Umpolung Ala^B Reagents for the Synthesis of Non-Proteogenic Amino Acids, Peptides and Proteins

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Abstract: Non-proteogenic amino acids and functionalized peptides are important motifs in modern drug discovery and used as mechanistic tools in chemical biology. Here, we show that functionalized amino acid synthons in the form of Ala^B can serve as universal building blocks in the synthesis of a diverse collection of modified amino acids, peptides, and proteins. First, we develop the synthesis of Ala^B from redox-active esters of aspartic acid resulting in a series of β-boronoalanine derivatives. Next, we show that Ala^B can be integrated into automated solid-phase synthesis of oligopeptides and proteins. Ala^B is compatible with common transformations used in preparative peptide chemistry such as native chemical ligation and radical desulfurization as showcased by total synthesis of boronoalanine-modified ubiquitin (Phe4→Ala^B). Furthermore, Ala^B reagents participate in Pd-catalyzed reactions, including C(sp^3)-C(sp^2) cross-couplings and macrocyclizations. Taken together, Ala^B synthons are practical reagents to access non-natural amino acids, modified peptides, proteins, and in the synthesis of cyclic/stapled peptides.

Peptides and proteins are important targets in modern drug discovery because of their ease of synthesis, low toxicity, and target selectivity.[1] Native peptides, however, can often show low bioavailability and short lifetimes rendering them suboptimal for clinical applications.[2] To address these challenges, non-proteogenic amino acids (NPAAs), a class of amino acids not encoded in the human genome, emerged as a valuable tool to increase structural diversity and improve pharmacokinetic properties.[3] Several strategies to access NPAAs are known including the Strecker reaction,[4] asymmetric hydrogenation,[5] conjugate addition,[6] biotransformations,[7] photoredox cross-electrophile coupling,[8] C-H activation[9], and phase-transfer alkylation.[10] Access to unnatural surrogates through any of these strategies complements studies on selective modifications of biologics that can be achieved through various handles tat can enable downstream functionalizations (Scheme 1A). One promising approach that can avoid competing reactions with innate groups are transformations based on umpolung of reactivity.[11] We recently reported the synthesis and applications of Ala^{Sn} reagents in the form of carbastannatrane **1**,[12] a member of a larger group of reagents where alanine's β-carbon is substituted with a metal or metalloid (Scheme 1B). Ala^M reagents represent a novel type of synthons that can be engaged in cross-coupling reactions through a reversal of polarity at the β-carbon. From a conceptual standpoint, these reagents can give rise to native as well as unnatural amino acids based on a formal alanine derivatization. Several members of this family are known including organogermanium Ala^{Ge} **2**,[13] organoboron Ala^B **3**,[14] organosilane Ala^{Si} **4**[14j, 15] organozinc Ala^{Zn} **5**,[16] organolithium,[14d, 14e] and organonickel Ala^{Ni}[17] derivatives (Scheme 1B). Unlike α-aminoboronic acids, which have an established position as protease inhibitors,[18] boronoalanine Ala^B has only recently emerged as a tool to interrogate protein function[19] and as a potential Taspase1 inhibitor.[14g] Several methods are known to access Ala^B either as a racemic material derived from conjugate addition to dehydroalanine (Dha)[14j,14m,14u,19-20] or in enantiomerically pure form serine through a multistep synthesis from asymmetrically protected 2-amino-1,3-propanediol[14d] and auxiliary-directed C-H activation of alanine amides[15f] (Scheme 1C). Furthermore, despite its synthetic appeal, Ala^B has not been used in cross-coupling reactions with carbon-based electrophiles. Here we show that Ala^B in the form of BMIDA boronate (MIDA = *N*-methylimidoacetic acid) and BPai (Pai = 1*S*,2*S*,3*R*,5*S*-(+)-pinanediol) ester can be readily accessed from aspartic acid and serve as convenient reagents in the synthesis of functionalized amino acids, cyclic peptides, and proteins through a Pd-catalyzed Suzuki-Miyaura C-C cross-coupling. These novel reagents are stable under strongly acidic and basic conditions, compatible with current protein synthesis protocols and addresses an important synthetic gap in the preparation of functionalized amino acids and peptide-based therapeutics.

In our initial investigations into the synthesis of Ala^B we diverted from 1,4-conjugate additions to Dha because the stereochemistry at the resulting α-carbon can be difficult to control[14u] and extensive optimizations may be required to achieve practical selectivities.[20-21] Instead, we opted to develop a more direct synthesis from L-aspartic acid which presents several benefits in respect to the scalability and functional group compatibility (Scheme 2). We first converted aspartic acids **10** and **11** into redox active esters followed by light-mediated decarboxylative borylation and trans ligation with (+)-pinanediol or MIDA

to give **12-15** in acceptable yields (41-70%).[22] Further investigations into the optical purity of the resultant amino acids by acylation with α-methoxy-α-trifluoro-methylphenylacetic acid[23] revealed ~3% epimerization (for details, see the SI). We hypothesized that the boronic acid intermediate activates the carboxylate ester effectively rendering the α-proton prone to soft enolization. On the basis on this proposal, we opted to avoid the boronic acid intermediate, and we investigated complementary conditions described by Aggarwal[24] and Baran.[25] Because the photoredox reaction with B_2Cat_2 **19** affords the boronic ester and the catechol ligand can easily be exchanged, we opted to use these conditions in the preparation of **12**. To our delight, subjecting redox-active esters to the photoredox conditions followed by pinanediol delivered Ala^B (**12**) with no detectable epimerization (^{19}F NMR of the Mosher amide) and in excellent yields. The optimized protocol was then applied to the synthesis of Boc and Fmoc-protected Ala^B in the form on BPai and BMIDA esters. BMIDA ester **15** was also readily converted into a free acid suitable for oligopeptide/protein synthesis (vide infra).

To better understand chemical behavior of Ala^B reagents, we conducted in silico conformational analysis of three representative Ala^B structures in the form of Pin and Pai esters (Table 1A). Two Lewis basic sites located either at the α-amine (such as amides or carbamates) or the carboxylic acid (and its derivatives) can compete for boron's empty *p* orbital. Computational data indicate that both Pin and Pai esters prefer structures **21** and **22** over other conformers not stabilized by direct C=O→B interactions. Among two boracycles **21** and **22**, five-membered isomers **22** are favored for all computed structures with the carboxylate ions existing almost exclusively in the cyclic form **22**.[14a] Larger pinanediol groups are more efficient in shielding the boron center, which is also reflected in lower stabilization energies among the two isomers (entries 1-3 vs. 4-6). This study also allowed us to evaluate the effects of intramolecular carbonyl activation on acidity of α-protons (Table 1B). In each case, the α carbonyl protons carry increased positive charge consistent with our observations that epimerization can be observed for some Ala^B amino acids. Interestingly, boron coordination has little effect on the computed atomic charges of the carbonyl group. However, intramolecular activation of the boron towards transmetalation becomes important in the subsequent studies on Pd-catalyzed C-C coupling of these C(sp^3) nucleophiles (vide infra).

Several features of Ala^B and its derivatives are important to highlight. Ala^{BMIDA} and Ala^{BPai} are convenient reagents in solution-phase and automated solid-phase synthesis of oligopeptides and proteins (Scheme 3). We first demonstrated that boron remains intact under acidic conditions required to cleave peptides from the resin (95% TFA) after solid-phase synthesis (**23** and **24**). Similarly, the effects of boron substitution on the efficiency of macrocylization are minimal, as demonstrated in total synthesis of boronoalanine analog of pseudostellarin G **24**.[26]

To further highlight the utility of Ala^B in the synthesis of proteins, we prepared modified ubiquitin **28** with Phe4 mutated into Ala^B (Scheme 3B).[27] We selected this target because it allows to test the compatibility of Ala^{BMIDA} with denaturating as well as reducing conditions of native chemical ligation[28] (NCL; conversion of **26** into **27**) and radical desulfurization (**27**→**28**).[29] In a retrosynthetic manner, modified ubiquitin **28** was dissected into two fragments Met1-Phe45 (**26**) and Cys46-Gly76 (**29**) that were joined by the NCL. Ala^{MIDA} was incorporated into peptide **26** (assembled through Fmoc-SPPS on the methyl Dawson MeDbz linker)[30] that allowed for *in situ* generation of a thioester intermediate. To our delight, boronoalanine survived several high-temperature (90 °C) couplings and basic Fmoc deprotection conditions with piperidine/DBU. The union of oligopeptides **26** and **29** could be accomplished with a thioester generated from mercaptophenylacetic acid (MPAA), but this step required additional experimentation to achieve optimal yields. We observed that hydrolysis of the peptide backbones well as formation of transient structures where the MIDA group exchanged with intramolecular Lewis basic sites such as amides forming a boraheterocyle (dehydro-**27**) were the main issues. We hypothesized that the formation of boronic acid anhydrides and the concomitant loss of MIDA group could be controlled by pH. We found that under basic conditions (pH 7.6), substantial cleavage of **27** was observed (72%; conversion into truncated **27**) and the intramolecular boronic acid anhydride constituted the remainder of the material. By adjusting pH to 6.4, we were able to improve the yield of **27** to 89% and reduce the fragmentation to ~11%, but we also observed that the MIDA group was removed during HPLC purification resulting a dehydrated boronic acid. Next, in order to complete the chemical synthesis of **28**, we subjected **27** to radical desulfurization with glutathione (GSH)[31] as the H-atom donor and reduced Cys46 to Ala46 in 33% isolated yield after HPLC purification. Gratifyingly, these conditions showed no cleavage or truncation of **28**. The isolated material shows the formation of dehdro-**28** (MS) due to loss of water from boronic acid whereas the HPLC trace indicates a single entity. Collectively, these results demonstrate that Ala^B itself can serve as a unique NPAA and is compatible with the current methods for chemical synthesis of proteins.

With several Ala^B derivatives in hand, we next investigated if these primary nucleophiles could participate in Pd-catalyzed Suzuki-Miyaura cross-coupling reactions (Table 2). Curiously, previous studies did not report if Ala^B could participate in either radical or two-electron C-C bond-forming processes. The initial optimization studies identified BPai ester **30** with $PdCl_2$ (10 mol%), K_2CO_3 (3 equiv.), Ag_2O (2 equiv.) in 1,4-dioxane at 100 °C and a monodentate phosphine ligand as a promising catalytic system (entries 1-5). JackiePhos (entry 2) optimized to promote rapid transmetalation of Ala^{Sn} reagents furnished low yield of **32** (15%),[12] whereas SPhos (entry 3) and XPhos (entry 4) resulted in encouraging albeit suboptimal yields (47-48%). Based on these initial screening studies, we next turned to diphosphines, among which dppf was identified as the most efficient ligand (entries 6-14). We found that moderate yields were obtained for reactions with K_2CO_3 (entry 5) and Cs_2CO_3 (entry 8) but slight improvement was observed for K_3PO_3 and KF (entries 6 and 7). Pd(II) as the precursor for this transformation was

consistently superior when compared to the efficiency of the reaction catalyzed by Pd(0) (entry 9) as well as to the conditions where excess of the electrophile **31** was used (entry 10). Because even after 24 h 4-bromobiphenyl **31** was not completely consumed, we elected to change silver additive to Ag_2CO_3 (entry 11), extend the reaction time to 48 h (entry 12) and increase the amount of Ala^B to 2 equivalents (entry 13) which resulted in 83% yield of alanine derivative **32** (entry 14). Under the optimized conditions, we modified the solvent and used THF, which allowed us to lower the reaction temperature to 70 °C. Other solvent systems including MeCN or 2-methyltetrahydrofuran gave lower yields, but a 4:1 mixture of THF and H_2O was compatible with the substrate but afforded **32** in lower yields (56%; for details, see the SI).

Scheme 4 lists the scope electrophilic coupling partners in reactions with Ala^{Bpai}-containing peptides. We found little discrimination between aryl bromides and iodides as well as the electronic nature of the aryl electrophiles (electron-rich and electron-poor) with 52-85% yields across various halides (**34a-k**). 2,7-Diiodonaphtalene **34f** afforded doubly coupled product in acceptable yield when excess (3 equiv.) of dipeptide **33** was used. Boronoalanine coupling was also applied to late-stage diversification of small natural products and drugs (Scheme 4B). Several commercially available drugs which were pre-functionalized with aryl electrophiles such as sulfadimethoxine (**34l**), indomethacin (**34m** and **34n**), steroids (**34o**, **34r**, **34t**, **34u**) and esters of ibuprofen (**34q**) and σ-tocopherol (**34u**) were easily incorporated into amino acid **33** under the standardized conditions in 47-86% yield. Along similar lines, various amino acids such as tyrosine (**34w**), lysine (**34x**), and serine (**34y**) participated in reactions with 4-bromo-*N,N*-dimethylbenzamide (Scheme 4C). Finally, coupling with Ala^{BPai} tripeptide was achieved in 56% yield when excess of 4-bromo-1,1'-biphenyl was used (**34z**).

To further demonstrate the potential of Ala^B in the synthesis of functionalized peptides, we investigated reactions forming macrocyclic oligopeptides (Scheme 5). Cyclic peptides occupy a privileged role due to their propensity to inhibit protein-protein interactions[32] and their ability to permeate cell membranes while remaining resistant to degradation when compared to their acyclic congeners.[33] Direct macrolactamization is the established strategy to form cyclic/stapled peptides, but other disconnections, particularly those that introduce unnatural linkages, need to consider the inherent limitations originating from oligopeptide preorganization. The generality of the transition metal-catalyzed conditions has unlocked a new standard for late-stage, chemoselective reactions of peptides.[34] Transition metal-catalyzed reactions can take advantage of C-H activation to overcome the unfavorable cyclization but require specialized directing groups on the *N*-terminus to guide the sp^3 activation.[34] Based on previous literature precedent[34a] we opted for short peptide sequences with 3-bromophenylalanine and Ala^B occupying the terminal positions (Scheme 5). We established that during the synthesis of linear peptides **34** and **35**, Ala^{BPai} was stable to repeated LiOH saponifications to give the corresponding carboxylic acid that were subsequently elaborated into peptides **34** and **35** (for details, see the SI). These peptides were then subjected to the optimized conditions (Table 2, entry 14) and furnished **37** in 50-64% yield after HPLC purification. Macrocyclization with Ala^{BPai} from either end of the peptide were equally successful indicating that the attempted substrates show strong conformational bias toward the formation of a macrocycle. This disconnection represents a selective and directing-group-free strategy to introduce disubstituted aryl macrocycles into peptides and further demonstrates the utility of Ala^B as a universal building block.

In summary, we described a novel type of umpolung peptide reagents that features boronoalanine. Because BMIDA and BPai analogs are readily available in enantiomerically pure forms, incorporation of these synthons into oligopeptides and proteins that require treatments under acidic, basic, radical, and reductive conditions is now possible. Furthermore, the primary boronic esters can partake in a Pd-catalyzed cross-coupling with C(sp^2) electrophiles significantly expanding the chemical space of amino acids and potentially oligopeptides and proteins. In a broader sense, Ala^M reagents unlock new opportunities to functionalize peptides and proteins based on umpolung of reactivity.

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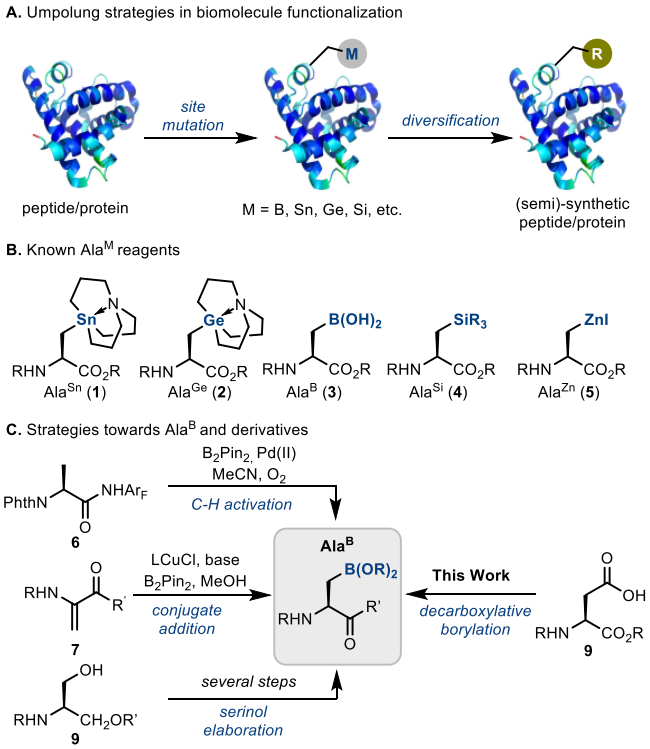
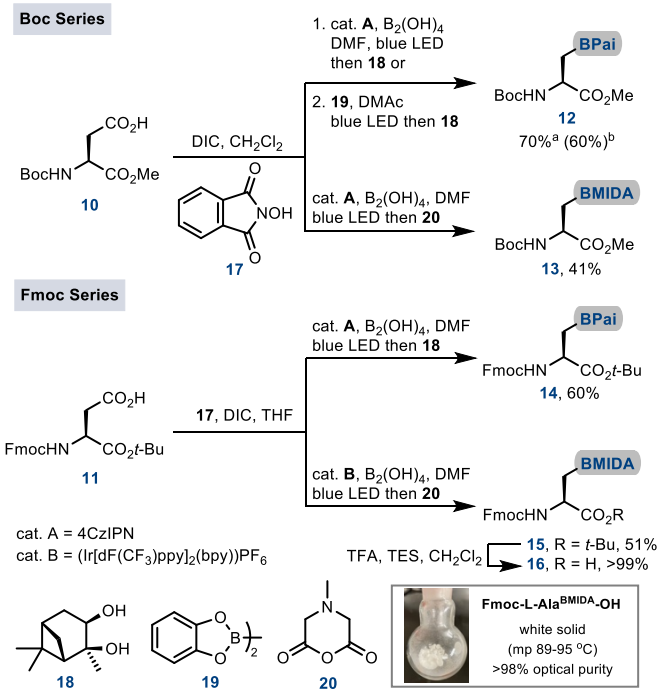
Scheme 1.


A. Umpolung strategies in biomolecule functionalization
site mutation
diversification
M
R
peptide/protein
M = B, Sn, Ge, Si, etc.
(semi)-synthetic peptide/protein
B. Known Ala^M reagents
Ala^Sn (1)
Ala^Ge (2)
Ala^B (3)
Ala^Si (4)
Ala^Zn (5)
C. Strategies towards Ala^B and derivatives
B2Pin2, Pd(II)
MeCN, O2
C-H activation
6
LCuCl, base
B2Pin2, MeOH
conjugate addition
7
Ala^B
This Work
decarboxylative borylation
9
several steps
serinol elaboration
9

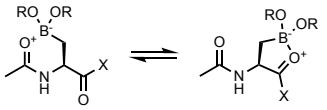
Scheme 2. Synthesis of Ala^{BPai} and Ala^{BMIDA} reagents.



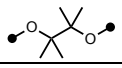
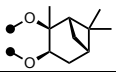
[a] Refers to conditions 1. [b] Refers to conditions 2.

Table 1. Conformational preferences of Ala^{B}.

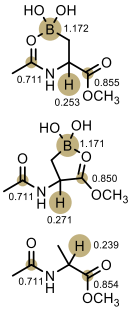
A.



21 22

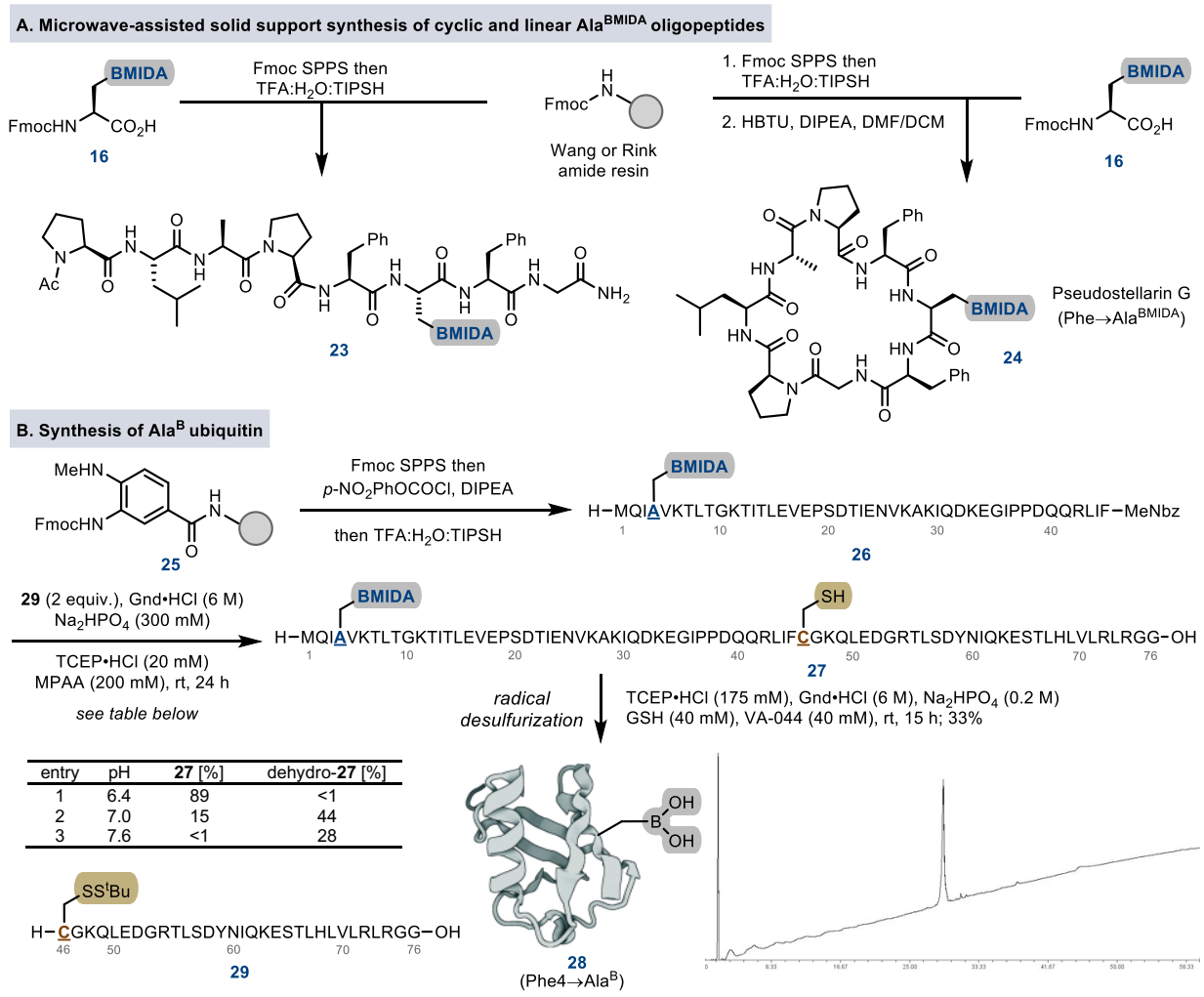
Entry	R	X	Relative Energy ΔG {ΔH} (kcal·mol^{-1})
1		NH_2	-3.2 {-2.4}
2		OMe	-3.9 {1.4}
3		O^-	-7.3 {-4.3}
4		NH_2	-2.8 {-0.87}
5		OMe	-0.65 {-1.4}
6		O^-	-7.9 {-7.8}

B.



A. Calculated at M06-2X/6-311+G(d,p) (SDM THF)//B3LYP-GD3(J3)/6-31(d) (SDM THF) level of theory (298 K, 1 atm). Structures **21** used as the reference (0.0 kcal·mol^{-1}). B. Atomic NBO charges for the lowest energy conformers calculated at M06-2X/6-311+G(d,p) (SDM THF)//B3LYP-GD3(J3)/6-31(d) (SDM THF).

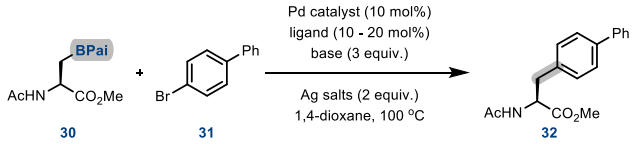
Scheme 3. Elaboration of **Ala^{B}** into peptides and proteins.[a]



entry	pH	**27** [%]	dehydro-**27** [%]
1	6.4	89	<1
2	7.0	15	44
3	7.6	<1	28

[a] LC conditions: Agilent EC-C18 Poroshell, 40 °C, 1 mL/min; 5-65% MeCN/H_2O with 0.05 % TFA over 1 h. Abbreviations: DIPEA=diisopropylethylamine; Gnd=guanidine; GSH=glutathione reduced; HBTU=(2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; MPAA=mercaptophenylacetic acid; SPPS=solid-phase peptide synthesis; TCEP=(tris(2-carboxyethyl)phosphine; TFA=trifluoroacetic acid; TIPSH=triisopropylsilane; VA-044=2,2'-azobis[2-(2-imidazolin-2-yl)propane]dihydrochloride.

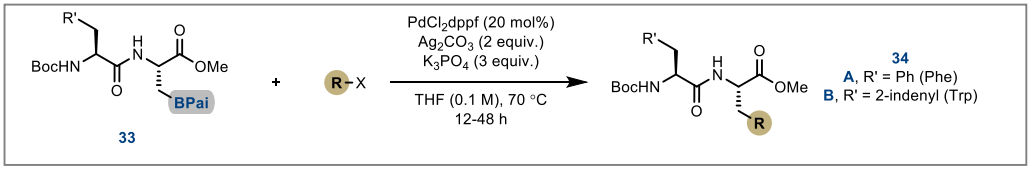
Table 2. Reaction optimization for C-C cross-coupling of Ala^{BPai}.



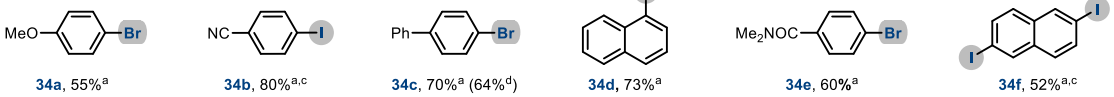
Entry	Pd Catalyst	Ligand	Base	Ag Salts	Yield [%]
1	$PdCl_2$	PPh_3	K_2CO_3	Ag_2O	36
2	$PdCl_2$	JackiePhos	K_2CO_3	Ag_2O	15
3	$PdCl_2$	SPhos	K_2CO_3	Ag_2O	47
4	$PdCl_2$	XPhos	K_2CO_3	Ag_2O	48
5	$PdCl_2$	Davephos	K_2CO_3	Ag_2O	28
6	$PdCl_2$dppf		K_3PO_4	Ag_2O	47
7	$PdCl_2$dppf		KF	Ag_2O	45
8	$PdCl_2$dppf		Cs_2CO_3	Ag_2O	11
9	$Pd_2(dba)_3$	dppf	K_3PO_4	Ag_2O	30
10	$PdCl_2$dppf[a]		K_3PO_4	Ag_2O	37
11	$PdCl_2$dppf		K_3PO_4	Ag_2CO_3	63
12[b]	$PdCl_2$dppf		K_3PO_4	Ag_2CO_3	63
13[d]	$PdCl_2$dppf		K_3PO_4	Ag_2CO_3	73
14[b,d]	$PdCl_2$dppf		K_3PO_4	Ag_2CO_3	83 (81)[c,e]

[a] 1.0:1.5 ratio of **30** and **31**. [b] Reaction time 48 h. [c] Isolated yield. [d] 2.0:1.0 ratio of **30** and **31**. [e] THF used instead of 1,4-dioxane. dppf = 1,1'-bis(diphenylphosphino)ferrocene.

Scheme 4. Scope of Suzuki cross-coupling with Ala^{B}.



A. Electrophile scope



34a, 55%[a]

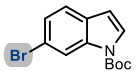
34b, 80%[a,c]

34c, 70%[a] (64%[d])

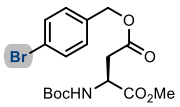
34d, 73%[a]

34e, 60%[a]

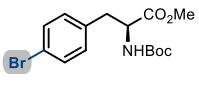
34f, 52%[a,c]



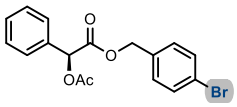
34g, 85%[a]



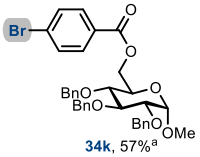
34h, 73%[a]
from aspartic acid



34i, 66%[a]
from phenylalanine

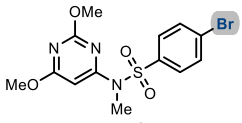


34j, 63%[a]
from (S)-(+)-mandelic acid

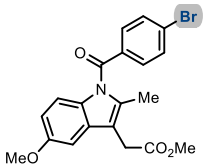


34k, 57%[a]
from (D)-glucose

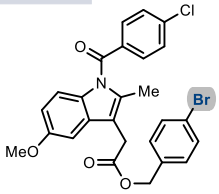
B. Late-stage functionalization of pharmaceutical agents and bioactive molecules



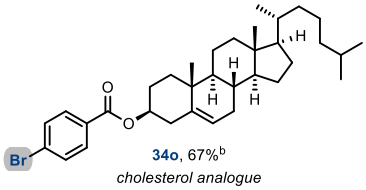
34l, 56%[a]
sulfadimethoxine analogue



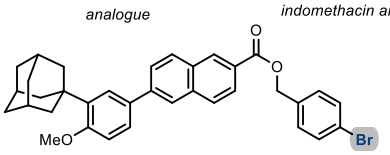
34m, 65%[a]
indomethacin analogue



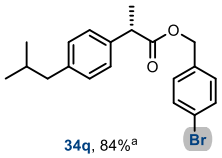
34n, 86%[a]
indomethacin analogue



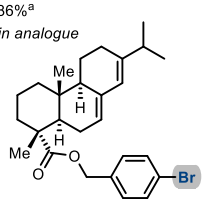
34o, 67%[b]
cholesterol analogue



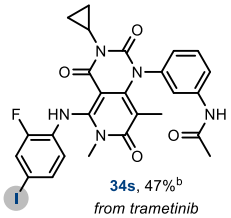
34p, 70%[a]
adapalene analogue



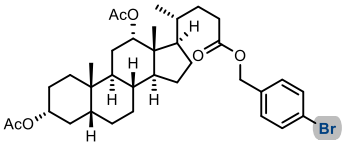
34q, 84%[a]
ibuprofen analogue



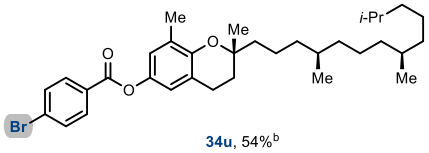
34r, 72%[b]
from abietic acid



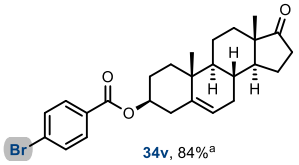
34s, 47%[b]
from trametinib



34t, 76%[b]
deoxycholic acid analogue

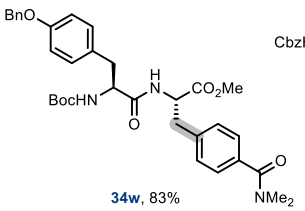


34u, 54%[b]
-tocopherol analogue

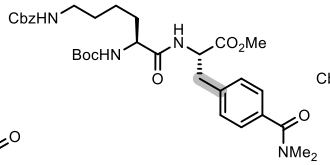


34v, 84%[a]
dehydroepiandrosterone analogue

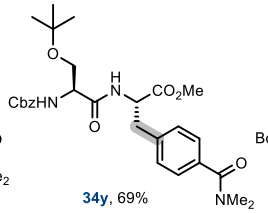
C. Amino acid diversity



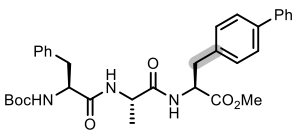
34w, 83%



34x, 59%



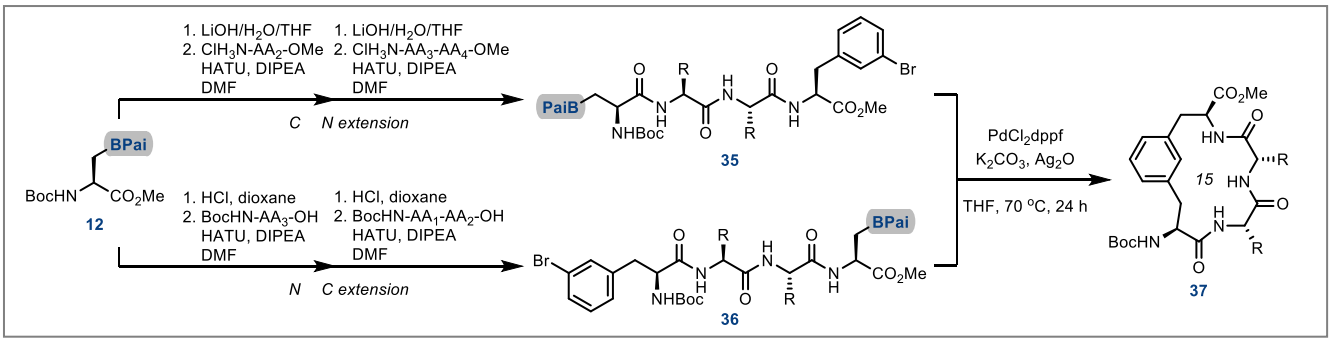
34y, 69%



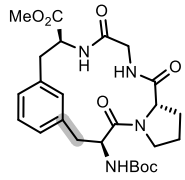
34z, 63%

[a] Refers to **34A** series. [b] Refers to **34B** series. [c] K_2CO_3 at 60 °C; [d] $PdCl_2dppf$ (30 mol%), 72 h.

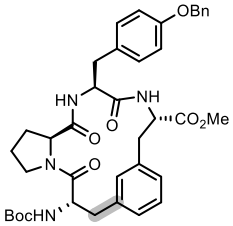
Scheme 5. Macrocyclization of Ala^{B} oligopeptides.



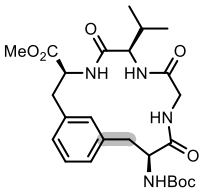
Macrocyclization scope



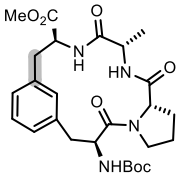
37a, 61%[a,b]



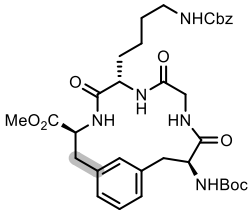
37b, 53%[a,b]



37c, 54%[a,b]



37d, 61%[a,c]



37e, 50%[a,c]

[a]$PdCl_2dppf$ (10 mol%), Ag_2O (2 equiv.) K_2CO_3 (3 equiv.) THF (50 mM). [b]Macrocycle derived from **35** (Boc-AlaB^{Pai}-AA$_2$-AA$_3$-Phe(*m*-Br)-OMe). [c]Macrocycle derived from **36** (Boc-Phe(*m*-Br)-AA$_2$-AA$_3$-AlaB^{Pai}-OMe).