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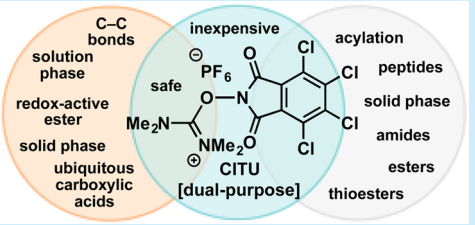
CITU: A Peptide and Decarboxylative Coupling Reagent

Justine N. deGruyter,[†,⊥] Lara R. Malins,[†,⊥] Laurin Wimmer,[†] Khalyd J. Clay,[†] Javier Lopez-Ogalla,[†] Tian Qin,[†] Josep Cornella,[†] Zhiqing Liu,[‡] Guanda Che,[‡] Denghui Bao,[‡] Jason M. Stevens,[§] Jennifer X. Qiao,[∥] Martin P. Allen,[∥] Michael A. Poss,[∥] and Phil S. Baran*[,†]

[†]Department of Chemistry, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, United States
[‡]Asymchem Laboratories, (Tianjin) Co., Ltd., TEDA, Tianjin 200457, P. R. China
[§]Chemistry and Synthetic Development, Bristol-Myers Squibb, 1 Squibb Drive, New Brunswick, New Jersey 08901, United States
[∥]Department of Discovery Chemistry, Bristol-Myers Squibb, P.O. Box 5400, Princeton, New Jersey 08543, United States

Supporting Information

ABSTRACT: Tetrachloro-*N*-hydroxyphthalimide tetramethyluronium hexafluorophosphate (CITU) is disclosed as a convenient and economical reagent for both acylation and decarboxylative cross-coupling chemistries. Within the former set of reactions, CITU displays reactivity similar to that of common coupling reagents, but with increased safety and reduced cost. Within the latter, increased yields, more rapid conversion, and a simplified procedure are possible across a range of reported decarboxylative transformations.



C−C bond formation via cross-coupling and C−N bond formation via amide bond construction remain the most useful reactions in the modern organic chemist's arsenal.[1] The objective of this work was to develop an inexpensive and safe multipurpose reagent that would conveniently enable both acylation of heteroatoms—with particular interest in solid- and solution-phase peptide synthesis—and decarboxylative cross-coupling reactions. Herein, we report the realization of this goal with the invention of CITU (**1**, Figure 1), a reagent that successfully blends high reactivity in both reaction manifolds with a safety profile that is particularly attractive for industrial applications.

Over the past year and a half, a variety of decarboxylative cross-coupling reactions have emerged which exploit simple activating agents for the formation of redox-active esters (RAEs)—so named for their ability to accept an electron from a suitable metal catalyst. Tetrachloro-*N*-hydroxyphthalimide (TCNHPI) esters, forged through activation of carboxylic acids with TCNHPI and *N*,*N*-diisopropylcarbodiimide (DIC), are preferred RAE substrates in several of the cross-coupling protocols.[2] One limitation of this chemistry is the long activation periods and large excesses of activating agents that are required for the efficient conversion of peptide-based substrates. Inspired by historical advances in peptide coupling reagents,[3] the promising TCNHPI motif was modified to incorporate a tetramethyluronium moiety,[4] a cationic core commonly found in similar reagents. Thus, tetrachloro-*N*-hydroxyphthalimide tetramethyluronium hexafluorophosphate (CITU) was invented. Synthesized from the reaction of tetramethyluronium chloride and TCNHPI (itself derived from a nontoxic flame retardant) in the presence of mild base, CITU can be safely made on multikilogram scale (Figure 1). The reagent features great versatility in reaction scope, performing well in peptide coupling reactions as compared to industry standards. Extensive epimerization studies (vide infra) indicate a negligible degree of epimerization at the α-stereocenter in solution, while epimerization on solid phase is both minimal and comparable with that of similar reagents (e.g., OxymaPure/DIC, PyAOP, HATU). Additionally, CITU has proven to be a powerful activator in the suite of recently reported nickel-catalyzed decarboxylative cross-coupling methods, forming adducts that readily engage with various zinc reagents in alkylation,[2a] arylation,[5] alkenylation,[2c] and alkynylation[2d] reactions. Formation of the requisite redox-active ester is efficient, mild, and can be similarly achieved in either solid- or solution-phase reactions. When applied to peptide substrates, the CITU-mediated cross-coupling approach enables direct introduction of

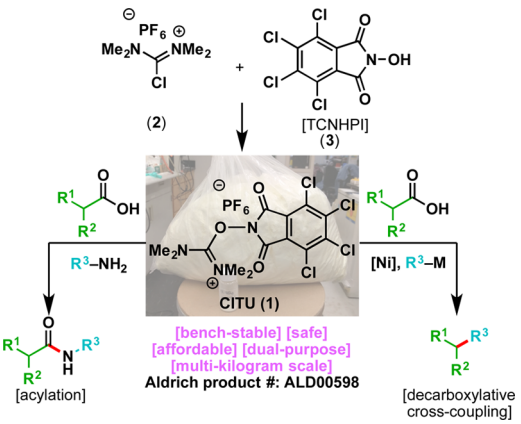


Figure 1. CITU: a dual-purpose activating reagent.

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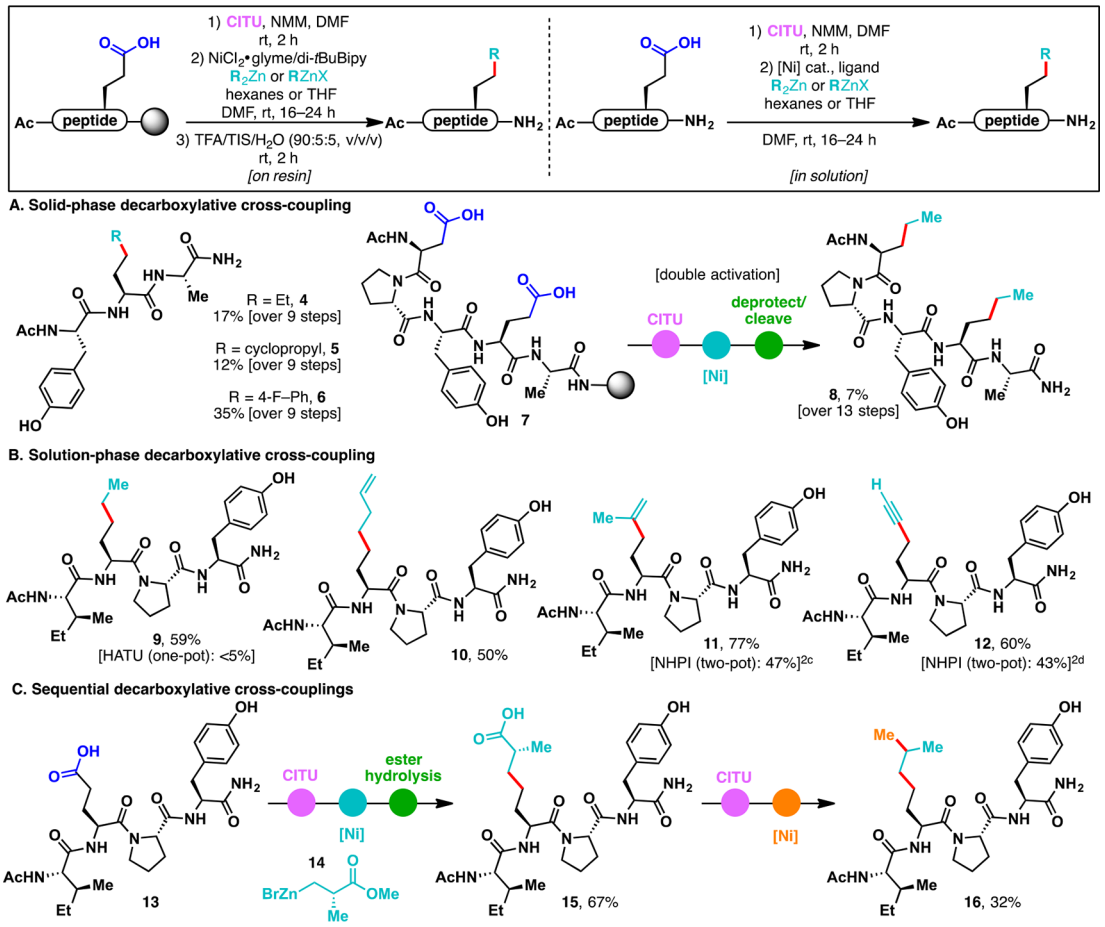
Scheme 1. CITU Activation and Decarboxylative Cross-Coupling



non-natural side chains onto native sequences, a challenge that has captivated synthetic chemists for decades.[6]

Investigations into the reactivity of CITU began with the construction of tri- and tetrapeptides assembled under standard Fmoc-solid-phase peptide synthesis (Fmoc-SPPS) conditions (Scheme 1A). Solid-phase cross-couplings necessitated orthogonal protection of the embedded glutamic (Glu) or aspartic (Asp) acids, and incorporation of allyl esters allowed for selective deprotection of the acid moieties while retaining the resin linkage and other acid-labile protecting groups. Once unmasked, a solution of CITU and *N*-methylmorpholine (NMM) in DMF was added to the resin-bound acid, which was then agitated at room temperature for 2 h. Following the activation period, the newly formed RAE was treated with a solution of the nickel–ligand complex and the desired zinc reagent for 16–24 h. While care was taken to exclude water from the SPPS vessel, no specialized equipment or skill is required for successful cross-coupling, a testament to the robust nature of the TCNHPI ester. Of note, one-pot, tandem activation (**8**) of Glu and Asp side-chains within a single peptide was accomplished in moderate yield (7% over 13 steps); judicious choice of orthogonal acid protecting groups should allow for sequential introduction of distinct groups when greater diversity is desired.

For solution-phase substrates (Scheme 1B), Glu was incorporated with the standard acid-labile *tert*-butyl ester protecting group, which was readily removed under the acidic conditions employed for resin-cleavage. Subsequent HPLC purification afforded the tetrapeptide **13** (shown in Scheme 1C) in excellent yield (79%). As with the solid-phase variant, solution-phase side-chain decarboxylative cross-couplings proceeded smoothly at room temperature, with alkylation (**9** and **10**), alkenylation (**11**), and alkynylation (**12**) all readily accessible pathways. Of note, formation of the TCNHPI ester of **13** did not proceed after 24 h with TCNHPI/DIC, while the NHPI ester required 16 h before complete consumption of the starting material was observed. In contrast, conversion to the TCNHPI active ester is complete after 2 h at ambient temperature when using CITU. Finally, tetrapeptide **13** was activated under standard CITU conditions and then subjected to nickel-catalyzed cross-coupling with the chiral zinc reagent **14**. Complete ester hydrolysis was observed under the reaction conditions to afford the free acid **15**, with the chiral center intact. A second CITU-mediated activation–alkylation sequence of the resulting carboxylic acid provided **16** in 32% yield. One drawback to the use of CITU, however, is that decarboxylative Suzuki cross-couplings[7] do not proceed, presumably due to inhibition by the PF_6^- counterion.

To evaluate efficiency as an acylating agent (Scheme 2A), CITU was next used to assemble select endogenous opioid peptides, manually in the case of hemorphin-4 (**17**) and spinorphin (**18**), and both manually and via automated SPPS for *β*-neoendorphin (**19**) and Leu enkephalin (**20**) (see the Supporting Information for details). Rubiscolin-6 (**22**), a *δ* opioid peptide, was similarly synthesized, albeit in lower yield; premature capping of the resin-bound amine with CITU hydrolysis products accounts for the mass balance in most cases. Further, CITU performed well in the synthesis of **21**, a hexapeptide recognized for its notoriously "difficult" to

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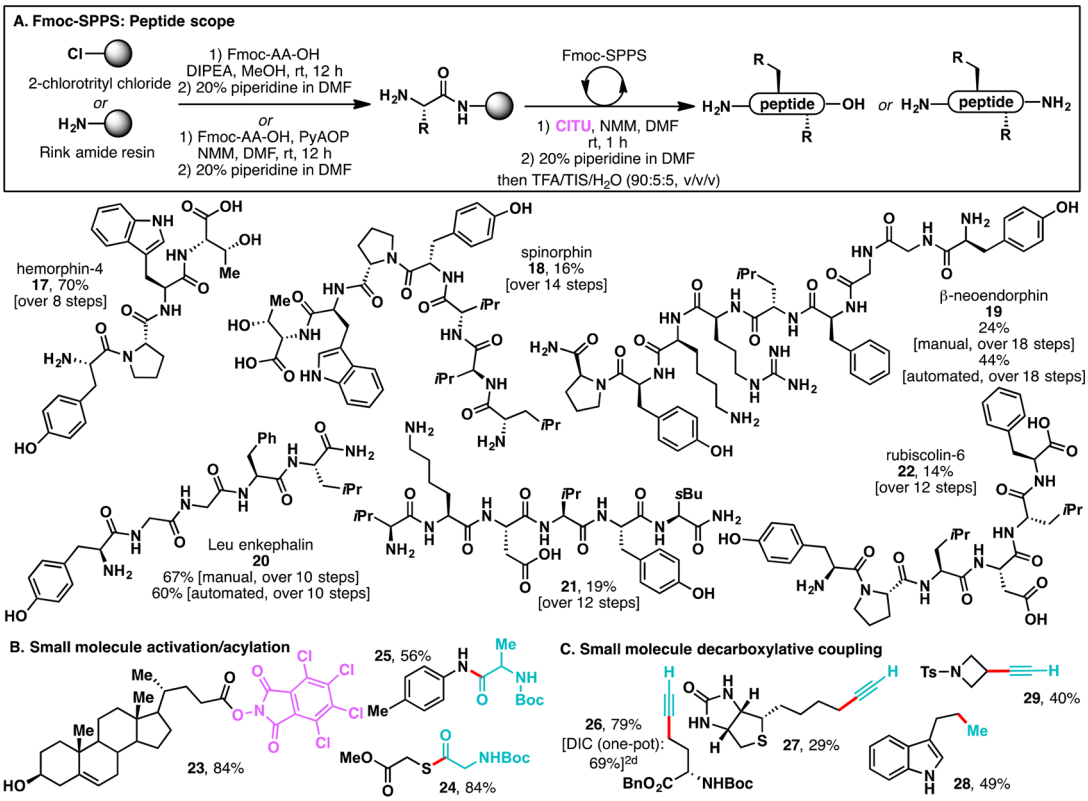
Scheme 2. Peptide Acylation and Small Molecule Functionalization



synthesize sequence.[8] Activation of small molecules (Scheme 2B) to form TCNHPI ester (**23**), thioester (**24**), and racemic amide (**25**) proceeded with similar facility, with yields ranging from fair to excellent. Notably, only 50% of the TCNHPI ester **23** was formed when using TCNHPI/DIC, a sharp contrast to the 84% conversion observed with CITU. CITU-mediated decarboxylative cross-coupling of several small molecules was also achieved (Scheme 2C), providing access to a variety of intriguing alkynyl substitutions, including that of bis-protected Glu (**26**),[2d] biotin (**27**), and the *N*-tosyl azetidine (**29**). Interestingly, alkylation of indole-3-propionic acid proceeded in good yield (**28**, 49%), with no protection of the indole core required.

Comprehensive solution-phase epimerization studies were undertaken (Figure 2A, see the SI for detailed analysis) for the synthesis of dipeptide Fmoc-AlaPhe-NH_2 (**30**) using a variety of solvents, bases, and additives. Of the 48 experiments conducted, only two instances of epimerization were identified, both of which used *N*-methylpiperidine as the base (max of 1.33% epimerization). Comparison of solid-phase degrees of epimerization with CITU to that of industry-standard coupling reagents utilized a fragment coupling approach for tripeptide Phth-AlaValPhe-NH_2 (**31**) and the iterative assembly of tripeptide H-GlyCysPhe-NH_2 (**32**), a substrate commonly employed in similar studies (Figure 2B, see the SI for experimental protocols).[9] For the latter, studies indicate that CITU performs better than comparable "all-in-one" reagents, but slightly worse than several that require additives (e.g., DIC). However, when cost and convenience are considered, the slight decrease in dr should not deter widespread process- and laboratory-scale implementation.

Given the explosive properties of benzotriazole-based coupling reagents,[10] differential scanning calorimetry (DSC) data were obtained (see the SI for experimental details) to assess the safety profile of CITU. The temperature of onset was established at 170 °C, with a decomposition heat of −509 J/g.

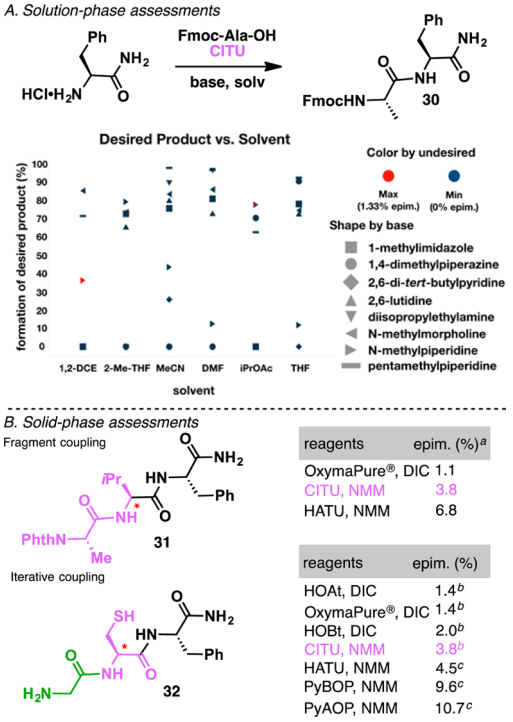


Figure 2. Epimerization studies. (a) Percent epimerization determined by analytical HPLC (UV trace, see the SI for details). (b) Average of values obtained in triplicate experiments as determined by analytical HPLC (UV trace, see the SI for details). (c) Percent epimerization determined by analytical HPLC (mass integration, see the SI for details).

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The data revealed no correlation when run through the Yoshida explosivity prediction model, signifying that the compound is unlikely to be shock sensitive and/or explosion propagating. For comparison, TBTU—one of the most structurally similar of the common coupling reagents—is classified as an explosive with an energy release of 401 kJ/mol, a value nearly 1.5 times higher than that of CITU (277 kJ/mol).[10a] Importantly, the reagent has been prepared in kilogram batches from tetrachlorophthalic anhydride, a factor which all but eliminates both cost and safety concerns.[10c] CITU is bench-stable when stored as a solid at ambient temperature; however, as with many comparable reagents, stock solutions (including those frequently employed in automated peptide synthesizers) should be freshly prepared every 2 days to avoid degradation and hydrolysis.

CITU, now commercially available,[11] is an inexpensive, dual-purpose reagent that enables both solid- and solution-phase acylation and decarboxylative cross-coupling reactions. It is best suited for practitioners seeking a safe alternative to the standard array of coupling reagents, particularly on large scale. While CITU is not recommended for use in the previously disclosed decarboxylative Suzuki cross-coupling reaction,[7] it can be readily employed for the analogous Ni-catalyzed Negishi alkylation, arylation, alkenylation, and alkynylation reactions, as well as for solution- and solid-phase acylations.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.7b03121.

Detailed experimental procedures and analytical data for linear peptides and small molecules (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: pbaran@scripps.edu.

ORCID

Lara R. Malins: 0000-0002-7691-6432

Phil S. Baran: 0000-0001-9193-9053

Author Contributions

⊥J.N.D. and L.R.M. contributed equally.

Notes

The authors declare no competing financial interest.

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(11) CITU is now available in the MilliporeSigma catalog (ALD00598).

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