Rapid Mechanochemical Synthesis of Amides with Uronium-Based Coupling Reagents, a Method for Hexa-amidation of Biotin[6]uril

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ABSTRACT: Solid-state reactions using mechanochemical activation have emerged as solvent-free atom-efficient strategies for sustainable chemistry. Herein we report a new mechanochemical approach for the amide coupling of carboxylic acids and amines, mediated by combination of (1-cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylaminomorpholinocarbenium hexafluorophosphate (COMU) or *N*,*N*,*N*′,*N*′-tetramethylchloroformamidinium hexafluorophosphate (TCFH) and K_2HPO_4. The method delivers a range of amides in high 70–96% yields and fast reaction rates. The reaction protocol is mild, maintains the integrity of the adjacent to carbonyl stereocenters, and streamlines isolation procedure for solid amide products. Minimal waste is generated due to the absence of bulk solvent. We show that K_2HPO_4 plays a dual role, acting as a base and a precursor of reactive acyl phosphate species. Amide bonds from hindered carboxylic acids and low-nucleophilic amines can be assembled within 90 min by using TCFH in combination with K_2HPO_4 or *N*-methylimidazole. The developed mechanochemical liquid-assisted amidation protocols were successfully applied to the challenging couplings of all six carboxylate functions of biotin[6]uril macrocycle with phenylalanine methyl ester, resulting in an 80% yield of highly pure hexa-amide-biotin[6]uril. In addition, fast and high-yielding synthesis of peptides and versatile amide compounds can be performed in a safe and environmentally benign manner, as verified by green metrics.

Keywords: mechanochemistry, solvent-free chemistry, amides, peptides, amide coupling reagents, macrocycle, hemicucurbituril, green metrics

INTRODUCTION

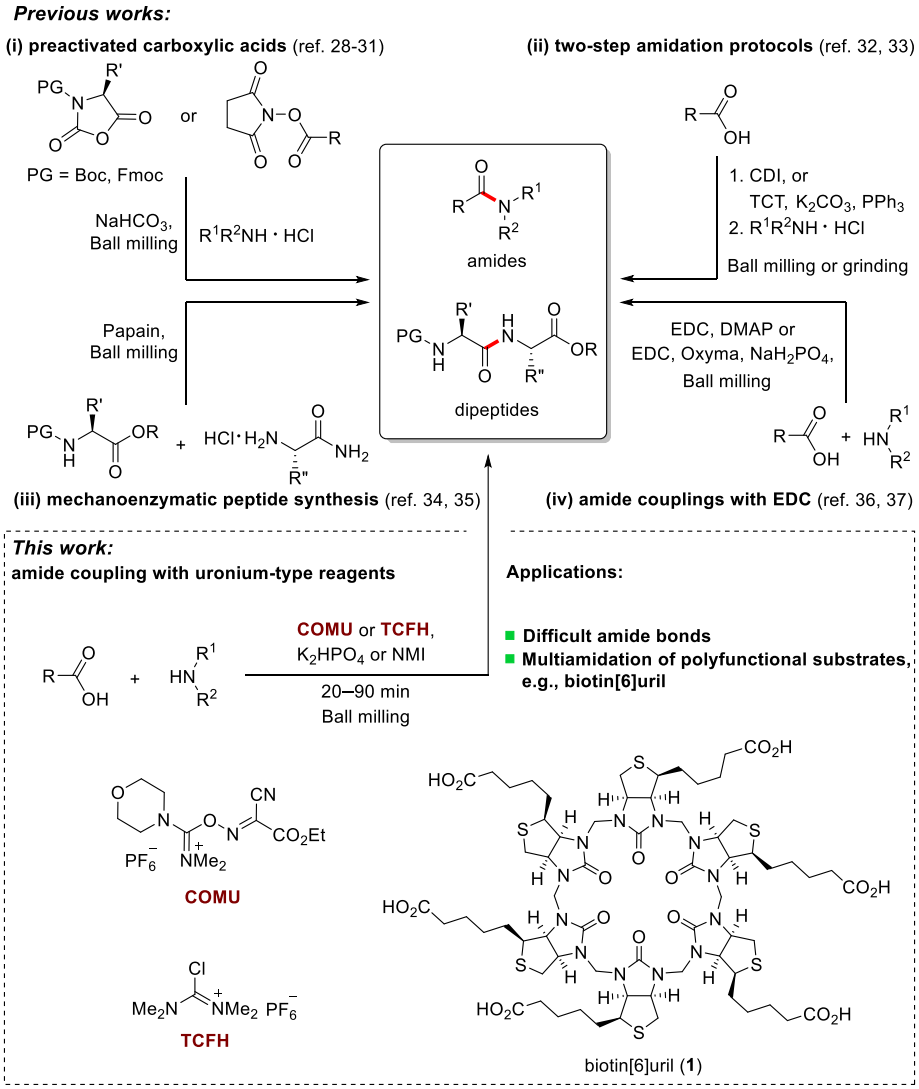
Amide bond is widespread in both natural compounds and artificial materials. It occurs in molecules fundamental to life, such as peptides and proteins, as well as in synthetic polymers and in a massive array of pharmaceuticals. In fact, amide preparation from carboxylic acids and amines

represents the most frequently applied chemical transformation in drug production and comprises about 25% of the current medicinal chemistry synthetic toolbox.[1] As a consequence of its wide usage, the development of sustainable amidation methods was listed among the top green chemistry research priorities by the American Chemical Society Green Chemistry Pharmaceutical Roundtable (ACS GCIPR) in 2007[2] and has been retained in the recent revision.[3] Although direct condensation of carboxylic acids and amines with water as a single by-product can be considered a "green" landmark in the field, it remains impractical because of the process's harsh reaction conditions (T > 100 °C) and limited substrate scope.[4–6] A robust method of amide synthesis commonly requires prior activation of a carboxylic function to replace OH with a better leaving group.[7–9] Notably, this is also the case in biosynthetic pathways, including the translation process and non-ribosomal enzymatic transformations.[10–13] For laboratory and industrial use, vast numbers of amide coupling reagents, performing in situ activation of carboxylic acid, have been developed in the quest for faster, milder, and high-yielding amidation protocols.[14,15] Low atom economy of these reagents and accompanying safety issues are their major drawbacks, which has incited the development of alternative approaches.[16–19] Important advancements have thus far followed traditional solution-based approaches; however, solvent is actually responsible for 80−90% of mass consumption in a typical chemical process and also plays a major role in overall toxicity.[20] In this way, solvent greatly outperforms the contributions of reagents themselves. Hazardous solvents like DMF and DCM are preferred in industrial amide synthesis, reinforcing both environmental and safety concerns.[17,21] Therefore, the application of solvent-free techniques represents an efficient way to improve the overall process mass intensity and to prevent generation of hazardous waste. Recent advances in mechanochemistry and its related fields have established solvent-free reactions as environmentally friendly tools to perform chemical transformations that are no less efficient than the conventional solution-based chemistry.[22–24]

In the area of amide synthesis, the benefits of solvent-free techniques have not remained unnoticed and have been previously demonstrated in numerous studies (Scheme 1).[25–27] For example, mechanosynthesis of various amides and peptides has been performed from a series of activated carboxylic acid derivatives, such as *N*-carboxyanhydrides;[28,29] *N*-hydroxysuccinimide esters;[30] *N*-acyl benzotriazoles.[31] *N*-Acyl imidazoles[32] and acyloxytriazine esters[33] have been produced mechanochemically from carboxylic acids prior to reacting with amines. Notably, even papain enzyme can catalyze the formation of peptides from the corresponding amino acid building blocks

under solvent-free conditions.[34,35] In addition, direct coupling of amines with carboxylic acid has been demonstrated by using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) as a coupling reagent.[36,37] In general, EDC-mediated transformations have shown remarkably short reaction times (typically within 10-30 min), high yields, and simple work-up protocols.

Scheme 1. Overview of the State-of-Art Mechanochemical Amidation Approaches and Outline of the Current Work



Following these prominent earlier contributions,[24-33] we aimed to further expand the scope and synthetic utility of the mechanochemical amidation methods. The current research was impelled by three objectives: First, most of the amide coupling reagents are simply not efficient enough for a range of substrates,[8] which require expansion of the established one-step mechanochemical

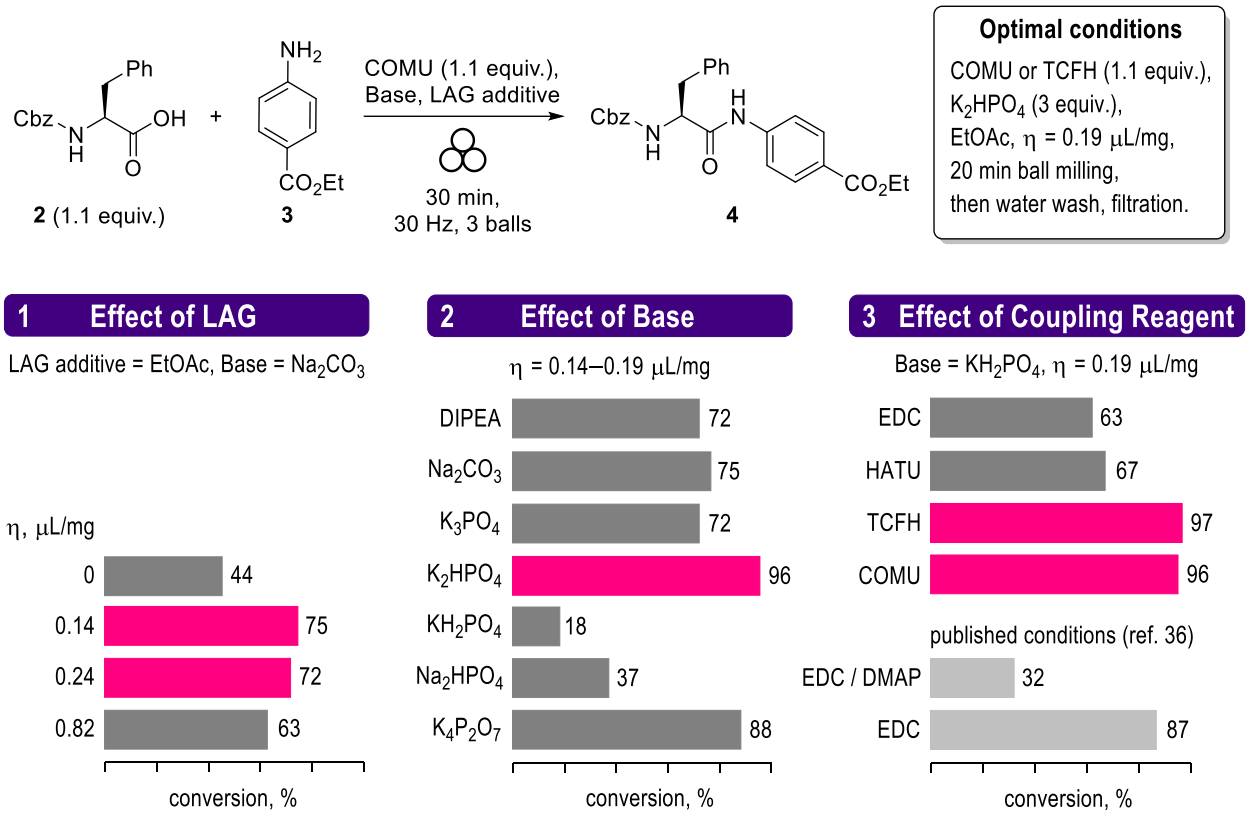
amidation protocols beyond the previously applied EDC; for that purpose, in this work we mapped the coupling efficiency of uronium-type reagents (COMU and TCFH, Scheme 1) on several carboxylic acid/amine pairs. Second, the scope of previously published mechanochemical approaches was evaluated based mainly on peptide synthesis, while the challenging couplings of sterically hindered carboxylic acids and low-nucleophilic amines remained virtually unproven; here we demonstrated that such difficult amide bonds can also be assembled under solvent-free conditions. Implementation of the two objectives mentioned above was required as a prerequisite for the third objective as our ultimate goal. Due to the interest of our group in the synthesis and supramolecular applications of macrocyclic host molecules,[38–43] we required a robust procedure for amide-functionalization of biotin[6]uril macrocycle (**1**),[44–46] to access the family of modified biotin[6]uril hosts. Despite the apparent ease of such a transformation, it also presented a substantial challenge: six-fold stepwise amidation of carboxylate groups in **1** is inevitably accompanied by accumulation of the "failed" underfunctionalized products if incomplete coupling occurs at each step. Limited solubility of **1** in the common organic solvents dictates additional practical inconvenience of the traditional solution chemistry; in fact, only dipolar aprotic solvents like DMF can be used. Here we showed that application of solvent-free techniques, additionally reinforced with the reactive uronium-type amide coupling reagents, allows the desired functionalization of **1** in a high-yielding, scalable, and sustainable manner, avoiding harmful solvents or significant reagent excess.

RESULTS AND DISCUSSION

Development of Mechanochemical Amidations with Uronium-Type Reagents. At the outset, amide coupling of Cbz-protected L-phenyl alanine (**2**) and ethyl 4-aminobenzoate (benzocaine, **3**), mediated by COMU as a representative "green" uronium-type amide coupling reagent,[47–49] was selected as a model process (Scheme 2). We aimed to screen and compare the results of various reaction conditions, including the evaluation of coupling efficiency for different coupling reagents beyond the COMU itself, to reveal the most promising hits in terms of product yield and green chemistry requirements. The choice of aromatic amine **3** was dictated by its reduced nucleophilicity in comparison with aliphatic amines, additionally attenuated by an electron-withdrawing ethoxycarbonyl group. We expected that suppressed reactivity of **3** in the carbonyl addition reactions would facilitate more reliable differentiation of various coupling conditions. Use of

phenyl alanine derivative **2** as coupling counterpart provided an additional opportunity to examine the resistance α-stereocenter towards its possible epimerization, as commonly encountered in peptide synthesis.[9,15]

Scheme 2. Optimization Experiments



Conversion of **3** into **4** according to ^{1}H NMR analysis of the crude reaction mixtures

The test reactions were run in a Form-Tech Scientific FTS1000 shaker mill operating at 30 Hz by using 14 mL zirconia-coated milling jars, 3 × 7 mm zirconia milling balls and typical solid reactants loading around 0.3−0.4 g (including 0.2 mmol of amine **3** as a limiting substrate). After 30 min milling time, a sample of the crude reaction mixture was treated with $CDCl_3$, followed by separation of insoluble inorganic materials. The conversion of amine **3** into amide **4** was determined by ^{1}H NMR analysis (see Supporting Information for the details). The amide coupling reagent, base, and amount of liquid additive needed to assist the grinding process (Scheme 2) were identified as the three most crucial parameters affecting the yield of amide **4**, as described below.

The addition of a small volume of liquid constitutes an efficient method to enhance the performance of solvent-free mechanochemical reactions, known as liquid-assisted grinding (LAG).[22,24] The ratio of the volume of liquid (μL) added to the amount of solid present (mg) is denoted as η (μL/mg).[50] A value of $\eta = 0$ generally corresponds to dry grinding, but in a typical LAG process, η is usually between 0 and 1.[24] Although LAG cannot be described as a totally solvent-free technique, it requires a minimal amount of liquid, especially advantageous if a green solvent is used. Among the latter,[20,51,52] ethyl acetate appears to be the most promising and chemically compatible candidate to act as a LAG additive in COMU-mediated amide coupling. In our experiments (Scheme 2, Chart 1), the addition of ethyl acetate indeed showed a pronounced effect on the yield of amide **4**, generated in the mixture of solid reactants **2** and **3**, with COMU reagent and sodium carbonate (ca. 10 equiv.) as a base. Although dry grinding provided a rather modest outcome (44% conversion), LAG resulted in a markedly improved reaction performance, with the optimal η value in a range of 0.14−0.24 μL/mg, while the further increase of η led to slightly diminished conversion values.

The choice of base is also important in amide coupling. State-of-the-art solution approaches commonly apply non-nucleophilic tertiary amines, e.g. *N*,*N*-diisopropylethylamine (DIPEA).[15] However, the use of cheap and non-toxic inorganic salts, e.g. $NaHCO_3$, K_2CO_3, NaH_2PO_4,[28,30,33,37] insoluble in common organic solvents, can be considered as an additional advantage of mechanochemical reactions. In our hands (Scheme 2, Chart 2), replacement of DIPEA with Na_2CO_3 gave similar conversion values (72% vs 75%). For further process optimization, a range of readily available phosphate salts, with notably distinct pK_a values, were screened. Among them, potassium pyrophosphate $K_4P_2O_7$ and dipotassium phosphate K_2HPO_4 provided the best outcomes, especially the latter (96% conversion). Generally, the performance of phosphate salts does not correlate with Brønsted basicity of the respective anions. Although the poor outcome with KH_2PO_4 (only 18% conversion) in comparison with K_2HPO_4 (96%) could be probably connected with the significantly reduced base strength of the former (respective pK_a values 2.12 vs 7.21; pK_a of RCO_2H is typically about 4−5 in aqueous media),[53] much more basic K_3PO_4 (pK_a 12.32) also afforded amide **4** with reduced efficiency (72%). Surprisingly, the counter-cation effect (Na^+ vs K^+) also had a prominent impact on reaction outcome (37% vs 96%, for Na_2HPO_4 and K_2HPO_4 respectively). These results clearly indicate that the effect of an inorganic base on a solid-state reaction is more intricate than trivial proton transfer.

Finally, amide coupling reagents are essential for attaining high yields. The selection of coupling reagent was governed by considering chemical (substrate scope, reactivity); safety; and environmental issues. Uronium salts are advantageous because of their prominent reactivity and efficient reaction rates,[8,14] but the most commonly applied triazole-based reagents, such as HBTU and HATU, possess dangerous explosive properties[54] and pose significant health risks.[55] COMU was introduced as a safe and "greener" replacement.[47,48,56] To our delight, COMU also noticeably exceeded the coupling efficiencies of HATU and EDC in our experiments (Scheme 2, Chart 2), delivering a high 96% conversion. TCFH can be considered as an even more reactive alternative with better atom economy, affording a high 97% yield of amide **4** within only 10 min.

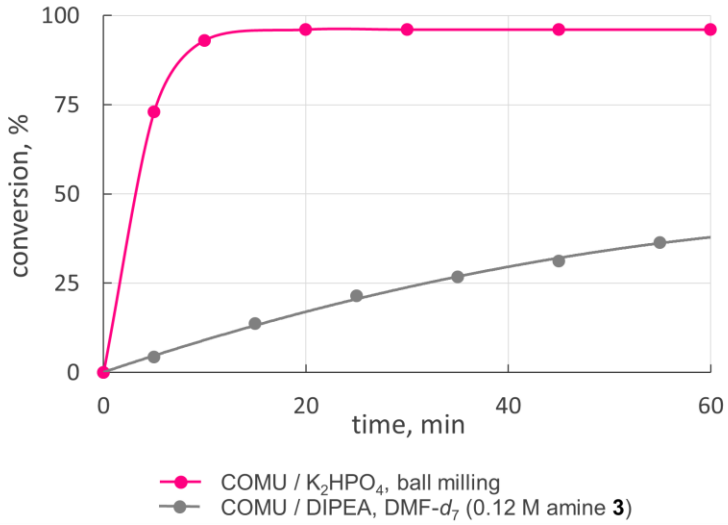


Figure 1. Accumulation of amide **4** over time in mechanochemical (purple) and solvent-based (gray) reactions.

The mechanochemical amidation with COMU/K_2HPO_4 was also rapid, reaching the maximal conversion within 20 min (Figure 1; see the Supporting Information for further detail), far surpassing the rate of the solution-based process (in DMF-d_7, Figure 1). The latter reached the maximal 70% conversion after approximately 20 h (see the Supporting Information). Concurrently, about 30% of COMU reagent degraded due its well-known hydrolytic instability in DMF solutions, which is often referred as the main disadvantage of COMU.[57,58] Evidently, this drawback can be fully eliminated under solvent-free conditions.

After achieving these results in the optimization experiments, we formulated the optimal experimental procedure as follows: COMU or TFCH (1.1 equiv.) as coupling reagents; K_2HPO_4 (3

equiv.) as base; ethyl acetate as LAG additive, and 20 min milling time. The amount of solid base (3 equiv.) was adjusted to keep η within the optimal range (~ 0.2 μL/mg), but not less than 2 equiv. required according to the reaction stoichiometry. Furthermore, an additional equivalent of K_2HPO_4 was required to release free amine when ammonium salt was used as the starting material. Isolation of pure amide **4** was achieved with a high 96% yield by simple water wash and filtration since all by-products are water soluble. No detectable racemization of the chiral center in **4** occurred during the synthesis, as was established by the chiral phase HPLC chromatography (see the Supporting Information).

Green Chemistry Metrics Comparison. The advantages and drawbacks of the developed mechanochemical amidation methods were further revealed and compared with the solution-based reaction by analyzing the respective green metrics (Table 1). The metrics were calculated and assessed by marking them with red, orange, or green flags by following the Clark's unified metrics toolkit (see Supporting Information).[59] Atom economy (AE), reaction mass efficiency (RME), and process mass intensity (PMI) are defined as follows:[59]

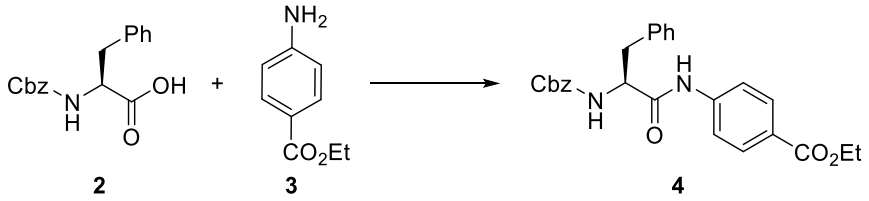
$$\mathrm{AE} = \frac{\text{molecular weight of product}}{\text{total molecular weight of reactants}} \times 100$$

$$\mathrm{RME} = \frac{\text{mass of isolated product}}{\text{total mass of reactants}} \times 100$$

$$\mathrm{PMI} = \frac{\text{total mass in a process}}{\text{mass of product}}$$

First, isolated yields and product purity were much better in mechanochemical reactions, due to the higher conversion and more facile isolation procedure discussed above. Atom economy was a bit higher for the TCFH-mediated reaction because of lower molecular weight of TCFH. RME reflects both product yield and atom economy issues and was lower for the solution-based reaction. Comparison of PMI values clearly shows that mechanochemical reactions produce far less waste. Excluding mass-extensive work-up procedures, solvent occupied 84% of PMI for the solution-based reaction and only about 15% (LAG additive) for the mechanochemical conditions. Furthermore, sustainable solvents like water and ethyl acetate were used in the latter, in contrast with toxic DMF.

Table 1. Comparison of Green Metrics for Mechanochemical and Solution-Based Amidation Reactions



2 3 4

Metrics	Mechanochemistry		Solution[a]
	COMU / K_2HPO_4	TCFH / K_2HPO_4	COMU / DIPEA in DMF
Yield (%)[b]	96	92	70
Atom economy (%)[c]	50	60	50
RME (%)	46	53	35
PMI (total), including:	196.3	203.7	1464.7
PMI (reactants)	3.4	3.1	3.7
PMI (solvent)	0.6	0.6	18.9
PMI (work-up)	192.3	200	1442.2[d]
Solvent choice	EtOAc, water	EtOAc, water	DMF
Work-up, isolation	filtration	filtration	chromatography
Health and safety			
Main hazard statements[e]	H302, H312	H360	H226, 312, 332, 360

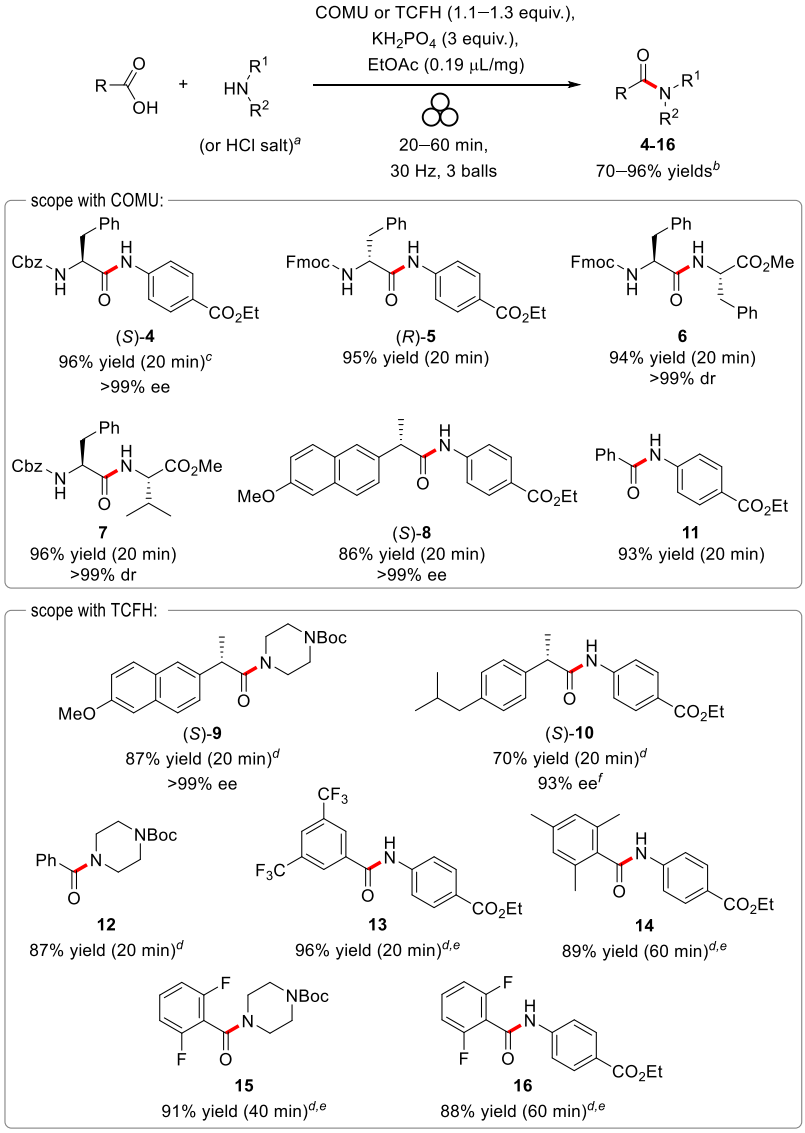
[a] Following the published protocol, see ref. [49]. [b] Yield of isolated product. [c] Including coupling reagent. [d] Excluding column chromatography. [e] See Supporting Information for additional safety data.

To determine the safety risks, a combination of physical, health, and environmental threats must be assessed, which can be done with the help of MSDS[60] and further available safety data.[54] DMF, for instance, is a flammable (H226), acute toxic (H312, 332), as well as a reproductive toxin (H360) and can thus be cited as the main hazard contributor for the solution-based process, which therefore received a red flag. For the mechanochemical reactions, the TCFH-mediated process was given a red flag due to the production of tetramethylurea by-product (reproductive toxin, H360). On the other hand, exothermic decomposition with a thermal onset of 127 °C can be considered as the main hazard of COMU, according to a recent study.[54] However, this property produced an orange flag, since COMU-mediated mechanochemical amidation protocol operates at room temperature. To conclude, although the developed mechanochemical amidation conditions cannot be considered totally safe, the risks are minimal because of its room temperature operation and relatively low

amount of produced waste, as opposed to the solution-based reaction (see Supporting Information for additional safety considerations).

Substrate Scope for mapping reactivity with COMU and TCHF. Having established the optimal conditions, substrate scope and limitations was briefly examined on a range of amine and acid coupling partners (Scheme 3).

Scheme 3. Substrate Scope for Mechanochemical Amidation with COMU/K_2HPO_4 and TCFH/K_2HPO_4 Systems.



[a] Amine hydrochloride salt was used for preparation of peptides **6** and **7**. [b] Yields of isolated products. [c] Obtained in 92% yield and >99% ee with TCFH. [d] Isolated by column chromatography. [e] With 1.3 equiv. of TCFH. [f] 94% ee with COMU.

The substrate scope included, besides other, N- and C-protected amino acids and pharmaceutically relevant starting materials (e.g. (*S*)-naproxen, (*S*)-ibuprofen, benzocaine **3**, *N*-Boc-protected piperazine). In addition to the foremost example of Cbz-masked amide (*S*)-**4** comprehensively described above, its Fmoc-protected analogue (*R*)-**5** was obtained in a high 95% yield by using the COMU-mediated reaction. Following the same protocol, dipeptides **6** and **7** with sterically hindered amino acid residues (phenylalanine and valine) were flawlessly prepared in high yields. No detectable epimerization of the stereocenters was noted in these cases. Coupling of (*S*)-(+)-6-methoxy-α-methyl-2-naphthaleneacetic acid [(*S*)-naproxen] with amine **3** provided a more demanding test for stereochemical integrity, since 2-arylpropionic acids are prone to easy epimerization.[61–64] The amide product (*S*)-**8** was obtained from (*S*)-naproxen with a high 86% yield and excellent stereochemical purity (>99% ee). This was also the case in the TCFH-mediated reaction, which showed high reactivity and a subtle amount of epimerization for (*S*)-**9**. On the other hand, amidation of (*S*)-ibuprofen (98% ee) produced (*S*)-**10** with slightly degraded optical purity (93–94% ee). Crude amides **10**, **12**, **15** appeared as oils immediately following the milling, which eventually enabled a chromatographic isolation for these cases (see Supporting Information).

One advantage of TCFH over COMU-mediated amide coupling is the higher reactivity of the former reagent, which makes it more suitable for less reactive substrates. This property was explicitly revealed during the amidation of sterically hindered *ortho*-substituted benzoic acids. Thus, coupling of benzoic acid with benzocaine **3** proceeded well under the COMU-mediated protocol, furnishing amide **11** in a 93% yield after 20 min of milling time. Conversely, 2,4,6-trimethylbenzoic acid under the same conditions produced only 22% of the target amide **14**, without any further improvement, even when a longer milling time (up to 60 min) was applied. After the brief optimization studies (see Supporting Information), we found that a slight excess (1.3 equiv.) of more reactive TCFH and at least 60 min of milling time are required to attain a high 89% yield of **14**. Moreover, chromatographic purification of **14** was necessary to separate mesitoic anhydride impurity. Diminished reactivity was also observed for 2,6-difluorobenzoic acid, furnishing amides **15** and **16** in reactions with *N*-Boc piperazine and low-nucleophilic amine **3** in acceptable yields after milling times of 40 and 60 min, respectively. On the other hand, coupling of the same amines with benzoic and 3,5-bis(trifluoromethyl)benzoic acids proceeded flawlessly, producing amides **12** and **13** with excellent yields and brief reaction times.

Activating Effect of Phosphate Salts. During the optimization studies, the enhancement of yields with dipotassium phosphate and potassium pyrophosphate was especially notable (Scheme 2, Chart 2). We speculated that phosphate salts could additionally contribute to the activation of the carboxyl substrate **2** via the formation of acyl phosphate intermediates containing a "high-energy" phosphoester bond, prone to easy nucleophilic amine attack.[65,66] Interestingly, the same pathway is also involved in the ATP-dependent biosynthesis of amide bond-containing biomolecules.[11] The plausibility of our assumption is further supported by existing literature showing that acyl phosphates can be indeed generated in solution by the DCC-mediated coupling of carboxylic acids with phosphate salts.[67–69] To confirm the credibility of our hypothesis, mechanochemical synthesis of acyl phosphates from carboxylic acids and phosphate salts, mediated by COMU and TCFH, was attempted.

As expected, a 20-min ball milling of COMU (1.1 equiv.) with acetic acid (1 equiv.) and K_2HPO_4 (3 equiv.) yielded 60% of acetyl phosphate **17**, which was confirmed by NMR analysis of the freshly obtained reaction mixture in D_2O solution (Figure 2). Acetyl phosphate **17** displayed a singlet signal at $\delta = -2.1$ ppm in ^{31}P NMR, which rapidly disappeared after the addition of morpholine, both in D_2O solution and in the solid state (see Supporting Information). In the ^{13}C NMR spectrum, carbonyl group **17** showed a doublet signal at $\delta = 168.1$ ppm ($J_{CP} = 8.8$ Hz), due to its coupling with the neighboring phosphorus.[65] Significantly lower yields of **17** were attained with K_3PO_4 or with TCFH as coupling reagent (Figure 2). The reaction of acetic acid with $K_4P_2O_7$ produced acetyl pyrophosphate **18** in a 50% yield, according to ^{31}P-NMR analysis. As a result of the non-equivalence of phosphorus atoms in **18**, a pair of doublet signals appeared in ^{31}P NMR, at $\delta = -5.0$ and -17.9 ppm (*d*, $J_{PP} = 21.7$ Hz), thus confirming its structure.[67] As an extra example, the generation of acyl phosphate **19** (50% yield, $\delta = -7.6$ ppm in ^{31}P NMR) was also successful from Cbz-masked phenyl alanine **2**, which was similar to the acetic acid outcome.

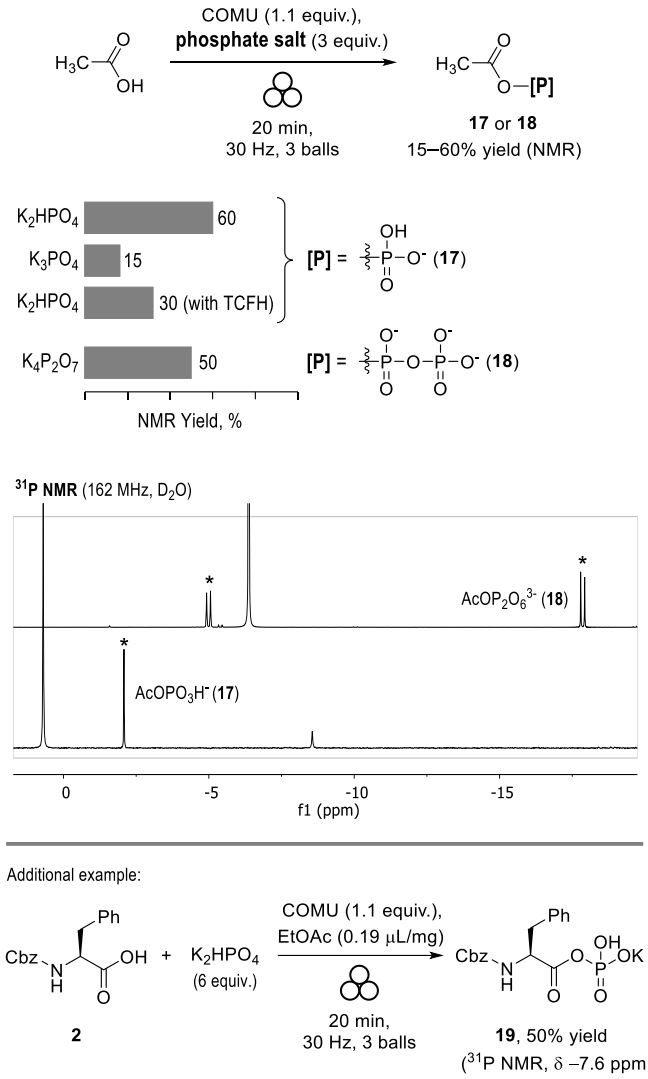
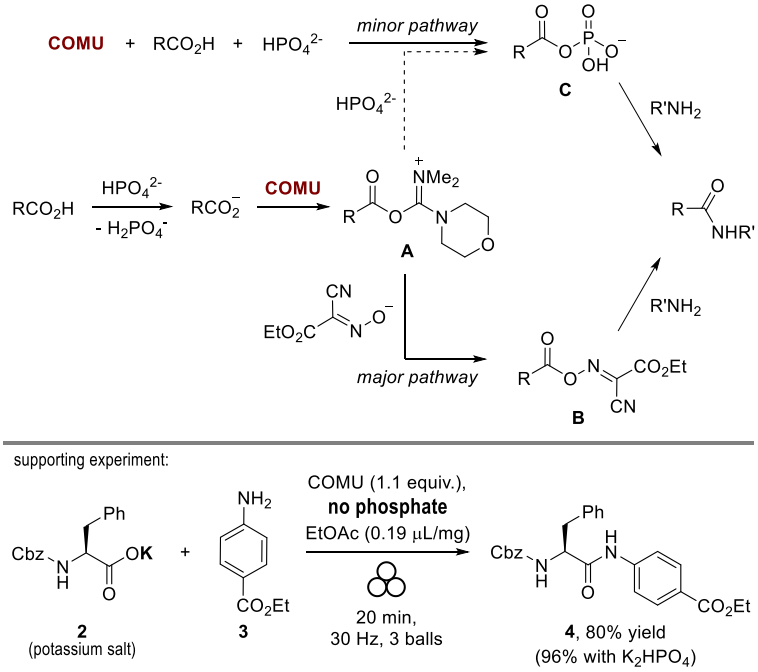


Figure 2. Mechanochemical generation of acyl phosphates **17**, **19** and acetyl pyrophosphate **18**. Signals of **17** and **18** in the traces of ^{31}P NMR spectra are marked with asterisks. Other signals belong to inorganic phosphates (see Supporting Information).

These results clearly indicate that generation of acyl phosphates can indeed take place in these newly developed mechanochemical amidation approaches and could account for the observed enhanced efficiency of K_2HPO_4 and $K_4P_2O_7$ additives. To evaluate the contribution of the acyl phosphate pathway (Scheme 4, via intermediate **C**) against the manifested activated ester pathway (via intermediates **A** and **B**),[48,70] the following experiment was undertaken. Amide coupling reaction of potassium salt of **2** with amine **3** without the phosphate salt additive afforded amide **4** in 80% yield, 16% lower than that obtained with K_2HPO_4. It was concluded from these results that in the amidation reaction leading to **4**, K_2HPO_4 acts primarily as a base performing deprotonation

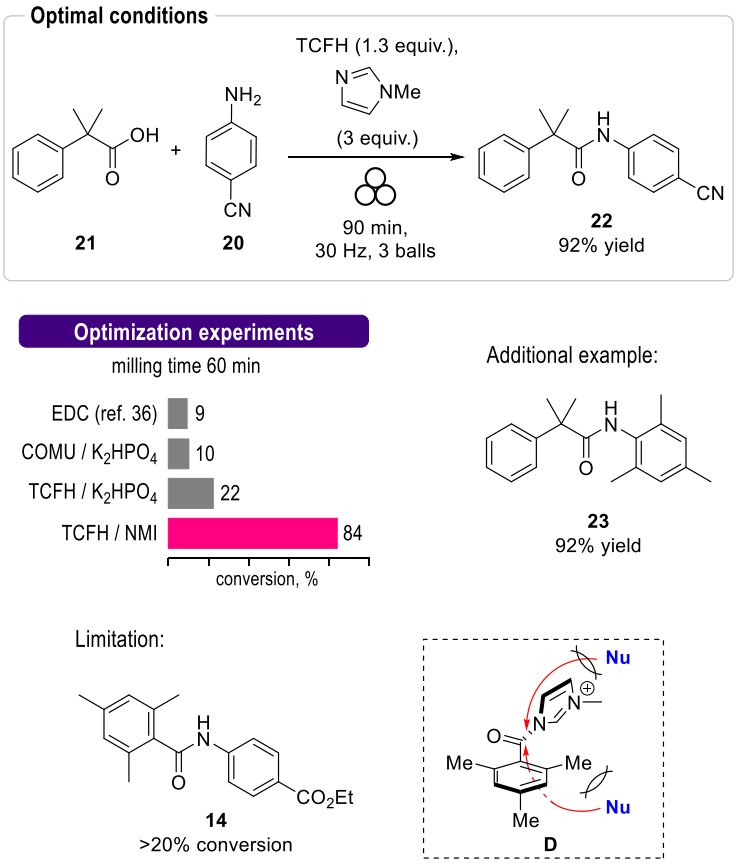
of **2**, but it also contributes at least 16% to the formation of amide **4** via acyl phosphate **19**. This estimation correlates well with the results of other optimization experiments (Scheme 2, Chart 2). For example, K_3PO_4 produced a rather low 15% yield of acetyl phosphate **17** (Figure 2), which also agrees with the lower conversion to amide **4** in the comparison with K_2HPO_4. The acyl phosphate pathway probably contributes less in the case of the more reactive TCFH reagent, which also produced a rather low 30% yield of **17** (Figure 2). The exact mechanistic sequence leading to acyl phosphates **C** from COMU, RCO_2H and K_2HPO_4 remains unclear but may include the reaction of acyl uronium intermediate **A** with HPO_4^{2-} anion (Scheme 4) or, alternatively, the initial formation of uronium phosphate[71] by the reaction of COMU with K_2HPO_4.

Scheme 4. Plausible Mechanistic Pathways Leading to Amide Product



Challenging Amide Bond Formation. As shown above, the coupling of low nucleophilic amine **3** with sterically hindered mesitoic acid could be efficiently mediated by the TFCH/K_2HPO_4 reagent system (Scheme 3). In accordance with existing literature,[61,72] we selected the coupling of electron-deficient 4-aminobenzonitrile **20** with 2-methyl-2-phenylpropanoic acid **21** (Scheme 5), an even more arduous way to test the performance of mechanochemical amidation protocols. Brief screening of various coupling conditions was undertaken, and conversion to amide product **22** was determined by ^{1}H NMR analysis after 60 min of milling time (Scheme 5).

Scheme 5. Mechanochemical Coupling of Hindered Carboxylic Acids and Poor Nucleophilic Amines



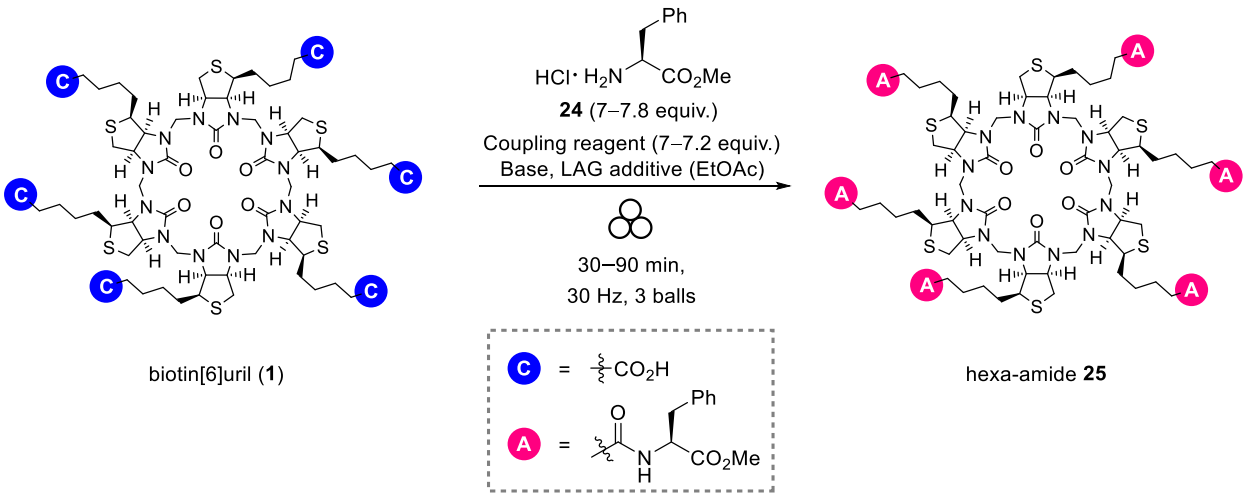
The use of EDC alone,[36] or the COMU/K_2HPO_4 system, yielded only a low ~10% conversion. Combination of TCFH and K_2HPO_4 delivered a noticeably better outcome but still failed to raise the conversion above 22%. According to the recent study of Beutner et al.,[61] *N*-methylimidazole (NMI) and TCFH reagent combined provided a high yield of **22** in solution, due to in situ generation of reactive *N*-acyl imidazolium ions. To our gratification, same combination of reagents also worked well under the solvent-free conditions, affording respectable 84% conversion after a 60–min reaction time. Finally, a slight excess (1.3 equiv.) of TCFH reagent, along with a bit longer milling time (90 min), allowed us to obtain pure amide **22** in 92% isolated yield after an aqueous work-up (see Supporting Information). Following the same reaction protocol, the coupling of **21** with sterically hindered 2,4,6-trimethylaniline was performed and furnished the corresponding amide **23** with a 92% yield. Notably, high yields of amides **22**, **23** were attained in a rather efficient reaction time of 1.5 h, in significant contrast with the solution-based reaction (21 h for amide **22**).[61]

Surprisingly, the same highly reactive combination of reagents failed to render amide **14** from mesitoic acid with yields exceeding 20%. This was also the case in the CD_3CN solution (see Supporting Information). We found that the reaction was stopped due to the formation of sterically bulky and therefore non-planar *N*-acyl imidazolium **D**, which, in contrast to the analogous species produced from benzoic acid, was totally inert towards the subsequent reaction with amine **3** (see Supporting Information for further detail). Inertness of **D** could be explained by the efficient steric shielding of the carbonyl group with both neighboring mesityl and imidazolyl moieties, preventing attack of a nucleophile along the Bürgi–Dunitz trajectory (Scheme 5). This stands in sharp contrast to the successful TCFH/K_2HPO_4-mediated transformation, where the less sterically crowded intermediate species are expected to form (e.g. mesitoyl chloride, uronium or phosphate).

Amide Coupling of Biotin[6]uril. As a part of our ongoing efforts towards the development of new chiral supramolecular receptors,[38–43] we needed an expedient synthetic procedure for derivatization of biotin[6]uril (**1**),[44] easily available in multigram quantities by HCl-catalyzed condensation of formaldehyde with D-biotin. The starting macrocyclic molecule, notable for its anion binding properties, common for the cucurbituril family,[73–77] satisfies 6 carboxylic functions, which could be conveniently coupled with various amines, thus providing facile access to a library of diversely functionalized chiral macrocyclic receptors. Although amide coupling of carboxylates in **1** might appear simple, unencumbered by any steric or electronic influence, full amidation of **1** is challenging because it proceeds via six consecutive steps. For example, if a high 97% yield were produced during each step, the fully functionalized product would eventually generate only a $(0.97)^6 \cdot 100\% = 83\%$ yield, while the rest of the produced material would contain a set of "failed" underfunctionalized molecules, thus necessitating time-consuming, laborious and mass-inefficient chromatographic purification. The situation resembles the synthesis of oligopeptides and oligonucleotides, in which an extremely high coupling efficiency (>99% per coupling step) is required to attain reasonable yields and high purity of long-chain oligomers, and it is customarily achieved by using an excess of highly reactive coupling reagents.[56] The low solubility of **1** in the environmentally benign and volatile organic solvents, compatible with the conventional amidation protocols (e.g. ethyl acetate), constitutes an additional restriction of the solution-based chemistry. We believed that the high coupling efficiency observed under the solvent-free conditions would allow us to perform the desired functionalization in a high-yielding, and scalable manner without using an excess of reagents, toxic solvents, or laborious purification.

As a convenient model reaction for this study, we selected the amide coupling of **1** with methyl ester of phenyl alanine **24** (used as HCl salt, see Scheme 6). At its outset, this task required us to explore the performance of different amide coupling conditions. Only a slight excess of amine **24** and a coupling reagent (7–7.8 equiv., which is 1.16–1.3 equiv. per CO_2H group of **1**) were applied in the optimization experiments. It was expected that more reactive combinations of reagents would deliver higher yields of the hexa-amide product **25**. Based on our previous findings, the order of coupling efficiency for the different reagent systems can be roughly plotted as follows: EDC ~ COMU/K_2HPO_4 < TCHF/ K_2HPO_4 << TCHF/NMI. Although such generalizations must be made with care since the coupling performance is substrate-dependent[8,78] and exceptions are possible (e.g. case of amide **14** above), this preliminary reactivity plot provided a helpful guide.

Scheme 6. Derivatization of biotin[6]uril (1) via six-fold amidation with L-phenylalanine methyl ester (24).



Outcomes of the test reactions were analyzed by HPLC (Figure 3, see Supporting Information for further detail) and quantified by calculating HPLC area percentage for the hexa-amide product **25** (S_{rel}, Table 2), relative to underfunctionalized compounds.

Table 2. Amide coupling of biotin[6]uril with phenylalanine methyl ester 24

Entry	Reaction conditions [a]		Liquid chemicals (additives, solvents)	η, µL/mg	Time, min	S_{rel}, %[b]
1	**Ball milling**	COMU / K_2HPO_4	EtOAc	0.19	90	16
2		TCFH / K_2HPO_4	EtOAc	0.19	90	16
3		EDC / DMAP[c]	CH_3NO_2	0.25	90	55

4		TCFH / NMI	NMI	0.29	90	52
5		TCFH / NMI[d]	NMI	0.32	60	86
6		TCFH / NMI[d] / NaCl[e]	NMI	0.16	60	73
7		TCFH / NMI[d]	NMI, DMF	0.53	60	97
8		TCFH / NMI[d]	NMI, heptane	0.64	60	84
9		**TCFH / NMI**[d]	**NMI**, **EtOAc**	**0.64**	**60**	**98**
10	**Slurry stirring**	TCFH / NMI[d]	NMI, EtOAc	0.64	60	95
11	**Solution** (DMF)	HATU, DIPEA	DIPEA, DMF	2.1	60	68
12		TCFH, NMI[d]	NMI, DMF	2.4	60	98

[a] Reaction conditions: biotin[6]uril (50–70 mg, 0.03–0.05 mmol), **24** (7 equiv.), coupling reagent (7 equiv.), base (18 equiv.), unless other specified. [b] HPLC area percentage of hexa-amide **25**, relative to other amide products. [c] 12 equiv. of DMAP was used, following the published procedure, see ref.[36]. [d] With 7.2 equiv. of TCFH, 7.8 equiv. of **24** and 21 equiv. of NMI. [e] NaCl was used as grinding additive.

These initial experiments (Table 2, entries 1–4) clearly indicated that complete hexafunctionalization of **1** is difficult to perform. Thus, both the COMU and TCFH/K_2HPO_4 systems produced a mixture of phenylalanine-derivatized biotin[6]urils, containing all possible products from mono to hexa-amide **25**, the latter displaying a rather low 16% contribution (entries 1 and 2; Figure 3A). The use of EDC/DMAP combination (entry 3),[36] was more successful in this case, primarily producing a mixture of penta- and hexa-amides (Figure 3B). The highly reactive TCFH/NMI combination (entry 4) generated hexa-amide **25** as its main reaction product, but it was noticeably contaminated with underfunctionalized compounds (52% HPLC area, Figure 3C).

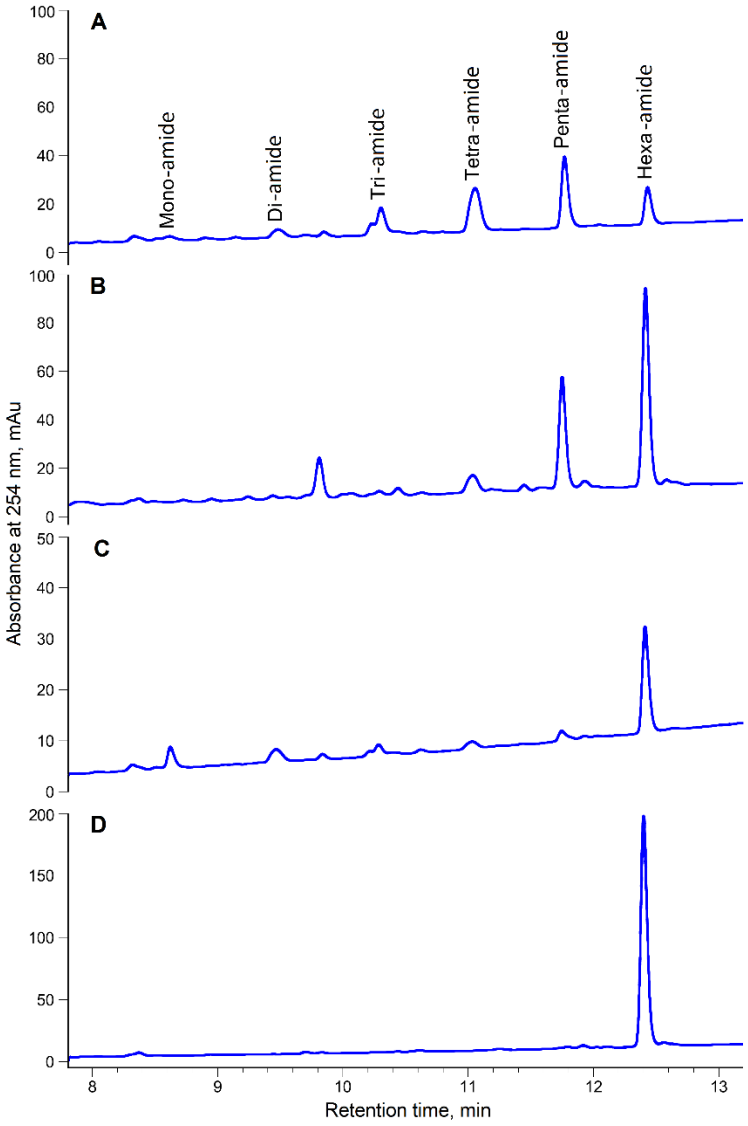


Figure 3. Derivatization of biotin[6]uril via amide coupling with phenylalanine methyl ester **24**. Traces of HPLC chromatograms for the selected reaction mixtures: **A**) COMU/K_2HPO_4; **B**) EDC/DMAP; **C**) TCFH/NMI; **D**) TCFH/NMI with EtOAc as LAG additive.

Notably, at least 90–min milling times had to be applied, since samples taken after 30 and 60 min still showed incomplete conversion (see the Supporting Information). Although the FTS1000 shaker mill could hypothetically achieve long milling, we considered any time longer than 1.5 h as impractical; therefore, our next goal was to adjust the reaction parameters accordingly, in order to reach at least 90% conversion within a 1.5–h reaction time.

Applying a slightly greater excess of TFCH (1.2 equiv. per carboxylate) and NMI (3.5 equiv. per carboxylate) noticeably improved the yield of the target product, **25** (86% HPLC area, entry 5),

and also shortened the reaction time. For further improvement, a screening of optimal η and LAG additive was performed. Since NMI is a liquid, and liquid tetramethylurea is produced, the addition of solid NaCl was attempted to reduce the initial η to 0.16 μL/mg. However, this distinctly reduced the yield of the product (73% HPLC area, entry 6). On the other hand, the addition of a few drops (ca. 35–50 μL) of solvents noticeably improved the outcome (entries 7 to 9) and was best when polar solvents like DMF or EtOAc were added (entries 7 and 9). These results clearly indicate that the nature of LAG additive plays an important role[37] and can substantially increase reaction rate, a probable result of the favorable interactions of the polar reactants with the mobile surface layers of LAG additive and improved mass transfer.[24] The outcome with EtOAc was especially remarkable, providing **25** with the best purity (98% HPLC area, Figure 3D). Since the reaction mixture visibly liquefied as the reaction progressed (due to the generation of tetramethylurea), slurry stirring was also tried instead of the ball milling (entry 10) and resulted in slightly reduced coupling efficiency.

Solution-based amide couplings were performed in DMF (entries 11 and 12) for the comparison with mechanosynthesis. Homogeneous solutions were obtained with an amount of solvent (ca. 0.5 mL) comparable to the weight of solid reactants (ca. 0.24 g), what kept η at around 2 μL/mg. In the DMF solution, HATU was another frequently used and highly reactive uronium-based amide coupling reagent that produced a rather modest outcome (entry 11). Conversely, the coupling efficiency of the TCFH/NMI combination in DMF solution (entry 12) was virtually the same as in the DMF-free transformation to a solid state (entry 9). Importantly, a bulk amount of harmful solvent was fully avoided in the latter.

Under the optimal reaction conditions (entry 9), the desired hexa-amide product **25** was isolated in a nearly quantitative yield and 95% HPLC-purity (relative to all other peaks) after the simple water wash and filtration. Purity of product was further increased (99% according to HPLC) by following the simple purification protocol (filtration of chloroform solution via Celite®, and then precipitation with hexane from EtOAc solution [see Supporting Information]). The same amide coupling reaction was also successful at loadings that were 3 times higher (150 mg of **1** per milling jar, 300 mg total), creating **25** in 80% isolated yield and 99% HPLC purity, albeit with a longer milling time (90 min).

CONCLUSIONS

In conclusion, we have developed a new mechanochemical approach for the direct synthesis of amides from carboxylic acids and amines by employing uronium-type amide coupling reagents (COMU, TCFH) and K_2HPO_4 as a base. The reaction protocols demonstrated fast reaction rates (typically within 20 min), generally high yields, an absence of noticeable epimerization for stereogenic centers adjacent to carbonyl group, and a simple isolation procedure for solid amide products. In addition to faster rates of solvent-free amide couplings in contrast to the solution-based protocols, the absence of solvent eliminated reagent compatibility issues (e.g. COMU and DMF), greatly reduced the amount of waste generated, and significantly attenuated safety risks. The dual role of K_2HPO_4, as both a base and an activating reagent for a carboxylic acid substrate, was also manifested. The rapid formation (within 60–90 min) of amide products was observed for even challenging coupling partners, such as sterically hindered carboxylic acids and poor nucleophilic amines, within the TCFH/K_2HPO_4 and TCFH/NMI reagent systems. However, full amidation of polyfunctionalized substrates, e.g., biotin[6]uril, was found to be especially challenging, even though the single amide bond itself is not difficult to form. Highly reactive coupling conditions (TCFH/NMI), prolonged reaction times (60–90 min), and suitable LAG additives (EtOAc) are essential for producing the hexa-amide **25** in high yield and purity. This efficient and environmentally benign synthetic methodology is useful for preparation of analogues of a new supramolecular host **25**, as well as for synthesis of peptides and amides, which could be used in various fields of applied chemistry.

Notes

The authors declare no competing financial interest.

ASSOCIATED CONTENT

Supporting Information: experimental protocols, additional experimental data, copies of NMR spectra, HPLC chromatograms.

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ABBREVIATIONS

AE, Atom economy; CDI, *N*,*N*'-carbonyldiimidazole; COMU, (1-Cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholinocarbenium hexafluorophosphate; DCC, dicyclohexylcarbodiimide; DCM, dichloromethane; DIPEA, *N*,*N*-diisopropylethylamine; DMAP, 4-dimethylaminopyridine; DMF, *N*,*N*-dimethylformamide; EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide; HATU, Hexafluorophosphate Azabenzotriazole Tetramethyl Uronium; HBTU, Hexafluorophosphate Benzotriazole Tetramethyl Uronium; HPLC, High Performance Liquid Chromatography; LAG, Liquid Assisted Grinding; MSDS, Material safety data sheet; NMI, *N*-methylimidazole; Oxyma, ethyl cyanohydroxyiminoacetate; PMI, process mass intensity; RME, reaction mass efficiency; TCFH, *N*,*N*,*N*′,*N*′-tetramethylchloroformamidinium hexafluorophosphate; TCT, 2,4,6-trichloro-1,3,5-triazine.

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