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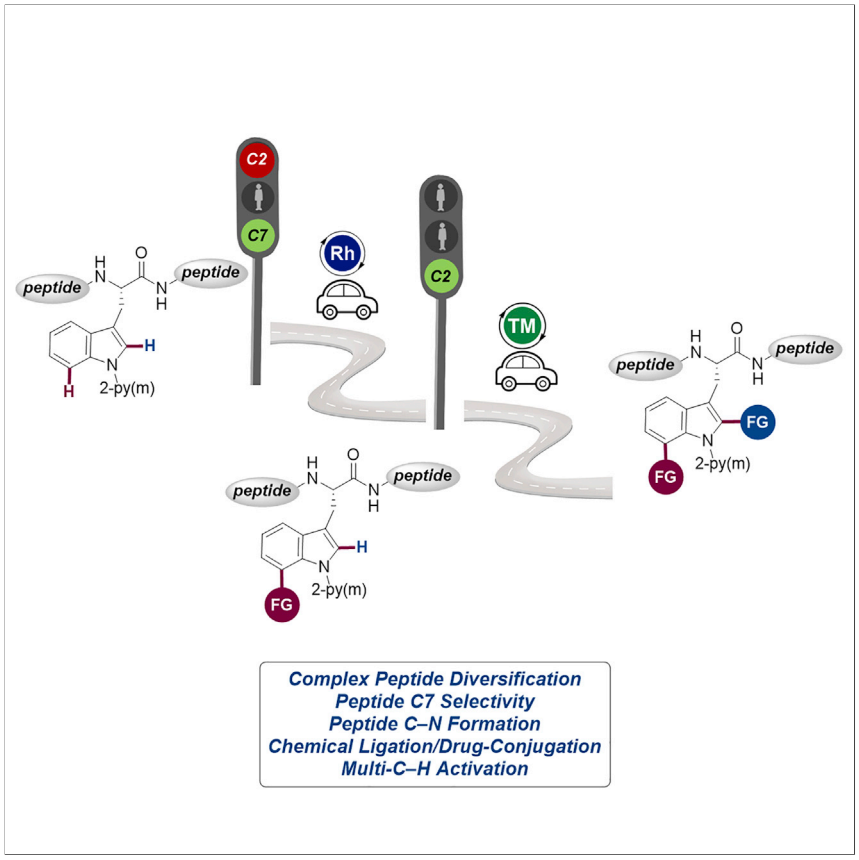
Peptide Late-Stage Diversifications by Rhodium-Catalyzed Tryptophan C7 Amidation



Herein, we disclose the first late-stage peptide C–H nitrogenations through unprecedented rhodium(III)-catalyzed peptide C7 diversification. Thus, peptides were directly transformed through position-selective C7 amidations with easily accessible 1,4,2-dioxazol-5-ones. Structurally complex peptides featuring a wealth of functional groups underwent C7-C–H amidations to deliver selectively modified natural product and drug hybrids.

Wei Wang, Jun Wu, Rositha Kuniyil, Adelina Kopp, Rafaely Nascimento Lima, Lutz Ackermann

lutz.ackermann@chemie.uni-goettingen.de

HIGHLIGHTS

Position-selective peptide diversification by tryptophan C7 modification

Late-stage peptide C–N bond formation

2-fold C–H activation for peptide multi-diversification

Bioorthogonal modification of functionalized, structurally complex peptide

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Wei Wang,[1,3] Jun Wu,[1,3] Rositha Kuniyil,[1] Adelina Kopp,[1] Rafaely Nascimento Lima,[1] and Lutz Ackermann[1,2,4,*]

SUMMARY

The late-stage diversification (LSD) of structurally complex peptides has emerged as a powerful platform for molecular engineering and drug discovery. Besides traditional peptide modifications, such as native chemical ligations or cross-couplings with prefunctionalized substrates, during recent years, C—H activation has gained considerable momentum as a robust and step-economical strategy for late-stage peptide modifications, thus far predominantly for the formation of C—C bonds. Although C—N bond formations represent established strategies in medicinal chemistry and drug discovery, methods for direct amidations of tryptophan and tryptophan-containing peptides are rare and severely limited to the activated C2 position. In contrast, we herein disclose the first example of direct late-stage peptide C—H amidation reaction and the unprecedented late-stage peptide diversification on tryptophan C7 position in a highly site-selective manner. Moreover, this strategy sets the stage for sequential double C(7)—H/C(2)—H modifications, further improving the peptide structural complexity.

INTRODUCTION

Late-stage diversification (LSD) of peptides has shown increased importance in drug discovery and pharmaceutical industries.[1–3] Non-natural peptides often exhibit improved properties as compared to their parent analogs,[4–6] and, as a consequence, the precise modification of structurally complex peptides is of central significance. However, peptide syntheses thus far largely relied on classical approaches, such as solid-phase peptide synthesis (SPPS) and asymmetric synthesis.[7,8] While palladium-catalyzed cross-coupling reactions have, hence, greatly contributed to peptide modifications through a tag and modify approach,[9–11] these conventional couplings require double substrate prefunctionalization, and thus lengthy synthetic procedures. In sharp contrast, catalyzed C—H activation has recently emerged as a complementary strategy for step-economical late-stage modifications.[12–18] C—H activation has, thus, been recognized as an enabling tool for late-stage peptide functionalizations,[19–22] with major contributions by Albericio and co-workers,[23] Lavilla and co-workers,[24] Shi and co-workers,[25] Wang and co-workers,[26] Yu and co-workers,[27,28] and Ackermann and co-workers[29–37] In this context, the functionalization of tryptophan-containing peptides has attracted considerable interest, due to tryptophan's low natural abundance as well as its unique impact on biological events.[38] However, all tryptophan C—H activations are severely limited to the activated C2 position (Figure 1B), which is in sharp contrast to the predominance of C7-substituted indoles/tryptophans in numerous bioactive agents and important structurally complex peptide-natural products (Figure 1A).[39–41] Despite the

The Bigger Picture

Peptides represent biopolymers of key relevance to biochemistry, medicinal chemistry, natural product synthesis, and pharmaceutical industries, among others. Traditional peptide syntheses thus far largely relied on lengthy functional group interconversions. Resource-economic C–H activation has surfaced as a transformative platform for peptide modifications but continues to be restricted to tryptophan modifications at the C2-position. In sharp contrast, C7-selective peptide modifications have now been realized by versatile rhodium catalysis. The catalyzed C–H amidations occurred with outstanding levels of C7 site selectivity on highly functionalized peptides. These findings unravel the unique potential of biorthogonal late-stage diversification for the direct assembly of structurally complex peptides for biology, molecular syntheses, and drug discovery.

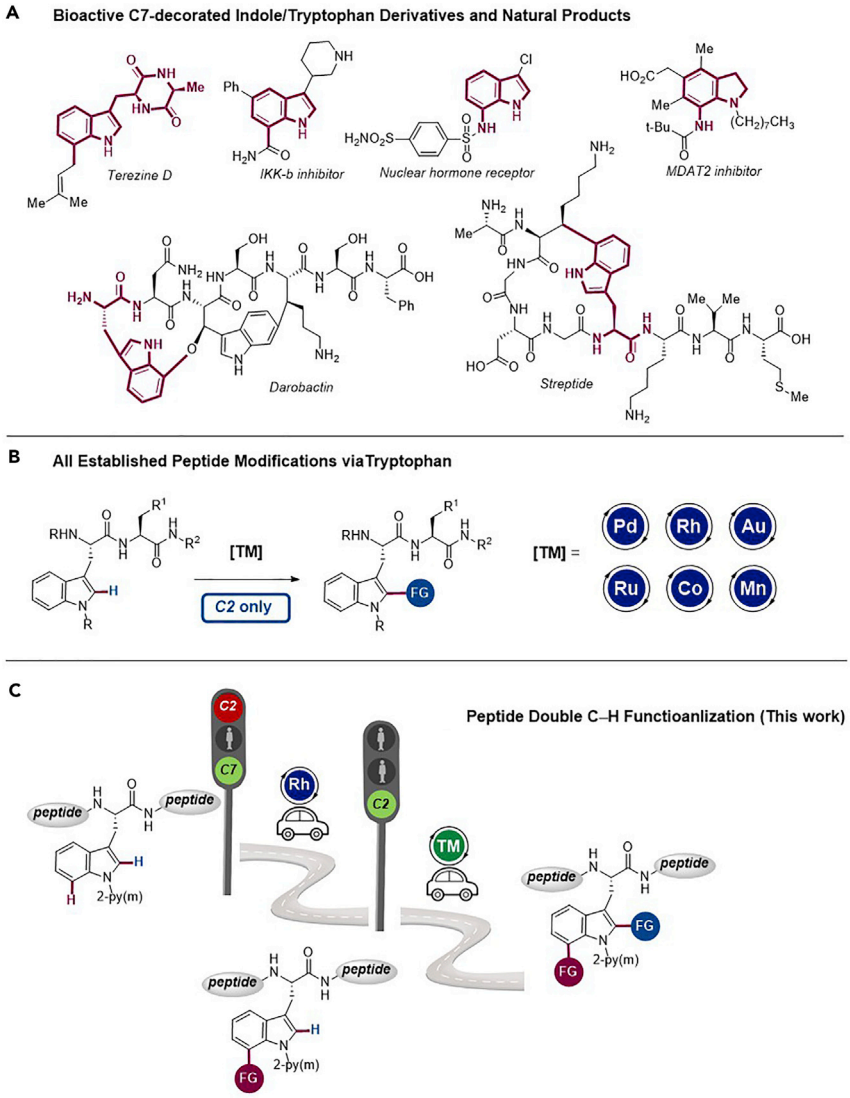

Figure 1. Double Tryptophan C(7)—H/C(2)—H Activation for Late-stage Peptide Diversifications

(A) The bioactive tryptophans and indole derivatives featuring C7 decoration.

(B) All the established tryptophan and tryptophan containing peptide modification strategies by transition metal catalysis give C(2)–H functionalizations.

(C) Site-selective C(7)–H functionalizations enabled by rhodium catalysis. Also, sequential double C(7)–H/C(2)–H activation for peptide diversifications is viable.

significance of C7-decorated tryptophan peptides, to the best of our knowledge, no chemical strategy was developed for the direct late-stage tryptophan-peptide C(7)—H diversification to date; few examples of C7 modifications are largely restricted to simple indole and tryptophan. This restricted not only the synthesis of C7 decorated tryptophan peptides but also hindered the access to the peptide structural complexity. In line with the very recent C(7)—H activation discoveries of elegant metal-free and rhodium-catalyzed C(7)—H activations by Shi and co-workers,[42,43] as well as the indole C(7)—H amination/amidation by Chang and co-workers,[44,45] tryptophan C(7)—H activations by Movassaghi and co-workers [46] and Ma and co-workers,[47,48] among others,[49,50] we hence questioned whether C7 activation for the diversification of a structurally more complex peptide would indeed be viable,

[1]Institut für Organische und Biomolekulare Chemie, Georg-August-Universität Göttingen, Tammannstrasse 2, 37077 Göttingen, Germany

[2]DZHK (German Center for Cardiovascular Research), Potsdamer Strasse 58, 10785 Berlin, Germany

[3]These authors contributed equally

[4]Lead Contact

*Correspondence: lutz.ackermann@chemie.uni-goettingen.de

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ideally amide bond formation, which is the most important reaction for both chemistry and biology field.[51–55] To this end, for the first time, we report on a highly selective and robust direct late-stage peptide amidation reaction, as well as the first late-stage tryptophan-peptide C7 diversification in a racemization-free manner (Figures S1 and S2). Moreover, sequential double C—H activation, C7 amidation, and subsequent C2 functionalization were devised, thus allowing late-stage modification for peptide multifunctionalization toward structurally complex peptides (Figure 1C).[40,41]

RESULTS AND DISCUSSION

Optimizations

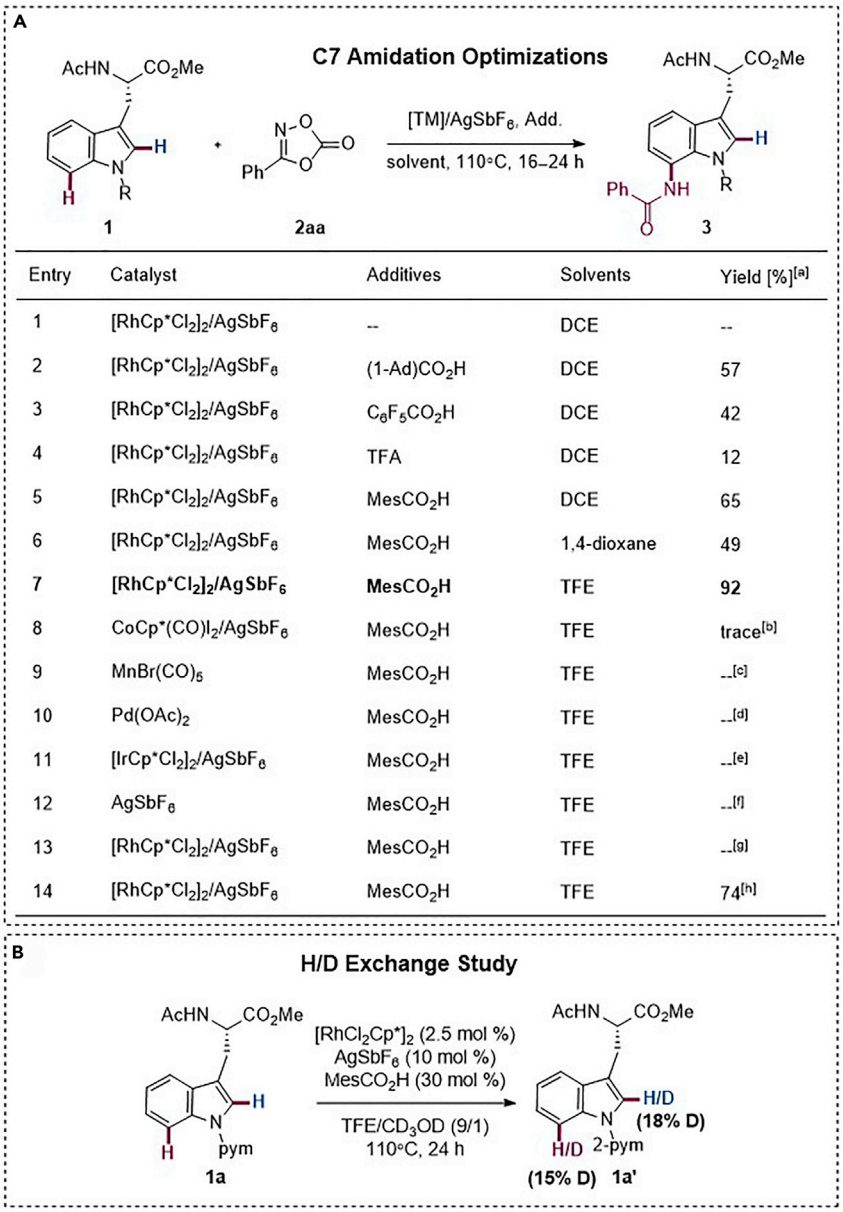
To access drug-relevant tryptophan peptides, we initiated our research in C—N bond formation employing 3-phenyl-1,4,2-dioxazol-5-ones as the amidating reagent,[56–61] which is easily accessible from various commercially available carboxylic acids. Intriguingly, considerable optimization studies revealed that unprecedented C(7)–H amidations were accomplished under rhodium(III)[62–64] catalysis. Next, we investigated the rhodium catalyst's efficacy under various reaction conditions (Figure 2A; Table S1). Acid additives were found to be crucial for the C7 amidation (Figure 2A, entries 1–5), with $MesCO_2H$ proving to be optimal (Figure 2A, entry 5). Among various solvents, 2,2,2-trifluoroethanol (TFE) was the reaction medium of choice (Figure 2A, entries 6 and 7). Omitting the rhodium(III) catalyst or the use of other typical transition metal complexes shut down the reaction entirely (Figure 2A, entries 8–12). *N*-Substituents other than pyrimidyl on the indole did not lead to a superior reaction outcome (Figure 2A, entries 13, 14). To rationalize the C7 selectivity, we tested whether an H/D exchange occurred on the tryptophan **1a** in the presence of $[D]_4$-MeOH in the absence and the presence of the dioxazolone **2aa** (Figures 2B and S4–S7), which unexpectedly unraveled a reversible C–H activation at both the C7 and C2 positions.

Mechanism Insights

However, the C–H amidations with indole substrate resulted in preferential C2 amidation, while tryptophan underwent C7 amidation with excellent site selectivity, indicating an increased impact of steric[65,66] hindrance of the peptide backbone (Figure S3). In order to gain insights into the exact reaction mechanism of the rhodium-catalyzed C–H amidation of tryptophan, density functional theory (DFT) calculations were performed at the PBE0-D3(BJ)/6-311++G(d,p), def2-TZVP(Rh), SDD(Rh)+-SMD(TFE)//TPSS-D3(BJ)/6-31+G(d), def-TZVP (Rh), and SDD(Rh) levels of theory (Figures 3, S12, and S13). The initial decarboxylation step features an activation barrier of 22.5 kcal mol^{-1} for the C7 selective C–N formation; whereas, for the C2 pathway, an activation energy of 25.4 kcal mol^{-1} is required, disfavoring the key decarboxylation step at the C2 position.

Tryptophan C7 Diversifications

With the optimized reaction conditions in hand, we explored the scope of the tryptophan diversification using a representative set of 1,4,2-dioxazol-5-ones **2**. Both aryl and alkyl dioxazolones were thus well tolerated (Figure 4A). Electron-rich and electron-poor arenes **2ab–2ae** were also compatible, with electron-withdrawing substituents leading to better yields. Furthermore, fluorene was introduced successfully by the C7-amidation to yield product **3ag**. Notably, iodide-substituted dioxazolone **2ag** was characterized by an excellent chemo-selectivity, without any cross-coupling products being observed. Beside arenes, heterocyclic thiophene **2ah** was efficiently converted within the site-selective amidation. Importantly, tryptophan with a free carboxylic acid selectively gave the C7 amidated product **3aj**,



Entry	Catalyst	Additives	Solvents	Yield [%][a]
1	$[RhCp^*Cl_2]_2/AgSbF_6$	--	DCE	--
2	$[RhCp^*Cl_2]_2/AgSbF_6$	$(1\text{-Ad})CO_2H$	DCE	57
3	$[RhCp^*Cl_2]_2/AgSbF_6$	$C_6F_5CO_2H$	DCE	42
4	$[RhCp^*Cl_2]_2/AgSbF_6$	TFA	DCE	12
5	$[RhCp^*Cl_2]_2/AgSbF_6$	$MesCO_2H$	DCE	65
6	$[RhCp^*Cl_2]_2/AgSbF_6$	$MesCO_2H$	1,4-dioxane	49
7	**$[RhCp^*Cl_2]_2/AgSbF_6$**	**$MesCO_2H$**	TFE	**92**
8	$CoCp^*(CO)I_2/AgSbF_6$	$MesCO_2H$	TFE	trace[b]
9	$MnBr(CO)_5$	$MesCO_2H$	TFE	...[c]
10	$Pd(OAc)_2$	$MesCO_2H$	TFE	...[d]
11	$[IrCp^*Cl_2]_2/AgSbF_6$	$MesCO_2H$	TFE	...[e]
12	$AgSbF_6$	$MesCO_2H$	TFE	...[f]
13	$[RhCp^*Cl_2]_2/AgSbF_6$	$MesCO_2H$	TFE	...[g]
14	$[RhCp^*Cl_2]_2/AgSbF_6$	$MesCO_2H$	TFE	74[h]

Figure 2. Optimization of Tryptophan C7 Amidation and H/D Exchange Study

(A) Reaction conditions for optimization: **1** (0.2 mmol), **2aa** (0.4 mmol), $[RhCp^*Cl_2]_2$ (2.5 mol %), $AgSbF_6$ (10 mol %), additive (15 mol %), solvent (0.4 mL), under N_2, R = 2-pyrimidyl; [a] yield of isolated products; [b] $CoCp^*(CO)I_2$ (10 mol %) as the catalyst; [c] $MnBr(CO)_5$ (10 mol %) as the catalyst, no silver salts; [d] $Pd(OAc)_2$ (10 mol %) as the catalyst, no silver salts; [e] [IrCp*Cl2]2 (2.5 mol %) as the catalyst; [f] [RhCp*Cl2]2 (0 mol %); [g] R = pivaloyl; [h] R = 2-pyridyl. DCE = 1,2-dichloroethane.

(B) Identification of reversible C–H activation process at both the tryptophan C7 and C2 position by H/D exchange study.

illustrating the robustness of this reaction to override the simple amide bond formation. Various alkyl dioxazolones could also be employed, ranging from aryl substituents (**2ai–2ak**) to olefins (**2al**). Intrigued by the compatibility of alkyl groups, we employed the reaction for the direct chemical ligation with various amino-acid-derived dioxazolones, thus enabling new disconnections toward dipeptides (Figure 4B).

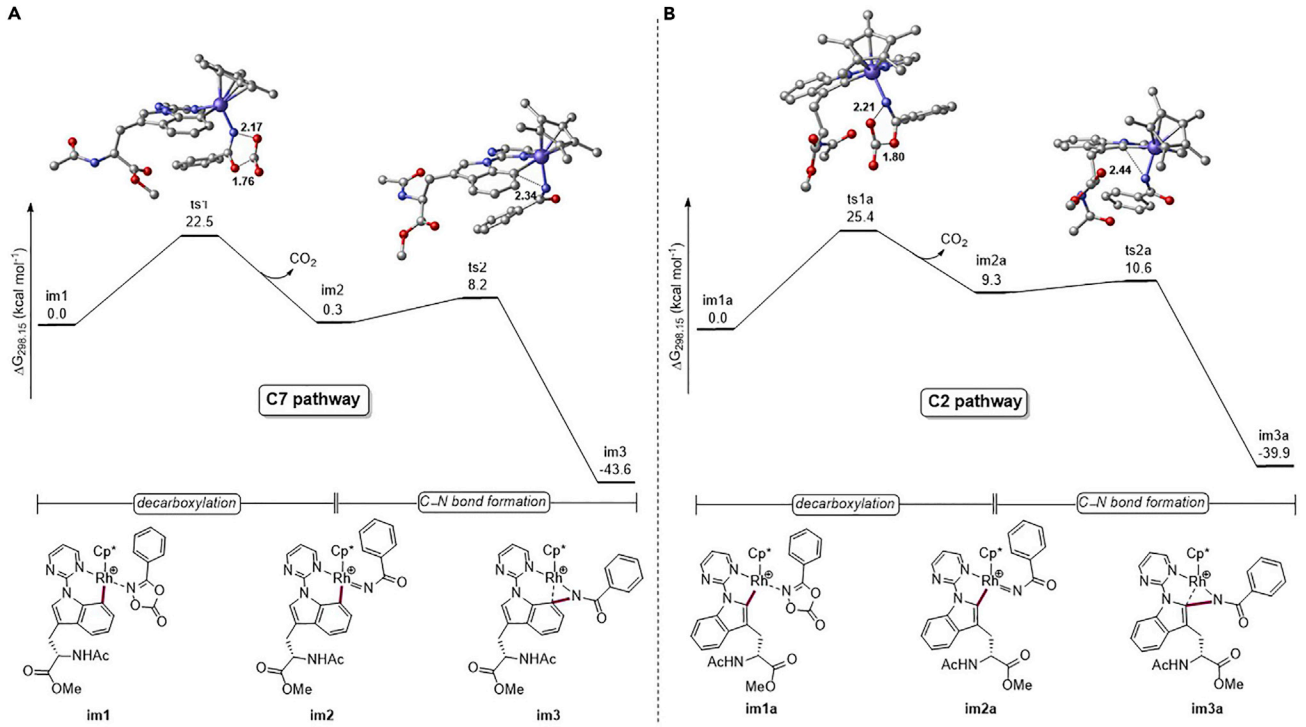


Figure 3. DFT Calculation Studies of the Mechanism on Tryptophan C7 and C2 Amidation Site Selectivity

(A and B) Computed Gibbs free energy profile in kcal mol^{-1} for the decarboxylation and C–N bond formation elementary steps at the PBE0-D3(BJ)/ 6-311++G(d,p),def2-TZVP(Rh),SDD(Rh)+SMD(TFE)//TPSS-D3(BJ)/6-31+G(d),def2-TZVP(Rh),SDD(Rh) level of theory, (A) for the C7 amidation, (B) for the C2 amidation of tryptophan **1a**. In the computed transition state structures, non-relevant hydrogen atoms are omitted for clarity. The bond lengths in transition state (TS) are given in Å.

Both α-amino acids (**2ba** and **2bb**) and β-amino acids (**2bc** and **2bd**) were introduced to the C7 position of tryptophan. The *N*-pyridyl group could be removed in a traceless manner, delivering *NH*-free product **3c** under racemization-free conditions (Figures 4C and S8).

Pyrimidyl Transformations

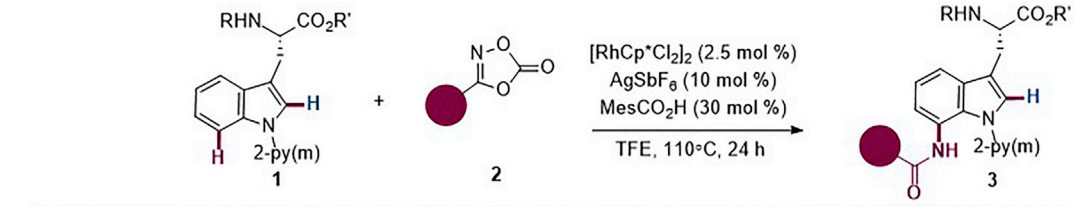
Next, we developed a novel hydrogenation of the thus-obtained product **3ao**, which selectively occurred at the *N*-pyrimidyl substituent to furnish C7 tetrahydropyrimidine **4a** and hexahydropyrimidine **4b** by the judicious choice of the reaction time (Figures 5, S9, and S10). Thereby, the hydrogen-bond acceptor was transformed into a donor motif, which should prove instrumental for medicinal chemistry programs.

Tryptophan Bio-conjugations

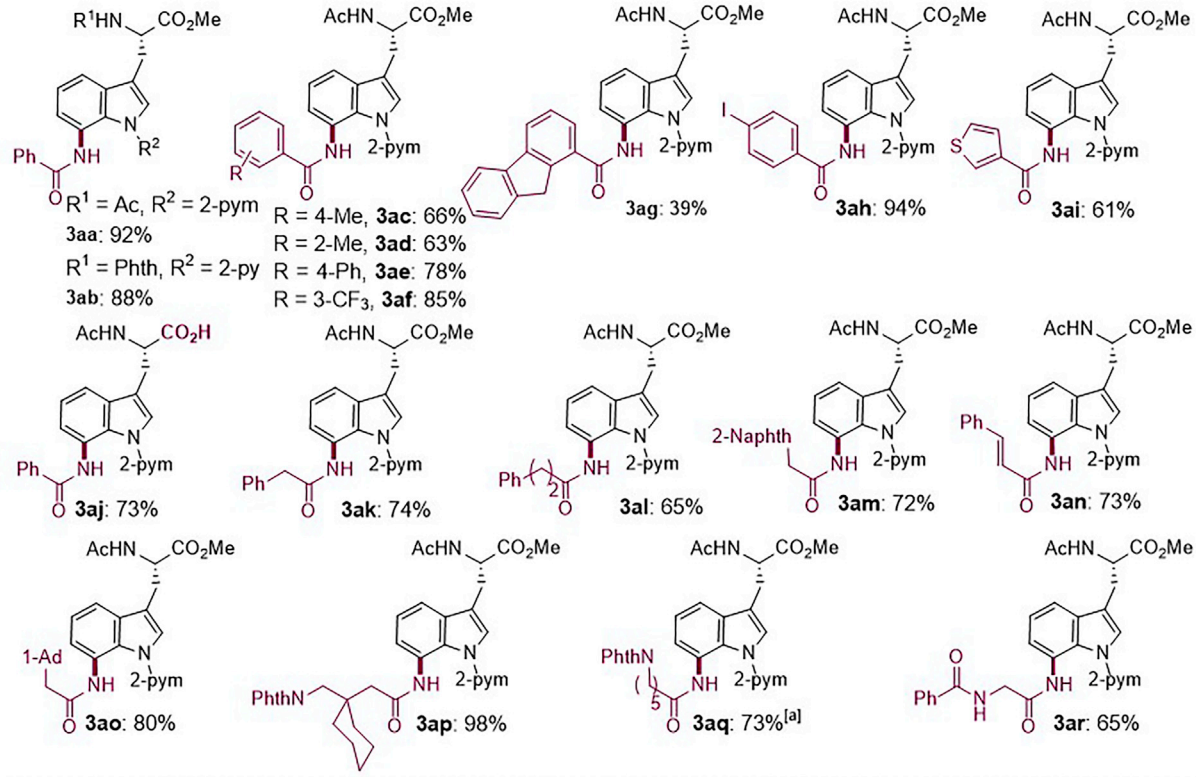
We studied the amidation for the bio-conjugation to form versatile amino-acid-natural product and -drug hybrids in a chemo- and site-selective manner (Figure 6). Hence, hybrid conjugates **5a–5f** with citronellic acid, erucic acid, probenecid acid, ibuprofen, dehydrocholic acid, and (-)menthol were selectively assembled, reflecting the versatility of our approach toward structurally complex drugs and natural products.

Peptide Double C—H Functionalizations

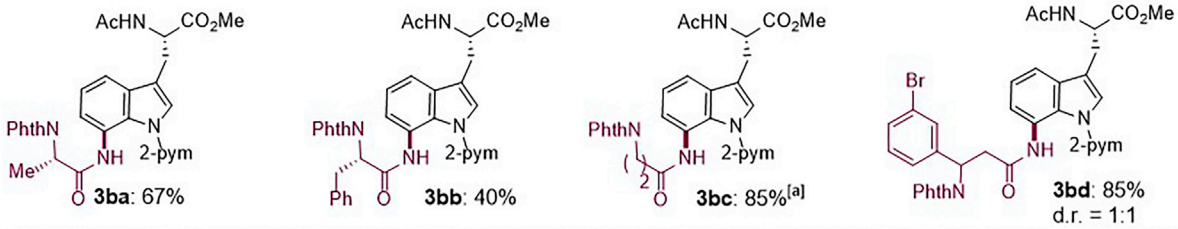
Next, we tested the implementation of our C7 modification for successive double C–H activations at C7 and then C2 (Figure 7). 2-fold rhodium-catalyzed



A **Tryptophan Diversifications**



B **Tryptophan C7 Chemical Ligation**



C **Traceless Removal of Pyridyl Group**

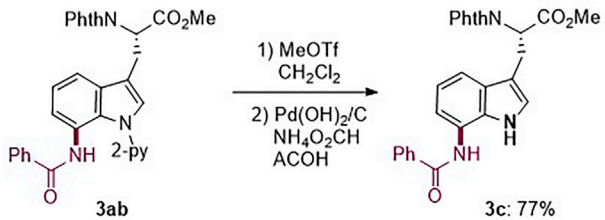


Figure 4. Tryptophan C7 Diversifications by Rhodium Catalysis

(A) Tryptophan C7 diversifications by various dioxazolones.[a] Using 1.0 equiv. dioxazolone for the reaction.

(B) Direct tryptophan C7 chemical ligations using various amino-acid-derived dioxazolones.

(C) Traceless removal of the tryptophan *N*-pyridyl group for generation of C7 amidated native tryptophan.

amidations with 1,4,2-dioxazol-5-ones, thus, selectively yielded di-amidated products **7a** in excellent yields under otherwise identical reaction conditions. TsN_3 was employed in successive amidations under rhodium catalysis as well, thus delivering the di-amidated tryptophan **7b**. Furthermore, successive C–H alkynylation was realized under manganese catalysis[36] for the di-functionalization toward amino acid **7c**, which should prove instrumental for subsequent diversifications by click reactions. Moreover, a manganese-catalyzed C(2)–H allylation[31] reaction took place successfully, furnishing peptides **7d** and **7e** with erucic acid amide on tryptophan C7 and allyl group on C2, both of which contain synthetically useful alkenyl groups.

Late-Stage Peptide Diversifications

The C7 amidation was thereafter probed for the envisioned peptide diversifications (Figure 8). Dipeptides, tripeptides, hexapeptides, and macrocyclic peptides site-selectively gave the C7 amidation products **8**. The reaction retained its unprecedented C7-selectivity irrespective of the peptidic amino acid sequence. Functional groups, such as a free hydroxyl of serine and tyrosine, proved compatible, thus giving the desired amidated peptides **8k** and **8l**, and **8o** and **8p**, respectively. The free amide of asparagine was also tolerated with rhodium(III) catalyst, delivering C7-functionalized peptides **8q** and **8r**. The free amine group of lysine was released in a one-pot manner upon efficient C7 installation to deliver peptide **8s**. Moreover, the disulfide-containing peptide was efficiently transformed into the C7 modified peptide **8m**, which provided access to free-cysteine peptide **8n**. Notably, peptide-natural product hybrids **8c**, **8j**, **8l**, and macrocyclic peptide **8t** were obtained by this strategy. Furthermore, the late-stage C7 amidation reaction delivered hexapeptide **8u**, heptapeptide **8v**, and octapeptides **8w** and **8x** as derivatives of Leuprorelin without decompositions, importantly with free C terminus and various functional amino acid side chains compatible, such as serine, tyrosine, and glutamine, thereby highlighting the potential to assemble structurally complex peptides. Meanwhile, peptides with

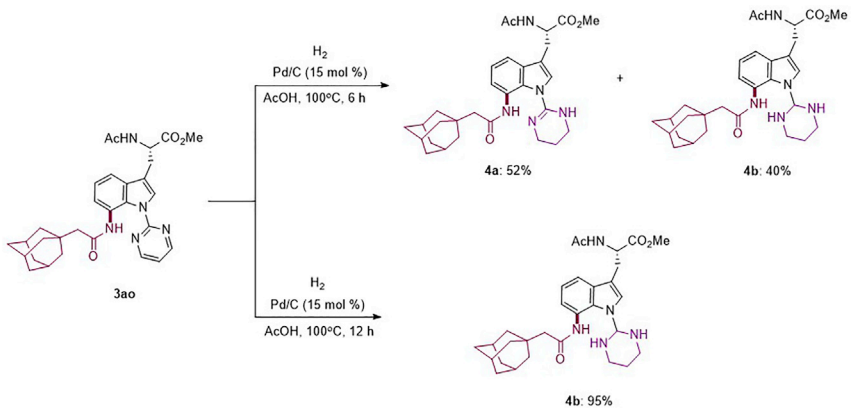


Figure 5. Pyrimidyl Transformation by Chemo-selective Hydrogenation

Selective hydrogenation of the pyridyl group was realized by palladium catalysis under ambient H_2. Hydrogen-bond donors **4a** and **4b** were thereby obtained selectively.

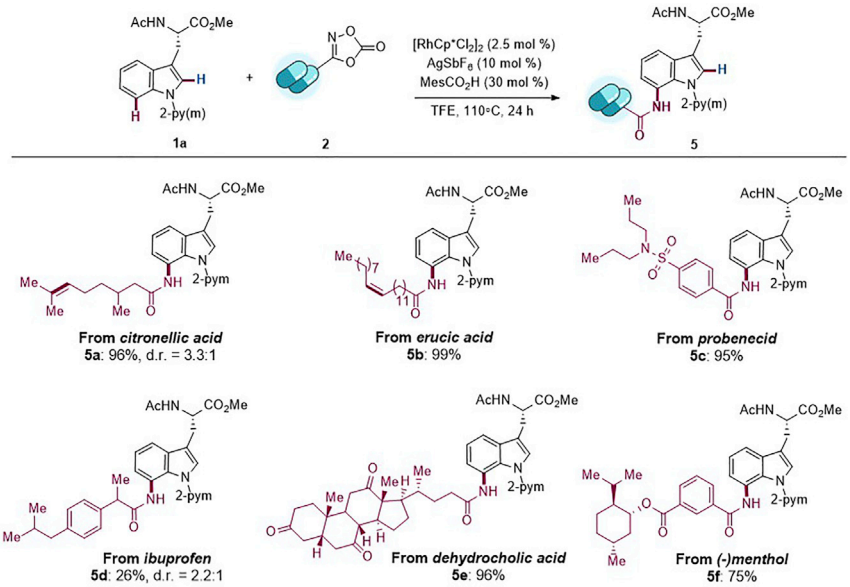


Figure 6. Tryptophan C7 Conjugations for the Assembly of Natural Product Hybrids

Various natural products were site-selectively transformed into tryptophan C7 amidation products by rhodium catalysis, accessing novel amino-acid-natural product hybrids.

amino acids, such as histidine and arginine, proved not compatible (Figure S11). Overall, direct modifications of more structurally complex peptides are of great interest to be further explored using C–H activations.

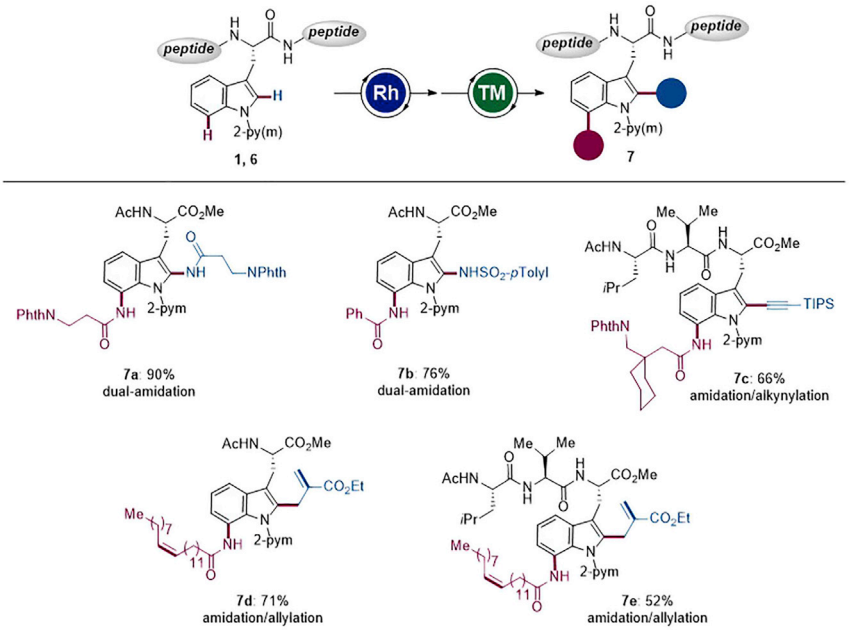


Figure 7. Peptide Sequential Double C–H Activations

Sequential peptide diversifications were established by C7 amidation reaction and successive C(2)—H activations. Double amidation reactions were employed for di-functionalized products **7a** and **7b**. Manganese-catalyzed alkynylation successively installed an alkynyl group on the C7-amidated peptide to afford **7c**. Through the C7 amidation and manganese-catalyzed allylation, **7d** and **7e** were obtained, with both internal and terminal alkenyl groups. For detailed information, see Supplemental Information.

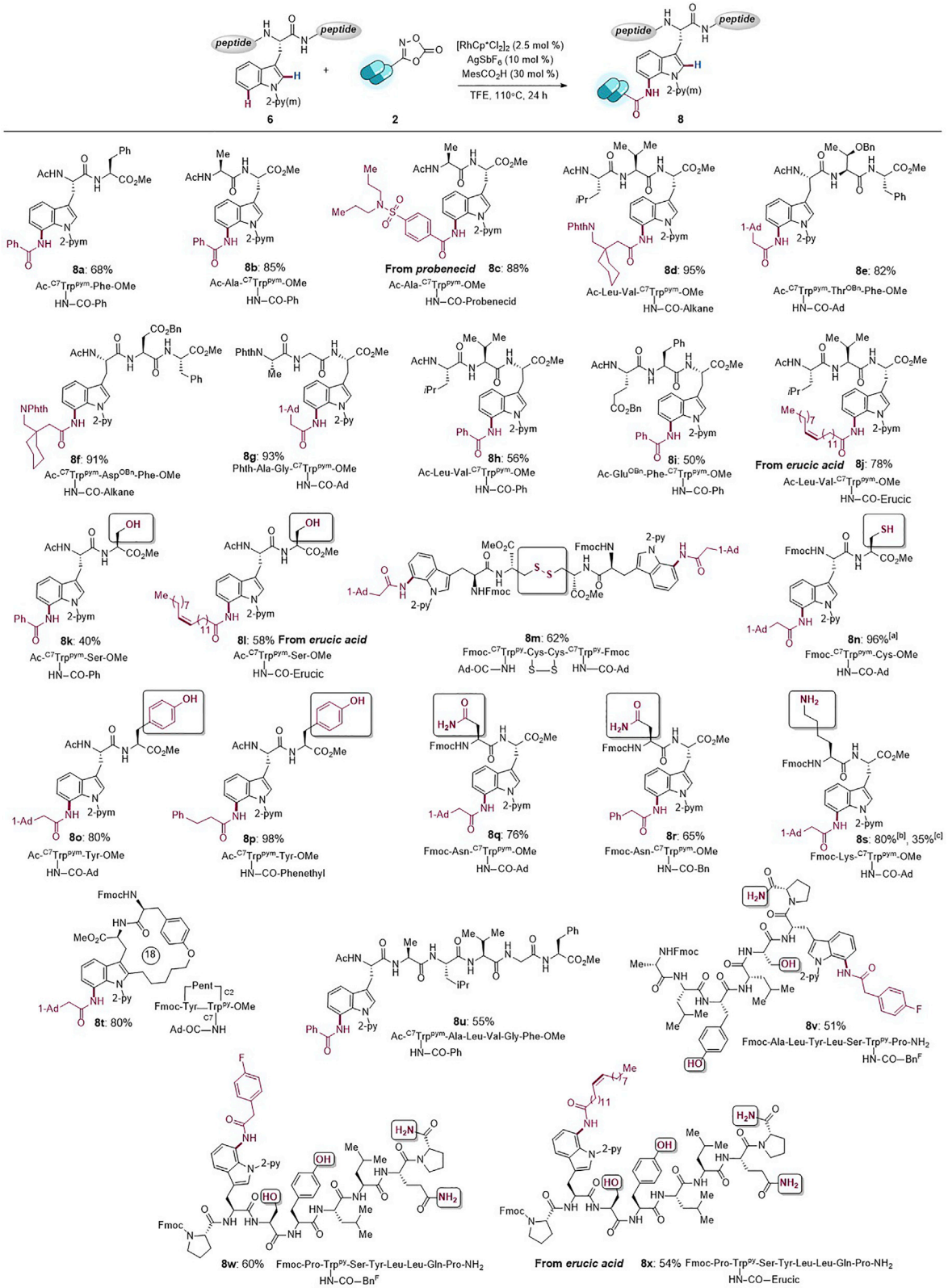

peptide
peptide
2-py(m)
6
2
[RhCp*Cl2]2 (2.5 mol %)
AgSbF6 (10 mol %)
MesCO2H (30 mol %)
TFE, 110°C, 24 h
peptide
peptide
2-py(m)
8
8a: 68%
Ac-C7Trppym-Phe-OMe
HN–CO-Ph
8b: 85%
Ac-Ala-C7Trppym-OMe
HN–CO-Ph
From probenecid 8c: 88%
Ac-Ala-C7Trppym-OMe
HN–CO-Probenecid
8d: 95%
Ac-Leu-Val-C7Trppym-OMe
HN–CO-Alkane
8e: 82%
Ac-C7Trppym-ThrOBn-Phe-OMe
HN–CO-Ad
8f: 91%
Ac-C7Trppym-AspOBn-Phe-OMe
HN–CO-Alkane
8g: 93%
Phth-Ala-Gly-C7Trppym-OMe
HN–CO-Ad
8h: 56%
Ac-Leu-Val-C7Trppym-OMe
HN–CO-Ph
8i: 50%
Ac-GluOBn-Phe-C7Trppym-OMe
HN–CO-Ph
From erucic acid 8j: 78%
Ac-Leu-Val-C7Trppym-OMe
HN–CO-Erucic
8k: 40%
Ac-C7Trppym-Ser-OMe
HN–CO-Ph
8l: 58% From erucic acid
Ac-C7Trppym-Ser-OMe
HN–CO-Erucic
8m: 62%
Fmoc-C7Trppy-Cys-Cys-C7Trppy-Fmoc
Ad-OC–NH HN–CO-Ad
8n: 96%[a]
Fmoc-C7Trppym-Cys-OMe
HN–CO-Ad
8o: 80%
Ac-C7Trppym-Tyr-OMe
HN–CO-Ad
8p: 98%
Ac-C7Trppym-Tyr-OMe
HN–CO-Phenethyl
8q: 76%
Fmoc-Asn-C7Trppym-OMe
HN–CO-Ad
8r: 65%
Fmoc-Asn-C7Trppym-OMe
HN–CO-Bn
8s: 80%[b], 35%[c]
Fmoc-Lys-C7Trppym-OMe
HN–CO-Ad
8t: 80%
Fmoc-Tyr—Trppy-OMe
Ad-OC–NH
8u: 55%
Ac-C7Trppym-Ala-Leu-Val-Gly-Phe-OMe
HN–CO-Ph
8v: 51%
Fmoc-Ala-Leu-Tyr-Leu-Ser-Trppy-Pro-NH2
HN-CO–BnF
8w: 60% Fmoc-Pro-Trppy-Ser-Tyr-Leu-Leu-Gln-Pro-NH2
HN–CO–BnF
From erucic acid 8x: 54% Fmoc-Pro-Trppy-Ser-Tyr-Leu-Leu-Gln-Pro-NH2
HN–CO–Erucic

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Figure 8. Scope of Tryptophan-Peptide Diversifications
Dipeptide, tripeptide, cyclic peptide, hexapeptide, heptapeptide, and octapeptide with versatile peptide sequence and functional amino acid side chains were probed for the C7 amidation reaction under rhodium catalysis with various dioxazolones, yielding the C7 functionalized tryptophan-containing peptides in good to excellent yields. [a] From **8m**. [b] Sequential synthesis. [c] One-pot synthesis.

Conclusions

In summary, we have developed a robust and position-selective C7 amidation by rhodium-catalyzed late-stage peptide modification. The installation of versatile functional groups on tryptophan-containing peptides was hence achieved with unprecedented C7 site selectivity, contrasting with all known ruthenium, gold, palladium, cobalt, and manganese-catalyzed C–H peptide transformation. Sequential double C–H activations were established for the synthesis of di-functionalized C7/C2 peptides, substantiating the great potential for medicinal chemistry and drug synthesis. Remarkably, the C7 amidation enabled the assembly of hybrid conjugates and allowed for the LSD of structurally complex peptides.

EXPERIMENTAL PROCEDURES

Resource Availability

Lead Contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Prof. Dr. Lutz Ackermann (lutz.ackermann@chemie.uni-goettingen.de).

Materials Availability

Catalytic reactions were carried out under N_2. Reactions were performed using standard Schlenk techniques. 1,2-dichloroethane was stirred with CaH_2 for 8 h and distilled under N_2 atmosphere. *N*-pyridyl and *N*-pyrimidyl indoles, tryptophans, and tryptophan-containing peptides were prepared by standard peptide-coupling methods, and the 1,4,2-dioxazol-5-ones **2** were derived from carboxylic acids and prepared according to previously described methods (see Supplemental Experimental Procedures). Other chemicals were obtained from commercial sources and were used without further purification.

Data and Code Availability

All of the data supporting the findings of this study are presented within the article and Supplemental Information. All other data are available from the Lead Contact upon reasonable request.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.chempr.2020.10.026.

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AUTHOR CONTRIBUTIONS

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The authors declare no competing interests.

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