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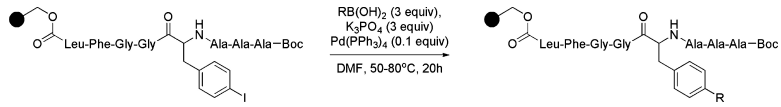

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Effectiveness of the Suzuki−Miyaura Cross-Coupling Reaction for Solid-Phase Peptide Modification

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The Suzuki−Miyaura (SM) cross-coupling reaction has recently become one of the most efficient methods for C−C bond construction opening a wide range of opportunities in organic synthesis. This study focused on the evaluation of the use of the SM reaction to modify peptides using a solid-phase synthesis approach, an avenue that was still not investigated intensively. We used as a peptide model [Ala^{1,2,3}, Leu^{8}]Enk linked to a polystyrene support on which it was previously assembled. The aromatic residues Tyr^{4} and Phe^{7} of [Ala^{1,2,3}, Leu^{8}]Enk were respectively substituted with *p*-iodo-Phe, and an SM-related strategy was developed. Results indicated that the reaction conditions involving K_3PO_4 or Na_2CO_3 (base), DMF (solvent), $Pd(PPh_3)_4$ (catalyst), and temperatures ranging from 50 to 80 °C during 20 h were found as optimal. Finally applying those optimal conditions, a series of [Ala^{1,2,3}, Leu^{8}]Enk analogs modified at Tyr^{4} or Phe^{7} positions was synthesized using diverse boronic acid derivatives.

Introduction

The use of transition metal-catalyzed reaction in organic synthesis allows C−C bond constructions and, thus, provides a key step to build more complex molecules from simple precursors.[1] One of the most efficient methods for C−C bond construction is the Pd-catalyzed Suzuki−Miyaura (SM) cross-coupling reaction (or Suzuki coupling). The SM reaction consists of the coupling of a boronic acid derivative with an aryl or vinyl halide or triflate, in the presence of a palladium catalyst and a base. The main advantages of the Suzuki coupling are the mild reaction conditions, its environmentally safer character in comparison to most other organometallic reagents, and the commercial availability of large sets of boronic acids.[2] Moreover, the manipulation and removal of byproduct containing boron are easier compared to other organometallic reagents, especially in large-scale synthesis applications.[3] After the original work of Suzuki and Miyaura,[4] the cross-coupling reaction opened a very wide range of opportunities in organic synthesis focusing on the preparation of several druglike molecules. For instance, one of the first SM applications in the pharmaceutical industry was the synthesis of Losartan, an angiotensin II receptor antagonist.[5] This palladium-catalyzed reaction was also used as a key step in the preparation of various COX-II inhibitors, such as Rofecoxib and Etorocoxib, and also in the synthesis of the steroidal abiraterone acetate that is a potent inhibitor of human cytochrome $P450_{17\alpha}$.[6] Furthermore, total syntheses of Tylophora alkaloids Cryptopleurine, (−)-Antofine, (−)-Tylophorine, and (−)-Ficuseptine C, using palladium-catalyzed cross-coupling reactions for the formation of the biphenyl scaffold was demonstrated.[7] Suzuki coupling was also described as an efficient method for the construction of an A−B biaryl system in the total synthesis of antibiotic Vancomycin.[8] Recently, SM coupling was used successfully for studying structure–activity relationships (SAR) of bioactive compounds. As a matter of fact, a report described the solid-phase synthesis of nonpeptidic endothelin receptor antagonists by applying the Suzuki reaction.[9] Furthermore, a series of 13 derivatives of 5-substituted nicotinic acid was prepared in solid-phase with high yield and purity using the Suzuki coupling method.[10]

Also, the SM cross-coupling reaction was used to generate unusual amino acid building blocks that were further incorporated in peptide structures to generate new molecules. For example, Collier et al.[11] found that an organoborane reagent was an excellent surrogate of the homoalanine anion and that it can be transformed into a range of unusual α-amino acids. Additionally, a general and efficient method to prepare C4-substituted dipeptide reverse-turn mimetics (unsaturated and saturated azabicyclo alkane amino acid derivatives) was developed by Zhang et al.[12] by using the Suzuki coupling reaction. A Pd-catalyzed coupling reaction of pinacolborane also allowed Nakamura et al.[13] to develop a practical method for the synthesis of 4-borono-L-phenylalanine from L-tyrosine or 4-iodo-L-phenylalanine derivatives. Moreover, with microwave irradiation, unprotected 4-aryl phenylalanines were prepared in high yields within 5–10 min as free amino acids from the cross-coupling of 4-borono phenylalanine with aryl halides.[14]

Solid-phase synthesis via the Suzuki reaction of biarylalanine derivative libraries was also described.[15] The application of the SM cross-coupling reaction to 4-iodo-L-phenylalanine-based peptides was first investigated by Kotha and Lahiri,[16] opening a new approach to produce unusual modified phenylalanine peptides. For example, dityrosine cross-linked peptide dimers were synthesized successfully.[17]

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Also, surface protein modifications via the SM coupling reaction were shown to to be possible in aqueous solution under mild conditions.[18] Furthermore, a study reported the solid-phase synthesis of a library of peptidomimetic inhibitors for the hepatitis C virus NS3 protease using the Mitsunobu and Suzuki cross-coupling reaction.[19] Therefore, the literature shows that the SM coupling reaction is used frequently in association with different synthesis strategies. However, up to now, there is only one recent publication describing the application of the SM reaction to modify a tripeptide at the solid-phase synthesis step.[20]

Recently, solid-phase organic synthesis (SPOS) has become the groundwork for the combinatorial synthesis of druglike small organic molecule libraries. Solid-phase synthesis exhibits several advantages over solution methods, and during the past decade, several groups have made efforts to transfer to solid support techniques that were originally developed for liquid phase.[21] In this respect, SPOS became the method of choice for the synthesis of various peptides. Because of their large spectrum of activities, peptides are among the most important bioactive compounds with applications in biomedical fields, as well as in nanosciences.[22] However, in most cases, because of poor bioavailability and metabolic susceptibility, modified peptides must be developed to be used, for instance, as drugs. Thus, to enhance receptor specificity and affinity and stability as well as biological activity of peptides, SAR studies through the development of peptide libraries proved to be an efficient method. Many strategies for the incorporation of chemical modifications were used, including the introduction of an aromatic ring, a heterocyclic moiety, or another small molecule fragment into a peptide chain, in order to produce conformational constraints that can provide new physicochemical properties or either mimic or produce unusual secondary structures in a peptide.[22a] In this line of view, the application of the SM cross-coupling reaction to solid-phase peptide synthesis would offer a novel tool to generate peptide libraries useful for SAR studies. Thus, [Ala^{1,2,3}, Leu^8]Enk (Ala-Ala-Ala-Tyr-Gly-Gly-Phe-Leu) was chosen as a model peptide to evaluate the applicability of the SM reaction in solid-phase peptide chemistry.

In the present study, we describe the effectiveness of a solid-phase SM reaction strategy to modify octapeptide-containing aromatic amino acids ([Ala^{1,2,3}, Leu^8]Enk). The reaction conditions were first studied and then optimized by focusing on the Tyr^4 position. Thereafter, the solid-phase SM reaction was applied to generate [Ala^{1,2,3}, Leu^8]Enk analogs modified at positions Tyr^4 and Phe^7.

Results and Discussion

Enkephalin analogs, N-α-Fmoc-[Ala^{1,2,3}, Phe(pI)^4, Leu^8]Enk and N-α-Fmoc-[Ala^{1,2,3}, Phe(pI)^7, Leu^8]Enk, were initially assembled on a Wang functionalized polystyrene resin using a BOP/DIEA coupling strategy. In parallel, N-α-Fmoc-[Ala^{1,2,3}, BiP^4, Leu^8]Enk and N-α-Fmoc-[Ala^{1,2,3}, BiP^7, Leu^8]Enk were also synthesized as standard peptides. Prior to the SM reaction, a small amount of peptide was cleaved and analyzed by RP-HPLC and MALDI-TOF MS. Results showed that all crude peptides were synthesized successfully with high purity (over 90%) and with the expected mass.

Our first attempts of Suzuki couplings were carried out with N-α-Fmoc-[Ala^{1,2,3}, Phe(pI)^4, Leu^8]Enk-resin using 3 equiv of phenylboronic acid, 0.1 equiv of catalyst, 3 equiv of base in various solvents, at 80 °C for 20 h. After cleavage, MS analysis indicated the presence of four compounds corresponding to the starting material N-α-Fmoc-[Ala^{1,2,3}, Phe(pI)^4, Leu^8]Enk and the expected product N-α-Fmoc-[Ala^{1,2,3}, BiP^4, Leu^8]Enk in combination with their N-α-deprotected counterparts, H_2N-[Ala^{1,2,3}, Phe(pI)^4, Leu^8]Enk-OH and H_2N-[Ala^{1,2,3}, BiP^4, Leu^8]Enk-OH. Furthermore, from the HPLC analysis, we found that H_2N-[Ala^{1,2,3}, Phe(pI)^4, Leu^8]Enk-OH and H_2N-[Ala^{1,2,3}, BiP^4, Leu^8]Enk-OH were the major compounds in the crude peptide preparation (Figure 1). Consequently, this data showed that the Fmoc protecting group was not stable to the Suzuki coupling conditions that were used, presumably because of the particular basic conditions and the high temperature. We did not attempt to carry out the reaction with the free N-terminal function because several studies suggested that the binding of unprotected amino groups ($-NH_2$) to metal centers can retard the catalytic cycle.[23] Instead of synthesizing a new peptide with Ala^1 being introduced under the Boc form, we removed the Fmoc moiety and replaced it with a Boc group using the $(Boc)_2O$ anhydride (Scheme 1). Moreover, a recent study reported that H_2N-Phe(pI)-OH anchored to a Wang linker was not totally stable to Suzuki conditions because of the base-catalyzed hydrolysis.[24] Thus, the reaction was attempted a second time but with a Boc protection approach at the N-terminus. Results showed that this scheme is compatible with the SM reaction. Also, to confirm the stability of the linker under the SM conditions, the solution after Suzuki coupling was analyzed by MALDI-TOF MS and HPLC. No trace of peptide was found in the SM solution showing that N-α-Boc-[Ala^{1,2,3}, Phe(pI)^4, Leu^8]Enk supported on a Wang polystyrene resin is an adequate peptide-resin model to determine the optimal reaction conditions (Scheme 1). Optimization was achieved on small peptide-resin samples, and results were analyzed according to conversion rates to the desired product and formation of byproduct. Usually, the experiments were carried out only once, and therefore, no statistical analysis was achieved.

Optimization of Solid-Phase Suzuki−Miyaura Reaction Conditions. Effect of Base. In the Suzuki catalytic cycle, a base is used to generate a more reactive borate through coordination of a hydroxide ion with the boron atom that will react with a Pd complex in a transmetalation process.[25] Previous results reported that the purity and selectivity of products obtained from a coupling reaction depend on the base and the catalyst used.[4] Furthermore, the base must be chosen according to the solvent used. Strong bases such as NaOH, KOH, or $NaOCH_3$ perform well in water or THF/H_2O solvent systems,[26] whereas weaker bases such as K_2CO_3, K_3PO_4, and Na_2CO_3 are generally used in DMF.[25b] These bases can be used as aqueous solutions, or as suspensions in organic solvent.[2f] To evaluate which base would give the highest level of coupling, phenylboronic acid

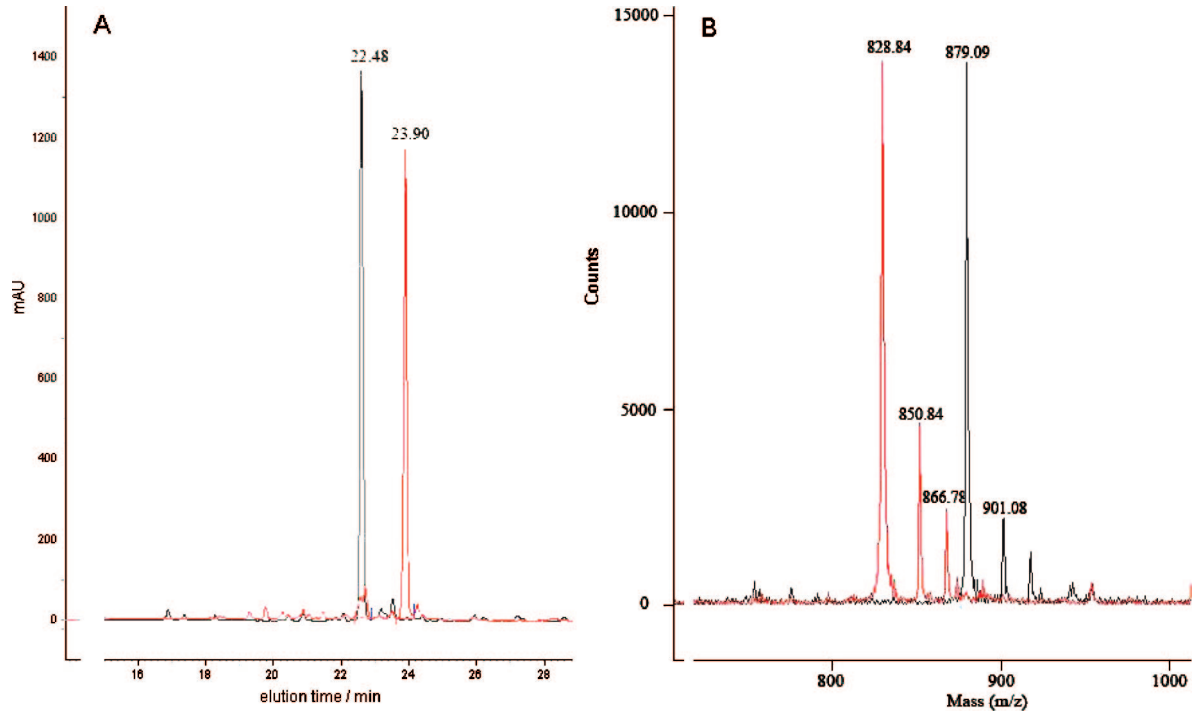
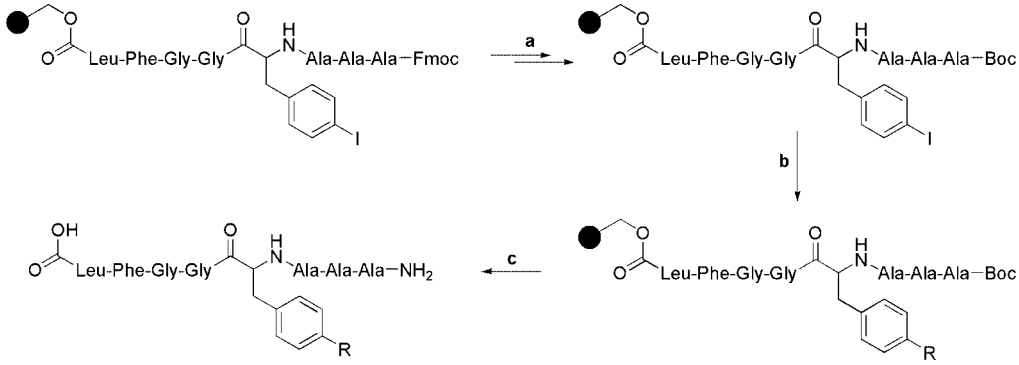


Figure 1. PLC (A) and MALDI-TOF MS (B) profile of the initial product (in black) and of the reaction mixture obtained after the SM reaction and the subsequent acid cleavage (in red). Theoretical masses of [Ala^{1,2,3}, BiP^4, Leu^8]Enk and [Ala^{1,2,3}, Phe(*p*-I)^4, Leu^8]Enk are 827.6 and 877.4, respectively.

Scheme 1. General Procedure for Solid-Phase Modification of [Ala^{1,2,3}, Phe(*p*I)^4, Leu^8]Enk via Suzuki Cross-Coupling Reaction[a]



[a] Conditions: (a) (i) piperidine/DMF (1:4) 2 min, then 15 min, rt; (ii) $(Boc)_2O$ (3 equiv), DIEA (3 equiv), DMF, 2 h, rt; (b) $RB(OH)_2$ (3 equiv), base (3 equiv), catalyst (0.1 equiv) in an organic solvent at 50–80 °C, for 20 h; (c) TFA/H_2O (95:5).

and N-α-Boc-[Ala^{1,2,3}, Phe(*p*I)^4, Leu^8]Enk-resin were used in the presence of $Pd(PPh_3)_4$ at 55 °C. DMF was chosen as the solvent because of its good swelling properties for polystyrene resin. Results showed that coupling reactions performed very well in DMF with K_3PO_4 and Na_2CO_3 with yields of 88% and 75%, respectively. With a stronger base such as NaOH, the conversion was only 33% whereas with weaker bases, such as KOAc and $CsHCO_3$, 31% and 35% of coupling occurred, respectively. Because all these bases, except for NaOH, were insoluble and used as suspensions in DMF, we evaluated if a phase-transfer reagent could increase the rate of conversion. To do so, a suitable amount of a quaternary salt, *n*-Bu_4NI, was added to the reaction mixture. However, this approach did not improve the yields (data not shown). Similarly, a DMF-soluble organic base, DIEA, was used, but it did not give satisfactory results with a poor reaction level and significant amounts of byproduct. No further attempts to identify other suitable bases were made, although it is known that compounds such as Cs_2CO_3[27] are described in the literature as often giving good yields. Thus, from our results, K_3PO_4 and Na_2CO_3 appeared as the more appropriate to pursue the study.

Effect of Solvent. To investigate the effect of solvent on the rate of the Suzuki coupling, different organic solution conditions were used (DMF, NMP, DMSO, THF, and 1,4-dioxane). All reactions were performed at 55 °C in the presence of K_3PO_4, and $Pd(PPh_3)_4$ for 20 h. Data showed that the best results were obtained with DMF (conversion at 88%), whereas no trace of the derived product was found in the crude cleavage solution when using THF as solvent. Even DMSO that was shown to accelerate the cross-coupling reaction in solution phase gave only 14% conversion. As well, NMP, which is known as an excellent solvent in SPPS, and a mixture of NMP and DMSO (4:1) or NMP and DMF (1:1) did not lead to satisfactory yields. Those results might reflect the swelling properties of polystyrene and the peptide solvation capacity of each solvent.

Table 1. Influence of Catalyst and Reaction Time on the Conversion Rate of Reaction of N-α-Boc-[Ala[1,2,3], Phe(*p*I)[4], Leu[8]]Enk with Phenylboronic Acid Using Solid-Phase SM Coupling Conditions[a]

no.	catalysts	time (h)	conversion (%)[a]
1	$Pd(PPh_3)_4$	4	28
2	$Pd(PPh_3)_4$	6	30
3	$Pd(PPh_3)_4$	12	66
4	$Pd(PPh_3)_4$	20	88
5	$Pd(PPh_3)_4$	48	91[b]
6	$PdCl_2(PPh_3)_2$	20	86[b]
7	$PdCl_2/PCy_3$[c]	20	45

[a] The percentage of coupling conversion was determined by analytical RP-HPLC of the crude product (λ = 212 nm). [b] Unknown byproducts were also formed. [c] Ratio: 1 equiv/2 equiv.

Effect of Temperature. In order to determine the optimal temperature for the solid-phase modification of N-α-Boc-[Ala[1,2,3], Phe(pI)[4], Leu[8]]Enkephalin, the cross-coupling reaction was performed at temperatures ranging from 25 to 90 °C in the presence of $Pd(PPh_3)_4$ and K_3PO_4 in DMF for 20 h. Results showed that the SM reaction performed well at temperatures between 55 and 90 °C, with yields varying from 75% to over 90%. On one hand, although at 90 °C the conversion of the iodo-peptide was very high (85%), many unknown byproducts were produced. On the other hand, at temperatures up to 40 °C, the conversion attained only 47% for iodo-peptide but no byproduct formation was observed. In fact, temperatures between 55 and 80 °C appeared optimal for producing the biaryl compound without generating a significant amount of byproduct. It must be noticed that at high temperatures (over 100 °C), the Boc-protecting group would not be stable. Therefore, our temperature conditions are mild enough to avoid the decomposition of the Boc-protecting group. It was then postulated that a stronger catalyst could be used concomitantly with low temperatures to carry out the Suzuki reaction while avoiding side-products. In this view, $PdCl_2(PPh_3)_2$ was used but no improvement of the yield was observed (data not shown).

Effect of Catalysts and Reaction Time. Cross-coupling reactions were performed in DMF with K_3PO_4, at 55 °C, but with different catalysts such as $Pd(PPh_3)_4$, $PdCl_2(PPh_3)_2$, and a mixture of $PdCl_2$ (1 equiv) and PCy_3 (2 equiv). The best results were obtained with $Pd(PPh_3)_4$ (conversion: 88%) (Table 1). $PdCl_2(PPh_3)_2$ gave similar results (86%), but significant amounts of unknown byproduct were also formed, while $PdCl_2/PCy_3$ gave only 45% conversion. Although $PdCl_2$ is considered as a weak catalyst, the latter results were somewhat surprising because Shen[28] established that a bulky and electron-rich phosphine, such as PCy_3, is a potent Pd catalyst ligand for the cross-coupling of boronic acid with aryl halides. Thus, according to Shen, tricyclohexylphosphine (PCy_3) would facilitate the oxidative addition step of the catalytic cycle and favor the formation of a catalytically active monophosphine Pd complex. In fact, a few studies had already shown that PCy_3 is a very efficient ligand for Suzuki couplings in mild conditions.[29] Therefore, in this work, the weak activity of $PdCl_2/PCy_3$ catalyst might come from the poor solubility of $PdCl_2$ in DMF. Its replacement with another palladium salt such as $Pd(OAc)_2$ might overcome this problem. Nevertheless, with the commercial availability of $Pd(PPh_3)_4$ and its potent catalytic activity, this material was considered as highly satisfactory for the study.

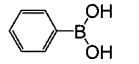
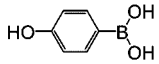
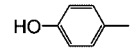
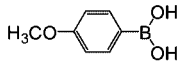
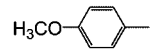
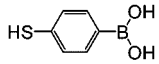
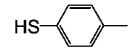
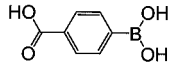
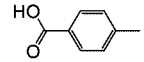
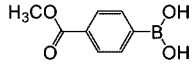
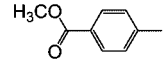
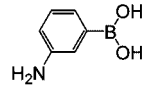
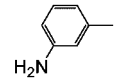
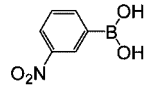
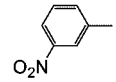
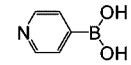
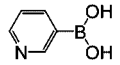
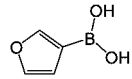
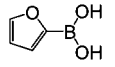
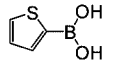
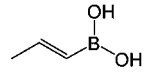
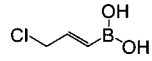
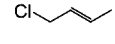
Using the base, solvent, catalyst, and temperature-optimized conditions for solid-phase peptide modification via SM coupling, we also evaluated the time course of the reaction. Results showed that only 30% of peptide conversion was completed in the first 6 h of reaction, whereas after 12 h, the reaction yield had reached 66%. At 20 h, 88% of biaryl compound was obtained, and longer time (48 h) did not increase significantly the yield of conversion (Table 1). In fact, with longer reaction times, side-reactions were generally accelerated. To reduce the reaction time and the byproduct formation, the SM reaction could be carried out under microwave conditions.[30] However, it was not checked if such conditions would be compatible with a peptide such as the octapeptide of this study. Hence, from these results, optimized conditions were found to be DMF as the solvent, K_3PO_4 or Na_2CO_3 as the base, and $Pd(PPh_3)_4$ as the catalyst. The reaction can be carried out at 80 °C for 20 h.

Solid-Phase Synthesis of an Enkephalin Analog Library Using the Suzuki−Miyaura Reaction. Because the conversion of N-α-Boc-[Ala[1,2,3], Phe(*p*I)[4], Leu[8]]Enk to N-α-Boc-[Ala[1,2,3], BiP[4], Leu[8]]Enk was successfully achieved using the optimized conditions of the SM reaction described above, we applied these conditions using various boronic acid derivatives, for the preparation of an Enk analog library containing modifications at position Tyr[4] or Phe[7]. In fact, as a preliminary study, the investigation was extended to several substitutions with boronic acids containing both electron-donating and electron-withdrawing moieties. A summary of the data obtained with the Enk analogs modified at position 4 is given in Table 2. On one hand, from these results, it seems that the presence of a strong electron-withdrawing substituent, such as a NO_2 group, would improve the extent of conversion (88%, entry 8). On the other hand, as shown with the opposite behavior of methoxyphenylboronic acid (entry 3, no reaction) and hydroxyphenylboronic acid (entry 2, 73%), in two compounds bearing electron-donating groups, the electronic properties of the substituents on the aromatic ring cannot explain the reactivity of the boronic acid derivative. This was also illustrated in other studies[31] in which no unambiguous correlations could be made according to the electronic properties of the substituting functions.

Sulfur-containing boronic acids were also evaluated. All attempts to carry out the reaction with mecaptophenylboronic acid were unsuccessful (entry 4), probably due to the strong thiophilicity of palladium resulting in a poisoning effect by the sulfur atom.[32] Moreover, the catalyst poisoning effect was also observed in the presence of nitrogen atoms.[33] Thus, the SM coupling of 3-aminophenylboronic acid gave only 14% conversion with $Pd(PPhe_3)_4$ as a catalyst, and many byproduct were also formed (entry 7). Finally, no peptide conversion via the SM reaction was achieved with carboxyphenylboronic acid unless its acid function was protected with a methyl ester. This protection improved the conversion from 0% to 87% without any detectable trace of hydrolysis.

We also investigated the conditions for synthesizing some heteroaromatic derivatives of H_2N-[Ala[1,2,3], Leu[8]]Enk-OH.

Table 2. Modification of [Ala[1,2,3], Phe(pI)[4], Leu[8]]Enk at Tyr[4] and Phe[7] Positions via Solid-Phase SM Reaction[c]

Entry	Boronic acid	Base	Catalyst	R	Conversion (%)[a] at Tyr[4]	Conversion (%)[a] at Phe[7]
1		K_3PO_4	Tetrakis		87	88
2		K_3PO_4	Tetrakis		73	61
3		K_3PO_4	Tetrakis $PdCl_2(PPh_3)_2$		0	0
4		Na_2CO_3	Tetrakis $PdCl_2(PPh_3)_2$		0	0
5		Na_2CO_3	Tetrakis $PdCl_2(PPh_3)_2$		0	0
6		K_3PO_4	Tetrakis		87	70
7		Na_2CO_3	Tetrakis $PdCl_2(PPh_3)_2$		14[b]	nd[b]
8		Na_2CO_3	$PdCl_2(PPh_3)_2$		88	84
9		Na_2CO_3	Tetrakis $PdCl_2(PPh_3)_2$		0	0
10		Na_2CO_3	$PdCl_2(PPh_3)_2$		93	90
11		K_3PO_4 Na_2CO_3	Tetrakis		5	5
12		Na_2CO_3	Tetrakis		60[b]	36[b]
13		Na_2CO_3	Tetrakis		72[b]	66[b]
14		K_3PO_4	Tetrakis		12	5
15		K_3PO_4	Tetrakis		81	79

[a] As determined by analytical RP-HPLC of the crude product (λ = 212 nm). [b] Significant amounts of unknown byproducts were also formed; nd, not determined. [c] Conditions: $RB(OH)_2$ (3 equiv), K_3PO_4 (3 equiv), $Pd(PPh_3)_4$ (or $PdCl_2(PPh_3)_2$, (0.1 equiv), DMF, 80°C, 20 h.

It was found that 3-pyridineboronic acid gave 93% of conversion in the presence of $PdCl_2(PPh_3)_2$ (entry 10), while the 4-pyridineboronic acid (entry 9) was completely unreactive under the same conditions. These results were somewhat surprising and could not be explained solely by an increased electronegativity of the nitrogen atom when located at a para position. Nonetheless, a similar phenomenon was also observed in previous studies, but this time with 2-bromopyridine.[31a,33,34] As mentioned before, the presence of a sulfur atom can retard the reaction rate by poisoning the Pd-catalyst. Surprisingly, 2-thiopheneboronic acid gave an excellent yield of conversion (entry 13) under our SM conditions. However, it was observed that significant amounts of unknown byproduct were also formed. The same observation was also found with 2-furanboronic acid although this compound seemed to be less active than the previous one (entry 12). On the other hand, it was found that 3-furanboronic acid remained almost unreacted under the coupling conditions (entry 11). The similarity between these results and those obtained with pyridine boronic acid derivatives (entry 9 and 10) suggests that the position of the electronegative atom within the cyclic structure influences the SM reaction. However, further studies would be needed to outline a putative mechanism. In this study, we also carried out the SM reaction of iodo-peptide-resins with alkylboronic acid. Two boronic acid derivatives were studied, *trans*-2-chloromethylvinylboronic acid and 2-methylvinylboronic acid. Results showed that the halogen-substituted *trans*-2-chloromethylvinylboronic acid was easy to couple, whereas the nonsubstituted 2-methylvinylboronic acid was coupled to an extent of only 12%. Hence, it seems that the optimized conditions for phenylboronic acid compounds might not be satisfactory for all boronic acids. In this case, longer reaction times might be helpful to produce the conversion. However, it is probably better to avoid a large increase of temperature because side-products were observed at 90 °C and above.

For all boronic acid derivatives that were used in this study, similar results were obtained for the solid-phase

modification of N-α-Boc-[Ala[1,2,3], Leu[8]]Enk-resin at the Phe[7] position (Table 2). These data show that in the model peptide that was used, the vicinity of the polystyrene matrix, as observed with Phe[7], had no major steric hindrance effect on the formation of the activated-boronic acid−palladium−ligand complex.

Conclusion

In summary, we have developed a convenient strategy for the solid-phase modification of peptides through the SM cross-coupling reaction. Because peptides contain reactive groups that could interfere with the application of the SM reaction, it is more appropriate to perform the reaction on solid-phase while the peptide is still bearing all the protecting groups. It was found that the Fmoc moiety was not stable to the conditions used for the Suzuki reaction and had to be replaced with a Boc-protecting group. The methodology for the SM solid-phase modification of peptides was then optimized through the study of the influence of temperature, base, catalyst, solvent, and reaction time on the reaction yield. It was found that the best results were obtained with $Pd(PPh_3)_4$ as catalyst, 2 M aqueous solution of K_3PO_4 (or Na_2CO_3) as base, DMF as solvent, and temperature ranging from 50 to 80 °C for 20 h.

This general procedure can be used for the solid-phase synthesis of large libraries of modified phenylalanine peptide analogs for SAR studies. As an example, using optimized conditions, a series of [Ala[1,2,3], Leu[8]]Enk analogs modified at position Tyr[4] or Phe[7] was synthesized. High variability in the conversion rates was observed according to the boronic acid derivative coupled via the SM reaction. Additional studies are actually performed with more complex peptides to verify the compatibility of the SM method with peptide sequences containing reactive amino acids such as methionine, cysteine, histidine, and tryptophan. The development of SM cross-coupling reaction conditions suitable for all peptide sequences would then result in a new tool available for postsynthesis peptide modifications.

Experimental Details

Materials. All Fmoc-protected amino acids such as Leu, Phe, Gly, Ala, and Tyr(*t*-Bu) were obtained from Matrix Innovation Inc. (Montreal, QC), except for Phe(*p*I) and Wang resin that were from ChemImpex International (Wood Dale, IL). Benzotriazol-1-yl-oxy-tris(dimethylamino)-phosphonium hexafluorophosphate (BOP) and diisopropylethylamine (DIEA) were also purchased from Matrix. Biograde trifluoroacetic acid (TFA) was obtained from PSIG (Montreal, QC). Acetonitrile (ACN), *N*,*N*-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), tetrahydrofuran (THF), 1,4-dioxane, and dichloromethane (DCM) were obtained from Fisher Scientific (Nepean, ON). All other compounds such as the boronic acid derivatives, bases, palladium catalysts, and *N*-methylpyrrolidone (NMP) were purchased from Sigma-Aldrich (Oakville ON).

Peptide Synthesis. All peptide-resins were synthesized using a semiautomatic homemade solid-phase synthesizer, according to a protocol using standard Fmoc chemistry. The first amino acid (Leu) was fixed to the Wang resin using 2,6-dichlorobenzoyl chloride and pyridine following a method previously published by Sieber.[35] The loading of the resin was determined as 0.54 mmol/g. Then, the coupling of each amino acid (3 equiv) was performed in DMF using BOP (3 equiv) and DIEA (3 equiv) as reagents for 30 min. Deprotection of the Fmoc-protecting group was achieved with 20% piperidine in DMF. After the last amino acid (Ala) coupling, the Fmoc-protecting group was removed and replaced with a Boc-protecting group using $(Boc)_2O$ anhydride (20 equiv) and DIEA (20 equiv) coupling in DMF for 2 h at room temperature. Peptide-resin was dried under vacuum and stored at −20 °C until the SM reaction was applied.

General Procedure for Optimization of Solid-Phase Suzuki−Miyaura Reaction. A mixture of 10 mg [Ala[1,2,3], Phe(*p*-I)[4], Leu[8]]Enk linked to resin (1 equiv), phenylboronic acid (3 equiv), Pd-catalyst (0.1 equiv), and 2 M alkaline aqueous solution (3 equiv) in degassed organic solvent (2 mL) was heated and stirred for times varying from 4 to 48 h. After the reaction, the peptide-resin was thoroughly washed with DMF (×3), H_2O (×3), MeOH (×2), DMF (×3), and then DCM (×3). The modified peptides were deprotected and cleaved from the solid support using TFA containing 5% (v/v) water. The cleavage reaction was carried out for 2 h at room temperature. The solution was then filtered, and the resin was washed with an equal volume of TFA. After evaporation of TFA, a small volume of 60% ACN in water containing 0.06% TFA was added to the remaining material. Crude peptides were then precipitated in deionized water and lyophilized. The coupling reaction was monitored by HPLC and MALDI-TOF MS. The conversion rate was determined using the area under the curve corresponding to the desired product as a function of the total area under the curve corresponding to the starting peptide (measured at $\lambda = 212$ nm).

Peptide Characterization. Crude lyophilized peptides were analyzed by analytical reverse-phase HPLC using a Phenomenex Jupiter Proteo C_{18} (4 μm; 90 Å) column (250 mm × 4.60 mm) connected to a Beckman 128 solvent module coupled to a Beckman 168 PAD detector (Beckman Couter, Inc., Fullerton, CA). The flow rate was at 1.0 mL/min, and the elution of the peptide was carried out with a linear gradient of 0−60% B in 30 min in which A was TFA 0.06% in water and B was ACN. The crude peptides were also characterized by MALDI-TOF mass spectrometry (Voyager DE spectrometer, Applied Biosystems, Foster City, CA). The laser was set at 337 nm, and an acceleration voltage of 25 kV was applied. The matrix for peptide inclusion and ionization was α-cyano-4-hydroxycinnamic acid.

Solid-Phase Synthesis of an Enkephalin Analog Library. A mixture of 200 mg iodo-peptide-resin (1 equiv), boronic acid derivative (3 equiv), $Pd(PPh_3)_4$ (0.1 equiv), and 2 M aqueous solution of K_3PO_4 (or Na_2CO_3) (3 equiv) in degassed DMF (20 mL) was stirred for 20 h at 80 °C. After successive washings with DMF (×3), H_2O (×3), MeOH (×2), DMF (×3), and DCM (×3), the modified peptides were cleaved from the solid support using TFA/water (95:5) for 2 h at room temperature. The solution was filtered, and the resin was washed with an equal volume of TFA. After evaporation of TFA, peptides were solubilized in a

small volume of 60% ACN in water containing 0.06% TFA. Crude peptides were then precipitated in deionized water and lyophilized. The coupling reaction was monitored by HPLC and MALDI-TOF MS. The coupling conversions were determined by analytical RP-HPLC of the crude product ($\lambda =$ 212 nm). All peptides resulting from a SM reaction of over 60% conversion were purified by semipreparative HPLC using a Vydac C18 10 μm (300 Å, 250 mm $\times$ 20 mm) column connected to a Series 1050 HP HPLC system. The reaction yields were determined based on the ratio of the obtained amount of material to the expected quantity of final product. Results showed that the actual yields of final products ranged from 10% to 56%.

Abbreviations. SM, Suzuki−Miyaura; SPOS, solid-phase organic synthesis; SPPS, solid-phase peptide synthesis; SAR, structure–activity relationships; Enks, Enkephalins; Met-Enk, methionine-enkephalin; Leu-Enk, leucine-enkephalin; Tyr(*t*-Bu), O-*tert*-butyl-tyrosine; Phe(*p*I), *p*-iodo-phenylalanine; BiP, 4-biphenylalanine; BOP, benzotriazol-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate; DIEA, diisopropylethylamine; TFA, trifluoroacetic acid; ACN, acetonitrile; DMF, *N*,*N*-dimethylformamide; DMSO, dimethylsulfoxide; THF, tetrahydrofuran; DCM, dichloromethane; NMP, *N*-methyl-2-pyrrolidinone; RP-HPLC, reverse-phase high-performance liquid chromatography; Fmoc, 9-fluorenylmethoxycarbonyl; MALDI-TOF, matrix-assisted laser desorption and ionization-time-of-flight; MS, mass spectrometry; $Pd(PPh_3)_4$ (tetrakis), (triphenylphosphine)palladium(o); $PdCl_2(PPh_3)_2$, dichlorobis(triphenylphosphine)palladium(II); PCy_3, tricyclohexylphosphine; *n*-Bu_4NI, tetrabutylammonium iodide; $(Boc)_2O$, di*tert*-butyl dicarbonate; Boc, *tert*-butyloxycarbonyl.

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References and Notes

(1) (a) Tsuji, J. *Palladium reagents and catalysts*; Wiley: Chichester, 1996. (b) Diederich, F., Stang, P. J., Eds. *Metal-catalyzed cross-coupling reaction*; Wiley-VCH: Weinheim, 1998.

(2) (a) Miyaura, N.; Yanagi, T.; Suzuki, A. *Synth. Commun.* **1981**, *11*, 513–520. (b) Suzuki, A. *Pure Appl. Chem.* **1991**, *63*, 419–422. (c) Martin, A. R.; Yang, Y. *Acta Chem. Scand.* **1993**, *47*, 221–230. (d) Suzuki, A. *Pure Appl. Chem.* **1994**, *66*, 213–222. (e) Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457–2483. (f) Stanforth, S. P. *Tetrahedron* **1998**, *54*, 263–303. (g) Miyaura, N. *Advances in Metal-organic Chemistry*; Libeskind, L. S., Ed.; Jai: London, 1998; Vol. 6, pp 187−243. (h) Suzuki, A. *Organomet. Chem.* **1999**, *576*, 147–168. (i) Suzuki, A. In *Organoboranes for Synthesis*; Ramachandran, P. V., Brown, H. C., Eds; ACS Symposium Series 783; American Chemical Society: Washington, DC, 2001; pp 80–93. (j) Suzuki, A. *J. Organomet. Chem.* **2002**, *653*, 83–90.

(3) Kotha, S.; Lahiri, K.; Kashinath, D. *Tetrahedron* **2002**, *58*, 9633–9695.

(4) (a) Miyaura, N.; Yamada, Y.; Suzuki, A. *Tetrahedron Lett.* **1979**, *20*, 3437–3440. (b) Miyaura, N.; Yamada, Y.; Suginome, H.; Suzuki, A. *J. Am. Chem. Soc.* **1985**, *107*, 972–980.

(5) Larsen, R. D. *J. Org. Chem.* **1994**, *59*, 6391–6394.

(6) King, A. O.; Yasuda, N. *Top. Organomet. Chem.* **2004**, *6*, 205–245.

(7) Furstner, A.; Kennedy, J. W. J. *Chem.−Eur. J.* **2006**, *12* (28), 7398–7410.

(8) Nicolaou, K. C.; Natarajan, S.; Li, H.; Jain, N. F.; Hughes, R.; Solomon, M. E.; Ramanjulu, J. M.; Boddy, C. N. C.; Takayanagi, M. *Angew. Chem., Int. Ed.* **1998**, *37* (19), 2708–2714.

(9) Lange, U. E. W.; Braje, W. M.; Amberg, W.; Kettschau, G. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1721–1724.

(10) Fernandez, J-C.; Sole-Feu, L.; Fernandez-Forner, D.; de la Figuera, N.; Forns, P.; Albericio, F. *Tetrahedron Lett.,* **2005**, *46*, 581–585.

(11) (a) Collier, P. N.; Patel, I.; Taylor, R. J. K. *Tetrahedron Lett.* **2001**, *42*, 5953. (b) Collier, P. N.; Campbell, A. D.; Patel, I.; Taylor, R. J. K. *Tetrahedron Lett.* **2000**, *41*, 7115.

(12) Zhang, J.; Xiong, C.; Wang, W.; Ying, J.; Hruby, V. J. *Org. Lett.* **2002**, *4* (23), 4029–4032.

(13) Nakamura, H.; Fujiwara, M.; Yamamoto, Y. *Bull. Chem. Soc. Jpn.* **2000**, *73*, 231–235.

(14) Gong, Y.; He, W. *Org. Lett.* **2002**, *4* (22), 3803–3805.

(15) (a) Nielsen, T. E.; Le Quement, S.; Meldal, M. *Tetrahedron Lett.* **2005**, *46*, 7959–7962. (b) Vázquez-Campos, S.; St.-Hilaire, P. M.; Damgaard, D.; Meldal, M. *QSAR Comb. Sci.* **2005**, *24* (8), 923–942.

(16) (a) Kotha, S.; Lahiri, K. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2887–2890. (b) Kotha, S.; Lahiri, K. *Biopolymers* **2003**, *69*, 517–528.

(17) (a) Yoburn, J. C.; Vranken, D. L. V. *Org. Lett.* **2003**, *5*, 2817–2820. (b) Skaff, O.; Jolliffe, K. A.; Hutton, C. A. *J. Org. Chem.* **2005**, *70* (18), 7353–7363.

(18) Ojida, A.; Tsutsumi, H.; Kasagi, N.; Hamachi, I. *Tetrehedron Lett.* **2005**, *46*, 3301–3305.

(19) Poupart, M.-A.; Cameron, D. R.; Chabot, C.; Ghiro, E.; Goudreau, N.; Goulet, S.; Poirier, M.; Tsantrizos, Y. S. *J. Org. Chem.* **2001**, *66* (14), 4743–4751.

(20) Haug, B. E.; Stensen, W.; Svendsen, J. S. *Bioorg. Med. Chem. Lett.* **2007**, *17* (8), 2361–2364.

(21) Thompson, L. A.; Ellman, J. A. *Chem. Rev.* **1996**, *96*, 555–600.

(22) (a) Sewald, N.; Jakubke, H. D. *Peptides: Chemistry and Biology*; Wiley-VCH: Weinheim, Germany, 2002. (b) Gao, X. Y.; Matsui, H. *Adv. Mater.* **2005**, *17*, 2037–2050.

(23) (a) Billingsley, K. L.; Anderson, K. W.; Buchwald, S. L. *Angew. Chem., Int. Ed.* **2006**, *45*, 3484–3488. (b) Itoh, T.; Mase, T. *Tetrahedron Lett.* **2005**, *46*, 3573–3577. (c) Wagaw, S.; Buchwald, S. L. J. *Org. Chem.* **1996**, *61* (21), 7240–7241.

(24) Colombo, A.; Fernandez, J. C.; de la Figuera, N.; Fernaez-Forner, D.; Forns, P.; Albericio, F. *QSAR Comb. Sci.* **2005**, *24* (8), 913–922.

(25) (a) Matos, K.; Soderquist, J. A. *J. Org. Chem.* **1998**, *63*, 461–470. (b) Echavarren, A. M.; Cardenas, D. J. *Metal-Catalyzed Cross-Coupling Reactions*, 2nd ed.; WILEY-VCH Verlage GmbH&Co. KGaA: Weinheim, 2004; ISBN 3-257-30518-1.

(26) Bumagin, N. A.; Bykov, V. V. *Tetrahedron* **1997**, *53* (42), 14437–14450.

(27) (a) Miyaura, N.; Ishiyama, T.; Sasaki, H.; Ishikawa, M.; Satoh, M.; Suzuki, A. *J. Am. Chem. Soc.* **1989**, *111*, 314. (b) Ishiyama, T.; Murata, M.; Miyaura, N. *J. Org. Chem.* **1995**, *60*, 7508–7510.

(28) Shen, W. *Tetrahedron Lett.* **1997**, *38*, 5575–5578.

(29) (a) Littke, A. F.; Dai, C.; Fu, G. C. *J. Am. Chem. Soc.* **2000**, *122*, 4020–4028. (b) McClure, M. S.; Glover, B.; McSorley, E.; Millar, A.; Osterhout, M. H.; Roschangar, F. *Org. Lett.* **2001**, *3* (11), 1677–1680.

(30) Zhang, W.; Chen, C. H. T.; Lu, Y.; Nagashima, T. *Org. Lett.* **2004**, *6* (9), 1473–1476.

(31) (a) Phan, N. T. S.; Khan, J.; Styring, P. *Tetrahedron* **2005**, *61*, 12065–12073. (b) Yamada, Y. M. A.; Takeda, K.; Takahashi, H.; Ikegami, S. J. *Org. Chem.* **2003**, *68*, 7733–7741.

(32) (a) Singhal, A.; Jain, V. K. *J. Organomet. Chem.* **1995**, *494*, 75–80. (b) Miyauchi, Y.; Watanabe, S.; Kuniyasu, H.;

Kurosawa, H. *Organometallics* **1995**, *14*, 5450–5453. (c) Ruiz, J.; Giner, J.; Rodriguez, V.; Lopez, G.; Casabo, J.; Molins, E.; Miravitlles, C. *Polyhedron* **2000**, *19*, 1627–1631. (d) Savarin, C.; Srogl, J.; Liebeskind, L. S. *Org. Lett.* **2000**, *2*, 3229–3231. (e) Redon, R.; Torrens, H.; Wang, Z.; Morales-Morales, D. *J. Organomet. Chem.* **2002**, *654*, 16–20.

(33) Feuerstein, M.; Doucet, H.; Santelli, M. *J. Organomet. Chem.* **2003**, *687*, 327–336.

(34) (a) Feuerstein, M.; Doucet, H.; Santelli, M. *Tetrahedron Lett.* **2001**, *42* (33), 5659–5662. (b) Parry, P. R.; Wang, C.; Batsanov, A. S.; Bryce, M. R.; Tarbit, B. J. *Org. Chem.* **2002**, *67* (21), 7541–7543. (c) Molander, G. A.; Biolatto, B. J. *Org. Chem.* **2003**, *68* (11), 4302–4314.

(35) Sieber, P. *Tetrahedron Lett.* **1987**, *28* (49), 6147–6150.

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