine in acetonitrile at 20 °C for 12 h gave *O*-acylisoserine and *O*-acylisothreonine dipeptides without racemization (Scheme 20, Part (b)). *O*-Acylisotyrosine tripeptides were also prepared in yields of 74–91% by reacting tyrosine-containing protected dipeptides with *N*-Pg-(α-aminoacyl)benzotriazole in the presence of DBU in acetonitrile at 20 °C for 12 h.[24]




**Scheme 19.** Preparation of Conjugates of Plant Hormones. *(Ref. 104)*




**Scheme 20.** Synthesis of *S*- and *O*-acyl Isopeptides. *(Ref. 6,9,24,110)*




**eq 4** *(Ref. 6,109,110)*

### 8.2. Ligation at a Distance

#### 8.2.1. S to N Acyl Migration

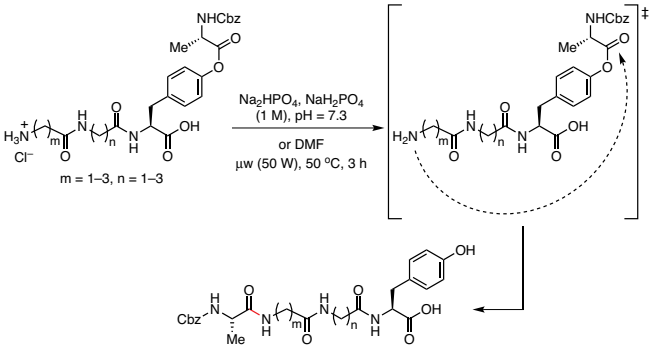
The S to N acyl migration through various cyclic transition states was investigated by carrying out the ligation experiment on mono-isopeptides under microwave irradiation (50 W) at 50 °C for 1–3 h using 1 M $NaH_2PO_4$–$Na_2HPO_4$ phosphate buffer to maintain pH 7.3 (**eq 4**). The rates and yields of long-range S to N acyl transfers were found to depend significantly on the size of the macrocyclic transition state (TS), with the rates qualitatively following the TS ring-size trend 5 > 10 > 11 > 14, 16, 17 > 12 > 13, 15, 19 > 18 >>> 9 > 8.[6,109,110]

#### 8.2.2. O to N Acyl Migration

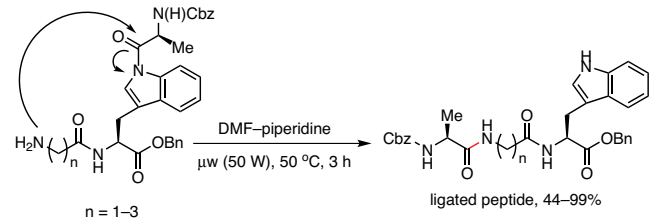
The chemical ligation of serine isopeptide through O to N acyl transfer via 8- and 11-membered-ring transition states occurs without the use of an auxiliary group (**eq 5**).[108] In contrast, threonine isopeptide failed to undergo acyl migration even under more basic conditions and longer reaction times.[112] Chemical ligation studies of tyrosine isopeptides (μw, 50 W, 50 °C, 3 h, using 1 M phosphate buffer and DMF–piperidine) via 12- to 19-membered-ring cyclic transition states showed that intramolecular O to N acyl transfer occurs with 12- to 14-membered-ring TS's under basic conditions and with 15- to 19-membered-ring TS's in aqueous media.[108]

#### 8.2.3. N to N Acyl Migration

Tryptophan isopeptides with α-, β-, or γ-amino acid units were synthesized, and the acyl migration from the indole nitrogen to the terminal $NH_2$ was studied under microwave irradiation. Intramolecular acyl transfer through 10-, 11-, and 12-membered-ring transition states was favored over that through a 7-membered-ring TS, and acyl migration occurred more readily in basic, nonaqueous media relative to aqueous buffered conditions (**eq 6**).[107]




**eq 5** *(Ref. 108)*




**eq 6** *(Ref. 107)*

## 9. Conclusions and Comparison with Alternative Methodologies

### 9.1. Carboxyl Group Activation by Isolation of an Intermediate

The most obvious method for activating the carboxyl group of an amino acid for peptide bond formation at room temperature or below is by forming the corresponding acid chloride.[113] This type of activation has been carried out with chlorinating reagents such as pivaloyl chloride,[114] phthaloyl dichloride,[115] thionyl chloride,[116] and oxalyl chloride.[117] However, an amino acid chloride bearing an acid labile protecting group can easily racemize through the oxazolone, which limits the application of acid chlorides despite their high reactivity and low cost. Amino acid fluorides are less moisture-sensitive than acyl chlorides, but the fluorinating reagents are expensive and hazardous, and the peptide-forming reactions require purification by chromatography.[118]

The acyl azide method of peptide coupling was developed about 100 years ago. It is not attractive for routine use because it involves four distinct steps, including two stable intermediates that require purification.[119] An additional side reaction that occurs at higher temperature is rearrangement of the acyl azide into the alkyl isocyanate, which can react with nucleophiles to yield a peptide urea that is difficult to remove from the product.[120] Recently, El-Faham and Albericio published a review on the use of different peptide coupling reagents including benzotriazoles.[121]

### 9.2. Carboxyl Group Activation without Isolation of an Intermediate

Besides acyl halides and acyl azides, other methods for peptide coupling include the use of various reagents, where the intermediates are not isolated. A traditional approach to form peptide bonds is the carbodiimide method, using dicyclohexylcarbodiimide (DCC). However, despite being compatible with solid-phase synthesis (SPS) that uses *tert*-butoxycarbonyl (Boc) chemistry, DCC is not compatible with the fluorenylmethoxycarbonyl (Fmoc) group. When DCC is utilized in solution, traces of the byproduct, DCU, are difficult to remove, even after passage through a chromatography column. Thus, DCC has been replaced by reagents such as diisopropylcarbodiimide (DIC), *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide (EDC), and *N*-cyclohexyl-*N*′-isopropylcarbodiimide (CIC), all of which are relatively soluble in DCM and therefore more suitable for Fmoc-SPS. Additives such as 1-hydroxybenzotriazole (HOBt), 1-hydroxy-7-azabenzotriazole (HOAt), *N*-hydroxysuccinimide (HOSu), and others increase the efficiency of carbodiimide-mediated reactions and decrease the degree of racemization.[122]

Phosphonium reagents were developed to avoid racemization and side reactions that can occur with carbodiimide reagents. Coste et al. introduced chloro- and bromotris(dimethylamino)phosphonium hexafluorophosphate (CloP and BroP) as peptide-coupling reagents with noticeable racemization in the Young test.[123] HOBt may be used in combination with (benzotriazol-1-yloxy)tris(dimethylamino)-phosphonium hexafluorophosphate (BOP) to suppress racemization. However, the intermediates formed in the coupling reaction are highly unstable and BOP is reported to be highly carcinogenic.[124]

The search for better coupling reagents based on DCC led to carbonyl diimidazole (CDI).[125] Rapoport introduced the imidazolium

reagent 1,10-carbonylbis(3-methylimidazolium) triflate (CBMIT) by bis-methylating CDI with methyl triflate.[126] This reagent showed no sign of racemizing the amino acid residues in the presence of $CuCl_2$ or $Cu(OTf)_2$. However, CBMIT is moisture-sensitive and, due to its polarity, the method is restricted to polar solvents such as nitromethane.[127] The reactivity of these reagents also increases in the presence of additives like HOAt, HOBt, or DMAP.

Aminium or uronium reagents such as *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HBTU) and *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU) all react directly with the amine moiety of the amino acid residue to give a guanidine side product, which terminates the peptide chain.[128]

Benzotriazole offers an extremely useful alternative to all the above methods by affording a versatile range of coupling procedures under the mild conditions required to avoid racemization.

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**Eric Scriven** is a native of Wales, U.K. After working at BISRA and Esso Ltd, he attended the University of Salford and graduated in 1965 with a degree in chemistry. He obtained his M.Sc. Degree from the University of Guelph, and his Ph.D. degree in 1969 from the University of East Anglia (with Prof. Katritzky). After postdoctoral years at the University of Alabama and University College, London, he was appointed Lecturer in Organic Chemistry at the University of Salford. There, his research interests centered on the reactivity of azides and nitrenes. He joined Reilly Industries in 1979, where he served as Director of R&D from 1991 to 2003. He is currently at the University of Florida. He has edited two books: *Azides and Nitrenes: Reactivity and Utility* (1984) and *Pyridines: from Lab to Production* (2013). He and Professor H. Suschitzky were founding editors of *Progress in Heterocyclic Chemistry* that has been published annually since 1989. He collaborated with Professors Katritzky and Rees as Editors-in-Chief of *Comprehensive Heterocyclic Chemistry II* (1996), and with Professors Katritzky, Ramsden, and Taylor on the third edition of the work. Currently, he is Publishing Editor of *ARKIVOC*, an online journal of organic chemistry free to readers and authors.

**Alan R. Katritzky** was educated at Oxford and Cambridge (Lecturer and Founder Fellow of Churchill College). Founder Dean of the School of Chemical Sciences at East Anglia from 1962, he transferred in 1980 as inaugural Kenan Professor to the University of Florida. His research in heterocyclic chemistry has covered inter alia *N*-oxides, benzotriazole methodology, electrophilic and nucleophilic substitutions, computational QSPR relationships, and peptide chemistry. He holds 14 honorary doctorates from 10 Eurasian countries and associate or foreign memberships of five national academies. He is Cavalieri Ufficiale (Italy) and Honorary Fellow of St. Catherine's College, Oxford, and of the Polish and Italian Chemical Societies. Over 1,000 graduate students and postdocs have trained in his group. He created the not-for-profit Arkat USA, Inc., which organizes the Flohet Conferences and publishes the open-access journal *ARKIVOC*. Contributions to the secondary literature include editing *Comprehensive Heterocyclic Chemistry* (40 volumes in 3 editions), *Advances in Heterocyclic Chemistry* (106 volumes), *Handbook of Heterocyclic Chemistry* (3rd edition, 2010), and *Heterocycles in Life and Society* (2nd edition, 2011).

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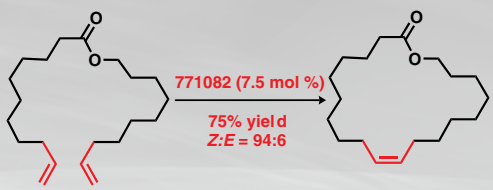


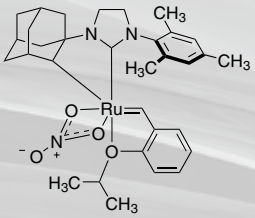

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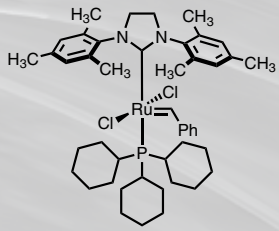

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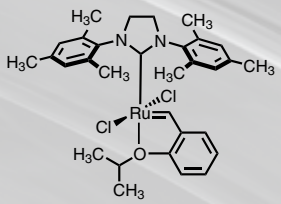

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Marx, V. M.; Herbert, M. B.; Keitz, B. K.; Grubbs, R. H. *J. Am. Chem. Soc.* **2013**, *135*, 94.

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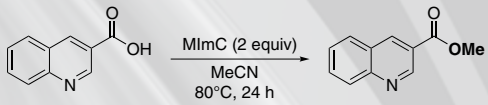
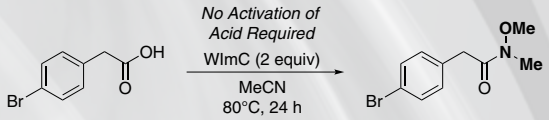
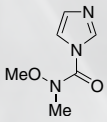



91% yield

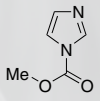
## Weinreb Amide Synthesis



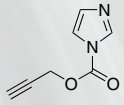

92% yield



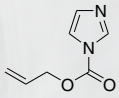
748838
**WImC**



748730
**MImC**



748781
**PImC**



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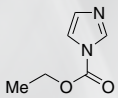
**AllylImC**



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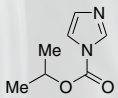
**EtImC**



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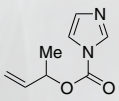
**IPlmC**



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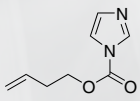

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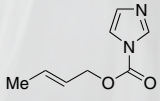

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(2) Heller, S. T.; Fu, T.; Sarpong, R. *Org. Lett.* **2012,** *14,* 1970.

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# Recent Advances in the Prins Cyclization


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**Keywords.** Prins reaction; tetrahydropyrans; dihydropyrans; tetrahydrofurans; dioxanes; piperidines; azepines; lactones; spiro compounds; macrocycles; natural products.

**Abstract.** The Prins reaction is often a key step in the synthesis of various heterocyclic rings that are important structural components of many classes of biologically active compounds and natural products. This review presents and discusses recent significant applications of this important reaction, and offers insight into its mechanism and regio- and stereochemical outcomes.

## Outline



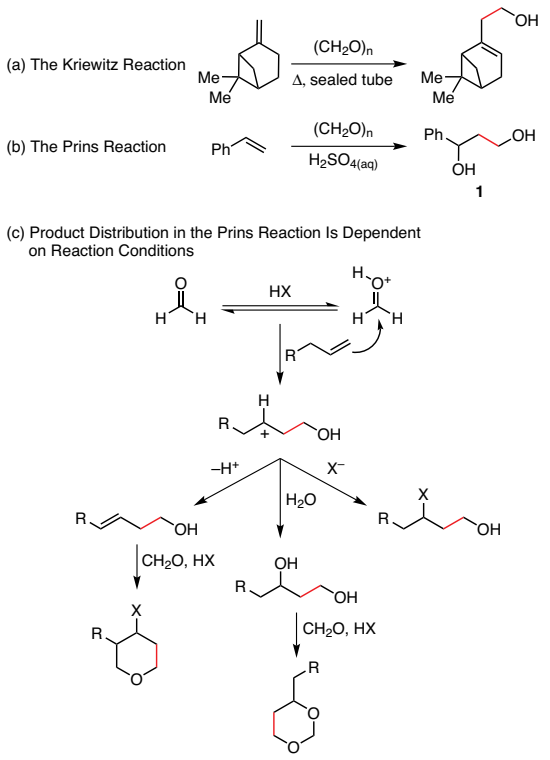
## 1. Introduction

The Prins reaction is often related to the Kriewitz reaction, an example of which is the reaction of β-pinene with paraformaldehyde to produce an unsaturated alcohol through a thermal ene rearrangement (**Scheme 1**, Part (a)).[1–3] When the reaction between an alkene and formaldehyde is conducted in the presence of an acid catalyst—such as the reaction of styrene with paraformaldehyde in the presence of aqueous sulfuric acid to give diol **1** (Scheme 1, Part (b))—it is called the Prins reaction. Numerous protic and Lewis acids are known to catalyze the reaction, and excellent reviews have been published on the early work.[1–3] The products generally obtained in this condensation are formed as complex mixtures of 1,3-dioxanes, 1,3-glycols, tetrahydropyrans, and allylic and homoallylic alcohols, with the composition of the mixture being dependent on the specific experimental conditions employed (Scheme 1, Part (c)). In the presence of water, the intermediate carbocation leads to the formation of 1,3-glycols and 1,3-dioxanes, while 3-alkyl-4-halotetrahydropyrans are obtained through the intermediacy of the homoallylic alcohols.

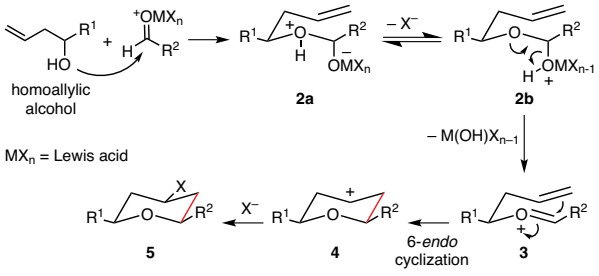
The Prins reaction plays a key role in the synthesis of such important product classes as dihydrofurans, dihydropyrans, piperidines, and oxabicyclo and spiro compounds. This review presents a survey and a discussion of pertinent and interesting recent developments relating to the stereochemical course and mechanism of the Prins reaction and to its advantageous application in organic synthesis.

## 2. Mechanistic Considerations

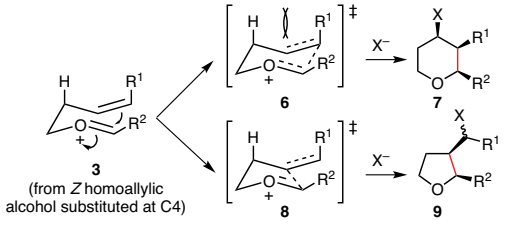
The Prins cyclization involves a homoallylic alcohol, an aldehyde, and a Lewis acid. The latter acts as catalyst and, depending on experimental conditions, it can also serve as a source of a nucleophilic anion. In the presently accepted mechanism, the reaction is initiated by complexation of the Lewis acid with the aldehyde, which activates the carbonyl carbon toward attack by the hydroxyl group of the alcohol, generating the hemiacetal intermediate **2**. Loss of the Lewis acid fragment from




**Scheme 1.** Condensation Reactions of Olefins with Paraformaldehyde and the Dependence of Product Distribution on the Reaction Conditions. *(Ref. 1–3)*




**Scheme 2.** General Mechanism of the Prins Cyclization. *(Ref. 2)*




**Scheme 3.** Regioselectivity in the Prins Cyclization. *(Ref. 4)*

the hemiacetal forms the key oxonium ion intermediate, **3**, which assumes the more stable chair conformation in which the substituents are pseudoequatorial. Subsequent 6-*endo* cyclization of **3** selectively leads to secondary tetrahydropyranyl carbocation **4**, which captures the halide to give rise to the 2,4,6-trisubstituted tetrahydropyran product (**Scheme 2**).[2]

### 2.1. Regioselectivity for 5- vs 6-Membered Rings

When the double bond geometry in the homoallylic alcohol is switched from *E* to *Z*, tetrahydrofurans can be formed in competition with tetrahydropyrans. This regioselectivity can be studied by examining the stereochemistry of intermediates present in the accepted mechanism of this reaction. Under the Prins cyclization conditions, the *Z* homoallylic alcohol reacts with the activated aldehyde to give rise to oxonium ion **3**. Two competing transition states can then be formed from **3**: six-membered-ring transition state **6** has a 1,3-diaxial interaction between H and the substituent $R^1$, while five-membered-ring transition state **8** has greater torsional and angular strains. When the $R^1$ substituent is sufficiently large, an increase in the activation barrier of the process results, which slows the formation of tetrahydropyran product **7** in favor of tetrahydrofuran product **9** (**Scheme 3**).[4]

### 2.2. Stereoselectivity of Nucleophile Capture at C4 of the Tetrahydropyran Ring

#### 2.2.1. Alder's Model: Equatorial Selectivity

On the basis of theoretical calculations employing Density Functional Theory (DFT), Alder and co-workers concluded that the all-cis 2,4,6-trisubstituted product of the Prins cyclization is favored by stabilization of the cationic intermediate **10** through hyperconjugation. When the hydrogen attached to the carbocation center is pseudoaxial, the empty p orbital of the positively charged carbon overlaps more efficiently with the coplanar $\sigma_{C-C}$ and $\sigma^*_{C-C}$ orbitals and with the orbital of the nonbonding electrons of oxygen. Nucleophilic attack thus occurs from the exo face (convex), leading to the 2,4,6-trisubstituted tetrahydropyran product with all three substituents in equatorial positions (**eq 1**).[5]

#### 2.2.2. Rychnovsky's Model: Axial Selectivity

Rychnovsky investigated the capture of bromide and iodide at C4 of **10** by reacting α-acetoxy ester **11** with TMSBr, AcBr, HBr, or TMSI and lutidine in dichloromethane. High axial stereoselectivity at C4 was observed for the resulting Prins cyclization product **16** (**Scheme 4**).[6] In contrast, when $SnBr_4$ was employed, the major product was the equatorial epimer **19**. In the proposed mechanism, some Lewis acids; such as TMSBr, AcBr, and HBr; act as donors of bromide by forming the intimately associated ion pairs **14** and **15**. The slightest movement (least motion pathway) in **15**, results in $Br^-$ attacking C4 in the axial position (endo attack) to form **16**. When $SnBr_4$ is employed as the Lewis acid, the in situ formed $[SnBr_5]^-$ in ion pair **17** is less nucleophilic than bromide, allowing separation of the ion pair by the solvent. In the resulting intermediate, **18**, exo (convex) attack leads to the formation of product **19** with an equatorial bromine at C4.

### 2.3. Diastereoselectivity of the Prins Cyclization

Substituents at C2 and C6 of tetrahydropyrans formed by the Prins cyclization are preferentially cis.[7a] Methodologies for forming the C2/C6 anti isomers are not well established. These isomers are present in some structures of natural products, such as the psymberins[7b] and the apicularens.[7c] Panek's group has succeeded in synthesizing enantiomerically pure *anti*-2,6-dihydropyrans by the Prins cyclization with the aid of TMSOTf.[7c–e]

Loh and co-workers investigated steric and electronic effects in the Prins cyclization of homoallylic *anti*-α-hydroxy esters, leading to 4-chloro-2,6-disubstituted THPs. This study demonstrated that groups with high electron density in the pseudoaxial position stabilize the oxonium ion by inductive electronic effects, and favor a transition state that forms the anti isomer, **20a**; while steric effects favor the transition state leading to the syn isomer, **20b**. In both cases, equatorial attack of the nucleophile is preferred (**Scheme 5**).[8]

## 3. Recent Synthetic Applications

Saturated six-membered-ring oxygen and sulfur heterocycles are features found in the structures of a variety of biologically important natural products such as polyether antibiotics, marine toxins, pheromones, and pharmaceutical agents. Tetrahydropyran is also the structural core of most carbohydrates, oligomers, and polymers, which play crucial roles in living organisms. It is therefore not surprising that considerable efforts have been expended toward developing facile and viable syntheses of tetrahydropyran-containing compounds.

### 3.1. Substituted 1,3-Dioxanes

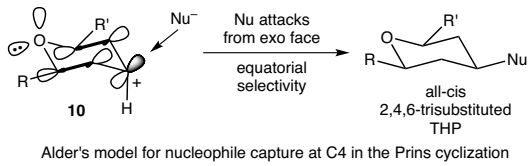
When olefins are condensed with aldehydes in aqueous solutions of mineral acid catalysts, alkyldioxanes (cyclic formals or acetals of 1,3-butanediols) and 1,3-butanediols are formed. The distribution of these two products varies with the concentration of the solution of the acid catalyst and the reaction temperature. Amrute et al. studied the catalytic activity of $MoO_3/SiO_2$ (7 wt %) in the Prins cyclization of a series of olefins with paraformaldehyde (2 equiv) in 1,2-dichloroethane at 80 °C.[9] 72–90% conversions and 96–100% selectivities were observed for the corresponding 4-alkyl- and 4-phenyl-substituted 1,3-dioxane products. Du and Tian synthesized 1,3-dioxanes in moderate-to-high yields from formalin (aqueous formaldehyde), styrene derivatives, and trifluoromethanesulfonic acid (TfOH).[10] The use of organic acid as catalyst for the Prins reaction was unprecedented. It is worth noting that this approach avoids the use of organic solvents by conducting the reaction in water, which makes it a more environmentally friendly process.

Yang and co-workers explored the use of water-stable and recyclable Brønsted acidic ionic liquids as environmentally benign catalysts for the Prins cyclization. The effectiveness of these ionic liquids was compared in the model reaction of styrene with formaldehyde at 94–96 °C, whereby [BMIM][$HSO_4$] was found to be the most effective catalyst (**eq 2**).[11] The 1,3-dioxane products were obtained in good yields, and the catalysts, after vacuum distillation at 80 °C, were recovered and reused in subsequent runs, thus reducing the risks to the environment by avoiding the use of organic solvents and enabling large-scale applications of the Prins cyclization.

### 3.2. Spiro and Bicyclic Tetrahydropyrans (THPs)

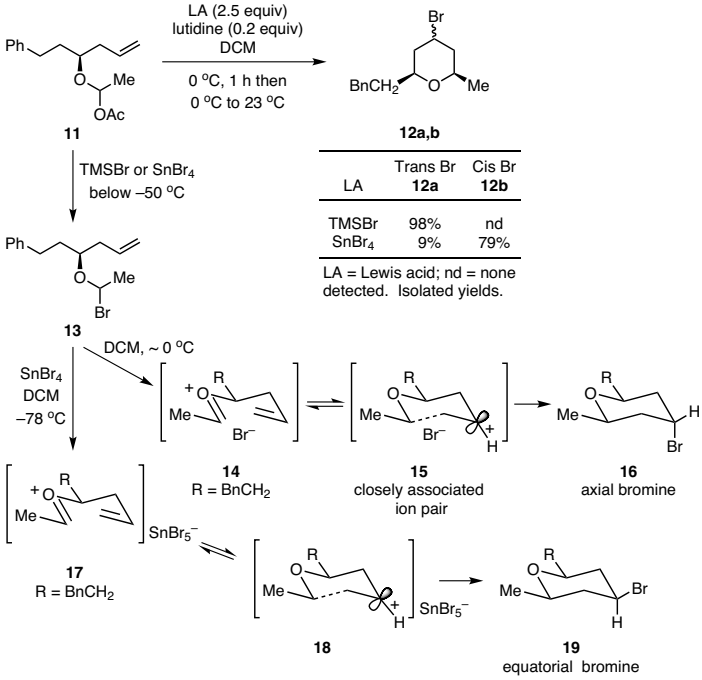
Gais and co-workers have reported a modular asymmetric approach to spiroketals, spiroethers, and oxabicycles that employs a spiro- or bicycloannulation of α-hydroxydihydropyrans. The synthesis included a stereoselective Ferrier-type O- and C-glycosidation, ring-closing metathesis, and stereoselective Prins cyclization as key steps. When α-hydroxydihydropyran **21** was treated with $TiCl_4$ in dichloromethane at –78 °C, the regioisomeric spiro ethers **22** and **23** resulted from a Prins cyclization (**eq 3**).[12]

Nakamura and co-workers have described a versatile method for the synthesis of 4-substituted 6-methyl-3-oxabicyclo[3.3.1]non-6-ene-1-methanol derivatives using a Prins-type cyclization reaction between aldehydes and O-protected or unprotected 4-methylcyclohex-




Alder's model for nucleophile capture at C4 in the Prins cyclization

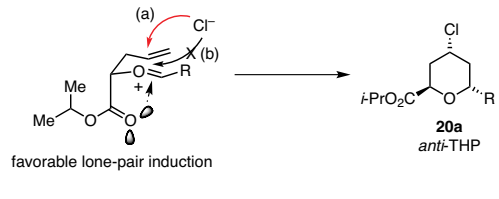
**eq 1** *(Ref. 5)*




| LA | Trans Br 12a | Cis Br 12b |
|---|---|---|
| TMSBr | 98% | nd |
| $SnBr_4$ | 9% | 79% |

LA = Lewis acid; nd = none detected. Isolated yields.

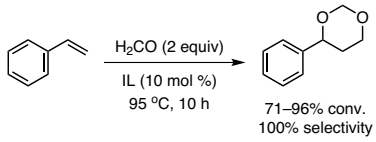
**Scheme 4.** Axial Selectivity in the Prins Cyclization. *(Ref. 6)*




(a) equatorial approach (favored); (b) axial approach (unfavorable).

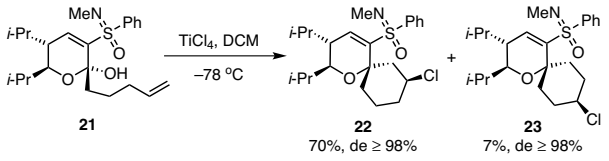
R = Cy, Et, $Et_2CH$, $BnCH_2$, $MeCH{=}CH_2$
Reaction conditions: $In(OTf)_3$ (20 mol %), TMSCl, DCM, 0 °C
56–62% yields; **20b:20a** = 44:56 to 51:49

**Scheme 5.** Diastereoselectivity of the Prins Cyclization. *(Ref. 8)*

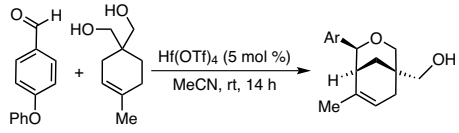



IL = $[HMIM]BF_4$, $[(CH_2)_4SO_3HMIM][HSO_4]$, $[(Ac)_2BIM]Br$, $[NMP][HSO_4]$, $[BMIM][HSO_4]$, $[BMIM][H_2PO_4]$

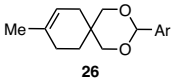
**eq 2** *(Ref. 11)*




**eq 3** *(Ref. 12)*

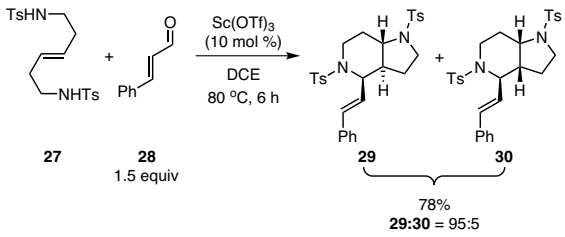



Six additional examples of aromatic aldehydes, 72–96%



**26** was the major product when $Zn(OTf)_2$, $Yb(OTf)_3$, and $La(OTf)_3$ were used: 75%, 66%, and 68% yield, respectively.

**eq 4** *(Ref. 13)*




**eq 5** *(Ref. 17)*

3-ene-1,1-dimethanol (**24**). Under the optimized reaction conditions employing hafnium triflate, various aldehydes, including functionalized benzaldehydes and heteroaromatic aldehydes, afforded the cyclization products in high yields (**eq 4**).[13] The zinc and lanthanum triflates form preferably spirodioxane **26.**

### *3.3. Nitrogen Heterocycles via the Aza-Prins Cyclization*

The Prins cyclization of homoallylic amines (the aza-Prins cyclization) takes place in a fashion similar to that of homoallylic alcohols, whereby the nonbonding electrons on the nitrogen initiate the sequence of reaction steps by attacking the electrophilic site of the aldehyde activated by an acid catalyst. The key intermediate of the aza-Prins cyclization is an iminium ion, in analogy to the oxonium ion. Piperidines are commonly found subunits in many biologically relevant molecules including alkaloids, and are attractive structural scaffolds for drug discovery.[14] Subba Reddy showed that the $BF_3$•$OEt_2$ catalyzed aza-Prins reaction of benzaldehyde and *N*-tosyl-3-butenamine (a homoallylic amine), in the presence of anisole as solvent and nucleophile, produces the *trans*-2,4-diarylpiperidine in 83% yield.[15,16] This is the first report of the preparation of 4-arylpiperidines via an aza-Prins–Friedel–Crafts reaction sequence.

The coupling of *E*- (**27**) and *Z*-3-hexene-1,6-ditosylamides with various aldehydes, including cinnamaldehyde (**28**) in the presence of 10 mol % $Sc(OTf)_3$ in 1,2-dichloroethane gave the corresponding trans- and cis-fused saturated pyrrolopyridines **29** and **30**, respectively, in good yields by an intramolecular aza-Prins cyclization (**eq 5**).[17] Other aromatic aldehydes such as benzaldehyde, *para*-anisaldehyde, and thiophene-2-carboxaldehyde were not effective substrates in the reaction. Ketones, such as cyclohexanone, failed to give the spiro-diaza bicyclic product. In contrast, aliphatic aldehydes; such as isovaleraldehyde (70%, trans:cis = 95:5), cyclohexanecarboxaldehyde (73%, trans:cis = 95:5), and propionaldehyde (66%); participated well in this reaction.

Camara et al. synthesized azepines fused to a naphthoquinone moiety by an intramolecular aza-Prins cyclization starting with an amino derivative, **31**, of lapachol (**Scheme 6**).[18] Products **32a** and **32b** were formed in 42% yield as a diastereomeric mixture, with a trans:cis ratio of about 7:3. The mechanism of C–C bond formation leading to **32a** and **32b** appears to resemble that of the intramolecular ene reaction between a carbonyl group and an alkene.[19] The authors proposed that the formation of intermediates occurs through a Prins reaction, via nucleophilic attack of $H_2O$ or MeOH at the isoprenyl double bond, possibly followed by a concerted attack onto the protonated carbonyl. The observed diastereoselectivity is possibly induced by steric hindrance of the 4-isopropyl and 3-hydroxyl groups, despite the fact that the resulting seven-membered ring is conformationally less restricted than the corresponding six-membered ring. This synthetic method is important, since there are very few publications on the synthesis of such heterocyclic systems, which are of great interest in the scientific community because of their pharmacological applications.[20,21]

3-Azabicyclo[3.3.1]non-6-enes are structural motifs in many natural products and, with the proper choice of substituents, can serve as templates for complexity-generating transformations. Krasavin and co-workers have reported a facile synthesis of this ring system in a diastereomerically pure form by an aza-Prins cyclization involving a δ,ε-unsaturated imine and an equivalent of $BF_3$•$OEt_2$ under microwave irradiation at 180 °C for 1 h (**Scheme 7**).[22] As in the mechanism proposed earlier by Overman for the aza-Prins cyclization employed in the total synthesis of (+)-nankakurines A and B,[23] it is believed that the pair of nonbonding electrons on nitrogen participate in a regiospecific

intramolecular deprotonation of the hydrogen vicinal to the initially formed carbocation in intermediate **A**.

Subba Reddy and co-workers[24] have reported the synthesis of 2-aryl- and 2-alkyl-4-amidopiperidines in good yields and high selectivities by an aza-Prins–Ritter tandem reaction employing a slight excess of triflic acid in MeCN at 0 °C. It was observed that in the absence of triflic acid, no aza-Prins cyclization occurred even in refluxing acetonitrile. Other Brönsted acids—such as acetic acid, formic acid, and trifluoroacetic acid—were tested and, in all cases, the reaction proceeded rapidly at 0 °C; however, triflic acid provided the best conversion.

### 3.4. Synthesis of Furan Derivatives

Substituted dihydrofuranones (γ-butyrolactones or GBLs) are important intermediates in synthetic organic chemistry, and are commonly found as structural fragments in natural products, receptor ligands, and drug molecules.[25] Compounds containing a GBL moiety exhibit pharmacological effects some of which are muscarinic (pilocarpine) and antimuscarinic (Kaiser lactones) activities, convulsant (picrotoxin, β-substituted GBL) and anticonvulsant (α-alkyl-substituted GBLs) activities, and the ability to modulate quorum sensing.[26]

Gao and Canney reported a novel and concise approach for the synthesis of structurally diverse, substituted 5-(2-hydroxyethyl)-3,3-dihydrofuran-2(3*H*)-ones. This method relies on a modified Prins reaction that employs a catalytic amount of $H_2SO_4$ in glacial HOAc. In the proposed mechanism, acetic acid captures the initially formed carbocation to provide a protected hydroxyl group. The remaining aliphatic alcohol is also protected by an intramolecular esterification, leading to a caprolactone intermediate. Sequential treatment of the resulting seven-membered-ring lactone with aqueous base and then acid affords the desired hydroxyethyl lactones in moderate-to-good yields (**Scheme 8**).[26]

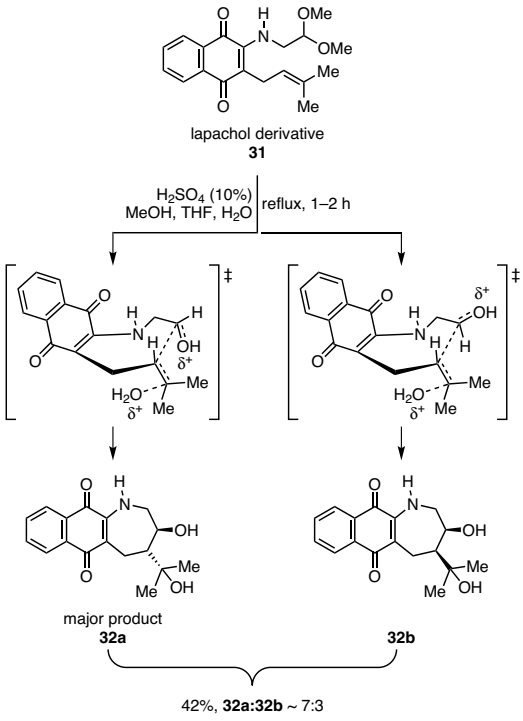
Two examples of the use of the Prins cyclization in the synthesis of furan structures fused with pyran rings (furopyrans) have recently been reported.[27,28] This ring system is commonly found in natural products such as flavonoids, pterocarpans, and catechins.[29] In the first example, 3-hexene-1,6-diol (**36**) was reacted with 4-bromobenzaldehyde in an intramolecular Prins cyclization in the presence of 10% TsOH in 1,2-dichloroethane to give the cis-fused bicyclic product **37** in 72% yield (**Scheme 9**, Part (a)).[27] In the second example, a substituted 2,6-dioxabicyclo[3.2.1]octane was similarly prepared by a tandem acetalization–intramolecular Prins cyclization starting with 4-pentene-1,2-diol (Scheme 9, Part (b)).[28] The overall process is catalyzed by a combination of $Sc(OTf)_3$ and TsOH and leads to good yields, high selectivities, and faster reaction times.

### 3.5. Synthesis of Halogenated Tetrahydropyrans: Halo-Prins Cyclization

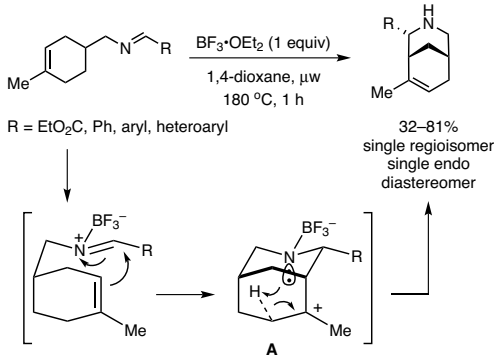
In analogy to the oxygen and nitrogen variants, the halo-Prins cyclization involves nucleophilic attack by halogen present in the reaction medium on the carbocation intermediate that arises from the oxonium ion formed after cyclization.

#### 3.5.1. Fluorinated THPs

The introduction of fluorine atoms into organic molecules alters in important ways their biological activity, solubility, hydrophobicity, metabolism, and bulk properties.[30–32] However, few methods for the synthesis of fluorinated pyranyl motifs are known and, of these, the ones that employ $BF_3$•$Et_2O$ and $Et_4NF$•5HF as both Lewis acids and fluorine sources successfully achieve the Prins cyclization of homoallylic alcohols into fluorinated pyranyl motifs.[33a–e] When $BF_3$•$Et_2O$ is utilized in stoichiometric quantities, it contributes fluoride ion to quench the intermediate carbocation, giving rise to the fluorinated products. As an example of this approach, O'Hagan and co-workers investigated the oxa- and aza-Prins reactions for the synthesis of 4-fluoropyrans and 4-fluoropiperidines starting from homoallylic alcohols and various aldehydes. The fluorinated THP products were obtained in good yields, but with only moderate diastereoselectivity.[34] This method was extended to the aza-Prins reaction that utilizes *N*-tosylhomoallylamines to generate the corresponding 4-fluoropyrrolidines.



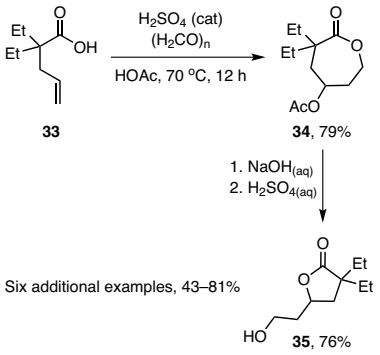

**Scheme 6.** Intramolecular Aza-Prins Cyclization of a Derivative of Lapachol. *(Ref. 18)*



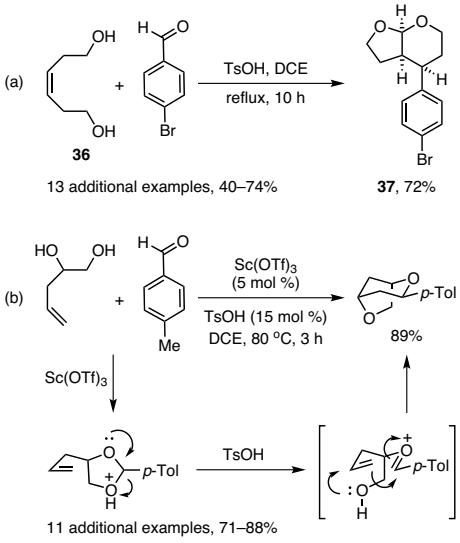

**Scheme 7.** Synthesis of Azabicyclo Compounds through an Aza-Prins Cyclization. *(Ref. 22)*

O'Hagan's group then investigated the Prins fluorination reactions under microwave conditions, and observed significantly reduced reaction times and higher conversions. However, there was a slight decrease in the diastereoselectivity of the reaction and, in some cases, an inversion of the diastereoselectivity. When a series of low-temperature (–20 °C) experiments were carried out in an attempt to improve the diastereoselectivity, dr increased from ~2:1 to 10:1 and yields remained good, but, not surprisingly, a significant increase in the reaction time was observed.

Prior to Loh and co-workers' recent disclosure,[35] all studies of the Prins fluorination reaction reported the almost exclusive formation of *cis*-2,6-disubstituted fluorinated di- or tetrahydropyrans. An efficient, highly diastereoselective synthesis of the *trans*-2,6-disubstituted




**Scheme 8.** Synthesis of Dihydrofuranones. *(Ref. 26)*




**Scheme 9.** Use of Unsaturated 1,6-Diol **36** for the Preparation of Furopyrans. *(Ref. 27,28)*

counterparts—useful in the development of new pharmaceuticals—would be highly desirable. Loh's group explored the Prins reaction of various allenic alcohols, e.g. **38**, with a variety of aldehydes using different Lewis acids (LAs) and fluorine sources.[35] Their research demonstrated that $BF_3$•$Et_2O$, acting both as an efficient Lewis acid and as a source of fluoride, gives the best results. The authors proposed a mechanism in which the Prins cyclization of the allenic alcohol takes place through a distorted chair transition state, in which a lone electron pair on the carbonyl oxygen of the ester group stabilizes the partial positive charge on the oxocarbonium carbon. This forces the carbonyl group to adopt an axial orientation, leading to the desired intermediate **40** and suppressing the generation of the undesirable intermediate **41**. In turn, intermediate **40** gives rise to the desired *trans*-2,6-disubstituted fluorinated dihydropyran **39**, selectively (**Scheme 10**).[35]

Saikia and co-workers have reported that $TiF_4$ can efficiently be employed for the strereoselective synthesis of substituted all-cis 4-fluorotetrahydropyrans via the halo-Prins cyclization.[36] A variety of aliphatic and aromatic aldehydes were reacted with a number of homoallylic alcohols to give good yields and high diastereoselectivities of the corresponding THPs. Moreover, acyclic and cyclic ketones were subjected to the reaction and found to be less reactive (5–6 h vs 2.5–4 h for the aldehydes), giving only moderate yields (50–70% vs 80–92% for the aldehydes). Cyclic ketones afford spiro compounds, as illustrated by the reaction of cyclohexanone, which leads to spirocyclic compound **42** in 70% yield (**eq 6**).[36]

### 3.5.2. Chlorinated and Brominated THPs

$InCl_3$ has been demonstrated to be an excellent Lewis acid for the insertion of a chlorine atom at the 4 position of THPs by the Prins cyclization. For example, the $InCl_3$-promoted diastereoselective Prins reaction of **43** with benzaldehyde led to the pentasubstituted tetrahydropyran derivative **44** (essentially as a single product) in which five stereogenic centers (up-down-up-down-up) were controlled (**Scheme 11**, Part (a)).[4,37,38] Subba Reddy and co-workers[39] reported another example of the effectiveness of $InCl_3$, whereby the synthesis of cis-fused hexahydro-1*H*-furo[3,4-*c*]pyran scaffolds containing chlorine proceeded smoothly under mild conditions (**Scheme 11**, Part (b)).[39]

$FeCl_3$ has been employed as an inexpensive, environmentally friendly, and stable Lewis acid to promote the halo-Prins cyclization of 3-buten-1-ol with several aldehydes.[40] The cyclization affords the corresponding *cis*-4-halo-2-alkyltetrahydropyrans in generally excellent yields.[40] The reaction works quite well with both aliphatic and aromatic aldehydes and, when $FeBr_3$ is employed, the 4-bromo-substituted analogue is formed. Liu and Loh have disclosed an efficient and highly stereoselective Prins cyclization leading to *cis*-2,6-dialkyl-3,4-dibromotetrahydropyrans from terminal vinyl bromides. This method employs $InBr_3$ as the Lewis acid and TMSBr as the source of bromide ion.[41]

Cascade reactions can be very powerful transformations in organic synthesis.[42] The first examples of a Mukaiyama aldol–Prins (MAP) cascade cyclization reaction were reported by Rychnovsky's group, whereby a very reactive allylsilane served as the internal nucleophile in a rapid and clean Prins cyclization.[43] Rychnovsky and co-workers also described the use of simple alkene substrates in MAP cyclizations and the importance of selecting the appropriate Lewis acid to promote the reaction. The attraction of such a sequence is that it forms two new C–C bonds, a ring, and three new stereogenic centers. Initially, the Mukaiyama aldol addition and Prins cyclization with the simple alkene **45** was evaluated using previously optimized conditions.[43] The reaction of **45** with 2.5 equiv of dihydrocinnamaldehyde in the presence

of $BF_3$•$OEt_2$ and 2,6-di-*tert*-butylpyridine (2,6-DTBP) at –78 °C led to the unexpected product **46** in 82% yield. The more powerful Lewis acids, $TiCl_4$ and $TiBr_4$, did not produce 1,3-dioxane **46** and gave the best yields of **47**. $TiBr_4$ was particularly effective and gave adduct **47** in 72% yield (**eq 7**).[44]

### 3.5.3. Iodinated THPs

The mild Lewis acidic nature of molecular iodine[45] has been exploited by Yadav and co-workers in the first direct and metal-catalyst-free Prins cyclization of homoallylic alcohols with aldehydes for the rapid synthesis of highly substituted iododihydro- and iodotetrahydropyrans in good yields and selectivities under neutral conditions.[46,47] Other reagents such as LiI, KI, and NaI failed to produce the desired product. Aliphatic, simple aromatic, and moderately activated aldehydes gave higher yields of products than strongly activated or deactivated aldehydes. This same research group reported a simple and metal-catalyst-free Prins cyclization for the synthesis of highly substituted tetrahydropyrans from sugar-based homoallylic alcohols and aldehydes using molecular iodine under neutral conditions.[46]

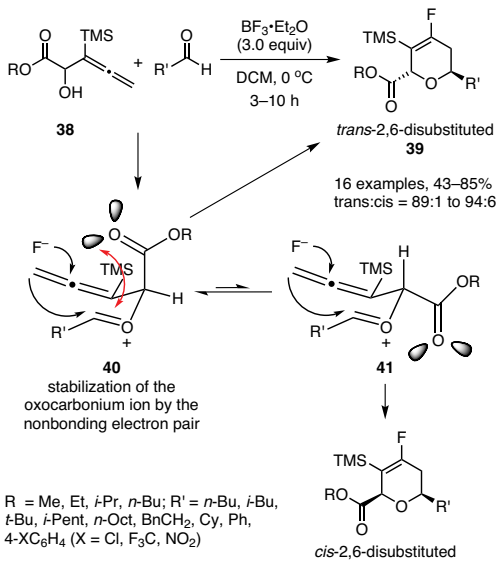
When silylated secondary homopropargylic alcohols were subjected to the same experimental protocol with aldehydes, highly substituted dihydropyrans were rapidly formed by a Prins cyclization (**Scheme 12**).[47] The reactions were completed in 3 hours or less, and the all-cis products were obtained in 72–90% yields. The cis selectivity presumably arises from an *E* oxocarbenium ion formed via a chair-like transition state. The optimal geometry of this oxocarbenium ion places the hydrogen atom at C4 in a pseudoaxial position, which favors equatorial attack of the nucleophile.

TMSI, generated in situ from TMSCl and NaI, has been employed as an iodide source in the Prins cyclization of homoallylic and homopropargylic alcohols with various ketones leading to 2,2-disubstituted 4-iodotetrahydropyrans, spirocyclic 4-iodotetrahydropyrans, and spirocyclic 4-iodo-5,6-dihydro-2*H*-pyrans.[48] In the presence of iodide, no trapping of the intermediate 4-tetrahydropyranyl carbocation by acetonitrile was detected.

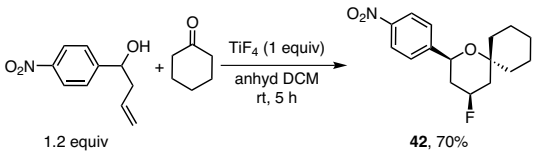
The synthesis of 4-iodotetrahydropyrans by the Prins cyclization can also be performed in the presence of a combination of $CeCl_3$•$7H_2O$ and LiI.[49] This approach can be applied to both aldehydes and ketones, but requires a higher temperature (reflux in dichloroethane), with the best results achieved with 1 equiv of $CeCl_3$. In contrast, CAN and $Ce(OTf)_3$ were not effective and led to hydroxylated side products. Gallium triiodide (35 mol %) has also been employed for the synthesis of 4-iodotetrahydropyrans at room temperature in 10–25 min and in 82–89% yields.[50]

## 3.6. Macrocyclization Involving the Prins Reaction

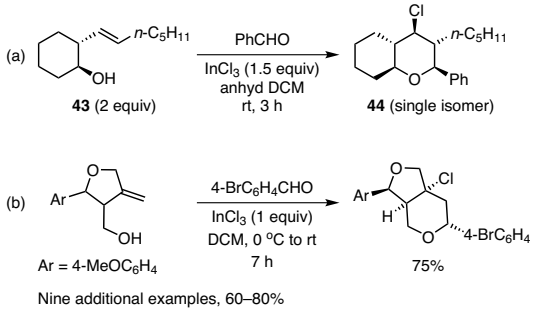
Oxacyclic macrodimers constitute an important class of natural products that possess a wide range of structural complexity and bioactivity,[51] and are popular targets for synthetic chemists.[52] Rychnovsky and co-workers introduced a new sequential dimerization–macrocyclization based on the Prins cyclization for forming symmetrical macrocycles.[53] This approach is illustrated by the optimized conditions in the example in **equation 8**. A variety of Lewis acids, including other Re and non-Re ones, were investigated and found to be inferior to $O_3ReOSiPh_3$. Other reaction parameters; such as temperature, concentration, and substrate scope; were also examined: Both acetals and aldehydes were found to be viable substrates, whereas increasing the reaction temperature to 40 °C did shorten the reaction times but did not improve the yields. The usefulness of this strategy was demonstrated in a successful synthesis of a model for clavosolide A, a marine sponge metabolite.



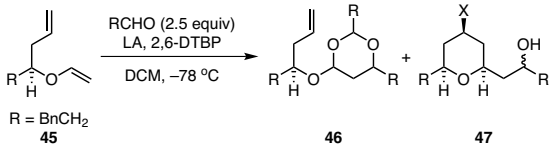

**Scheme 10.** Fluorinated Dihydropyrans by the Prins Cyclization of Allenic Alcohols. *(Ref. 35)*




**eq 6** *(Ref. 36)*




**Scheme 11.** Chlorinated THPs by the Prins Cyclization of Homoallylic Alcohols. *(Ref. 4,39)*

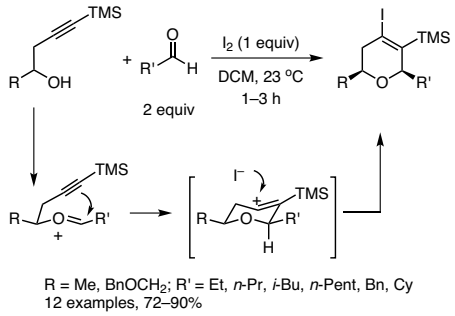



Six additional examples using $TiBr_4$: 53–82% of **47**, dr = 1:1 to 1.3:1

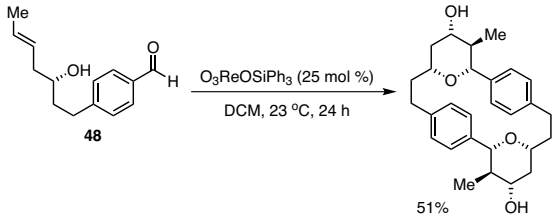
| LA | X | 46 | 47 |
|---|---|---|---|
| $BF_3$•$OEt_2$ | --- | 82% | nd |
| $TiCl_4$ | Cl | nd | 62% |
| $TiBr_4$ | Br | nd | 72% |

nd = not detected.

**eq 7** *(Ref. 44)*




**Scheme 12.** Prins Cyclization of Silylated Secondary Homopropargylic Alcohols with Aldehydes under Mild, Metal-Catalyst-Free Conditions. *(Ref. 47)*




**eq 8** *(Ref. 53)*

## 4. Conclusion

The Prins cyclization is an efficient reaction for the stereoselective synthesis of substituted tetrahydropyran rings, and has significantly advanced in the past few years, as demonstrated by the number of applications described in the literature. This reaction is, in some cases, the method of choice for the preparation of natural products and biologically active compounds that feature the tetrahydropyran moiety in their structures.

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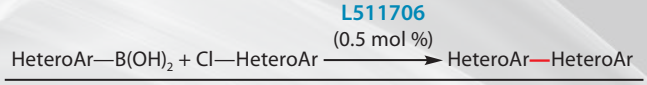


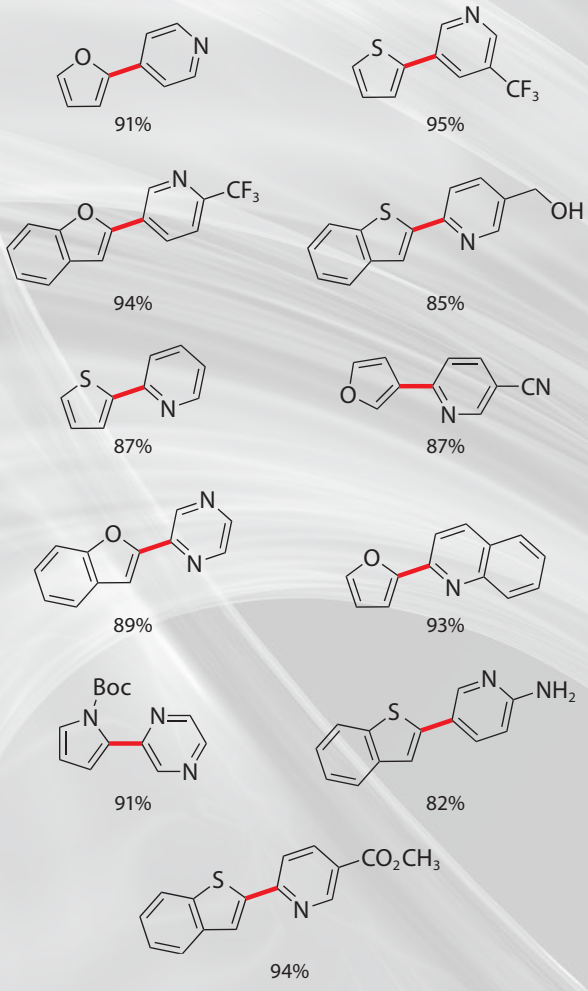


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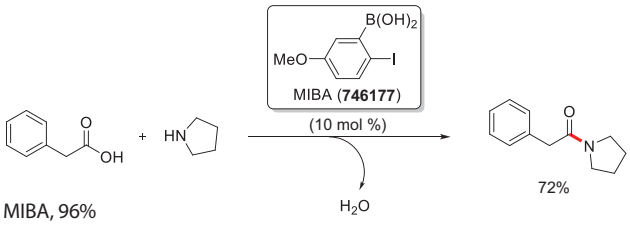


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## ABOUT OUR COVER

Detail from ***A View of the Mountain Pass Called the Notch of the White Mountains (Crawford Notch).*** Courtesy National Gallery of Art, Washington, DC.

Born and raised in England, Thomas Cole (1801–1848) immigrated with his family to the USA in 1818. Following a series of unremarkable jobs as engraver (England and Philadelphia) and itinerant portrait painter (Ohio), he moved, at 22, to Philadelphia to study at the Pennsylvania Academy of the Fine Arts. While he was largely self-taught, he benefitted from two trips to Western Europe, where he traveled extensively, met prominent artists such as Turner and Constable, and studied the Old Masters, such as Raphael. A founder of the Hudson River school of painting, he was a big influence on American landscape painters of the mid-19th century among whom is his pupil, Frederic Edwin Church. The subjects for many of his canvases were his beloved Hudson River valley, Catskill Mountains (New York), and White Mountains of New Hampshire.

***A View of the Mountain Pass Called the Notch of the White Mountains (Crawford Notch)*** (oil on canvas, 102 x 155.8 cm) is a wonderful example of Cole's desire to create a more sophisticated form of landscape painting that expresses a higher meaning.* This realistic and exquisitely detailed landscape, with its warm autumnal yellow and red colors and contrasting brightly lit and dark areas, embodies Cole's romance with America's unspoiled and beautiful wilderness and his dismay at its fast disappearance as a result of encroaching human activity (represented in the foreground by the cut trees, road, structures, and farming). By rendering the human figures small and on the move and the landscape majestic, he is reflecting on man's insignificance and fleeting existence vis-à-vis nature's timeless beauty and power.

**This painting was acquired by the National Gallery of Art, Washington, DC, through the Andrew W. Mellon Fund.**

** Cole has included elements in this painting that hint at what his message likely is. Can you guess what they are? To find out, visit* ***Aldrich.com/acta463***

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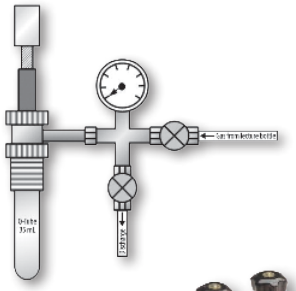

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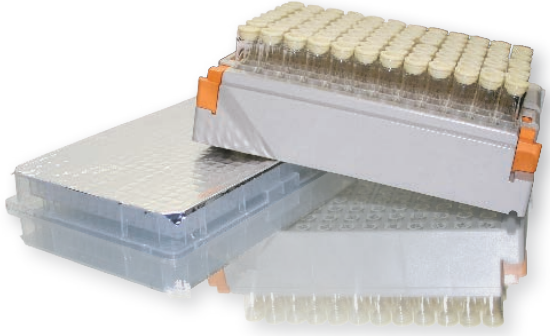

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# Scientist-Led High-Throughput Experimentation (HTE) and Its Utility in Academia and Industry


Prof. J. R. Schmink


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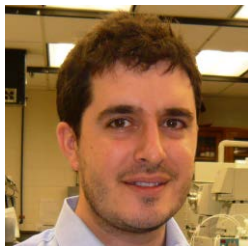
Dr. S. Berritt

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**Keywords.** High-Throughput Experimentation (HTE); catalysis; palladium catalysis; parallel microscale screening; Suzuki–Miyaura Reaction (SMR).

**Abstract.** High-Throughput Experimentation is emerging, in both the academic and industrial settings, as a powerful tool for developing new synthetic methodologies. This approach has the advantage of being highly transferable from one reaction type to another. The numerous variables associated with transition-metal-catalyzed methodology development complement HTE techniques perfectly. This report highlights recent (2009–2013) advances in the application of HTE in synthetic organic chemistry.

## Outline



## 1. Introduction

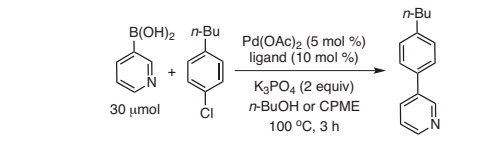
Developed in the 1980s to improve the efficiency of drug discovery and optimization, high-throughput methods involved a variety of experimental techniques in which thousands of compounds could be created rapidly using *parallel synthesis*, and very general conditions were employed to produce a collection of related organic compounds.[1] In contrast, high-throughput experimentation (HTE) was conceived as a valuable tool to accelerate the discovery and implementation of efficient new methodologies. Where parallel synthesis seeks to utilize previously developed protocols to make new compounds, HTE aims to rapidly develop the best conditions to effect a specific transformation, oftentimes on a very specific and/or problematic substrate or substrate class. In short, HTE (i) allows for the rapid refinement of existing protocols, (ii) assists in the development of completely new reaction parameters, and (iii) can afford non-obvious lead "hits" that might otherwise remain unexplored due to the number of individual reactions that would have to be conducted when more traditional approaches are employed.

To expedite the development of new synthetic methodologies, organic chemists have started to borrow screening approaches from their biochemistry (enzymatic catalysis)[2] and medicinal chemistry

(library synthesis)[3] colleagues. The past decade has seen numerous entries into the field that can be described broadly as "high throughput reaction optimization". While these meritorious works have contributed to continued scientific development, they generally do not report broadly applicable techniques or approaches. Past reports on high-throughput reaction optimization (HTRO) can be broken into two classes: the reports tend to be either reaction specific or reaction-type specific, or tend to rely on happenstance discovery. The broader chemistry community has been slow to adopt reaction-specific HTROs, because of the narrow range of potential applications. Similarly, "multidimensional"[4] or "accelerated serendipity"[5] approaches that rely upon chance discovery often yield interesting leads, but, for the chemist who is looking to develop a *specific transformation* in order to advance a particular project, they do not constitute a viable strategy. In contrast, the targeted, scientist-driven high-throughput experimentation platform is reliable, scalable, consistent across the platform, and is amenable to the entire range of reaction conditions that a synthetic organic chemist expects to have in his or her arsenal.

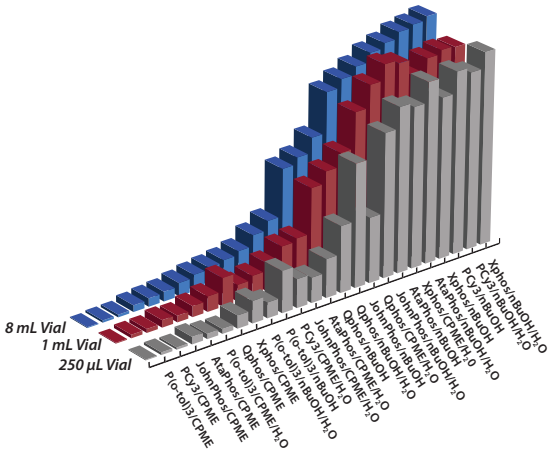
While enzymatic catalysis and library synthesis approaches seem obvious to build upon, the adaptations of these platforms are not as trivial as they first appear. Both of these approaches have one very powerful advantage: the platforms need to accommodate only *a single set of reaction conditions!* With few exceptions, enzyme catalysis is run in a buffered aqueous solution or in aqueous solution with an organic co-solvent at 5–37 °C. Similarly, library syntheses have as their variables only the coupling partners, and utilize the identical set of reaction conditions across the screen. In HTE, the array of requirements is inverted: A single transformation is examined across a screen with a diverse set of reaction conditions. This dynamic necessitated significant platform development in order to make the HTE screening platform as robust as possible and amenable to the widest range of potential reaction conditions.

It has been amply demonstrated that the reactivity of transition metals towards a specific and desired bond-forming event[6] can be attenuated, tuned, enhanced, varied, tweaked, or otherwise manipulated by altering the catalyst environment via the ligand type (N, P, O, etc.), the steric and electronic properties of the ligand, and even the peripheral reaction conditions such as solvent, base, temperature, additives, and co-catalysts. When one considers the diverse range of potential metals, ligands, solvents, and bases or other additives that are routinely employed in catalytic bond-forming events, it should be no surprise that chemists looking to rapidly optimize transition-metal-mediated synthetic approaches have embraced the HTE screening platform.




Ligand = $Ph_3P$, XPhos, JohnPhos, QPhos, $Cy_3P{\cdot}HBF_4$, A-$^{tg}$Phos
CPME = cyclopentyl methyl ether

Validation of HTE reaction scales rank-ordered conditions based on 0.16 mmol




**eq 1** *(Ref. 8a)*

## 2. The HTE Approach

In order to access any experimental conditions that could conceivably be used at the traditional gram scale, a *general* screening platform was needed that would fulfill the following requirements: (i) It must be able to accommodate the full range of conceivable reaction conditions routinely employed by the bench chemist. (ii) It must operate with high fidelity across the screen. (iii) The results obtained on the small scale (1–10 µmol) must scale with high correlation to synthetically useful scales (e.g., 1–100 mmol). (iv) The platform must be able to accommodate solvents ranging from hexane to HMPA. (v) It must operate across a range of temperatures from cryogenic conditions to temperatures that well exceed the boiling point of screened solvents. (vi) It must effortlessly accommodate homogeneous (easy) as well as heterogeneous and biphasic (not always so easy on a small scale) reaction conditions. (vii) The platform needs to be designed in such a manner as to allow for anhydrous conditions and for reactions to be run under an inert atmosphere or under an atmosphere of reactive gas such as $H_2$ or CO. (viii) Moreover, vial-to-vial fidelity must be achieved: When a reaction fails, there must be a reasonably high level of certainty that it does so because the conditions are non-ideal, not simply because the catalyst was incorrectly dosed, solvent was lost, mass transfer was poor, or the desired temperature on that particular location on the plate was not achieved. (ix) Finally, for this technology to be widely adopted and feasible, the reaction size must be scaled such that running 24, 96, 192, 384 reactions, and so on does not require an exorbitant outlay of reagents. Ideally, the chemistry should be developed using 1–5 mg per reaction.

In collaboration with chemists at Merck & Co., and using a relatively non-trivial Suzuki–Miyaura reaction (SMR)[7] at three different reaction scales (5 µmol in 250 µL vials, 20 µmol in 1 mL vials, and 0.16 mmol in 8 mL vials), we were able to validate that the platform indeed gives the same result on scale up. The 6x2x2 screen was carried out in triplicate utilizing six diverse ligands, two solvents, and with and without water, and resulted in good reproducibility (**eq 1**).[8a] The excellent reaction fidelity among the different reaction scales allows the chemist to conduct 24 discrete reactions at the 25 µmol scale, using only approximately 32 mg of material,[8b] and to be confident in the ability to scale the screening hits with similar results.

Often, HTE reaction optimization utilizes an iterative approach. An initial screen would usually aim to cast a wide net, exploring diverse conditions. Typically, qualitative or semi-quantitative analysis will yield preliminary hits, and, even though these early conditions may only provide the desired compound in <10% conversion, the initial results would be used for the second iteration of screens and would rapidly be improved upon.

## 3. Typical Situations Where HTE Excels

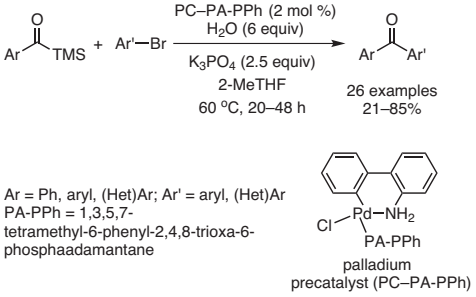
### 3.1. New Methodology Development

The third iteration of a series of sequential screens for the development of a new palladium-catalyzed cross-coupling of arylacylsilanes with aryl bromides is illustrated in **eq 2**.[9] The use of inorganic bases in anhydrous solvents, i.e. heterogeneous conditions, was needed to effect this transformation. Due to the significant work from prior development of robust dosing techniques, the scientists could confidently run reactions in 100 µL of solvent, accurately dosing 30 µmoles of $K_3PO_4$. By the third iteration, conditions had been developed to the point where a broad range of phosphine ligands could effect the desired reactivity to some extent, although one ligand stood out (1,3,5,7-tetramethyl-6-phenyl-2,4,8-trioxa-6-phosphaadamantane, PA-PPh), providing enhanced reactivity as compared to the other 95 ligands screened. Subsequent screens designed around this ligand ultimately led the authors to disclose a new methodology, reporting the synthesis of 26 diverse benzophenones.[9]

### 3.2. Logical Extensions of Known Reactions

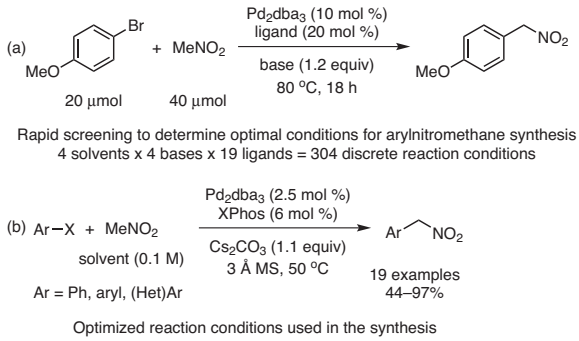
The palladium-catalyzed cross-coupling of the higher congeners of nitroalkanes with aryl halides is known.[10] However, the synthetically more useful cross-coupling of nitromethane with aryl halides gives low yields and multiple side products. Wishing to capitalize on the use of the arylnitromethane moiety for further synthetic manipulations, Kozlowski and co-workers carried out an HTE screen of 19 achiral phosphine ligands, four inorganic bases, and four solvents (**Scheme 1**).[11]

Interestingly, di-*tert*-butyl ligands that worked well in a prior nitroalkane coupling failed to give the desired product; rather, ligands possessing bis-cyclohexyl phosphines such as XPhos and BrettPhos gave good conversions to products. Correlating HPLC conversion with reaction purity (noting side products such as aldehyde formation and desbromination) XPhos, THF, and $K_3PO_4$ gave the best overall reaction profile. Further optimization resulted in nitromethane being utilized as the reaction solvent, allowing 19 diverse arylnitromethanes to be synthesized.

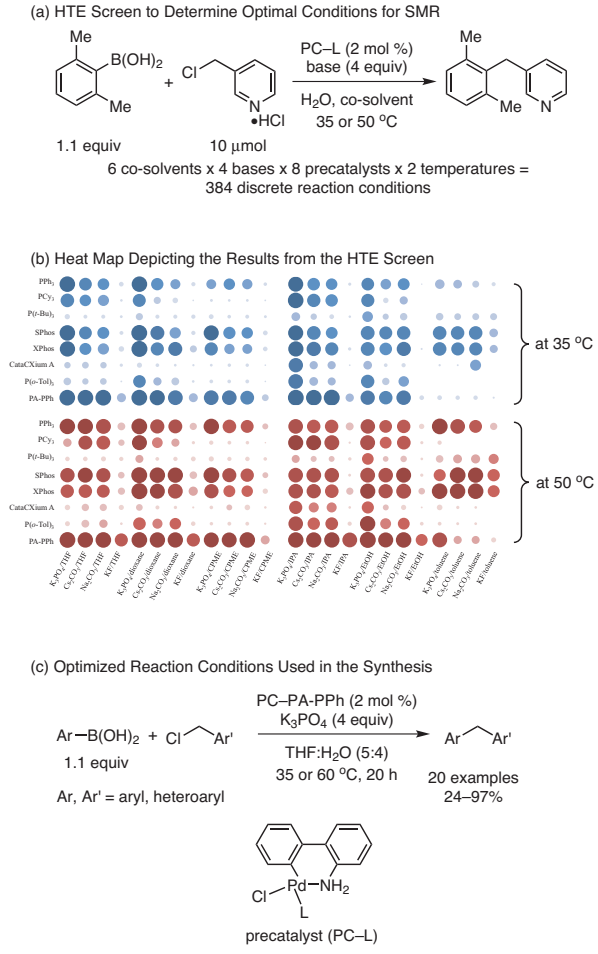



Initial screening of 95 ligands [Ar = Ph, Ar' = 4-MeOC$_6$H$_4$, [(C$_3$H$_5$)PdCl]$_2$ (2.5 mol %), ligand (10 mol %), $K_3PO_4$, 1,4-dioxane, 100 °C, 15 h] had identified the following ligands as being the best in effecting the conversion to 4-methoxybenzophenone: PA-PPh >> DPEPhos > QPhos > (2-Tol)$_3$P ~ dippf > (*t*-Bu)$_2$PPh > (4-Anis)$_3$P ~ CyPPh$_2$ ~ (Cy$_2$P)-Mes-Im ~ dppp ~ dppf

**eq 2** *(Ref. 9)*




**Scheme 1.** Palladium-Catalyzed Nitromethylation of Aryl Halides. *(Ref. 11)*




**Scheme 2.** Employing HTE for the Development of Suzuki–Miyaura Conditions to Cross-Couple Arylboronic Acids with Heteroarylmethyl Chlorides. 384 Reaction Conditions Were Screened, Providing the Range of Results Depicted in the Heat Map. *(Ref. 16)*

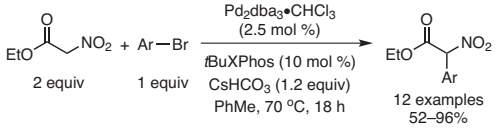
### 3.3. Refinement of Existing Transformations

The Suzuki–Miyaura reaction (SMR) is one of the most studied reactions of the past two decades.[12] It has become a workhorse in the industrial setting, and two recent reports indicate that, as of 2008, palladium catalysis represents over 60% of all C–C bond formations carried out by medicinal chemists.[13] While there are numerous examples that utilize SMR to generate all-carbon diarylmethane scaffolds,[14] there had been relatively few examples of basic *N*-heterocylic-halomethyl (*N*-Het-$CH_2X$) couplings.[15]

In an effort to synthesize diverse heterodiarylmethanes, Schmink and Tudge performed a multidimensional screen, focusing on conditions known to be amenable to SMR (**Scheme 2**).[16] They simultaneously screened for reactivity at two temperatures (35 and 50 ºC) using eight ligands, four inorganic bases, and six organic co-solvents. In order to maximize the operational simplicity, the authors utilized a "precatalyst" platform to deliver both the palladium and ligand in a single dosing event.[17,18] Assay yields determined by HPLC ranged from no reaction to quantitative conversion. The authors reported that 45 out of 384 sets of reaction conditions resulted in an assay yield of 90% or greater. Among these, each base and co-solvent is represented as well as seven out of the eight catalysts. After this single screen, the authors employed the optimized conditions—phosphaadamantyl precatalyst (PA-PPh) with THF as co-solvent and aqueous potassium phosphate as base—to synthesize 20 diverse heterodiarylmethanes.

### 3.4. Directed Serendipity

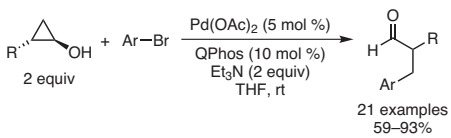
Bellomo et al. employed HTE techniques to improve upon a thermal cyclization protocol in the synthesis of pyrimidinone heterocycles.[19a] A previous synthesis of this compound class relied on a two-step protocol utilizing a Michael addition of an *N*-hydroxyamidine to an acetylynic diester with a subsequent thermal rearrangement to provide the core scaffold in 15–50% yields.[19b]




Ar = Ph, $XC_6H_4$ (X = 4-Cl, 4-MeO, 4-$F_3C$, 4-Me, 3-$F_3C$, 2-MeO, 4-Ac), 2-Np, 6-MeO-2-Np, benzofuran-5-yl, 1-methylindol-5-yl

Optimized Conditions for the Palladium-Catalyzed α-Arylation of Ethyl Nitroacetate

**eq 3** *(Ref. 21)*




α-Benzyl aldehydes by a tandem palladium-catalyzed cyclopropanol ring-opening–arylation

**eq 4** *(Ref. 25a)*

The development of a reaction discovery platform, containing highly practical pre-dosed compounds which might activate a reaction through a variety of different mechanistic pathways enabled the scientists to rapidly screen for best conditions. The diversified plate consisted of representative Brønsted and Lewis acids and bases, transition-metal catalysts, mono- and bidentate phosphorus and nitrogen ligands, oxidants, reductants, phase-transfer reagents, and miscellaneous additives such as molecular sieves and crown ethers. The platform allowed one chemist in a single day to set up 475 experiments and analyze the results using MISER chromatography,[20] a fast analytical technique. Employing this platform and microscale optimization techniques led to the identification of two high-quality catalytic systems for the construction of the highly functionalized, biologically important pyrimidinone core of HIV Integrase inhibitors.

## 4. Survey of Recent Applications

### 4.1. Cross-Coupling of Activated Methyl, Methylene, and Methine Carbon Atoms

#### 4.1.1. Ethyl Nitroacetates

Kozlowski and co-workers have utilized HTE to develop a useful strategy for synthesizing 2-aryl-2-nitroacetates by a palladium-catalyzed α-arylation of nitroacetates.[21] In light of the significant difficulties in coupling the highly acidic substrate, such as poor nucleophilicity and propensity to form O,O'-bound intermediates, initial attempts utilizing procedures based on literature precedents[22] met with little success. As the initial reaction showed some product formation, the authors conducted a comprehensive screen of 24 ligands, four bases, two solvents, and two palladium sources to further elicit the details of the reaction mechanism. Out of these 192 discrete combinations, surprisingly only 3 ligands gave rise to any product: BrettPhos, $Me_4$*t*BuXPhos, and *t*BuXPhos—with *t*BuXPhos providing the cleanest reaction profile (**eq 3**).[21] One final multidimensional HTE experiment expanded on solvent selection, palladium source,[23] temperature, and base (a total of 192 discrete combinations) to identify the best conditions for the reaction.[24] The *tert*-butyl and methyl nitroacetates also participated in this reaction.

#### 4.1.2. Arylnitromethanes

The related arylnitromethane moiety is synthetically useful, since the nitro group can be elaborated into synthetically valuable aromatic aldehydes and oximes. Kozlowski and co-workers have utilized parallel microscale screening to find a way to cross-couple aryl halides directly with nitromethane. This circumvented the two-step α-arylation–decarboxylation of nitroacetates. Employing dicyclohexylphosphine-type ligands and conducting the reaction in nitromethane as solvent provided good conditions to prepare 19 diverse arylnitromethanes in 44–97% yields (see Scheme 1, Part (b)).[11]

#### 4.1.3. Strain-Activated Carbon Atoms

Walsh's research group recently disclosed a route to synthesize α-benzyl aldehydes by employing a tandem palladium-catalyzed ring opening of cyclopropanols via a C–C cleavage step followed by arylation of the palladium homoenolate. HTE was used to develop room temperature conditions, and 21 examples were disclosed in 59–93% isolated yields (**eq 4**).[25a]

#### 4.1.4. Aryl-Activated Carbon Atoms

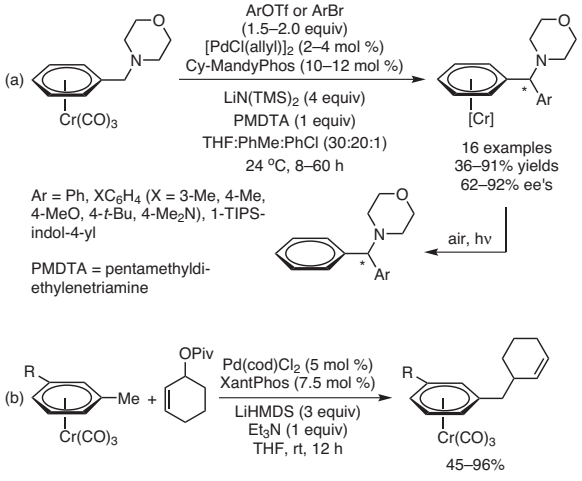
Building on earlier work detailing the α-arylation of ($\eta^6$-tolyl)$Cr(CO)_3$ complexes,[25b] Walsh and co-workers have employed HTE techniques to optimize the reaction parameters of the catalytic intermolecular arylation of weakly acidic $sp^3$ C–H bonds. Even though initial conversion was

low, Cy-MandyPhos provided the best enantioenrichment among the 192 chiral mono- and bidentate phosphine ligands that were screened. Further screening led to the optimal conditions, and the use TMEDA or PMDTA to break up lithium aggregates improved the reactivity and allowed aryl bromides and triflates to be coupled to the chromium-complexed arenes in yields of up to 91% and up to 92% ee. This strategy was utilized to carry out the first catalytic asymmetric arylation of activated benzylic amines (**Scheme 3**, Part (a)).[26] The authors employed HTE to screen for the best conditions to effect the palladium-catalyzed Tsuji–Trost allylic substitution reaction starting with the $\eta^6$-tolylchromium scaffold (Scheme 3, Part (b)).[27] They found that $Pd(cod)Cl_2$, XantPhos, and $LiHMDS/Et_3N$ were optimum conditions, with triethylamine as additive enhancing the reactivity and thus allowing the reaction to proceed at room temperature.[28] Demetallation of chromium was achieved under oxidative photolysis conditions.

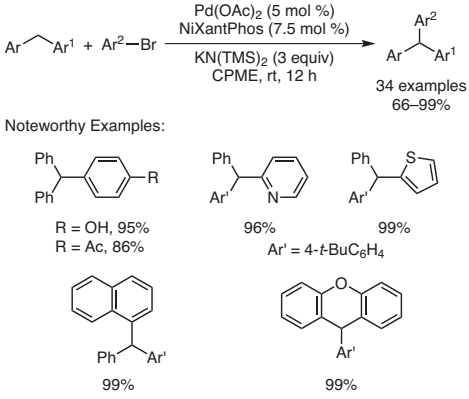
Application of sequential HTE screens to the chromium-free direct $C_{sp3}$–H arylation of *un*activated diarylmethanes by a deprotonative cross-coupling process (DCCP) led to the identification of one base and ligand combination (KHMDS, NiXantPhos) out of a possible 1344 (12 bases x 112 ligands). The protocol developed enabled the synthesis of a variety of sterically and electronically diverse aryl- and heteroaryl-containing triarylmethanes at room temperature (**eq 5**).[29] Remarkable chemoselectivity was observed in the presence of substrates that typically undergo O-, N-, enolate-, and $C_{sp2}$–H arylation.

The authors noted in the course of their investigation that LiHMDS and NaHMDS did not give rise to the triaryl product. Knowing from their earlier studies that amine additives increased reaction performance, the authors conducted a rapid HTE screen of mono- and polydentate Lewis base additives[30] that are known to improve synthetic performance. Polydentate ethers, 12-crown-5, 15-crown-5, diglyme, and the tetradentate amine HMTETA[31] gave the best hits and permitted the expansion of the scope of this reaction to heteroaryl bromides.[32a]

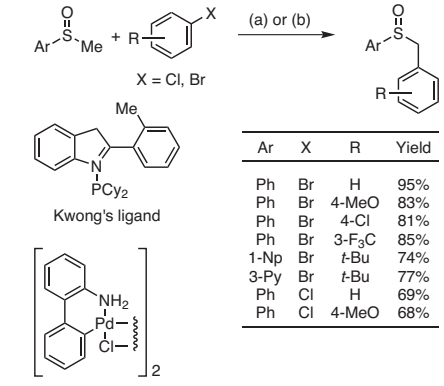
Walsh and co-workers have very recently reported a palladium-catalyzed direct α-arylation of unactivated sulfoxides with aryl halides (**eq 6**).[32b] Initial high-throughput experimentation explored strong bases and four solvents by employing the $Pd(OAc)_2$–NiXantPhos system previously employed for the arylation of diarylmethanes, whereby LiO*t*-Bu in CPME generated the desired product in moderate yield while other bases rendered lower yields or generated very messy reactions. The search for more active catalysts was continued with LiO*t*-Bu and CPME by testing a series of sterically and electronically diverse mono- and bidentate ligands. Of the 112 ligands examined, Kwong's indole-based phosphine[33] showed significant enhancement for this transformation. Under the optimized conditions, aryl bromides were cross-coupled successfully with sulfoxides. Initially aryl chlorides failed to undergo the desired cross-coupling. Modifying the protocol to include a water additive and the μ-chloro-dimer biphenylamino palladacyle allowed aryl chlorides to be coupled in moderate yields.




**Scheme 3.** Asymmetric α-Arylation of $\eta^6$-Benzylchromium Species. *(Ref. 26,27)*




**eq 5** *(Ref. 29)*

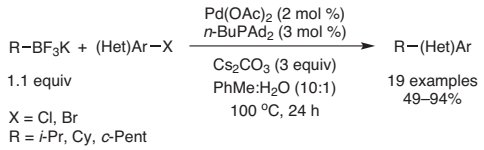



**eq 6** *(Ref. 32b)*

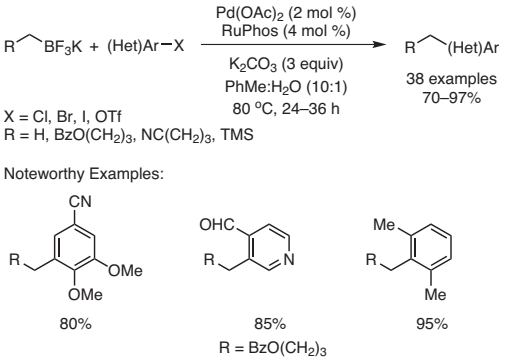
### 4.2. $C_{sp3}$–$C_{sp2}$ and $C_{sp2}$–$C_{sp3}$ Cross-Couplings Employing Boron-Based Nucleophiles

#### 4.2.1. Cross-Coupling of $C_{sp3}$ Organoboron Nucleophiles with $C_{sp2}$ Electrophiles

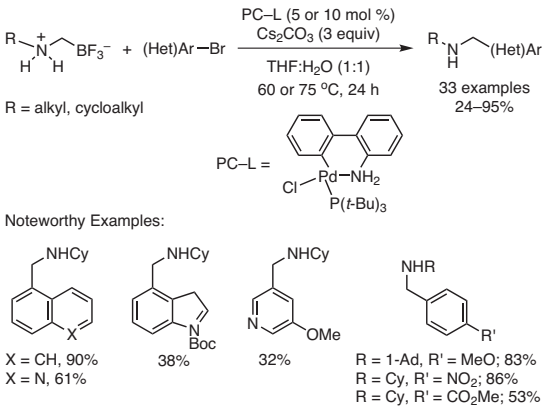
In the first comprehensive study of alkylboron, in particular secondary alkylboron, coupling to aryl chlorides, HTE was employed and led to the discovery of three catalyst systems capable of coupling secondary organotrifluoroborates with sterically and electronically demanding aryl chlorides and bromides. A ligand-dependent β-hydride elimination–reinsertion mechanism was implicated in the cross-coupling of more hindered substrates, leading in some cases to isomeric mixtures of coupled products (**eq 7**).[34]




**eq 7** *(Ref. 34a)*




**eq 8** *(Ref. 35)*

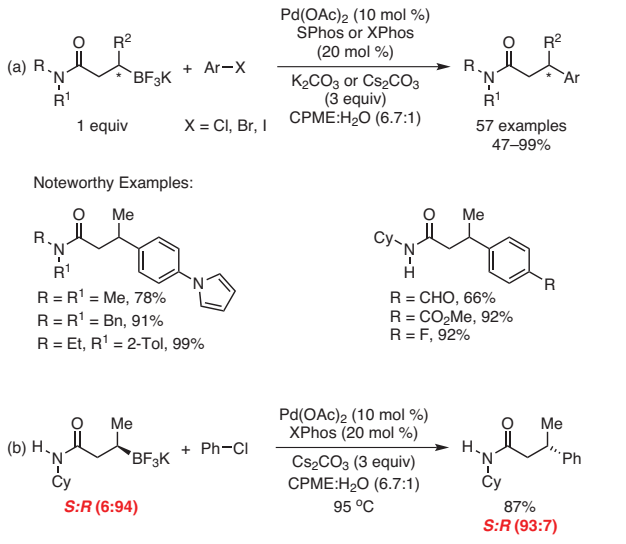



**eq 9** *(Ref. 37)*

HTE was utilized to develop two sets of conditions for the SMR of diversely functionalized primary alkyltrifluoroborates with a variety of aryl chlorides (**eq 8**).[35] These conditions were found to be amenable as well to coupling with aryl bromides, iodides, and triflates. The conditions that were previously identified through similar techniques to promote the cross-coupling of secondary alkyltrifluoroborates with aryl chlorides were not optimal for the primary alkyltrifluoroborates. Unlike the preceding examples, the pendant ether and acetal functionalities in the closely related alkoxyethyl and dialkoxyethyl trifluoroborates impacted their chemical reactivity enough that a unique set of conditions was developed, in which A-[ta]Phos was the best-performing ligand.

As an extension of their investigations of the coupling of a diverse range of aminomethyltrifluoroborates,[36] Molander's group applied HTE to the Suzuki–Miyaura reaction (SMR) of ammoniomethyltrifluoroborates with aryl and heteroaryl bromides in order to access the biologically relevant methylamine motif.[37] Until this report, this method was limited to the production of tertiary and primary amines. The optimum cross-coupling conditions included the inexpensive P(*t*-Bu)$_3$ aminobiphenyl palladium precatalyst in THF–$H_2O$ and $Cs_2CO_3$. This method offers a new way to access biologically relevant motifs and, together with the previously developed methods, allows access to all three classes of aminomethylarenes (**eq 9**).[37]

The same research group reported on the stereospecific cross-coupling of enantioenriched *nonbenzylic* secondary alkyltrifluoroborates. By employing Buchwald catalysts of SPhos or XPhos, they found that the high selectivity for product formation (over an undesired β-H elimination pathway) could be realized by intramolecular coordination of an ancillary amide carbonyl to the metal center in the diorganopalladium intermediate. Interestingly, the authors




**Scheme 4.** Cross-Coupling of Aryl Halides with Secondary Potassium Organotrifluoroborates Containing an Ancillary Amide That Is Believed to Lead to an Unanticipated Inversion of Stereochemistry at the Reactive Center. *(Ref. 34b)*

speculated that this ancillary group leads to *inversion* of configuration at the newly formed tertiary carbon center (**Scheme 4**).[34b]

Molander's group employed HTE techniques to develop a palladium-catalyzed SMR between potassium 1-(alkoxy or 1-acyloxy) alkyltrifluoroborates and aryl or heteroaryl chlorides, providing access to protected secondary alcohols in high yields (**eq 10**).[38] The authors surmised that, in this case, the β-hydride elimination pathway is avoided through the use of the benzyl protecting group and proposed that it stabilizes the diorganopalladium intermediate by coordination of the arene to the metal center. In contrast to the group's reports of inversion of configuration in the case of the ancillary amide group in the trifluoroborate, here the stereospecific cross-coupling proceeds with complete retention of stereochemistry.

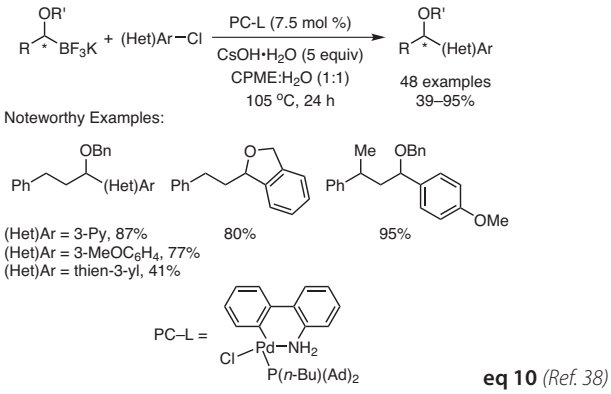
### 4.2.2. Cross-Coupling of $C_{sp2}$ Organoboron Nucleophiles with $C_{sp3}$ Electrophiles

Schmink and Tudge have employed HTE for the rapid development of a palladium-catalyzed SMR for the synthesis of highly functionalized nitrogen-containing diarylmethanes.[16] In almost all cases, highly efficient cross-couplings were observed at ambient temperature, and, throughout, only the most problematic substrates were coupled; i.e., those containing basic nitrogen atoms or containing multiple electron-withdrawing groups on the arylboronic acids. The broad substrate scope and respectable yields highlight the synthetic utility of this method. A study of the cross-coupling of alkyl electrophiles with potassium aryl- and heteroaryltrifluoroborates revealed that the $NiCl_2$•glyme–L-prolinol catalyst system was effective for the coupling of aryltrifluoroborates with primary or secondary chlorides (**eq 11**).[39] Nearly stoichiometric amounts of the organoboron species could be employed to cross-couple a large variety of challenging heteroaryl nucleophiles with satisfactory results. A second catalyst system, $NiBr_2$•glyme–bathophenanthroline, achieved the chemoselective reaction of $C_{sp3}$–Br bonds in the presence of $C_{sp2}$–Br bonds, and of $C_{sp3}$–Br/I bonds in the presence of $C_{sp3}$–Cl bonds.

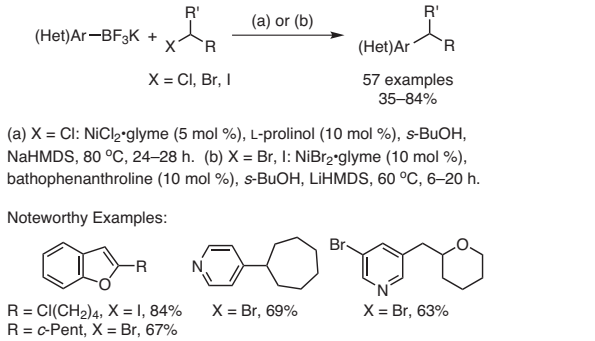
Molander's group disclosed very recently a protocol to synthesize α-(hetero)aryl esters and amides from organotrifluoroborate salts and α-chloro esters and amides by utilizing a Pd-catalyzed SMR (**eq 12**).[40] Using HTE, the authors developed conditions that avoid the use of strong base, do not necessitate an inert atmosphere or low-temperature formation of reagents, and do not require the use of a large excess of organometallic reagent. They discovered that addition of a catalytic amount of $Cu_2O$ improved yields when cross-coupling secondary amides with trifluoroborate salts. A variety of functional groups and heterocyclic compounds were tolerated.

## *4.3. Other Applications*

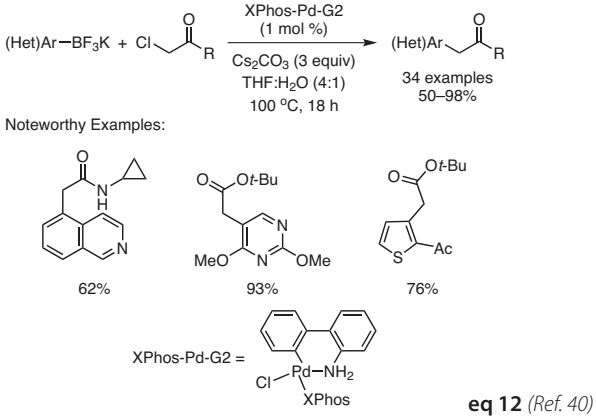
A simple and atom-economical synthesis of aryl- and heteroarylboronic acids (61 diverse examples) by a Pd-catalyzed direct borylation of aryl halides with tetrahydroxydiboron, $(HO)_2B–B(OH)_2$, or tetrakis(dimethylamino)diboron, $(Me_2N)_2B–B(NMe_2)_2$, has recently been reported by Molander and co-workers.[41a,b] The authors successfully combined this step with a Suzuki cross-coupling in an efficient one-pot synthesis of biaryls (26 examples).[41c] They later improved upon the methodology by developing an alternative direct borylation of halides and pseudohalides that utilizes a nickel catalyst at room temperature.[41d] Similarly, HTE permitted the development of successful reaction conditions for the synthesis of unsymmetrical diaryl ketones by a palladium-catalyzed cross-coupling of aryl bromides with acylsilanes (see eq 2).[9] Kozlowski and co-workers have employed HTE to optimize the reaction conditions of the palladium-catalyzed



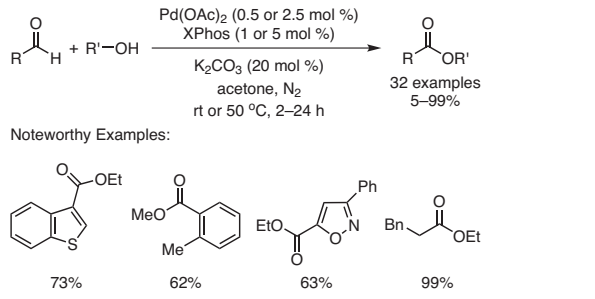

**eq 10** *(Ref. 38)*




**eq 11** *(Ref. 39)*




**eq 12** *(Ref. 40)*

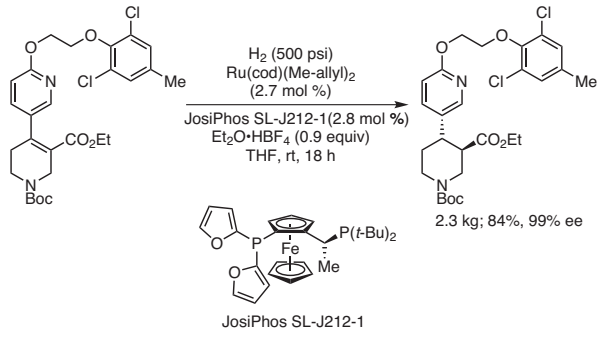



**eq 13** *(Ref. 43)*

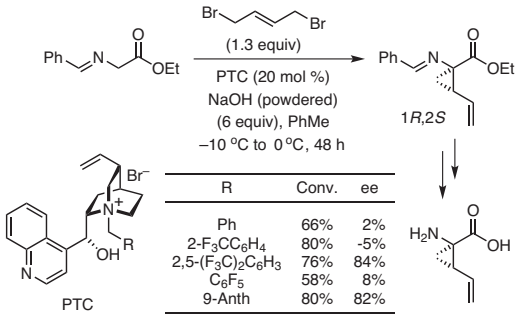
enantioselective Claisen rearrangement of propargyloxy indoles.[42] This led to a reduction of the reaction time from *5 days to 2 hours* with good yields (80–90%) but only moderate enantioselectivities (86–93% ee's) by using Au(III), (*R*)-BINAP, and $AgSbF_6$.

### 4.3.1. Palladium-Catalyzed Oxidative Esterification

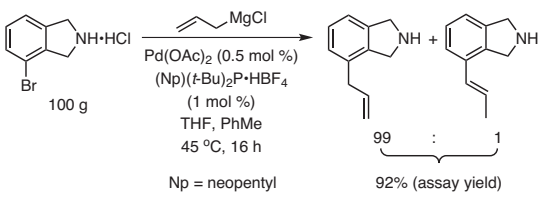
Capitalizing on the observation that aldehydes can be problematic substrates in palladium-catalyzed cross-coupling reactions leading, among others, to a small amount (<5%) of an ester side product, Tschaen et al. employed rapid screening to optimize the reaction conditions that select for ester formation from the aldehyde. Aliphatic and aromatic aldehydes were successfully converted into the corresponding esters using $Pd(OAc)_2$ and XPhos (**eq 13**).[43] The reaction utilizes a hydrogen-transfer protocol with acetone as the hydrogen acceptor, and provides an inexpensive and sustainable method for the conversion of aldehydes to esters that reduces the need for other oxidants.




**eq 14** *(Ref. 44)*




**eq 15** *(Ref. 45)*




**eq 16** *(Ref. 46)*

### 4.3.2. Asymmetric Hydrogenation

Molinaro and co-workers have developed a practical synthesis of Renin Inhibitor MK-1597,[44] a potent inhibitor of the renin receptor and possible lead in the treatment of hypertension. A key step in the synthesis utilizes an asymmetric hydrogenation of a tetrasubstituted ene-ester moiety. Employing microscale HTE, these researchers carried out >384 combinations of metal precursors, solvents, and chiral ligands, using 500 psi $H_2$ and identified the combination of Ru(cod)-(Me-allyl)$_2$, JosiPhos ligand SL-J212-1, and $HBF_4$•$OEt_2$ as giving the optimal results. Scaling up the optimized procedure provided 2.3 kg of the key intermediate in 84% isolated yield and 99% ee (**eq 14**).[44]

### 4.3.3. Phase-Transfer Catalysis

The scope of microscale HTE extends well beyond that of transition-metal-directed catalysis. Belyk, Xiang, and co-workers[45] utilized microscale HTE to develop a concise route to the ethyl ester of (1*R*,2*S*)-1-amino-2-vinylcyclopropanecarboxylic acid, an important structural motif in HCV NS3/4A protease inhibitors. The authors screened >100 different *cinchona* alkaloid derived phase-transfer catalysts (PTCs) for the dialkylation reaction between benzaldimine-protected ethyl glycinate and *trans*-1,4-dibromo-2-butene (**eq 15**),[45] and succeeded in identifying a cinchonidine-derived PTC that provided a 78% yield and 77.4% ee of the desired product on a multigram scale.

### 4.3.4. Kumada Coupling

High-throughput screening has permitted the rapid development of a practical and a cost-effective Kumada coupling on scale to synthesize 4-allylisoindoline from allylmagnesium chloride.[46] A general screen of palladium sources and readily available ligands was conducted, revealing $Pd(OAc)_2$ and (neopentyl)(*t*-Bu)$_2$P•$HBF_4$ as good candidates for this coupling and the mixed solvent system of THF and toluene to be crucial for suppressing olefin isomerization. The authors were able to conduct the transformation on a 100 g scale, with good yield and excellent regioselectivity (**eq 16**).[46]

## 5. Conclusions and Outlook

This review examined the last four years of the chemical literature dealing with parallel, microscale high-throughput screening. As the featured examples demonstrate, a wealth of chemistry has been developed in a rapid and cost-effective manner. Although several of the reported transformations are closely related, substrate variations have led researchers to develop a wide range of optimum reaction conditions. The simplicity of the screening tools allows even the inexperienced user to quickly set up an array of conditions to supplement the existing literature or develop novel chemistry. As more and more academic and industrial laboratories adopt HTE, the latter will become an increasingly invaluable asset.

## 6. Acknowledgements

The authors thank Professors Molander, Kozlowski, and Walsh and their respective research groups at the University of Pennsylvania. The authors are deeply indebted to Spencer D. Dreher, Shane W. Krska, and Matthew T. Tudge, at Merck & Co., for sharing with them this enabling technology. The authors acknowledge the National Science Foundation for financial support (NSF-GOALI 0848460).

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# Cross-Linked Enzyme Aggregates (CLEAs) in Organic Synthesis†


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**Keywords.** enzymes; immobilization; cross-linked enzyme aggregates; organic synthesis; green chemistry; sustainable processes.

**Abstract.** The immobilization of a wide variety of enzymes—hydrolases, oxidoreductases, lyases, and transferases—as cross-linked enzyme aggregates (CLEAs) and their applications in organic synthesis are reviewed. The examples are categorized on the basis of the functionality of the reaction product; e.g., alcohols, amines, carboxylic acids and derivatives, carbohydrates, amino acids and peptides, and even polyamides. The CLEA technology is a simple and effective way of immobilizing enzymes that offer several advantages when compared to the corresponding free enzymes, such as increased thermal stability, facile recovery and reuse, and high productivities as compared to carrier-bound enzymes. Selectivities are generally as high as, or even higher than, with the free enzymes. Co-immobilization of two or more different enzymes affords Combi-CLEAs which can be used effectively in one-pot, enzymatic cascade reactions.

## Outline

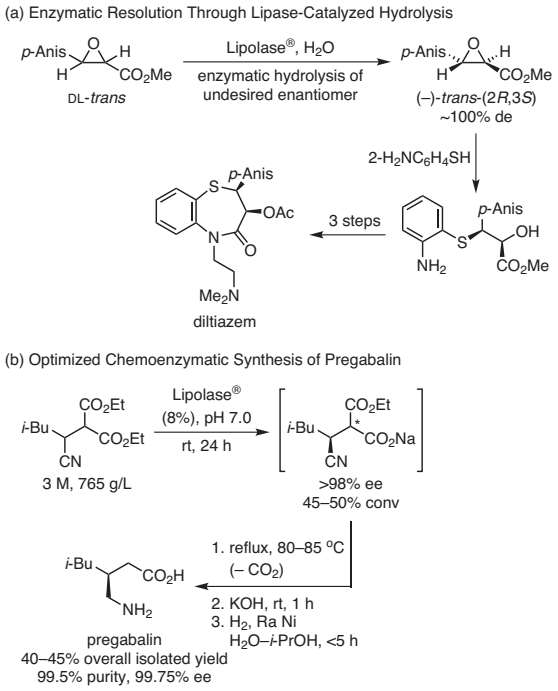


## 1. Introduction

Biocatalysis has emerged in the preceding two decades as a key enabling technology in the drive towards green and sustainable chemical manufacturing.[1,2] It is playing a decisive role, for example, in the synthesis of pharmaceuticals,[3] agrochemicals,[4] flavor and fragrances,[5] cosmetic ingredients,[6] and other fine chemicals.[7,8] Enzymes are Nature's sustainable catalysts. They are biocompatible, biodegradable, and are derived from renewable resources. Enzymatic processes are conducted under mild conditions (close to ambient temperature, atmospheric pressure, and physiological pH) in water, with high reaction rates and selectivities. Furthermore, the use of enzymes generally obviates the need for functional group protection and/or activation, affording synthetic routes that have fewer steps, generate less waste, and are more energy efficient than conventional organic syntheses. In short, enzymatic processes are more environmentally friendly, more cost-effective and, ultimately, more sustainable than many traditional organic syntheses.

This development was enabled by advances in modern biotechnology. Recombinant DNA techniques have made it, in principle, possible to produce virtually any enzyme for a commercially acceptable price.[9] By the same token, advances in protein engineering techniques, such as in vitro evolution,[10] have enabled the manipulation of enzymes such that they exhibit the required substrate specificity, activity, stability, pH profile, and other desirable properties. Natural enzymes are often highly selective catalysts, but they have evolved over millions of years to be able to convert their natural substrates in high rates in vivo. It is perhaps not surprising, therefore, that they are generally not sufficiently active or stable to sustain a high activity and productivity with non-natural substrates under more challenging conditions in vitro such as high substrate concentrations, non-aqueous media, and so forth. In

short, thanks to modern biotechnology, it is possible to produce most enzymes for commercially acceptable prices and to manipulate them in such a way that they exhibit the required properties.[11] This has made it possible to optimize an enzyme to fit a pre-defined optimum process, i.e., to be truly benign by design.[12]




**Scheme 1.** Successful Commercial Syntheses of (a) Diltiazem and (b) Pregabalin Using the Wild-Type Enzyme Lipolase®. *(Ref. 13,15)*

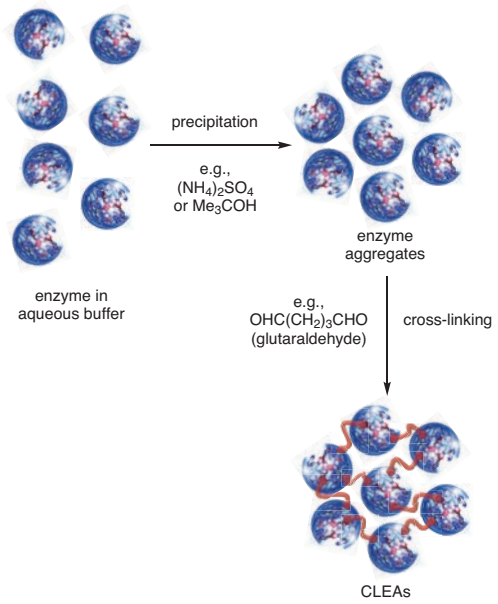



**Figure 1.** Preparation of Cross-Linked Enzyme Aggregates (CLEAs). *(Ref. 21)*

We note, however, that spectacular results have been obtained in some cases with wild-type enzymes without the need for protein engineering. An early example is provided by the enzymatic process for the synthesis of the key chiral intermediate in the manufacture of the antihypertensive drug, diltiazem. This process, developed and commercialized by DSM® Andeno in the 1980s, involves the highly enantioselective hydrolysis of a chiral glycidate ester catalyzed by *Thermomyces lanuginosus* lipase (EC 3.1.1.3), otherwise known as Lipolase® (**Scheme 1**, Part (a)).[13] The latter is an inexpensive, readily available enzyme which is used in a wide variety of industrial applications.[14] More recently, Pfizer employed the same enzyme to develop an extremely effective chemoenzymatic process for the manufacture of pregabalin (Scheme 1, Part (b)),[15] the active ingredient of the CNS drug Lyrica®. This second-generation route afforded a dramatic improvement in process efficiency by setting the stereocentre early in the synthesis (the golden rule of chirotechnology[16]) and enabling the facile racemization and re-use of the wrong enantiomer. The key enzymatic step was conducted at an impressive substrate concentration of 3 M (765 g/L) in a largely aqueous process with dramatically reduced organic solvent usage. Compared to the first-generation manufacturing process, the new process afforded a higher yield and a five-fold reduction in the E factor[17] from 86 to 17.

Notwithstanding the many benefits of biocatalysis, the industrial application of enzymes is often hampered by a lack of long-term operational stability and difficult recovery and re-use of the enzyme. These drawbacks can generally be overcome by immobilizing the enzyme.[18] In addition to more convenient handling of the enzyme, as a solid rather than a liquid formulation, immobilization allows for the facile separation of the enzyme from the product, thereby minimizing or eliminating protein contamination of the product. By the same token, this facilitates downstream processing and provides for the efficient recovery and re-use of the enzyme and, thus, its cost-effective use in batch or fixed-bed operation. Moreover, an immobilized enzyme cannot easily penetrate the skin and, therefore, it exhibits low or no allergenicity. A further benefit is generally enhanced stability, under both storage and operational conditions, e.g., towards denaturation by heat or organic solvents or by autolysis. Improved enzyme performance and re-use lead to higher catalyst productivities (kg product / kg enzyme), which determine the enzyme cost per kg product. Moreover, since most biocatalytic processes are performed under roughly the same conditions of (ambient) temperature and pressure, it is eminently feasible to integrate multiple steps into enzymatic cascade processes.[19] Co-immobilization of two or more enzymes then affords multifunctional solid biocatalysts capable of catalyzing such cascade processes.[20]

Methodologies for enzyme immobilization fall into three categories: (i) binding to a support (carrier), (ii) entrapment (encapsulation) in a carrier, and (iii) cross-linking. The use of a carrier inevitably leads to "dilution of activity", owing to the introduction of a large portion of non-catalytic ballast, ranging from 90% to >99%, which results in lower space-time yields and productivities. Consequently, there is an increasing interest in the use of *carrier-free immobilized enzymes*, as exemplified by cross-linked enzyme aggregates (CLEAs),[21,22] in industrial organic synthesis. CLEAs are prepared by precipitating the enzyme from aqueous solution, using a salt or a water miscible organic solvent or polymer, and the resulting enzyme aggregates are then cross-linked with a bifunctional reagent, usually glutaraldehyde (**Figure 1**).[21] CLEAs have several advantages in the context of industrial applications: (i) They do not require a highly pure enzyme; they can be prepared from very crude enzyme preparations, even directly from a crude cell-free lysate obtained from fermentation broth. (ii) Since they are carrier-free, they avoid the costs associated with the use of (often expensive)

carriers and exhibit high productivities and facile recovery and recycle. (iii) Furthermore, they generally have improved storage and operational stability with regard to denaturation by heat, organic solvents, and autolysis and are stable towards leaching in aqueous media.

This review surveys the applications of CLEAs in organic synthesis, which are organized by type of product formed, rather than by the class of enzyme employed. It covers the literature mainly over the last five years; for more information on the preparation and characterization of CLEAs, the reader is referred to other recent reviews.[21]

## 2. Carboxylic Acids

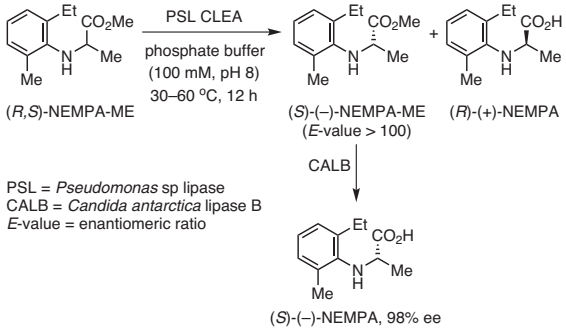
Carboxylic acids can be produced from the corresponding esters, amides, or nitriles in a variety of hydrolytic processes using the appropriate enzymes. For instance, Zhao et al. performed the kinetic resolution of the methyl ester of *N*-(2-ethyl-6-methylphenyl)alanine (NEMPA) in an interesting two-enzyme process.[23] Hydrolysis of the racemic ester, catalyzed by *Pseudomonas* sp. lipase (PSL, EC 3.1.1.3) afforded the *R* acid together with the *S* ester as remaining substrate in high enantioselectivity (*E*-value > 100) (**Scheme 2**).[23] Subsequent *Candida antarctica* lipase B (CALB, EC 3.1.1.3) catalyzed hydrolysis of the *S* ester afforded (*S*)-NEMPA with 98% ee. The latter is of interest as an herbicide intermediate. Immobilization of PSL as a CLEA resulted in a decrease in reaction time from 48 to 12 hours and improved thermal stability of the enzyme, with the highest activity of the CLEA being obtained at 60 °C. The high enantioselectivity (*E*-value > 100) of the CLEA was the same as that obtained with the free enzyme. Furthermore, after ten recycles, the CLEA still exhibited 80% of its initial activity.

A CLEA of the alkaline protease from *Bacillus licheniformis*, Alcalase® (EC 3.4.21.62), was used[24] to catalyze the enantioselective hydrolysis of racemic *N*-Boc 2-chlorophenylglycine methyl ester, affording the *S* acid in 34% isolated yield and 98% ee in 14 h compared to 63 h with the free Alcalase® (**Scheme 3**).[24] Moreover, facile recovery of the catalyst was possible and the workup was simplified. The product is an intermediate in the synthesis of the antithrombotic drug clopidogrel (Plavix®).

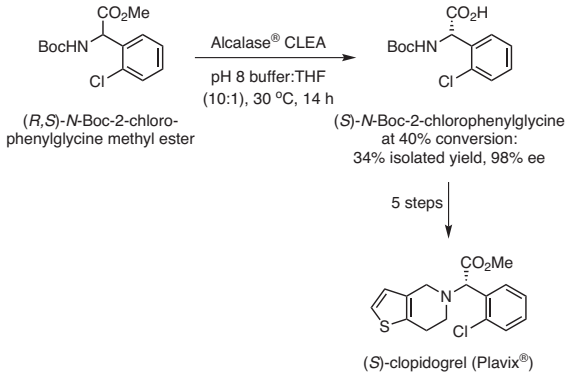
The stability of the lipase from *Serratia marcescens* (SML, EC 3.1.1.3) was significantly enhanced by immobilizing it as cross-linked co-aggregates with polyethyleneimine.[25] SML is highly enantioselective towards many industrially important chiral esters such as glycidyl butyrate, naproxen methyl ester, flurbiprofen ethyl ester, 4-hydroxy-3-methyl-2-(2-propynyl)cyclopent-2-enone acetate, and (±)-*trans*-3-(4'-methoxyphenyl)glycidic acid methyl ester.[25b] The latter is a key intermediate in the industrial synthesis of the cardiovascular drug diltiazem described above.

Kim and co-workers[26] have described the hydrolysis of a series of aliphatic amides catalyzed by a CLEA prepared from the crude fermentation broth of *Rhodococcus erythropolis* amidase (EC 3.5.1.4) by co-precipitation with bovine serum albumin (BSA) and cross-linking with glutaraldehyde. This technique is often used when the enzyme in question contains few lysine residues available for cross-linking. The CLEA exhibited enhanced stability compared to the free enzyme, and could be easily recovered by centrifugation while retaining 96% activity after 3 recycles.

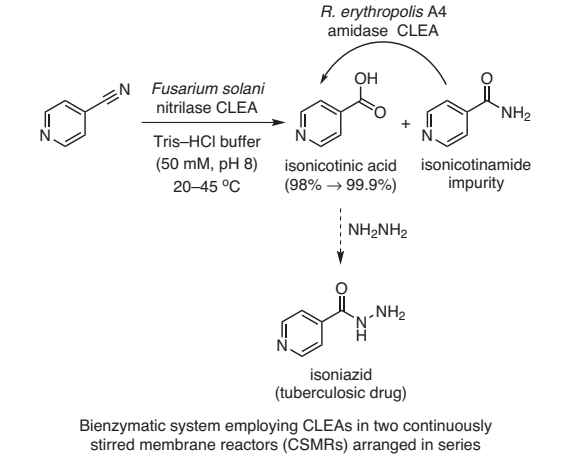
Malandra et al. employed a CLEA of the nitrilase (EC 3.5.5.1) from the fungus *Fusarium solani* in a continuously stirred membrane reactor (CSMR) for the highly chemoselective hydrolysis of 4-cyanopyridine to isonicotinic acid with minimal amide formation (**Scheme 4**).[27] The purity of the carboxylic acid product could be further increased (from 98% to 99.9%) by using two CSMRs in series, the first one containing the nitrilase CLEA and the second one containing an *R. erythropolis* A4




**Scheme 2.** *Pseudomonas* sp. Lipase Catalyzed Hydrolysis of NEMPA Methyl Ester. *(Ref. 23)*



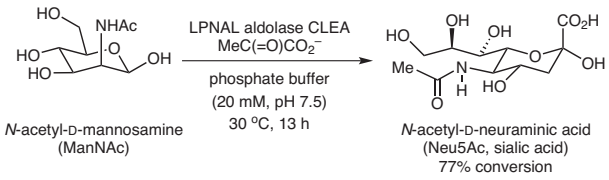

**Scheme 3.** Alcalase® CLEA Catalyzed Enantioselective Hydrolysis of an Ester Precursor of the Intermediate in the Synthesis of the Antithrombotic Drug Clopidogrel (Plavix®). *(Ref. 24)*



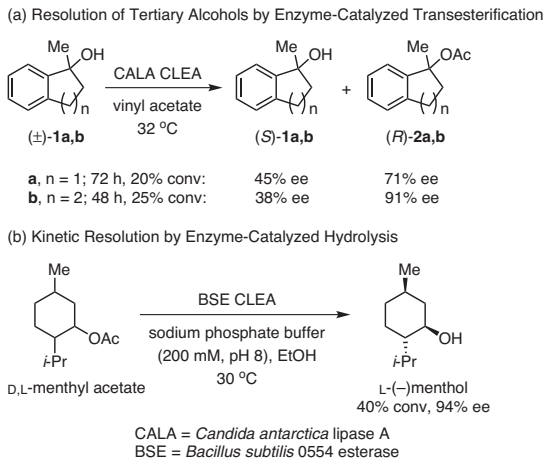

**Scheme 4.** Hydrolysis of 4-Cyanopyridine to Isonicotinic Acid Catalyzed by a *Fusarium solani* Nitrilase CLEA. *(Ref. 27)*

amidase CLEA to catalyze the hydrolysis of the small amount of amide byproduct. Isonicotinic acid is the precursor of tuberculosic drugs such as isoniazid and iproniazid.

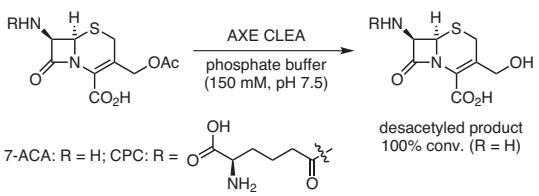
*N*-Acetyl-D-neuraminic acid aldolase (NAL, EC 4.1.3.3) is used industrially at basic pH for the condensation of pyruvate and *N*-acetyl-D-mannosamine (ManNAc) into *N*-acetyl-D-neuraminic acid (Neu5Ac, sialic acid), an advanced intermediate for GSK's antiviral drug Relenza® (zanamivir). Garcia-Garcia et al. prepared a robust CLEA from the *N*-acetyl-D-neuraminic acid aldolase from *Lactobacillus plantarum* (LPNAL, EC 4.1.3.3) by ammonium sulfate co-precipitation




**Scheme 5.** *N*-Acetyl-D-neuraminic Acid (Neu5Ac) Synthesis Utilizing an *L. planatarum* NAL CLEA. *(Ref. 28)*




**Scheme 6.** Resolution of Alcohols with Lipase and Esterase CLEAs. *(Ref. 30,31)*




**eq 1** *(Ref. 32)*

with BSA and cross-linking with glutaraldehyde, and applied it in the chemoenzymatic synthesis of Neu5Ac (**Scheme 5**).[28] The CLEA exhibited good activity and operational stability at alkaline pH, and at least ten cycles were possible without any significant loss of activity.

## 3. Alcohols

Optically pure chiral alcohols are often utilized as building blocks in the synthesis of fine chemicals and pharmaceuticals. They are readily prepared by enantioselective hydrolysis or (trans)esterification reactions using lipases (e.g., EC 3.1.1.3) and esterases (e.g., EC 3.1.1.1) in economical and environmentally benign procedures. Owing to steric hindrance, the enzymatic resolution of tertiary alcohols is problematic and only a few enzymes; notably pig liver esterase (PLE, EC 3.1.1.1), *Candida antarctica* lipase A (CALA, EC 3.1.1.3), and Alcalase®; can accept tertiary alcohols as substrates. CALA is a rather exceptional lipase in that it accepts bulky tertiary alcohols as substrates and, therefore, has considerable potential in organic synthesis.[29] Özdemirhan and co-workers showed that CALA CLEA is an effective catalyst for the resolution of aromatic-ring-fused cyclic tertiary alcohols by (trans)-esterification with vinyl acetate (**Scheme 6**, Part (a)).[30]

Zheng et al. investigated the enantioselective hydrolysis of DL-menthyl acetate using a *Bacillus subtilis* esterase (BSE, EC 3.1.1.1) CLEA as a highly active and enantioselective biocatalyst, affording L-menthol in 94% ee at 40% conversion (Scheme 6, Part (b)).[31] The thermal stabilities of the BSE CLEA at 30 °C and 50 °C were >360 and 14 times those of free BSE, respectively. After 10 cycles, the CLEA retained 92% of its initial activity and the authors concluded that the use of BSE CLEA could significantly decrease the manufacturing cost of L-menthol and would stimulate its practical applications.

A recombinant acetylxylan esterase (AXE, EC 3.1.1.72) from *Bacillus pumilus* was employed for converting cephalosporin C (CPC) and 7-aminocephalosporinic acid (7-ACA) into the corresponding desacetyl derivatives (**eq 1**).[32] The latter are advanced intermediates in the production of semi-synthetic cephalosporin antibiotics. CPC was a better substrate than 7-ACA for the AXE CLEA while 7-ACA was a better substrate than CPC for the free enzyme. This change in substrate specificity could be of industrial interest, allowing first deacylation of the 3-position side chain of CPC followed by removal of the 7-position side chain by D-amino acid oxidase and glutaryl-7-ACA acylase.

Enantiopure vicinal diols, such as (*R*)-1-phenyl-1,2-ethanediol, (*R*)-PED, are important intermediates in the synthesis of various pharmaceuticals.[33] They can be prepared by the enantioselective hydrolysis of epoxides catalyzed by epoxide hydrolases (EHs, EC 3.3.2.3).[34] For example, CLEAs of two novel epoxide hydrolases, MBEH-A and MBEH-B, isolated from mung beans (*Phaseolus radiatus*, now reclassified as *Vigna radiata*), were employed in an asymmetric hydrolysis of racemic styrene oxide to (*R*)-PED (**eq 2**).[35] Both enzymes catalyzed the enantioconvergent hydrolysis of styrene oxide by attacking the *S* isomer at the benzylic carbon with inversion of configuration and the *R* isomer at the terminal carbon with retention, resulting in a 100% theoretical yield of the *R* diol. The only difference between the two EHs is their opposite enantiopreference, which means that the optimum rate is achieved with a mixture of the two EHs. In aqueous buffer, the initial rate and the reaction yield (after 6 hours) were higher with the free enzyme than with the CLEA (50% vs 44%). However, the product ee significantly decreased over time under aqueous conditions, which was attributed to competing nonenzymatic hydrolysis. In contrast, in the biphasic *n*-hexane–phosphate buffer (1:1) system, the EH CLEAs resulted in a shorter reaction time (6 h vs 20 h) and a significantly higher enantioselectivity than the free enzyme

[97% (93% for the preparative run) vs 88%], presumably owing to suppression of nonenzymatic hydrolysis. Furthermore, the CLEA maintained its activity over 4 cycles, followed by a decrease to 53% over 8 cycles.

## 4. Carboxylic Acid Esters and Lactones

Carboxylic acid esters are found in fine chemicals, foods, fragrances, cosmetics, and pharmaceuticals. They are usually obtained by direct esterification (condensation) of a carboxylic acid and an alcohol, transesterification (transacylation, alcoholysis) of an ester and an alcohol, or by interesterification of two esters. Besides simple mineral acids and bases, these reactions are also catalyzed by enzymes, notably lipases (EC 3.1.1.3) and esterases (EC 3.1.1.1), often with high regio- and enantioselectivities. Esterification in high yield is only practical in an organic medium owing to an unfavorable equilibrium in water. In order to obtain optimum results, it is generally necessary to use immobilized enzymes in nonaqueous media.

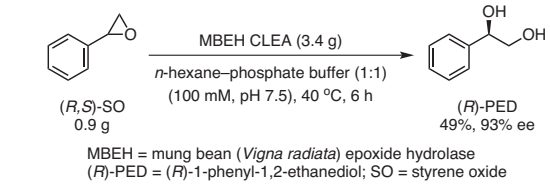
Kartal et al. employed a CLEA of *Candida rugosa* lipase (EC 3.1.1.3) in cyclohexane at 40 °C for the synthesis of ethyl esters of hexanoic, heptanoic, octanoic, and oleic acids.[36] The water byproduct was not removed so the ester yields were equilibrium-controlled and generally in the 80 to 90% range. Octanoic acid was the fastest reacting acyl donor, reaching equilibrium after 8 hours. In contrast, hexanoic acid required more than 40 hours to reach equilibrium. These results are clear examples of acyl donor chain length substrate specificity (preference) which is a common feature of all lipases. A similar, but less pronounced, specificity was observed for the alcohol substrate, with 1-butanol and 1-octanol reacting faster than ethyl alcohol and oleyl alcohol.

One disadvantage of a lipase-catalyzed direct esterification is that carboxylic acids are slow-reacting acyl donors. Ester synthesis is much faster when the acyl donor already is an ester such as in a transesterification or alcoholysis reaction. Thus, Yuryev and collaborators utilized *C. rugosa* lipase CLEA to catalyze the enantioselective transesterification of 2-chloro-3,3,3-trifluoropropanoic acid (CTFPA) methyl ester with ethanol in a packed bed reactor (**Scheme 7**, Part (a)).[37] At 48% conversion, the *R* isomer of the ethyl ester was isolated with 90% ee and the remaining methyl ester with 82% ee. These products are of interest as intermediates in the synthesis of herbicides.

Christakopoulos and co-workers[38] used a feruloyl esterase (EC 3.1.1.73) CLEA for the transesterification of methyl ferulate with excess 1-butanol in a mixture of *n*-hexane and water at 37 °C (Scheme 7, Part (b), R = H, R' = OMe). The feruloyl esterase CLEA prepared from commercially available Ultraflo® L afforded 97% 1-butyl ferulate after 6 days. Methyl sinapate (R = R' = OMe) and methyl caffeate (R = OH, R' = H) were also transesterified but methyl *p*-coumarate (R = R' = H) was unreactive.[39] In addition to 1-butanol, glycerol could also be used as an acyl acceptor.[40]

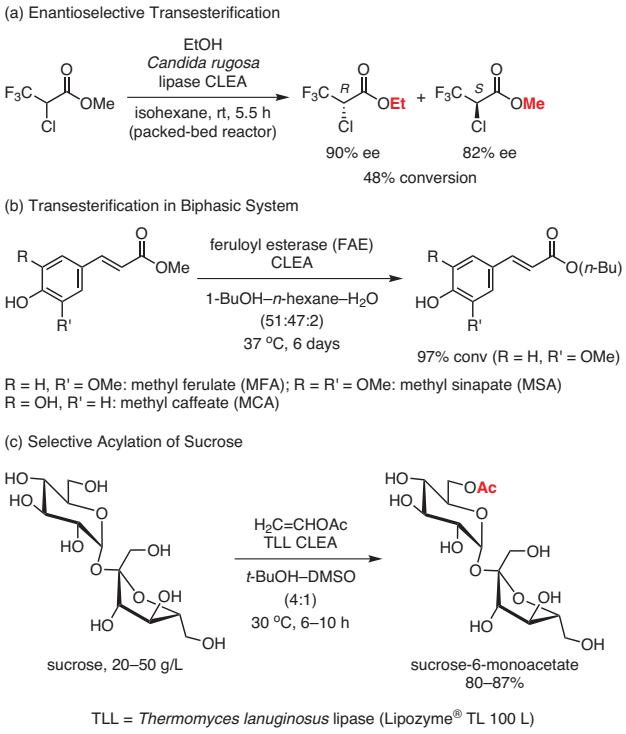
A transesterification is also an equilibrium reaction as the alcohol leaving group of the acyl donor competes with the substrate alcohol. This can be especially problematic when the substrate alcohol is poorly soluble in the reaction medium or when it is a much poorer acyl acceptor than the alcohol leaving group. One way to overcome this (kinetic) problem is by using a vinyl (enol) ester as the acyl donor.[41] This makes the transesterification irreversible because under normal conditions the vinyl alcohol leaving group tautomerizes to acetaldehyde. This can be a very useful strategy for the lipase-catalyzed esterification of sucrose (Scheme 7, Part (c)) with a CLEA from *Thermomyces lanuginosus* lipase (TLL, EC 3.1.1.3) in a 4:1 v/v mixture of *t*-BuOH and DMSO.[42] Below 50 g/L of sucrose, sucrose-6-monoacetate—a key intermediate in the preparation of sucralose—was obtained in approximately 80% yield. Interestingly, the TLL CLEA showed a better operational stability (more recycles) than the competing product, Lipozyme® TL IM.

Most enzymes, lipases and esterases included, are inactivated by low-molecular-weight alcohols, particularly by methanol and ethanol. This has hampered their use in solvent-free synthesis of methyl and ethyl esters for biodiesel (fatty acid methyl or ethyl esters). Lipase-catalyzed synthesis of biodiesel in principle offers a great advantage over the traditional sodium or potassium hydroxide catalyst in that a lipase can catalyze both transesterification of the triglycerides as well as esterification of the free fatty acids that inactivate a metal hydroxide catalyst. Han and Kim prepared a CLEA from a methanol tolerant lipase (M37, EC 3.1.1.3) from *Photobacterium lipolyticum* for




Enantioconvergent enzyme-catalyzed hydrolysis of styrene oxide

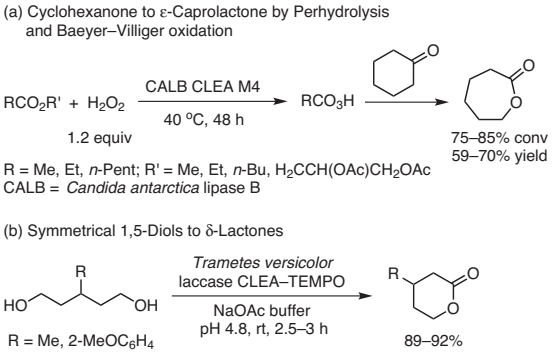
**eq 2** *(Ref. 35)*



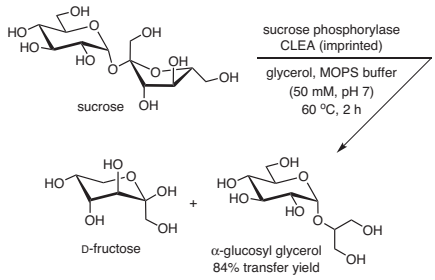

**Scheme 7.** Esterification Reactions: (a) *C. rugosa* Lipase CLEA Catalyzed Transesterification of CTFPA Methyl Ester, (b) Feruloyl Esterase CLEA Catalyzed Transesterification, and (c) TLL CLEA Catalyzed Acylation of Sucrose with Vinyl Acetate. *(Ref. 37–42)*

this purpose. The CLEA showed excellent stability in up to 60% v/v methanol, ethanol, 1-propanol, and 1-butanol. Without optimization of the methanolysis of olive oil (4:1 molar ratio of methanol over oil), they still obtained a 64% yield of biodiesel and the CLEA could be recycled with just over 70% retention of activity after 7 cycles.[43]

Some lipases also catalyze the perhydrolysis (i.e., the formation of a peroxy acid by acylation of hydrogen peroxide) of suitable acyl donors, which was demonstrated for the first time by Björkling and co-workers.[44] The in situ generated peroxy acid can be used for olefin epoxidation (Prilezhaev reaction) to afford an epoxide (oxirane), or for Baeyer–Villiger oxidation of ketones and cyclic ketones to esters and lactones, respectively. The latter reaction was studied by Chávez et al. who compared the performance of CALB CLEA (optimized for use in organic media) with Novozym® 435 in the chemoenzymatic Baeyer–Villiger oxidation of cyclohexanone to ε-caprolactone (**Scheme 8**, Part (a)).[45] On average, the catalytic performance of CALB CLEA was as good as Novozym® 435. Both catalysts accepted a wide range of inexpensive esters (e.g., ethyl and butyl acetate, ethyl propionate, methyl caproate, and triacetin) as acyl donors, which were simultaneously used as the solvent, affording 70–80% yields of caprolactone after 48 h at 40 °C. Cyclopentanone and all three isomers of methylcyclohexanone were also oxidized, but not cyclooctanone. Both catalysts were surprisingly stable, tolerating hydrogen peroxide concentrations up to 1 M.




**Scheme 8.** (a) Chemoenzymatic Synthesis of ε-Caprolactone by CALB CLEA Catalyzed Perhydrolysis and Baeyer–Villiger oxidation. (b) Oxidation of Symmetrical 1,5-Diols to the Corresponding δ-Lactones by *Trametes versicolor* Laccase CLEA–TEMPO. *(Ref. 45,46)*




transglucosylation of glycerol using an imprinted CLEA of sucrose phosphorylase from *Bifidobacterium adolescentis*

**eq 3** *(Ref. 50)*

An interesting example of ester synthesis using a somewhat surprising catalyst combination comprises the laccase CLEA–TEMPO catalyzed oxidation of diols to their lactones in aqueous medium in very good yields (Scheme 8, Part (b)).[46] In this system, the laccase enzyme (EC 1.10.3.2) catalyzed the formation of the oxoammonium ion of TEMPO, which then oxidized the primary alcohol to an aldehyde and further to the carboxylic acid (via the aldehyde hydrate). The acidic medium and the geometry of the hydroxy acid intermediate promoted subsequent lactone formation. The laccase CLEA showed improved operational stability with respect to the free enzyme and could be reused at least 3 times without significant loss of activity.

## 5. Carbohydrates

Carbohydrates are an extremely important class of molecules. They not only play a fundamental role in all living organisms but are also significant in the pharmaceutical, nutraceutical, cosmetic, food, and energy industries. The number of enzymes that act on the various types of carbohydrate molecules is vast, and many are used on industrial scale. A variety of these enzymes have been prepared as CLEAs[47] in order to increase the thermal stability, operational stability, or for use in the harsh environments of industrial processes.

Sucrose phosphorylase (EC 2.4.1.7), for example, is an important enzyme in the metabolism of sucrose. It catalyzes the reversible phosphorylation of sucrose into fructose and α-D-glucose-1-phosphate (G1P). Cerdobbel et al. prepared a CLEA of sucrose phosphorylase from *Bifidobacterium adolescentis* and showed that it had significantly improved properties compared to the free enzyme.[48] The process conditions for the production of G1P usually involve elevated temperatures of 60 °C or higher, and there are as yet no sucrose phosphorylases that can be utilized under such conditions.[49] By immobilizing the enzyme as a CLEA, the temperature optimum of the enzyme was raised to 75 °C compared to 58 °C for the free enzyme. This higher temperature profile was accompanied by an increase in the operational stability of the CLEA, which retained full activity after 1 week at 60 °C while the free enzyme lost 20% of its activity in just 16 h. The CLEA could be recycled at least 5–10 times without loss of activity. Sucrose phosphorylase is also able to catalyze transglucosylations whereby a glucose unit is transferred to a variety of carbohydrates and non-carbohydrate molecules. Desmet and co-workers studied the sucrose phosphorylase catalyzed transglucosylation of glycerol leading to α-glucosyl glycerol, a moisturizing agent used in cosmetics (**eq 3**).[50] They showed that an imprinted CLEA, formed by precipitating the enzyme in the presence of the product followed by cross-linking with glutaraldehyde, exhibited an increased specificity towards glycerol. The CLEA was extremely stable and eminently suitable for operation at 60 °C.

Similarly, Talekar et al. described the immobilization of *Saccharomyces cerevisiae* invertase (EC 3.2.1.26) as a CLEA which allowed for an increase in operational temperature from 40 °C to 70 °C in the conversion of sucrose into fructose and glucose to obtain inverted sugar syrup.[51] A porous CLEA[52] was also made by co-precipitation with starch and cross-linking followed by treatment of the resulting CLEA with an amylase to remove the occluded starch, creating a highly porous CLEA with a large surface area.

Trehalose is a disaccharide that is derived from starch and has many applications in the food, pharmaceutical, and cosmetic industries. Park and co-workers[53] have reported the production of trehalose from sucrose in a one-pot process using a combi-CLEA containing three enzymes: amylosucrase (AS, EC 2.4.1.4), maltooligosyltrehalose synthase (MTS, EC 5.4.99.15), and maltooligosyltrehalose trehalohydrolase (MTH, EC

3.2.1.141). The CLEA was recycled up to five times without any loss of activity.

Munguia's group employed a levansucrase (EC 2.4.1.10) from *Bacillus subtilis* for the synthesis of levan from sucrose and for the transfer of fructosyl moieties from sucrose to other acceptor molecules such as xylose. Levan, inulin, and other similar oligosaccharides—including fructosides containing xylose, galactose, glucose, and mannose—are alternative low caloric sweeteners and are of additional interest owing to their prebiotic properties. The levansucrase CLEAs performed the synthesis of the oligosaccharides and the transfructosylation as efficiently as the free enzyme and showed the highest reported specific activity for fructosyltransferases and glucosyltransferases (**Scheme 9**).[54]

Glucose amylase catalyzes the saccharification of starch through hydrolysis of α-(1,4) and α-(1,6) glycosidic bonds. Because the hydrolysis of the α-(1,6) branches is relatively slow using glucose amylase (EC 3.2.1.3), another enzyme, pullulanase (EC 3.2.1.41), is sometimes added to aid in this hydrolysis. Talekar and co-workers co-immobilized the two enzymes in a combi-CLEA, which exhibited a shift in optimum pH (from 5 to 7) and temperature (from 60 to 70 °C).[55] In a batch mode addition of starch, the combi-CLEA gave 100% conversion after 3 h, while the free enzyme gave only 30% after the same time interval. The combi-CLEA also had good stability, maintaining 90% of its initial activity after 8 recycles.

Hydrolysis of starch, cellulose, and other polysaccharides are important reactions in many industries including the food and energy industries. α-Amylase (EC 3.2.1.1) catalyzes the hydrolysis of starch to glucose and maltose. Talekar's group immobilized α-amylase with magnetic nanoparticles to produce a magnetic CLEA which showed activity comparable to that of the free enzyme.[56] Similarly, Xie et al. made a magnetic CLEA with improved stability and ease of separation from cellulase (EC 3.2.1.4) using magnetic chitosan microspheres.[57] β-Mannanase CLEA, an effective hemi-cellulase (EC 3.2.1.4), was produced with a porous structure after using macromolecules, such as dextran polyaldehyde and dialdehyde starch for cross-linking.[58] These CLEAs have an open, highly porous structure which renders them more suitable for conversion of macromolecular substrates in the synthesis of oligosaccharides.

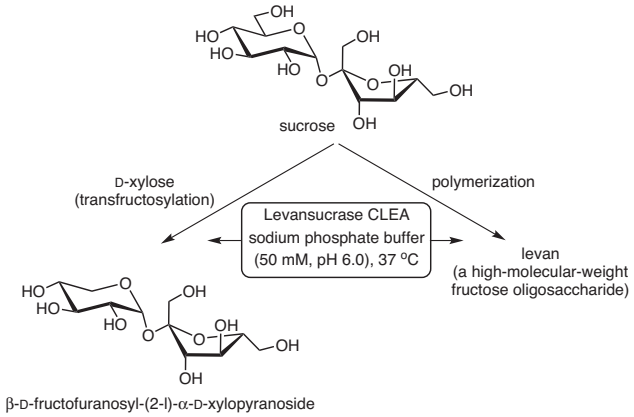
A *Penicillium decumbens* naringinase (EC 3.2.1.40) CLEA proved to be a highly effective catalyst for the conversion of the glycoside naringin into naringenin (**Scheme 10**, Part (a)).[59] Both compounds purportedly have interesting anti-inflammatory and anticancer properties.

Chen et al. showed that a CLEA of β-glucosidase (EC 3.2.1.20) from *Prunus domestica* seeds has a much higher productivity and improved thermal stability as compared to the free enzyme in the synthesis of salidroside (*para*-hydroxyphenethyl α-D-glucopyranoside) (Scheme 10, Part (b)).[60] The latter is used in Chinese traditional medicine and has recently been identified as having anticancer, antiviral, and cardio-protective properties.

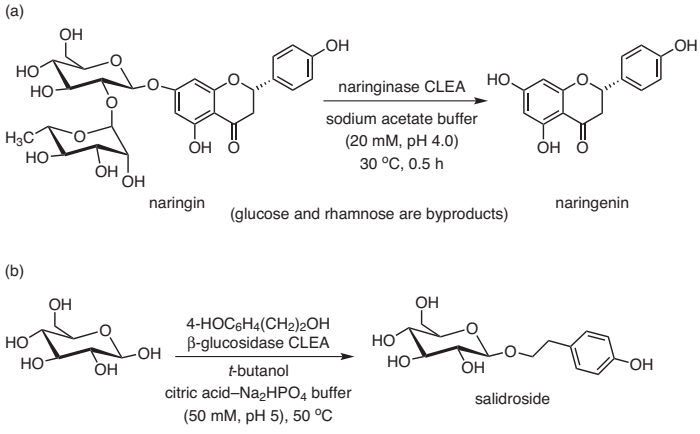
## 6. Amides

Nitrile hydratases (NHases; EC 4.2.1.84)[61,62] are Fe- or Co-dependent metalloenzymes that catalyze the addition of water to nitriles, a reaction of considerable industrial relevance. NHases often have a multimeric structure and are generally used as whole-cell biocatalysts because of limited stability of the enzymes outside the cell, possibly owing to dissociation of tetramers resulting in deactivation. In contrast, a CLEA prepared from a cell-free extract of a NHase isolated from an alkaliphilic bacterium showed excellent activity and stability in the conversion of acrylonitrile to acrylamide and was active with a variety of aliphatic nitriles.[63,64] This was attributed to cross-linking which prevented dissociation of the catalytically active tetramer. Moreover, the NHase CLEA was recycled 35 times with little loss of activity. A group of Co-containing NHases was recently identified,[65] and it showed a broad scope in the enantioselective hydration of chiral nitriles; the use of these enzymes in the form of CLEAs is currently being investigated. A CLEA prepared from a semi-purified *R. erythropolis* nitrile hydratase was most effective at producing the amides in the presence of an amidase inhibitor such as ammonium sulfate (360–800 mM) as the crude enzyme preparation also contained amidase activity. However, due to this activity, the CLEA was also able to favor carboxylic acid formation when conditions were adjusted.

The first studies of CLEAs[66] were conducted with penicillin G amidase (penicillin G acylase, PGA, EC 3.5.1.11), an industrially important enzyme used in the synthesis of semi-synthetic penicillin and




**Scheme 9.** Fructose Polymerization and Transfructosylation Employing Levansucrase CLEA. *(Ref. 54)*




**Scheme 10.** CLEA-Catalyzed (a) Hydrolysis of Naringin and (b) Synthesis of Salidroside. *(Ref. 59,60)*

cephalosporin antibiotics. Examples of these studies are the syntheses of ampicillin from 6-aminopenicillanic acid (6-APA) and cephalexin from 7-aminodesacetoxycephalosporanic acid (7-ADCA), respectively (**Scheme 11**).[67–70] The free enzyme has limited thermal stability and low tolerance to organic solvents, making it an ideal candidate for stabilization as a CLEA. Indeed, a PGA CLEA, prepared by precipitation with *tert*-butanol and cross-linking with glutaraldehyde, proved to be an effective catalyst for the synthesis of ampicillin in water, and maintained its high activity in organic solvents.[68] Similarly, Illanes et al.[69] used a partially purified PGA to produce a CLEA that was productive in both aqueous media and ethylene glycol–water (60:40, v/v). They also found that the degree of cross-linking had an influence, particularly on the activity recovery, thermal stability, and productivity of the CLEA in cephalexin synthesis,[70] all being higher at lower glutaraldehyde-to-enzyme ratios.

Youshko and co-workers observed an "ageing" effect on the properties of PGA CLEAs in the hydrolysis of D-phenylglycine amide and the synthesis of ampicillin.[71] The period of time between enzyme precipitation and cross-linking was found to influence the structural organization of the resulting CLEA. The "mature" CLEAs consisted of larger particles that were more effective in both the hydrolytic and synthetic processes. The authors suggested that aggregate size might regulate the extent of covalent modification and thereby the catalytic activity of the CLEAs.

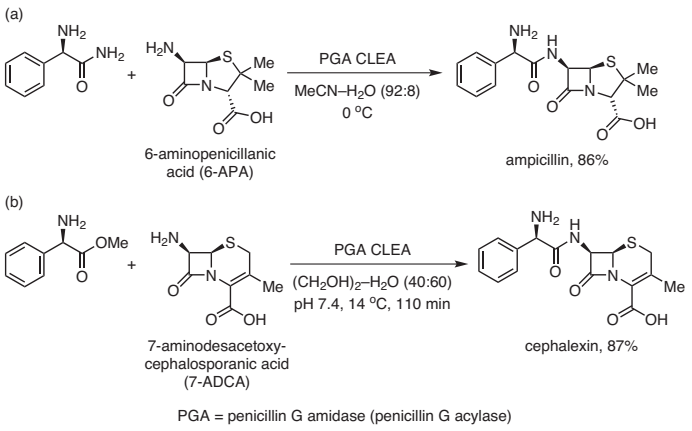
Polyamides, in particular high-added-value polyamides, are an important class of synthetic polymers the synthesis of which by use of enzymes as catalysts has recently attracted much attention. Poly-β-alanine or nylon-3, for example, is a silk-like polymer that is used as a stabilizer and in cosmetics, water purification, and construction. The current methodology for its synthesis involves the use of stoichiometric amounts of coupling reagents, difficult purification, side-product formation, and limited degrees of polymerization. Lipases (EC 3.1.1.3) are known to catalyze amide bond formation by aminolysis of esters, and Franssen and co-workers have employed CALB CLEA and *Pseudomonas stutzeri* lipase CLEA for the polymerization of several poly-β-alanine esters (**Scheme 12**, Part (a)).[72] For example, β-alanine methyl ester, afforded a 90% yield of a polymer with up to 53 polymer units, which is significantly higher than with the other immobilized forms of CALB. Similarly, other polyamides or nylons, with various aliphatic[73] or aromatic[74] moieties in the polymer backbone, were prepared from a range of diesters and diamines by utilizing a CLEA of a cutinase from *Fusarium solani pisi* (EC 3.1.1.74), which led to a higher maximal degree of polymerization than that observed with CALB in the form of Novozym® 435 (Scheme 12, Part (b)).

## 7. Amino Acids and Derivatives

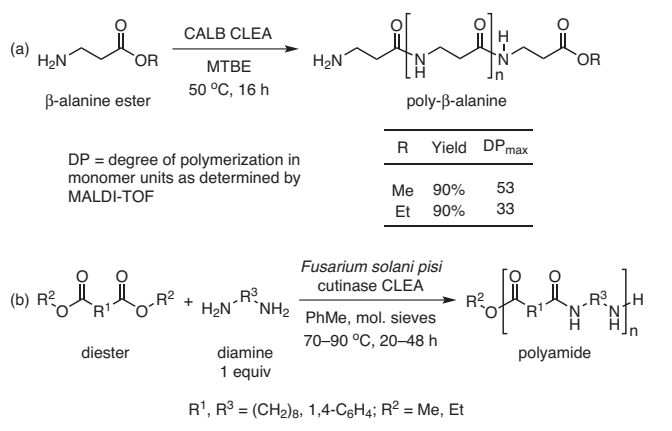
CLEAs have been successfully produced from a variety of enzymes that act on amino acids and peptides, such as amino acylase (EC 3.5.1.14),[75] phenylalanine ammonia lyase (EC 4.3.1.24),[76] tyrosinase (EC 1.14.18.1),[77] and papain (EC 3.4.22.2).[78] CLEAs produced from the alkaline protease of *Bacillus licheniformis* (Alcalase®, EC 3.4.21.62, also known as Subtilisin Carlsberg), an inexpensive laundry detergent enzyme, have received the most attention by far for this class of compounds, with a wide variety of their amino acid and peptide biotransformations having been described. Although Alcalase® CLEAs have been successfully applied in the selective hydrolysis of *C*-terminal *tert*-butyl esters of peptides without showing substantial endopeptidase activity,[79] most applications of Alcalase® CLEAs involve amino acid and peptide esterifications and amidations, as well as fully enzymatic peptide syntheses.

Generally, the popularity of enzymes in amino acid and peptide esterifications is related to their mild operating conditions during transformations with sterically demanding alcohols to orthogonally protected esters, thereby avoiding racemization and side reactions when sensitive substrates are employed. This is in sharp contrast to most chemical esterification methods. Furthermore, enzymes offer the interesting features of regioselectivity and enantioselectivity. Although high yields can be obtained in the esterification of amino acids and peptides by using lipases and esterases, these enzymes are usually not selective for the α-carboxylic acid moiety. Alcalase® CLEAs, however, not only show a high selectivity for the α-carboxylic acid moiety, but also have a broader substrate scope compared to other proteases such as papain (EC 3.4.22.2), chymotrypsin (EC 3.4.21.1), and α-chymotrypsin (EC 3.4.21.1).

Regioselective esterification of amino acids and peptides at the α position was demonstrated under continuous water removal using Alcalase® CLEAs. Methyl, ethyl, benzyl, allyl, 2-(trimethylsilyl)ethyl, and most notably *tert*-butyl esters of amino acids and peptides were




**Scheme 11.** Ampicillin and Cephalexin Synthesis with Penicillin G Amidase CLEA. *(Ref. 68,70)*

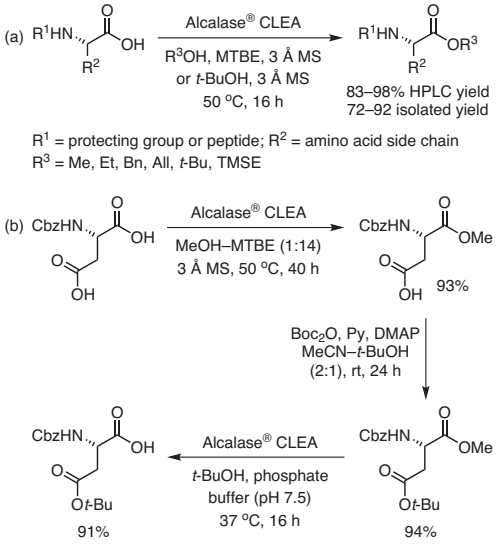



**Scheme 12.** Polyamide Formation Catalyzed by (a) Lipase and (b) Cutinase CLEAs. *(Ref. 72–74)*

produced under mild conditions in very high yields (**Scheme 13**, Part (a)).[80] An elegant example that exploits the regioselectivity of Alcalase® CLEAs in esterifications is the synthesis of β-protected aspartic acid and γ-protected glutamic acid via the selective α-hydrolysis of symmetrical aspartyl and glutamyl diesters or, for more demanding or costly alcohol moieties, in a three-step protocol involving enzymatic formation of the α methyl ester followed by chemical β-esterification and selective α-hydrolysis (Scheme 13, Part (b)).[81]

Besides the formation of esters, amide bonds on the *C*-terminus of amino acids and peptides can also be formed using Alcalase® CLEA in an aminolysis reaction of the corresponding free carboxylic acid or the methyl or benzyl ester with aromatic amines under nearly anhydrous conditions (**Scheme 14**, Part(a)).[82] Products of the aminolysis reaction are obtained in high chemical, enantio-, and diastereomeric purities without any racemization. For more challenging amino acids and peptides, activated enol esters can be used in Alcalase® CLEA catalyzed peptide coupling. These esters can be produced enzymatically in the same pot[83] or chemically via a ruthenium-catalyzed addition of a carboxylic acid to an alkyne.[84] L-Amino acid amides for use in peptide synthesis can also be produced from racemic natural and non-natural *N*-Boc-amino acid thio esters by Alcalase® CLEA catalyzed ammoniolysis and aminolysis in a dynamic kinetic resolution with simultaneous base-catalyzed racemization of the unconverted D-enantiomer (Scheme 14, Part (b)).[85]

More recently, two fully enzymatic methods for the synthesis of peptides via a novel *C*-terminal ester interconversion catalyzed by Alcalase® CLEA were published using two different *C*-terminal protection groups. In the first procedure, a *C*-terminal protected peptide *tert*-butyl ester was enzymatically converted, in quantitative yield, into a primary alkyl ester in the first step. The latter was then subjected to elongation using an enzymatic coupling with another amino acid *tert*-butyl ester (**Scheme 15**).[86] Both steps were catalyzed by Alcalase® CLEA. This fully enzymatic elongation strategy via *C*-terminal ester interconversion was successfully applied to the synthesis of biologically active peptides up to the pentamer level.

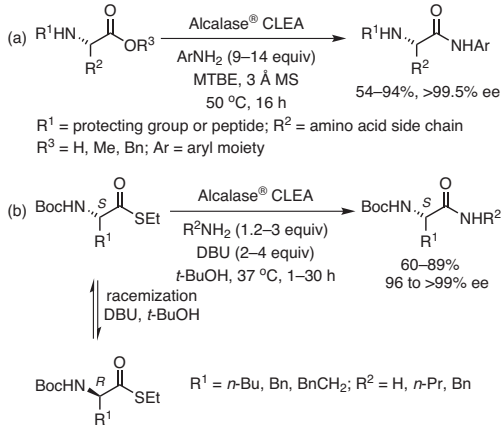



**Scheme 13.** Use of Alcalase® CLEAs in Amino Acid and Peptide Esterifications. *(Ref. 80,81)*

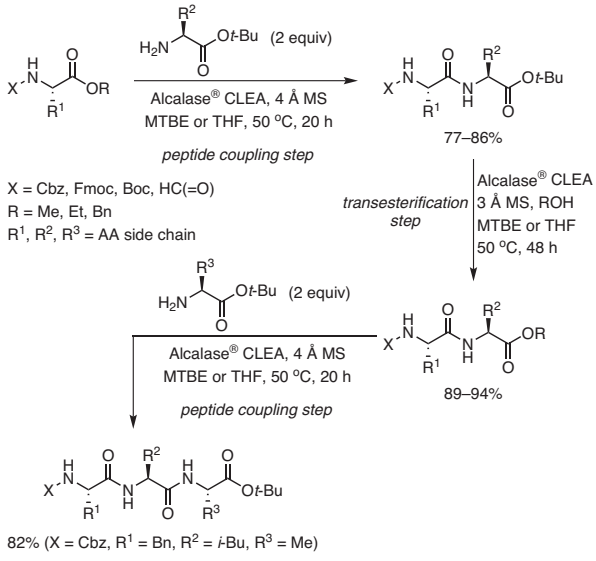
In the second procedure, Alcalase® CLEA converted the α-carboxyamide *C*-terminal protective group directly into the primary alkyl ester, which made possible a fully enzymatic *N*→*C* directed peptide synthesis of two different tripeptides.[87] In both of the procedures described here, a hydrolysis step and an esterification step are substituted by one Alcalase® CLEA catalyzed interconversion.

## 8. Cyanohydrins

Chiral cyanohydrins are interesting intermediates in organic synthesis, for example as precursors of α-hydroxy carboxylic acids and 1,2-amino alcohols. Hydroxynitrile lyases (HNLs, EC 4.1.2.10) catalyze the enantioselective hydrocyanation of a wide range of aldehydes. For

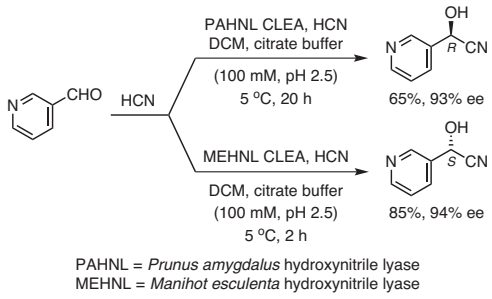



**Scheme 14.** Alcalase® CLEA in (a) the Synthesis of Amino Acid or Peptide Arylamides and (b) a Dynamic Kinetic Resolution of *N*-Boc-Amino Acid Thio Esters. *(Ref. 82,85)*

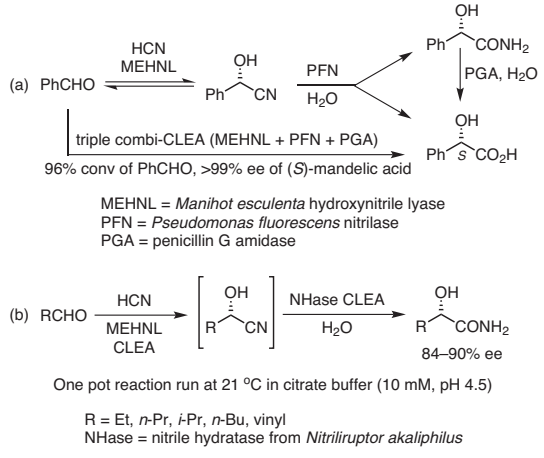



**Scheme 15.** Fully Enzymatic Peptide Synthesis in the *N*→*C* Direction Using Alcalase® CLEA. *(Ref. 86)*

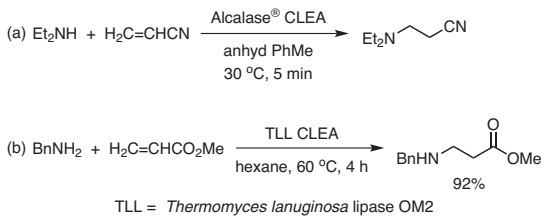
example, CLEAs prepared from the *R*-specific HNL from almonds, *Prunus amygdalus,* (PAHNL, EC 4.1.2.11) by cross-linking with glutaraldehyde[88] or dextran polyaldehyde[89] were highly effective catalysts for the hydrocyanation of aldehydes under microaqueous conditions and could be recycled several times without loss of activity. CLEAs were similarly prepared from the *S*-specific HNLs from *Manihot esculenta* and *Hevea brasiliensis*.[90,91] Because these CLEAs perform exceptionally well in organic solvents, they can afford higher enantioselectivities than is observed with the free enzymes owing to




**Scheme 16.** Asymmetric Synthesis of Cyanohydrins with Hydroxynitrile Lyase CLEAs. *(Ref. 92)*




**Scheme 17.** Combi-CLEAs for the Conversion of Aldehydes to Amides and Acids via the Cyanohydrins. *(Ref. 20b,94)*




**Scheme 18.** Synthesis of Functionalized Amines by Aza-Michael Additions Catalyzed by Alcalase® CLEA or Lipase CLEAs. *(Ref. 103,104)*

the essentially complete suppression of the competing non-enzymatic hydrocyanation under these conditions. For example, Roberge and co-workers obtained high enantioselectivities in the hydrocyanation of pyridine-3-aldehyde (**Scheme 16**).[92] The latter is a difficult substrate for enantioselective hydrocyanation owing to the relatively facile non-enzymatic background reaction as a result of the electron-attracting properties of the pyridine ring. The high enantioselectivities observed with the HNL CLEAs could not be obtained with the free enzyme or any other immobilized form of it.

Similarly, a CLEA was prepared from the relatively unknown *R*-selective hydroxynitrile lyase from *Linum usitatissimum* (LUHNL, EC 4.1.2.46) and utilized in the conversion of butanone to the (*R*)-cyanohydrin with 87% ee.[93] Interestingly, imprinting of CLEA by addition of the 2-butanone substrate prior to cross-linking enhanced its synthetic activity.

A combi-CLEA comprising the *S*-selective HNL from *Manihot esculenta* (MEHNL) and a non-selective nitrilase from *Pseudomonas fluorescens* catalyzed the one-pot conversion of benzaldehyde into (*S*)-mandelic acid[20a] The enantioselectivity is provided by the HNL, while the in situ conversion by the nitrilase serves to drive the equilibrium of the first step towards the product. However, substantial amounts of the corresponding (*S*)-amide were formed as byproduct, which led to the idea of using a third enzyme, PG amidase, to catalyze the hydrolysis of the amide. Consequently, a triple combi-CLEA prepared from these three enzymes catalyzed the formation of (*S*)-mandelic acid in 99% ee at 96% benzaldehyde conversion (**Scheme 17**, Part (a)).[94] Similarly, a combi CLEA comprising MEHNL and the alkaliphilic nitrile hydratase from *Nitriliruptor akaliphilus* catalyzed the one-pot conversion of aldehydes into (*S*)-α-hydroxycarboxylic acid amides (Scheme 17, Part (b)).[20b]

## 9. Amines

Chiral amines are important intermediates in the manufacture of fine chemicals and pharmaceutical intermediates,[95] and a variety of enzymes can be employed for their industrial synthesis. Examples of such enzymes are transaminases (EC 2.6.1.2);[96] amine oxidases;[97] imine reductases;[98] and various hydrolases such as lipases, proteases, and PG amidase for the resolution of racemic amines by enantioselective acylation,[99] including dynamic kinetic resolutions with in situ racemization catalyzed by transition metals.[100] Some of these reactions have been successfully performed with CLEAs, as exemplified by the use of PGA CLEA in the resolution of amines by acylation in an aqueous medium.[99d] Similarly, CLEAs of a transaminase[101] and an amine oxidase were recently prepared for use in amine resolutions.[102] Two interesting recent publications have reported the enzymatic aza-Michael addition of primary and secondary amines to acrylonitrile and acrylate esters using Alcalase® CLEA[103] and lipase CLEAs[104] as catalysts, respectively (**Scheme 18**).

## 10. Conclusions and Prospects

Immobilization of enzymes as cross-linked enzyme aggregates (CLEAs) is an extremely useful technique for enhancing their suitability for application under the often-harsh conditions of industrial organic synthesis. CLEAs are cost-effective, in that they are readily prepared in good yields from crude enzyme extracts, exhibit significantly increased stability, and are easy to recover and reuse. The technology has a broad scope and has been applied successfully to a plethora of industrially relevant transformations in the synthesis of pharmaceuticals, cosmetic ingredients, and other fine chemicals. We expect that the scope will be further extended in the future to even more enzymes, including

cofactor-dependent enzymes, such as ketoreductases, which up till now have received only scant attention. We also expect that CLEAs will find industrial applications in the production of chemicals from renewable raw materials, for example in the conversion of vegetable oils, fish oils, and a variety of carbohydrates from mono- to polysaccharides. Some of these conversions involve multi-enzyme processes, and the use of combi-CLEAs in enzymatic cascade reactions will reduce the number of unit operations and drive equilibrium reactions by removing the intermediate products. Another area where CLEAs are likely to be applied is in the synthesis of specialty condensation polymers, such as polyesters and polyamides, possibly from biomass-derived monomers. A few examples have already been reported (see Section 6) and we expect more to follow. In short, we believe that CLEAs have considerable potential that is yet to be broadly exploited in organic synthesis on both the laboratory and industrial scales.

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(†) CLEA is a trademark of CLEA Technologies B.V., registered in the Benelux countries.

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