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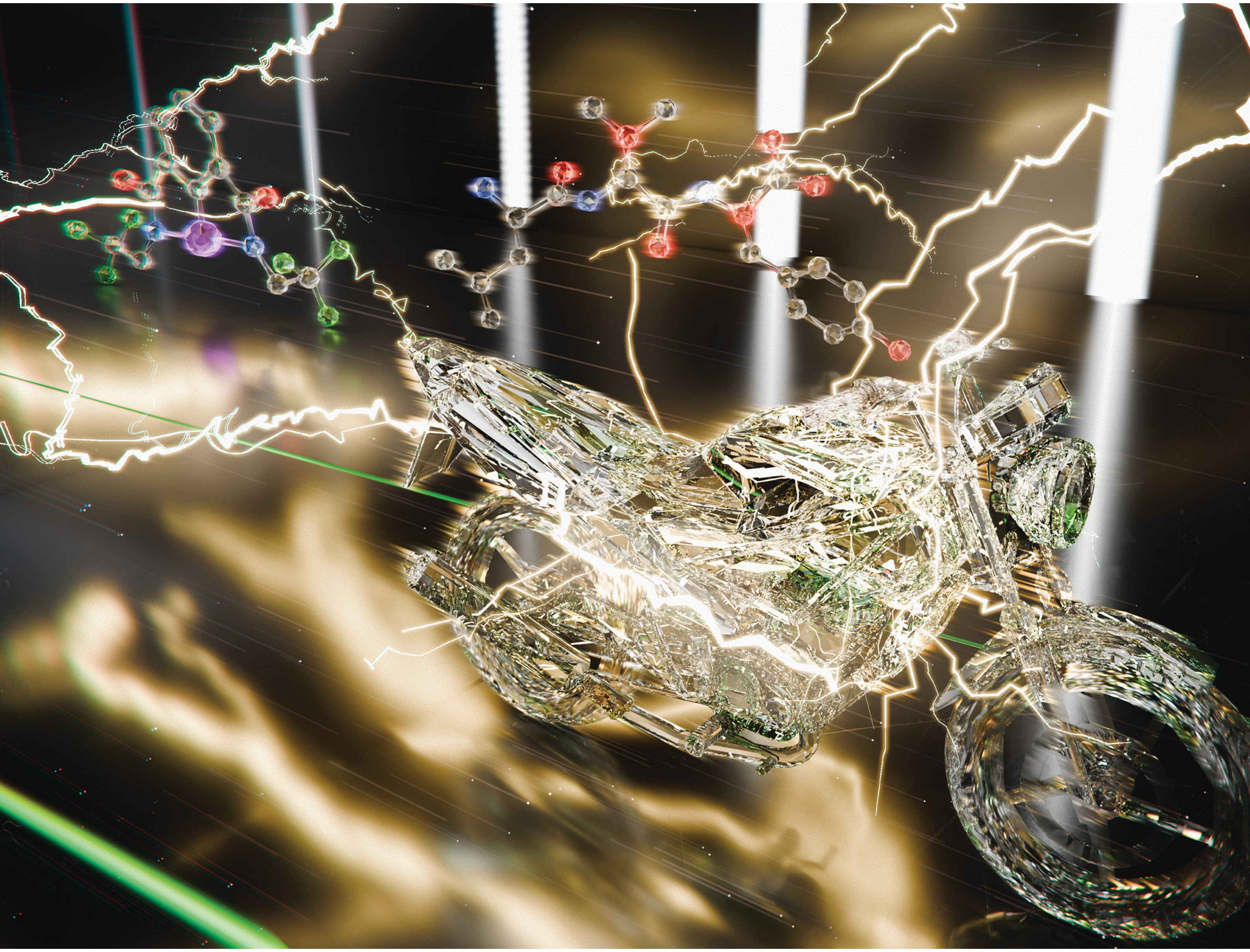

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Peptide coupling using recyclable bicyclic benziodazolone†

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We report a greener peptide coupling using bicyclic benziodazolone and triarylphosphine as coupling reagents. Bicyclic benziodazolone also works as a base and can be recovered as the corresponding iodine(I) compound after use, which can be converted to the original iodine(III) reagent by electrolytic oxidation.

The condensation reaction between a carboxylic acid and an amine provides the most straightforward approach to amide formation.[1] Nevertheless, since the amide condensation reaction does not smoothly proceed under mild conditions due to the formation of an ammonium carboxylate and low reactivity of the carboxylic acid, various coupling reagents have been developed for the amide condensation reaction including peptide coupling.[1c] In the synthesis of peptides, whose importance as candidates of medium molecular medicines has been increasing in recent years, uronium and phosphonium coupling reagents incorporating hydroxybenzotriazoles have been commonly used as well as the combination of carbodiimides with hydroxybenzotriazoles.[1c,2] However, although the hydroxybenzotriazoles are effective in avoiding epimerization of peptides, the benzotriazoles have been reported to exhibit explosive properties.[1c] In addition, at least stoichiometric amounts of coupling reagents are required, thereby generating stoichiometric or more amounts of byproducts as chemical waste.

In response to the above-mentioned issues, a variety of condensation catalysts have been developed for the amide formation. Since the first report of the catalytic amide condensation by Yamamoto and co-workers,[3a] arylboronic acids[3b–d] and the other organoboron catalysts[3e–h] have been well studied. However, although some of the catalytic methods have been demonstrated to be effective for oligopeptide synthesis,[3f] most of them require long reaction times (48–72 h) as well as desiccants (such as molecular sieves)[3b–d,f–h] or azeotropic removal of water.[3a,e] Furthermore, some of amino acids undergo epimerization under these catalytic conditions.[3d,f,g] Recently, other Lewis acid-catalyzed methods using the *in situ* generated silyl esters have been reported.[4] In these methods, although the active intermediate formed from the Lewis acid and the silyl ester produces peptide while avoiding epimerization, an excess amount of silylation reagent is required. Hence, the development of greener peptide synthesis processes is still desired.[1]

An alternative approach to waste reduction is to develop coupling reagents that can be recovered and reused. Considering that the conversion of phosphine oxide to phosphine can be carried out by various reduction methods[5] including electrolytic reduction,[5a–c] Chiba *et al.* very recently have developed the peptide coupling using triphenylphosphine under electrolytic conditions (Scheme 1a).[6] According to Chiba's report, electrophilic phosphine cations[7] generated by the electrochemical oxidation[7a] of triphenylphosphine serve as coupling reagents, activating carboxylic acids and facilitating the formation of amide bonds. In addition, the recovery of the phosphine oxide generated in this process has demonstrated the potential of phosphine as a recycling reagent.[6] Notably, since the electrolytic method performs oxidation and reduction by electron transfer at the electrodes, it is a greener method that does not emit waste derived from oxidants or reductants.[8] Unfortunately, basic additives have not been recovered, even though Chiba's method requires an excess amount of basic additive. Therefore, as part of our research on condensation reactions using hypervalent iodine reagents and phosphine,[9] we focused on bicyclic benziodazolone. The peptide coupling using the recyclable ester analogues benziodoxolones **1** has been vigorously developed by Zhang *et al.* (Scheme 1b).[10] According to

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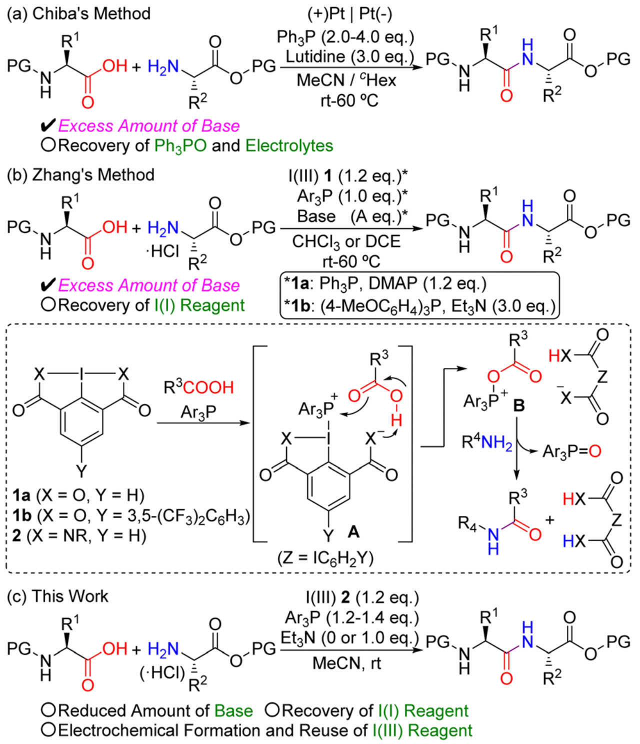
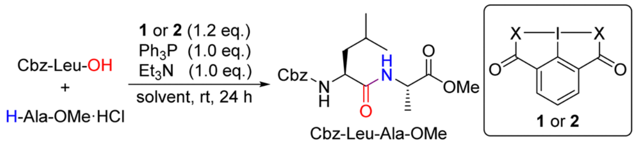


Scheme 1 Peptide coupling using oxidized phosphines.

Table 1 Optimization of conditions



Entry	1 or 2	Solvent	Peptide^a (%)
1	**2a** (X = N^{i}Pr)	DCE	84
2	**2a**	$MeNO_2$	78
3	**2a**	MeCN	74
4^b	**2a**	DCE	90
5	**2b** (X = N^{t}Bu)	DCE	76
6	**2c** (X = NCy)	DCE	81
7	**2d** (X = NMe)	DCE	74
8	**2e** (X = NCH_2CF_3)	DCE	26
9	**2e**	MeCN	90
10^{c,d}	**2e**	MeCN	97 (83)^e
11^{c,f}	**2a**	DCE	74
12^{c,f}	**2a**	MeCN	93 (71)^e
13^{f,g}	**2a**	MeCN	Quant. (48)^e
14^{c,f}	**1a** (X = O)	MeCN	52
15^{f,g}	**1a**	MeCN	30

^{a} Isolated yields of dipeptide products. ^{b} $(4\text{-}MeOC_6H_4)_3P$ instead of Ph_3P. ^{c} Temp.: 25 °C. ^{d} Ph_3P: 1.4 eq. ^{e} Values in parenthesis represent isolated yields of **3** (based on **1**). ^{f} Ph_3P: 1.2 eq. ^{g} Conditions: 40 °C, 5 h.

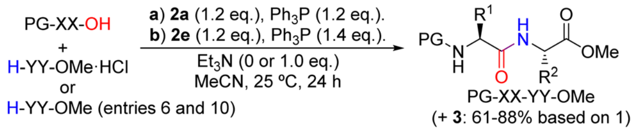
Zhang's reports, a carboxylate intermediate **A** (X = O) derived from **1** and phosphine reacts with carboxylic acid as a substrate to form acyloxyphosphonium intermediate **B**, which undergoes the nucleophilic acyl substitution reaction with an amine giving rise to a peptide. However, these reaction systems also require an excess amount of basic additive. This is probably not only due to slow ligand exchange between intramolecular ligands and phosphine,[10a,b] but also due to the low basicity of carboxylate **A** (X = O). In contrast to carboxylate, the corresponding amide anion **A** (X = NR) is expected to reduce the amounts of basic additives due to its strong basicity. In addition, since benziodazolones **2** can be chemically modified on nitrogen, it is easier to tune the reactivity of **2** than **1**. Herein, we report a greener peptide coupling using bicyclic benziodazolone **2** (Scheme 1c), which is recyclable by the electrolytic oxidation method[8g,h] and acts as a base and oxidant of phosphines.

Initially, the coupling reaction of *N*-benzyloxycarbonyl-L-leucine (Cbz-Leu-OH) with L-alanine methyl ester hydrochloride salt (H-Ala-OMe·HCl) was attempted in the presence of bicyclic benziodazolone **2a** (1.2 eq.) and Ph_3P (1.0 eq.) in various solvents at room temperature (Table 1). Note that Et_3N (1.0 eq.) was added to neutralize the hydrochloride of commercially available H-Ala-OMe·HCl. As a result, compared with relatively high polarity solvents (THF, DMF, DMSO) or toluene (Table S1 in ESI†), chlorinated solvents, $MeNO_2$ and MeCN showed good results (Table 1, entries 1–3). Especially, DCE gave the desired dipeptide Cbz-Leu-Ala-OMe in 84% yield (Table 1, entry 1), probably because the iodine reagent is relatively easy to dissolve and the Lewis acidity of iodine is hard to decrease. In addition, the use of $(4\text{-}MeOC_6H_4)_3P$ instead of Ph_3P increased the yield of the dipeptide to 90% (entry 4). Considering that phosphite esters did not afford the good results (Table S1 in ESI†), these results suggest that $(4\text{-}MeOC_6H_4)_3P$ having relatively high Lewis basicity (pK_a = 11.2)[11a] than Ph_3P (pK_a = 8.8)[11a] and phosphite esters[11b] would be more likely to form complexes with Lewis acid **2a**. However, since no significant improvement of the product yield was observed, the following study was conducted using the less expensive Ph_3P.

As for benziodazolones **2**, the ^{t}Bu-substituted **2b**, the cyclohexyl (Cy)-substituted **2c** and the Me-substituted **2d** only slightly reduced the yields of the dipeptides (Table 1, entries 5–7). However, the CF_3CH_2-substituted **2e** significantly reduced the product yield due to low solubility of **2e** in DCE (entry 8). Hence, when **2e** was used in MeCN, the yield of the dipeptide increased to 90% (entry 9). Furthermore, the increased amount of Ph_3P (1.4 eq.) at 25 °C led to the improved yield of the dipeptide up to 97% (entry 10). In this case, a simple extraction procedure allowed the recovery of iodoarene **3e** in 83%. Also, when **2a** was used in MeCN, the increased amount of Ph_3P (1.2 eq.) showed good result (the yield of dipeptide = 93%, the recovery of **3a** = 71%, entry 12) next to entry 10, even though the result did not change in DCE (entry 11). Under the similar conditions to entry 12, although the increased temperature to 40 °C afforded the dipeptides quantitatively for 5 h, the recovery of **3a** was reduced to 48% (entry 13). It should be mentioned that the use of benziodoxolones **1a** (entries 14 and 15) or other iodine(III) reagents (Table S1 in ESI†) instead of **2a** resulted in lower yields of the peptides. These results indicate that **2a** would easily undergo the ligand exchange with Ph_3P likely because of *trans* effect of benziodazolone[12] and/or N–I–N angle distortion (**2a**: N–I–N = 153.90° *vs.* **1a**: O–I–O = 155.21°).[9a,10a,b] In addition, considering that the yield of peptide decreased under elevated temperature conditions despite complete consumption of **1a** and Ph_3P (entry 15), the lower basicity of carboxylate **A** (X = O, Scheme 1b) would not smoothly promote the formation of acyloxyphosphonium intermediate **B** and thereby the carboxylate **A** decomposed under elevated temperature conditions.

Based on the above results (Table 1, entries 10 and 12), the formation of various peptides was examined using benziodazolone **2a** (1.2 eq., method **a**) or **2e** (1.2 eq., method **b**) in the presence of Ph_3P (1.2–1.4 eq.) in MeCN (Table 2). These methods could be applied to *N*-protected amino acids with *tert*-butoxycarbonyl (Boc, entry 4) and 9-fluorenylmethyloxy-carbonyl (Fmoc, entry 5) groups as well as Cbz groups (entries 1–3), and the desired dipeptides were obtained at 84–97%. In addition, Fmoc-Leu-OH reacted not only with tryptophan (Trp) having unprotected indole ring but also with serine (Ser) and tyrosine (Tyr) having unprotected OH groups, giving the corresponding dipeptides in 72–97% (entries 7–9). These results are noteworthy because the catalytic method required protection of these functional groups.[3f,g,4] The reaction with methionine (Met), which has methylsulfanyl group that is easily oxidized, also proceeded in good yield (82%, entry 11). On the other hand, glutamic acid (Glu) or lysine (Lys) showed good results (70% in both cases), albeit requiring protection of carboxyl and amino groups on the side chains (entries 12 and 13). Notably, good yields were obtained even when the free amino acid was used as a *C*-protected amino acid (entries 6 and 10), suggesting that Et_3N was not involved in the process other than neutralization of the hydrochloride salt.

Table 2 Substrate scope[a]



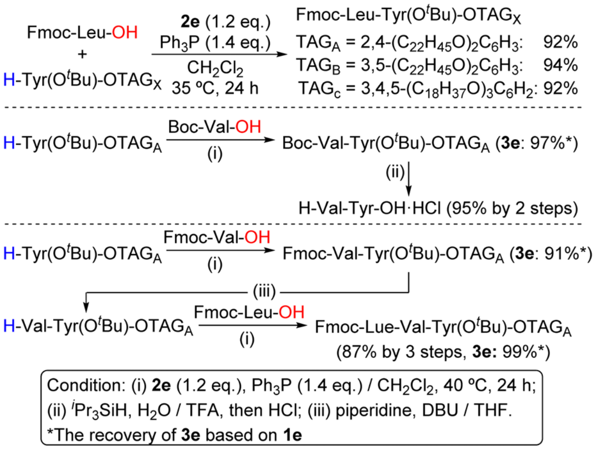
Entry	PG-XX-YY-OMe	Method	(%)[b]
1	Cbz-Leu-Ala-OMe	**a**	93
2	Cbz-Leu-Ala-OMe	**b**	97
3	Cbz-Leu-Phe-OMe	**b**	93
4	Boc-Leu-Ala-OMe	**a**	87
5	Fmoc-Leu-Ala-OMe	**a**	84
6	Fmoc-Leu-Phe-OMe	**a**	92
7	Fmoc-Leu-Trp-OMe	**b**	75[c]
8	Fmoc-Leu-Ser-OMe	**b**	72
9	Fmoc-Leu-Tyr-OMe	**b**	97[c]
10	Fmoc-Leu-Tyr-OMe	**a**	96
11	Fmoc-Leu-Met-OMe	**b**	82[c]
12	Fmoc-Leu-Glu(OMe)-OMe	**b**	70
13	Fmoc-Leu-Lys(Cbz)-OMe	**b**	70
14	Fmoc-Asn(NHTr)-Ala-OMe	**b**	88
15[d]	Fmoc-Tyr-Pro-OMe	**b**	80
16[d,e]	Fmoc-Pro-Phe-OMe	**b**	88[c]
17[d]	Fmoc-Val-Phe-OMe	**b**	80
18[d]	Fmoc-His(Trt)-Phe-OMe	**b**	84
19[d]	Fmoc-Phg-Ala-OMe	**b**	72[f]
20	Fmoc-Leu-Aib-OMe	**b**	92

[a] Et_3N (1.0 eq.) was added for H-YY-OMe·HCl, while Et_3N was not added for H-YY-OMe. [b] Isolated yield of dipeptides. [c] Diastereomer ratio > 99 : 1 by HPLC analysis. [d] $(4\text{-}MeOC_6H_4)_3P$ (1.2 eq.) instead of Ph_3P. [e] Temp.: 50 °C. [f] Diastereomer ratio > 98 : 2 by HPLC analysis.

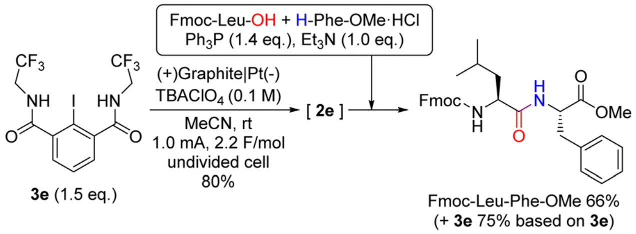
In the present methods, *N*-protected amino acids other than Fmoc-Leu-OH could also be used (entries 14–20). For example, trityl (Tr)-protected asparagine (Asn) and H-Ala-OMe·HCl gave the desired dipeptide in 88% yield under similar conditions (entry 14). In cases of bulky valine (Val), histidine (His) and the secondary amino acid proline (Pro) as a *N*- or *C*-protected substrate, the use of $(4\text{-}MeOC_6H_4)_3P$ at 25 or 50 °C was effective (80–88%, entries 15–18). In addition, non-natural amino acids aminoisobutyric acid (Aib) and α-phenylglycine (Phg) could also be converted to dipeptides in 92% and 72% yields, respectively (entries 19 and 20). The results of ^1H NMR spectra and/or chiral HPLC analysis (dr > 99 : 1 for entries 7, 9, 11 and 16; dr > 98 : 2 for entry 19) indicate that the obtained peptides are almost a single diastereomer (Fig. S1 and S2 in ESI†). These products contain the peptide derived from easily racemizable His (entry 18)[6] and Phg (entry 19),[4a] indicating an advantage of the present method. Note that, in all cases, the corresponding iodine(I) compounds **3** were recovered in good yields (61–88%).

Moreover, the present method could be applied to the soluble tag-assisted liquid-phase peptide synthesis (LPPS)[1c,d,6] as shown in Scheme 2. When benzyl group with long alkyl chains is used as a soluble tag, peptides protected with soluble tags dissolve in THF, CH_2Cl_2, and *c*-Hex and precipitate in polar solvents such as MeCN. In actually, the coupling reaction of Fmoc-Leu-OH with various tags-protected H-Tyr(O^{t}Bu)-OTAG$_X$ smoothly proceeded in CH_2Cl_2 at 35 °C under optimized conditions to give pure dipeptide in 92–94% yields simply by replacing the solvent to MeCN after filtering out **3e** insoluble in CH_2Cl_2. In particular, in the case of Fmoc-Leu-Tyr(O^{t}Bu)-OTAG$_A$, the recovery rate of **3e** was also good (95%). Boc-Val-Tyr(O^{t}Bu)-OTAG$_A$ could be prepared in a similar manner and was easily converted to valyltyrosine, which exhibits ACE inhibitory activity,[13] by deprotection. In addition, the tripeptide could be obtained in 86% yield by three steps including the similar coupling of H-Tyr(O^{t}Bu)-OTAG$_A$ with Fmoc-Val-OH, the deprotection by organic bases, and the coupling of *N*-unprotected dipeptide with Fmoc-Leu-OH.

Finally, we attempted the peptide coupling using *in situ* electrochemically generated iodine(III) reagent (Scheme 3).



Scheme 2 Soluble tag-assisted liquid-phase peptide synthesis.



Scheme 3 Peptide coupling using *in situ* electrochemically generated iodine(III) reagent.

Since 2-iodoisophthalamide **3e** could be converted into bicyclic benziodazolone **2e** up to 83% yield under electrolytic oxidation conditions (see ESI† for details), 1.5 equivalents of **3e** were used to conduct the peptide coupling of Fmoc-Leu-OH and H-Phe-OMe·HCl in the presence of Ph$_3$P (1.4 eq.) and Et$_3$N (1.0 eq.) in MeCN. Consequently, after the electrochemical generation of **2e** (80% yield determined by NMR analysis), the addition of the above-mentioned amino acids and additives afforded the desired dipeptide in 66% yield. Even in this case, the recovery rate of **3e** was still good (75%).

We have developed the peptide coupling using bicyclic benziodazolone and triarylphosphine. In this method, bicyclic benziodazolone was not only involved in the oxidation of the phosphine, but also served as the base. After the reaction, the iodine(III) reagent could be recovered as the iodine(I) reagent, which could be converted to the original reagent by electrolytic oxidation. In addition, the present method could be applied to the soluble tag-assisted LPPS as well as the procedure using the *in situ* electrochemically prepared iodine(III) reagent. Since phosphine oxide have been reported to be recoverable and convertible to the original phosphine by electrolytic reduction, this study provides the greener peptide coupling method.

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Conflicts of interest

There are no conflicts to declare.

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