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A New Oxyma Derivative for Nonracemizable Amide-Forming Reactions in Water

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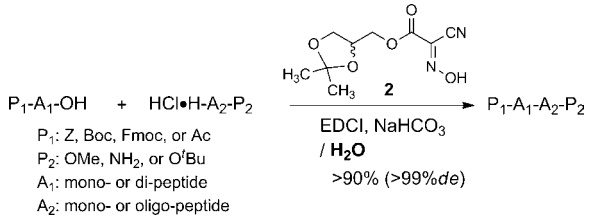
ABSTRACT



An Oxyma derivative, (2,2-dimethyl-1,3-dioxolan-4-yl)methyl 2-cyano-2-(hydroxyimino)acetate (2), displayed remarkable physicochemical properties as a peptide-coupling additive for peptide-forming reactions in water. Short peptides to oligopeptides could be synthesized by using 2, EDCI, and $NaHCO_3$ in water without measurable racemization. Significantly, a simple basic and acidic aqueous workup procedure can remove all reagents utilized in the reactions to afford only coupling products in consistently excellent yields.

The solid-phase peptide synthetic method has significantly contributed to basic and applied life sciences.[1] In parallel, peptide bond-forming reactions in the solution phase remain important C−N bond-forming processes in organic, inorganic, medicinal, and bioorganic chemistries.[2] To date, a large number of peptide coupling reagents and coupling additives have been developed, and a wide variety of peptide coupling reagents are now commercially available. In amide-forming reactions with α-amino acids, the use of a coupling additive such as benzotriazole derivatives (e.g., 1-hydroxy-7-azabenzotriazole (HOAt) and hydroxybenzotriazole (HOBt)) is essential to suppress racemization and improve the efficacy of peptide synthesis.[3] Recently, thorough evaluations of ethyl 2-cyano-2-(hydroxyimino)acetate, Oxyma (**1** in Table 1), for peptide synthesis have been reported by several research groups.[4] Oxyma is considered as an excellent nonexplosive replacement for HOBt and HOAt. Due to the fact that active esters of peptide-coupling additives are often the intermediates in peptide bond-forming reactions, the pK_a value of the peptide-coupling additive is one of the key factors that determines the reactivity of the additives and the stability of the active esters. The pK_a values of HOBt, HOAt, and Oxyma are 4.60, 3.28, and 4.60, respectively. Thus, effectiveness in suppressing racemization and increasing the reaction rate of peptide-forming reactions using Oxyma are believed to be similar to those of HOBt.[5] Suirós-Funosas et al. reported that dipeptide formation with Z-L-Phg-OH and H-L-Pro-NH_2 using the conditions of Oxyma (3 equiv) and *N,N′*-diisopropylcarbodiimide (DIC, 3 equiv) in DMF provided the desired peptide Z-L-Phg-L-Pro-NH_2 in 89.9% yield together with the racemized product (1.1%).[4c] In contrast, the same reaction with HOBt and DIC furnished the desired product in 81% yield with the racemized product, *Z*-D-Phg-L-Pro-NH_2 (9.3%).[6] These experimental results indicated that the degree of racemization in peptide-forming reactions with Oxyma can be minimized using nonbasic conditions with

(1) Merrifield, R. B. *Pure Appl. Chem.* **1971**, *50*, 643–653.
(2) (a) El-Faham, A.; Albericio, F. *Chem. Rev.* **2011**, *9*, 6557–6602. (b) Bray, B. L. *Nat. Rev.* **2003**, *2*, 587–593.
(3) Han, S.-Y.; Kim, Y.-A. *Tetrahedron* **2004**, *60*, 2447–2467 and references therein.
(4) (a) Khattab, S. N. *Bull. Chem. Soc. Jpn.* **2010**, *83*, 1374–1379. (b) El-Faham; Subirós-Funosas, R.; Albericio, F. *Chem.—Eur. J.* **2010**, *19*, 3641–3649. (c) Subirós-Funosas, R.; Prohens, R.; Barbas, R.; El-Faham, A.; Albericio, F. *Chem.—Eur. J.* **2009**, *15*, 9394–9403.
(5) Itoh, M. *Bull. Chem. Soc. Jpn.* **1973**, *46*, 2219–2221.
(6) The same reaction with EDCI, HOAt, NMM in DMF afforded Z-L-Phg-L-Pro-NH_2 in 95% yield with the racemized product (9.5%).

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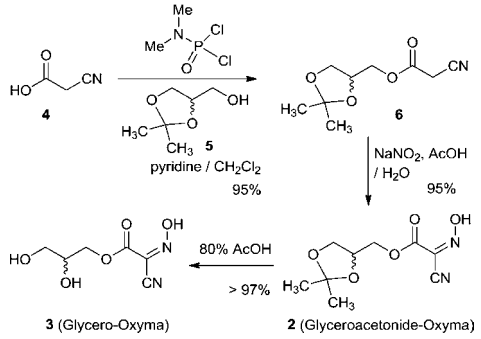
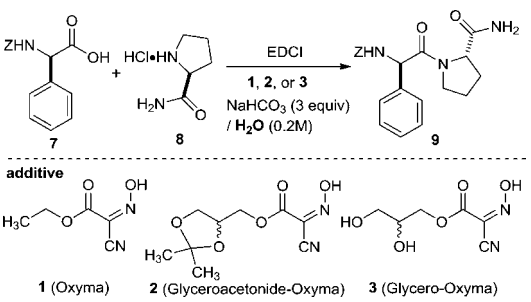
Scheme 1. Syntheses of the Oxyma Derivatives, **2** and **3**



salt-free amino acids or weakly basic conditions. To date, several attractive features of Oxyma in peptide chemistry have been reported using a conventional organic solvent such as DMF. Although peptide bond-forming reactions can often be performed in water containing organic solvents, no practical peptide coupling additive has been developed for the synthesis of oligopeptides in water.[7] Herein, we report the development of nonracemizable peptide-forming reactions of α-amino acids with glyceroacetonide-Oxyma derivative **2** in water.

Several Oxyma derivatives that increase water solubility were designed and synthesized. As summarized in Scheme 1, 2,3-dihydroxypropyl 2-cyano-2-(hydroxyimino)acetate (Glycero-Oxyma, **3**) could be synthesized in three steps from 2-cyanoacetic acid (**4**) with an excellent overall yield. The ester **6**, which was synthesized in a single step with *N, N*-dimethyl phosphoramidodichloridate,[8] was subjected to the hydroxyimination reaction using $NaNO_2$ and AcOH to furnish (2,2-dimethyl-1,3-dioxolan-4-yl)methyl 2-cyano-2-(hydroxyimino)acetate (Glyceroacetonide-Oxyma, **2**) in 95% yield.[9] Hydrolysis of the acetonide group of **2** yielded **3** in >97% yield. Several attempts at synthesizing other types of water-soluble Oxyma derivatives could not be achieved due to the fact that many water solubilizing groups such as the (*p*-hydroxyphenyl)-dimethylsulfonium group and dimethylaminoalcohols were not amenable to the hydroxyimination reactions (**6**→**2** in Scheme 1). Nonetheless, analyses of water solubility of **2** and **3** revealed that the glycerol and glyceroacetonide groups improved the water solubility 2.1 and >5 times greater than that of Oxyma in 0.2 M $NaHCO_3$ water solution (pH 8.3).[10] Thus, new Oxyma derivatives **2** and **3** are completely solubilized even at 0.1 M concentrations in water at a pH of 8.3. The effectiveness of the coupling yields and degree of racemization of Oxyma

Table 1. Yield and Diastereomeric Excess (*de*) of Z-L-Phg-L-Pro-NH_2 Isolated from the Reactions in Water with or without the Additives **1**, **2**, and **3**[a]



entry	additive	time (h)	yield (%)	*de* (%)[c]
1	**1**	2	25	>99
2	**2**	2	95	>99
3	**3**	2	5	>99
4	**3**	12	<10	>99
5	–[b]	2	45	75

[a] **7** (1.5 equiv), **8** (1.0 equiv), additive (1.2 equiv), EDCI (1.2 equiv), and $NaHCO_3$ (3 equiv) in H_2O (0.2 M concentrations). [b] No additive was added. [c] *de* was determined via HPLC and ^1H NMR analyses.

derivatives **2** and **3** was examined by an established model study using Z-L-Phg-OH and HCl•H-L-Pro-NH_2.[4c] In these studies we performed the reactions with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI)[11] and $NaHCO_3$ (3 equiv) in water solution. The yields and diastereomeric excess (*de*) of Z-L-Phg-L-Pro-NH_2 obtained with **1**, **2**, and **3** are summarized in Table 1.

The reaction with Oxyma **1** furnished the desired product **9** in 25% yield in 2 h with >99% *de* (entry 1).[12] Although an excellent *de* was attained, the product yield with **1** could not be improved even after extended reaction times. Interestingly, the same reaction with glyceroacetonide-Oxyma **2** provided **9** in 95% yield with greater than 99% *de* (entry 2). On the other hand, the reaction with glycero-Oxyma **3** did not provide a useful level of the product yield, but the *de* of the product was greater than 99% (entry 3). The product yield of **9** with **3** was not noticeably improved even after 12 h (entry 4). Due to the fact that the coupling reaction of **7** and **8** without the coupling additive (**1**, **2**, or **3**) furnished **9** in 45% with only 75% *de* (entry 5), the high *de*'s observed in entries 1–4 were attributed to the formation of oxime esters of **1**, **2**, or **3** (which are less racemizable or not at all) in water media at a pH of <8.3. However, the hydrophilic–hydrophobic balance of the coupling additive seems to be very important to achieve a high yielding amide coupling reaction between **7** and **8** in water.

To understand the scope and limitations of the peptide-forming reactions with EDCI, glyceroacetonide-Oxyma **2**,

(7) (a) Galanis, A. S.; Albericio, F.; Grøtli, M. *Org. Lett.* **2009**, *11*, 4488–4491. (b) Hojo, K.; Maeda, M.; Tanakamaru, N.; Kawasaki, K. *Protein Pept. Lett.* **2006**, *13*, 189–192. (c) Kouge, K.; Koizumi, T.; Okai, H.; Kato, T. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 2409–2418. (d) Kunz, H. *Angew. Chem., Int. Ed. Engl.* **1978**, *17*, 67–68 and references therein.

(8) Amancha, P. K.; Liu, H.-J.; Wei, T.; Shia, K.-S. *Eur. J. Org. Chem.* **2010**, *18*, 3473–3480.

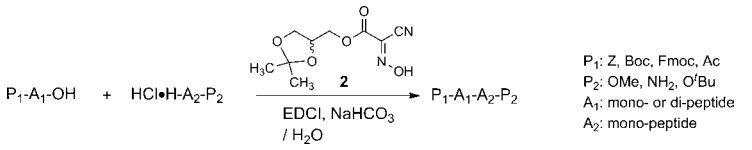
(9) Cheng, L. J.; Lightner, D. A. *Synthesis* **1999**, *1*, 46–48.

(10) Water solubility of **1**, **2**, and **3** was measured via the shake-flask method; **1** (14.90 mg/mL), **2** (31.5 mg/mL), and **3** (80 mg/mL), respectively.

(11) Kurzer, F.; Douraghi-Zadeh, K. *Chem. Rev.* **1967**, *67*, 107–152.

(12) The diastereomer was not detected by HPLC analysis (see Supporting Information).

Table 2. Syntheses of Di- and Tripeptides Using EDCI, Glyceroacetonide-Oxyme **2**, and $NaHCO_3$ in a Water-Based Solvent System[a]



entry	*N*-protected α-amino acid	*C*-protected α-amino acid	conditions[b]	time (h)	product	yield (%)	*de* (%)
1	Z-L-Tyr-OH	HCl•H-L-Ala-OMe	A	2	Z-L-Tyr-L-Ala-OMe	93	>99[c]
2	Boc-L-Tyr-OH	HCl•H-L-Ala-OMe	A	2	Boc-L-Tyr-L-Ala-OMe	95	>99[c]
3	Boc-L-Val-OH	HCl•H-L-Pro-NH_2	A	2	Boc-L-Val-L-Pro-NH_2	94	>92[d,c]
4	Fmoc-L-Tyr-OH	HCl•H-L-Ala-OMe	A	2	Fmoc-L-Val-L-Pro-NH_2	95	>99[c]
5	Fmoc-L-Val-OH	HCl•H-L-Pro-NH_2	A	2	Fmoc-L-Val-L-Pro-NH_2	94	>99[c]
6	Fmoc-L-Val-OH	HCl•H-L-Pro-NH_2	A	12	Fmoc-L-Val-L-Pro-NH_2	98	>99[c]
7	Boc-L-Lys($COCF_3$)-OH	HCl•H-L-Pro-NH_2	A	2	Boc-L-Lys($COCF_3$)-L-Pro-NH_2	93	>92[d]
8	Boc-L-Lys($COCF_3$)-OH	HCl•H-L-Ala-NH_2	A	2	Boc-L-Lys($COCF_3$)-L-Ala-NH_2	92	>92[d]
9	Boc-L-Val-OH	HCl•H-Gly-OMe	A	2	Boc-L-Val-Gly-OMe	95	>92[d]
10	Z-L-Phg-OH	HCl•H-L-Phe-OMe	A	4	Z-L-Phg-L-Phe-OMe	92	>92[d]
11	Z-L-Phg-OH	HCl•H-L-Phe-O^{t}Bu	A	12	Z-L-Phg-L-Phe-O^{t}Bu	40	>99[c]
12	Z-L-Phg-OH	HCl•H-L-Phe-O^{t}Bu	B	4	Z-L-Phg-L-Phe-O^{t}Bu	90	>99[c]
13	Z-L-Phg-OH	HCl•H-L-Val-O^{t}Bu	C	4	Z-L-Phg-L-Val-O^{t}Bu	90	>99[c]
14	Boc-L-Thr-OH	HCl•H-L-Ala-OMe	A	2	Boc-L-Thr-L-Ala-OMe	92	>92[d]
15	Boc-L-Tyr-OH	HCl•*N*-Me-Gly-OMe[g]	A	2	Boc-L-Tyr-*N*-Me-L-Ala-OMe	90	98[c]
16	Boc-L-Tyr-OH	HCl•*N*-Me-L-Ala-OMe	A	2	Boc-L-Tyr-*N*-Me-L-Ala-OMe	70	>99[c]
17	Boc-L-Tyr-OH	HCl•*N*-Me-L-Ala-OMe	A	6	Boc-L-Tyr-*N*-Me-L-Ala-OMe	92	>99[c]
18	Boc-L-Tyr-OH	HCl•H-L-Pro-OMe	A	2	Boc-L-Tyr-L-Pro-OMe	95	>99[c]
19	Ac-L-Ala-OH[e]	HCl•H-L-Ala-OMe	A	2	Ac-L-Ala-L-Ala-OMe	95	>92[d]
20	Ac-L-Phe-OH[e]	HCl•H-L-Ala-OMe	A	2	Ac-L-Phe-L-Ala-OMe	93	98[c]
21	Ac-L-Tyr-OH[e]	HCl•H-L-Ala-OMe	A	2	Ac-L-Tyr-L-Ala-OMe	93	>99[c]
22	Boc-L-Ala-D-(4-OH)Phg-OH[f]	HCl•H-L-Ala-OMe	A	2	Boc-L-Ala-D-(4-OH)Phg-L-Ala-OMe	95	>92[d]
23	Boc-L-Ala-L-Phe-OH	HCl•H-D-Val-OMe	A	2	Boc-L-Ala-L-Phe-D-Val-OMe	95	>99[c]
24	Boc-L-Ala-L-Phe-OH	HCl•H-D-Ala-OMe	A	2	Boc-L-Ala-L-Phe-D-Ala-OMe	95	>99[c]

[a] *N*-protected α-amino acid (1.5 equiv), *C*-protected α-amino acid (1.0 eqiv), **2** (1.2 equiv), EDCI (1.2 equiv), $NaHCO_3$ (3 equiv). [b] A: in H_2O (0.2 M). B: H_2O/DMF = 2:1 (0.2 M). C: Octyltrimethylammonium bromide (2 equiv) in water (0.2 M). [c] *de* was determined by HPLC. [d] *de* was determined by ^1H NMR analysis. [e] 3 equiv of acetylated α-amino acid was used. [f] (4-OH)Phg = 4-hydroxyphenylglycine. [g] *N*-Methylglycine methyl ester hydrochloride (Sar-OMe).

and $NaHCO_3$ in water, these conditions were applied to the syntheses of a wide variety of di- and tripeptides with partially protected α-amino acids. Selected examples are summarized in Table 2. *N*-Protected α-amino acids with commonly utilized carbamate protecting groups such as Cbz (Z), Boc, and Fmoc could be applied to the water-mediated amide-forming reactions; all reactions tested with methyl esters and *primary* amides of α-amino acids provided the desired dipeptides in >90% yield without detectable diastereomers (analyzed by ^1H NMR or HPLC) (entries 1−5).[13] The pH of the water-mediated peptide coupling reactions established in Table 1 is <8.3; thus, the formation of 9-methylene-9*H*-fluorene in the coupling reactions with Fmoc-L-Val-OH was not observed even with extended reaction times (entry 6). The dipetide-forming reactions of a hydrophilic α-amino acid, Boc-L-Lys($COCF_3$)-OH, with a hydrophilic or a hydrophobic *C*-protected amino acid provided the corresponding products in >92% yield with >92% *de* (determined by ^1H NMR) within 2 h (entries 7 and 8). We have investigated the feasibility of forming hydrophobic dipeptides in water media (entries 9−13). The coupling reaction between Boc-L-Val-OH and HCl•H-Gly-OMe in water gave Boc-L-Val-Gly-OMe in 95% yield with >92% *de* in 2 h. Significantly, the reaction of Z-L-Phg-OH with HCl•H-L-Phe-OMe occurred in water to furnish Z-L-Phg-L-Phe-OMe in 92% yield without detectable diastereomers in ^1H NMR after 4 h. On the other hand, the reaction of Z-L-Phg-OH with HCl•H-L-Phe-O^{t}Bu yielded the coupling product in only 40% yield even after 12 h, but the *de* of the product was >99% (entry 11). The low yield reaction of Z-L-Phg-OH with HCl•H-L-Phe-O^{t}Bu in water was dramatically improved by the addition of a phase-transfer catalyst, octyltrimethylammonium bromide (entry 13), or by using a mixture of H_2O and DMF (2:1) (entry 12).[14] The hydroxy group of Boc-L-Thr-OH

(13) The corresponding diastereomers were synthesized with *N*-protected D-α-amino acids for the ^1H NMR studies. In all ^1H NMR analyses of the products (entries 1−24), we did not observe the diastereomers. However, because of the detection limit of ^1H NMR spectroscopy, the *de* was tentatively determined to be >92% in Table 2.

(14) All reactions summarzied in Table 2 could also be performed using **2**, EDCI, and $NaHCO_3$ in H_2O/DMF (2/1).

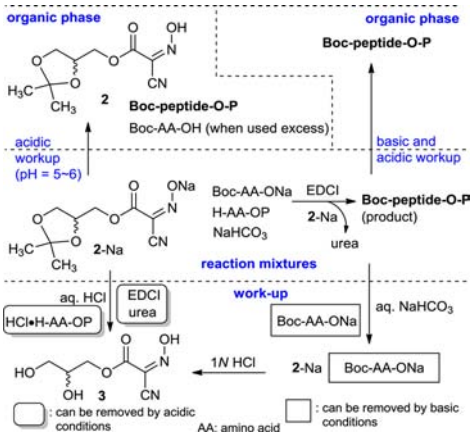


Figure 1. General workup procedures to extract coupling products from water media.

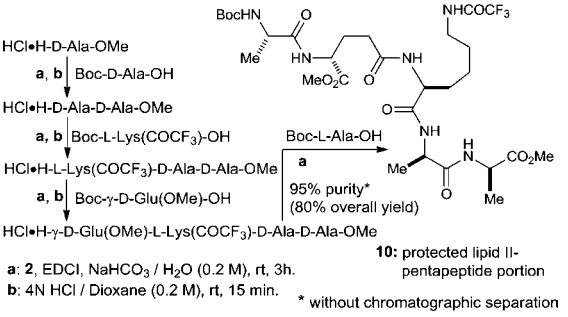
did not affect the coupling reactions in water (entry 14). The nucleophilicity of *secondary* amines of α-amino acids in peptide-forming reactions in water was also investigated (entries 15−18). HCl•*N*-Me-Gly-OMe could form the amide bond with Boc-L-Tyr-OH in 2 h to afford Boc-L-Tyr-*N*-Me-L-Ala-OMe in 90% yield with 99% *de*. The reaction rate of the coupling reaction of HCl•*N*-Me-L-Ala-OMe with Boc-L-Tyr-OH was slower than that of HCl•*N*-Me-Gly-OMe in water, but Boc-L-Tyr-*N*-Me-L-Ala-OMe was isolated in 92% yield after 6 h. As observed in Table 1, an excellent nucleophilicity of HCl•H-L-Pro-OMe in water could be confirmed by the reaction with Boc-L-Tyr-OH (entry 18).

One of the challenges in peptide syntheses of α-amino acids is achieving high-yielding reactions with *N*-acyl protected α-amino acids without racemization.[15] Several acetylated α-amino acids were examined for the coupling reactions with HCl•H-L-Ala-OMe in water (entries 19−21). Ac-L-Ala-OH could be coupled with HCl•H-L-Ala-OMe to afford Ac-L-Ala-L-Ala-OMe in 95% yield with >92% *de* (entry 19). The same reaction with standard conditions (HOAt, EDCI, NMM in DMF) furnished Ac-L-Ala-L-Ala-OMe in <5% yield with 75% *de* in 2 h. Similar to the result observed in entry 19, Ac-L-Phe-OH and Ac-L-Tyr-OH were reacted with HCl•H-L-Ala-OMe to yield the corresponding acetylated dipeptides in high yields without noticeable diastereomers (determined by HPLC analyses) (entries 20 and 21). Encouraged by the feasibility of peptide-forming reactions with *N*-acetylated α-amino acids, syntheses of tripeptides using the *N*-protected dipeptides were demonstrated via the water-mediated peptide-forming reactions (entries 22−24). Boc-L-Ala-D-(4-OH)Phg-OH was coupled with HCl•H-L-Ala-OMe to form Boc-L-Ala-D-(4-OH)Phg-L-Ala-OMe in

(15) Bodanszky, M.; Conklin, L. E. *Chem. Commun.* **1967**, 773–774.

(16) Lipid II is a membrane-anchored bacterial cell-wall precursor. The structure of *M. tuberculosis* lipid II represents GlcNAc-MurNAc-(L-Ala-γ-D-Glu(OMe)-L-Lys($COCF_3$)-D-Ala-D-Ala)-diphosphoryldecaprenol.

Scheme 2. Efficient Synthesis of **10** in Water



95% yield with >92% or >99 *de* (determined by ^{1}H NMR or HPLC).[13]

In glyceroacetonide-Oxyma **2**/EDCI mediated coupling reactions in water, **2** can be removed by basic aqueous workup or by hydrolysis of the acetonide of **2**. Excess starting materials (*N*- and *C*-protected amino acids), EDCI, and the urea byproduct are readily washed out by a standard aqueous workup procedure. Thus, protected oligopeptides synthesized via the conditions utilized in Table 2 can be isolated with high purity by basic and acidic aqueous workups (Figure 1). To date, we have demonstrated the syntheses of 4- to 18-mers of linear oligopeptides in water without chromatographic purifications. In most cases, syntheses of oligopeptides could be achieved in >90% overall yield using the Boc strategy. For example, an over 1 g quantity of a hydrophobic penptapeptide Boc-L-Ala-γ-D-Glu(OMe)-L-Lys($COCF_3$)-D-Ala-D-Ala-OMe (**10**), the peptide portion of lipid II,[16] was synthesized in water solution without chromatographic purifications (Scheme 2). In this synthesis the Boc groups of the intermediates were conveniently deprotected with 4 N HCl in dioxane. LC-MS and ^{1}H, ^{13}C NMR analyses of the crude product **10** revealed that purity of the product was concluded to be >95%.[12]

In conclusion, we have developed a new Oxyma derivative, (2,2-dimethyl-1,3-dioxolan-4-yl)methyl 2-cyano-2-(hydroxyimino)acetate (**2**), which displays remarkable physicochemical properties as a peptide-coupling additive in water media. Short peptides to oligopeptides could be synthesized by using **2**, EDCI, and $NaHCO_3$ in water without measurable racemization products. Significantly, simple basic and acidic aqueous workup procedures can remove all reagents utilized in the reactions to afford coupling products in high yield with excellent purity.

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Supporting Information Available. Experimental procedures and copies of NMRs. This is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.