CHEMISTRY

Late-stage stitching enabled by manganese-catalyzed C—H activation: Peptide ligation and access to cyclopeptides

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Bioorthogonal late-stage diversification of structurally complex peptides bears enormous potential for drug discovery and molecular imaging. Despite major accomplishments, these strategies heavily rely on noble-metal catalysis. Herein, we report on a manganese(I)-catalyzed peptide C—H hydroarylation that enabled the stitching of peptidic and sugar fragments, under exceedingly mild and racemization-free conditions. This convergent approach represents an atom-economical alternative to traditional iterative peptide synthesis. The robustness of the manganese(I) catalysis regime is reflected by the full tolerance of a plethora of sensitive functional groups. Our strategy enabled an expedient access to challenging cyclic peptides by a modular late-stage macrocyclization of structurally complex peptides.

INTRODUCTION

The late-stage diversification of biomolecules is of prime importance in biomolecular chemistry with immediate impact on academia and pharmaceutical industries (*1*). In this regard, nonnatural amino acids bear substantial potential, as they can modify the conformation of peptides, improve bioactivities, and diminish the hindrance from proteolytic degradation of native peptides (*2*). This holds particularly true for cyclic peptides, as they generally lack free N- and C-terminal residues that are essential for recognition by most proteolytic enzymes (*3*, *4*). Significant recent momentum was gained through transformative palladium-catalyzed cross-coupling of peptides (*5*–*7*). Despite major advances, this approach requires two pre-functionalized substrates, leading to multistep syntheses. As a consequence, late-stage diversification of amino acids and peptides (*8*, *9*) via noble metal-catalyzed C—H activation was developed by Lavilla/Albericio (*10*, *11*), Chen (*12*–*14*), Ackermann (*15*–*18*), and Yu (*19*–*22*), among others (*23*–*27*). While this regime enabled the assembly of challenging cyclic peptides, toxic and costly palladium catalysts were required, which, among others, prove detrimental because of their costs and trace metal impurities (*28*–*32*). In contrast, 3d transition metal-catalyzed C—H activation has gained considerable recent attention, with major advances by Earth-abundant, nontoxic manganese (*33*, *34*). Thus, manganese-catalyzed C—H activation has proved instrumental for efficient and selective C—H functionalization (*35*, *36*). In sharp contrast, base metal catalysis for peptide diversification and macrocyclization continues to be scarce (*37*–*40*). As part of our program on sustainable C—H activation (*41*, *42*), we now report on the first manganese(I)-catalyzed C—H activation for hydroarylations of structurally complex peptides with easily accessible propiolates. Notable features of our findings include (i) racemization-free hydroarylation of peptides by user-friendly manganese catalysis, (ii) facile synthesis of ligated and hybrid peptides, and (iii) an unprecedented strategy for the assembly of cyclic peptides embedded with an electrophilic α,β-unsaturated moiety (Fig. 1).

RESULTS

We commenced our studies by probing various reaction conditions for the envisioned hydroarylation of propiolate **2a** with tryptophan derivative **1a** (Table 1). Among a representative set of solvents, DME (1,2-dimethoxyethane) and 1,4-dioxane were identified as being optimal (entries 3 and 4). Gratifyingly, lowering the reaction temperature did not diminish the catalyst's efficacy (entries 7 and 8). Control experiments clearly demonstrated the importance of NaOAc, likely enabling carboxylate-assisted C—H cleavage (*42*–*44*), and the essential nature of the $MnBr(CO)_5$ catalyst and the 2-pyridyl directing group (entries 10 to 12). Notably, the peptide backbone was not essential for the C—H activation, because 1-(pyridin-2-yl)-1*H*-indole was efficiently converted under otherwise identical reaction conditions (entry 13). Changing the propiolate **2a** to the more complex serine-derived propiolate **2b** called for an elevated reaction temperature to ensure full conversion (entry 16). Notably, the reaction proceeded without erosion of the enantiointegrity of the tryptophan scaffold.

With the optimal reaction conditions in hand, we envisioned a straightforward approach for the synthesis of peptides via manganese(I)-catalyzed C—H activation for the stitching of peptidic fragments. This approach would offer a convergent ligation toward large peptides in sharp contrast to tedious traditional iterative peptide syntheses. Thus, a plethora of di- and tripeptides were efficiently synthesized in an atom-economical manner under exceedingly mild reaction conditions that did not jeopardize the tolerance of sensitive functional groups on the side chain of amino acids (Fig. 2). In addition, under the optimized reaction conditions, internal alkynes **2e** and **2f** proved viable substrates leading to tryptophan derivatives bearing trisubstituted olefins **3e** and **3f**. Furthermore, nitrogen-containing amino acids, such as tryptophan **3d** and protected arginine **3n**, were also well tolerated, albeit in the latter case with diminished *E*/*Z* ratio. In addition, aliphatic alcohols and phenols, found in serine, threonine,

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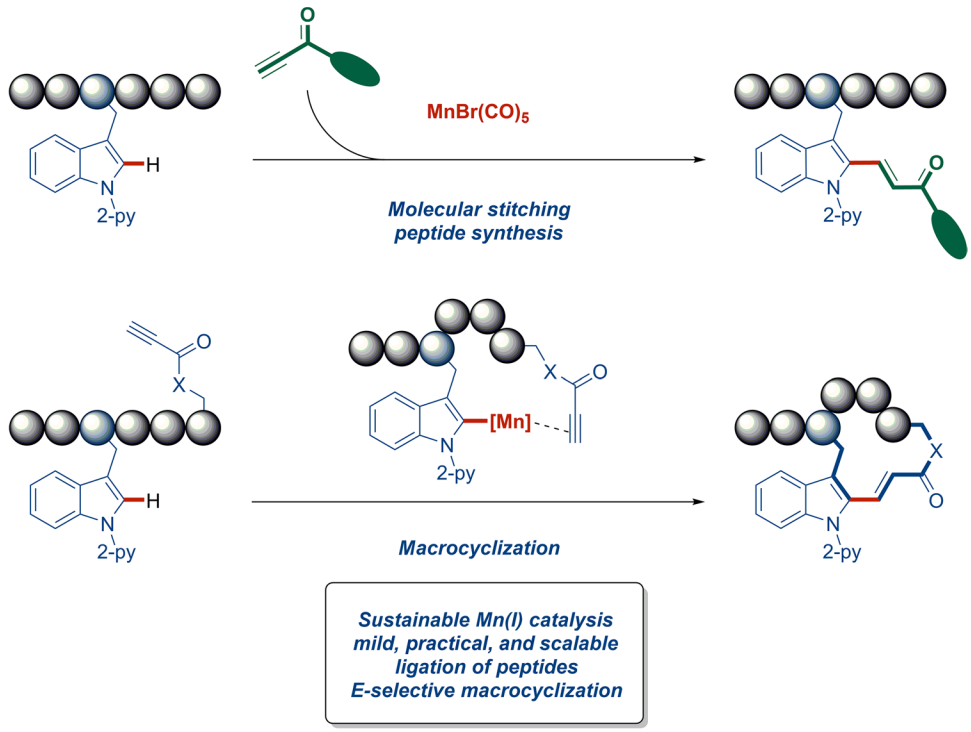
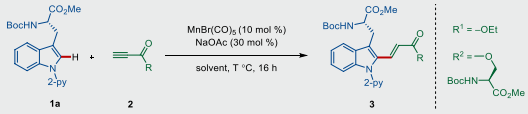


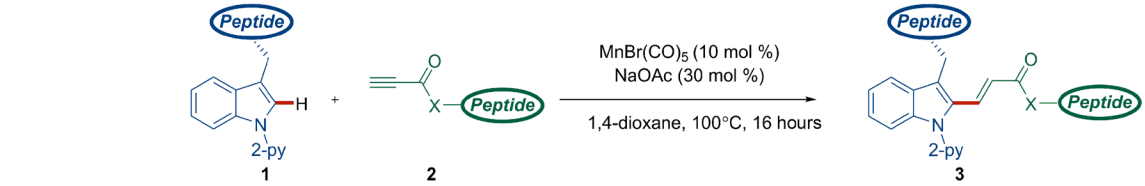
Fig. 1. Late-stage stitching and macrocyclization toward molecular complexity. A versatile approach toward site-selective peptide modifications via a ligation and macrocyclization of complex precursors, catalyzed by Earth-abundant manganese.

Table 1. Optimization studies for the hydroarylation with tryptophan 1a. Exploration of solvent, additive, and temperature effects on the hydroarylation of alkynes with tryptophan derivative **1a**.

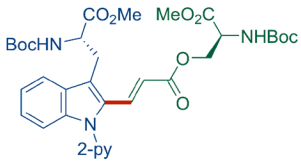


Entry	R	Solvent	Temperature (°C)	Yield (%)
1	R^1	toluene	100	37
2	R^1	DCE	100	83
3	R^1	DME	100	97
4	R^1	1,4-dioxane	100	98
5	R^1	DMF	100	–
6	R^1	H_2O	100	43
7	R^1	1,4-dioxane	80	97
8	**R^1**	**1,4-dioxane**	**60**	**97**
9	R^1	1,4-dioxane	40	58
10	R^1	1,4-dioxane	60	85*
11	R^1	1,4-dioxane	60	–†
12	R^1	1,4-dioxane	60	–‡
13	R^1	1,4-dioxane	60	97§
14	R^2	1,4-dioxane	60	86
15	R^2	1,4-dioxane	80	90
16	**R^2**	**1,4-dioxane**	**100**	**95**

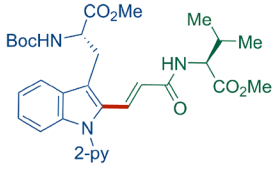
Reaction conditions: **1a** (0.15 mmol), **2** (0.23 mmol), $MnBr(CO)_5$ (10 mol %), NaOAc (30 mol %), solvent (0.6 ml), 16 hours. Yields of isolated product.
*Without NaOAc. †Without $MnBr(CO)_5$. ‡Boc-Trp-OMe (0.15 mmol) in lieu of **1a**. §1-(pyridin-2-yl)-1*H*-indole (0.15 mmol) in lieu of **1a.**



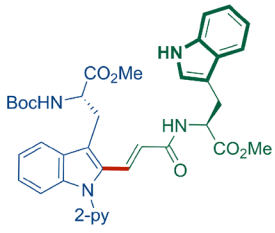
A *Assembly of dipeptides*



3b: 95%

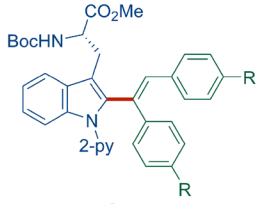


3c: 66%



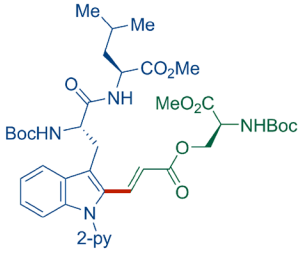
3d: 84%

B *Tryptophan bearing trisubstituted olefins*

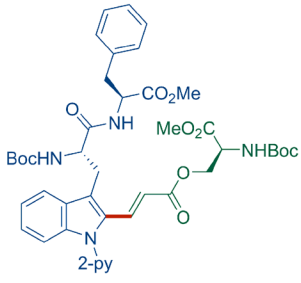


R = H (**3e**): 92%
R = Me (**3f**): 85%

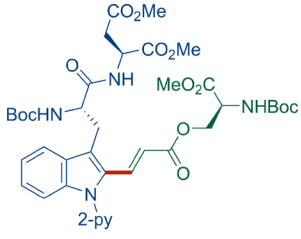
C *Assembly of tripeptides*



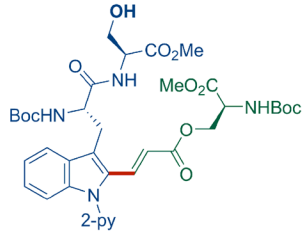
3g: 92%



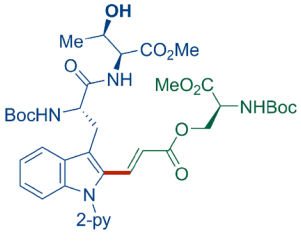
3h: 87%



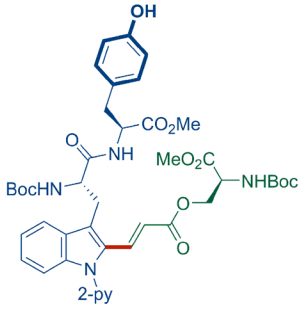
3i 84%



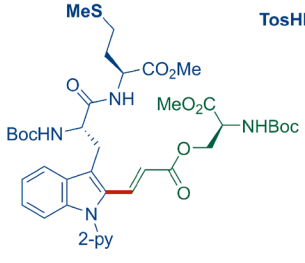
3j: 69%



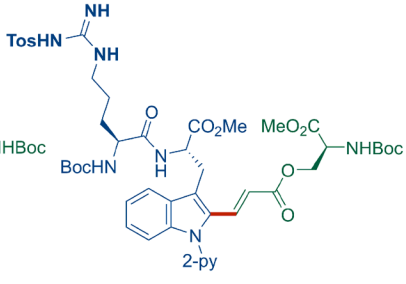
3k: 72%



3l: 67%

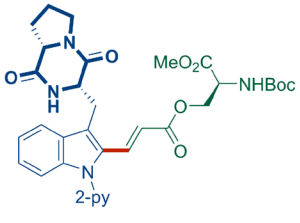


3m: 88%

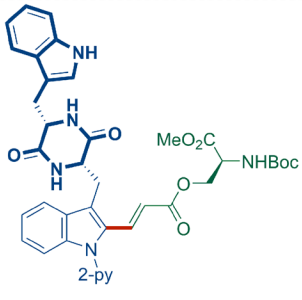


3n: 85% (*E*/*Z* = 85:15)

D *Ligation of diketopiperazines*



3o: 78%



3p: 72%

Fig. 2. Small peptide assembly by manganese catalysis. (**A**) Synthesis of dipeptides via a C—H peptidic coupling. (**B**) Disubstituted alkynes **2e** and **2f** as coupling partner. (**C**) Synthesis of tripeptides via a C—H peptidic coupling featuring various functional groups. (**D**) Ligation of brevianamide F and fellutanine A analogs.

and tyrosine, were fully tolerated, selectively furnishing the desired tripeptides **3j**, **3k**, and **3l**. Strongly coordinating and oxidation-prone thioether, present in methionine, did not affect the robustness of the manganese(I) catalysis, as tripeptide **3m** was obtained in 88% yield. Likewise, diketopiperazine derivatives, found in a plethora of natural products, **1o** and **1p** were efficiently converted to the desired tripeptides **3o** and **3p** in good yields.

Encouraged by the outstanding versatility of the manganese(I) catalysis manifold, we investigated whether more complex peptides could be viable substrates. Thus, tetra- and pentapeptides were readily accessed in excellent yields, showcasing the translational impact such method could have to biological sciences (Fig. 3). Thus, under mild and racemization-free reaction conditions, complex peptides containing tryptophan, tyrosine, protected serine, threonine, and

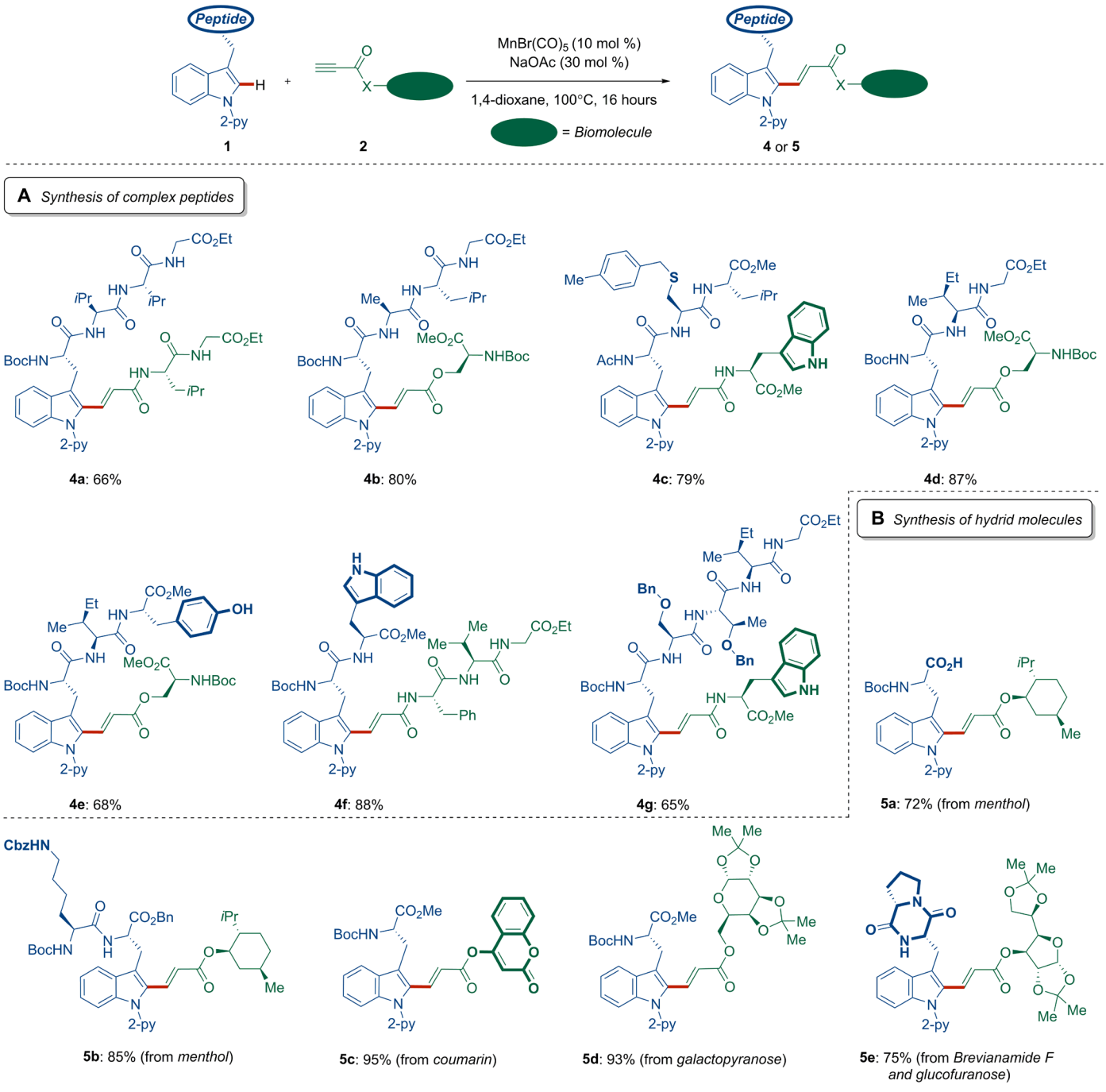


Fig. 3. Synthesis of complex hydrid molecules via C—H hydroarylation. (**A**) Synthesis of complex via a C—H coupling strategy. (**B**) C—H stitching of biomolecules toward hydrid architectures.

cysteine could be efficiently assembled. The ability to stitch fragments that bear two distinct functions is of utmost importance since new entities arise, featuring multifunctional molecules. Thus, hybrid peptides were selectively prepared bearing natural products including terpenes and sugars, as well as fluorescent tag coumarin in good to excellent yields.

The robustness and mild nature of the manganese(I) catalysis regime is clearly reflected on the gram-scale synthesis of tryptophan derivatives bearing the valuable electrophilic α,β-unsaturated ester (Fig. 4A). Thereafter, we envisioned a strategy for accessing *SH*-free cysteine-containing peptides bearing the reactive olefin that could engage in complementary postsynthetic manipulation (Fig. 4B). To this end, cystine-containing peptide **6** was used in a twofold C—H hydroarylation, where the disulfide acts as atom-economical protecting group of the thiol moiety. Subsequent, disulfide reduction swiftly yielded the free cysteine-containing peptide. Furthermore, the dimeric peptide **6** is destined for sequential C—H activation process, governed by the judicious choice of the reaction conditions and stoichiometry. Thus, highly functionalized tetrapeptide **8** was obtained in 67% overall yield after a sequential C—H hydroarylation and C—H allylation.

Inspired by the remarkable versatility of our manganese(I)-catalyzed C—H activation, we probed whether the manganese(I) catalysis can be used for the synthesis of challenging cyclic peptides. To avoid detrimental di- and oligomerization, we used high dilution conditions. Gratifyingly, under these conditions, a plethora of cyclic peptides were thereby obtained with excellent chemoselectivity furnishing 15- to 22-membered macrocycles (Fig. 5A). To exploit the full potential of our manganese(I)-catalyzed macrocyclization, we performed the challenging macrocyclization using either a linker at C terminus (Trp→C terminus) or the native N terminus (Trp→N terminus) to attach the key propiolate motif. Moreover, we recognized that the native functional groups of the side chains of the amino acids can be used for the installation of the propiolate moiety. Hence, cyclic peptides **10m** and **10n** were selectively obtained in good to excellent yields (Trp→Ser). Moreover, the traceless removal of the *N*-pyridyl group was accomplished by a selective methylation/hydrogenation protocol giving rise to *NH*-free tryptophan-containing cyclic peptide **11** (Fig. 5B).

DISCUSSION

Last, the thus-obtained cyclic peptides showed considerable anticancer activities against HCT116 cells. Small changes in the peptidic backbone had profound effect on the biocativity, as showcased by the enhanced activity of **10h** over **10j**. Further demonstrating the translational nature of the late-stage C—H activation regime (Fig. 6).

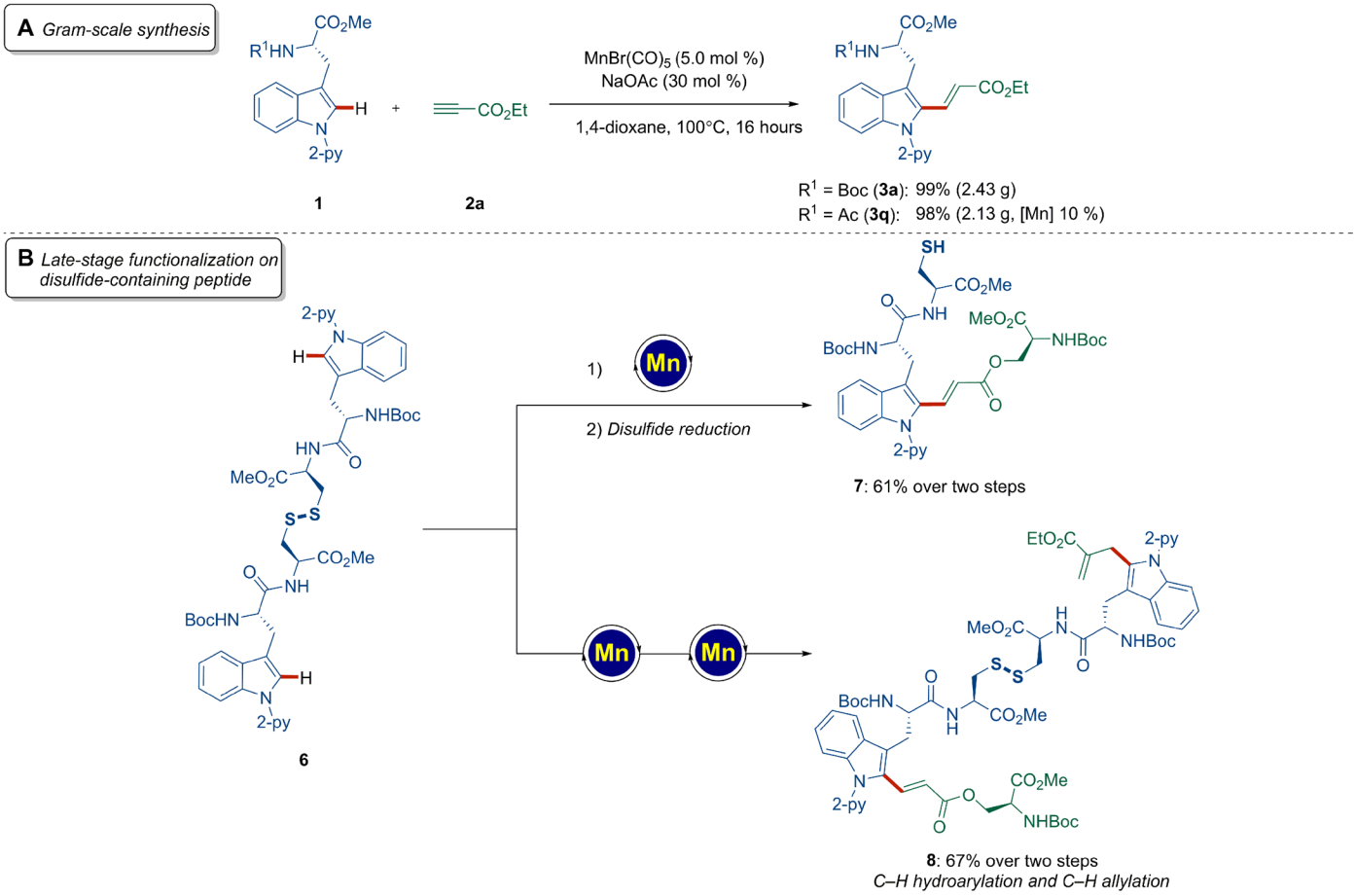


Fig. 4. Synthetic applications of the C—H hydroarylation. (**A**) Gram-scale synthesis. (**B**) Late-stage manipulation on the hydroarylated peptides.

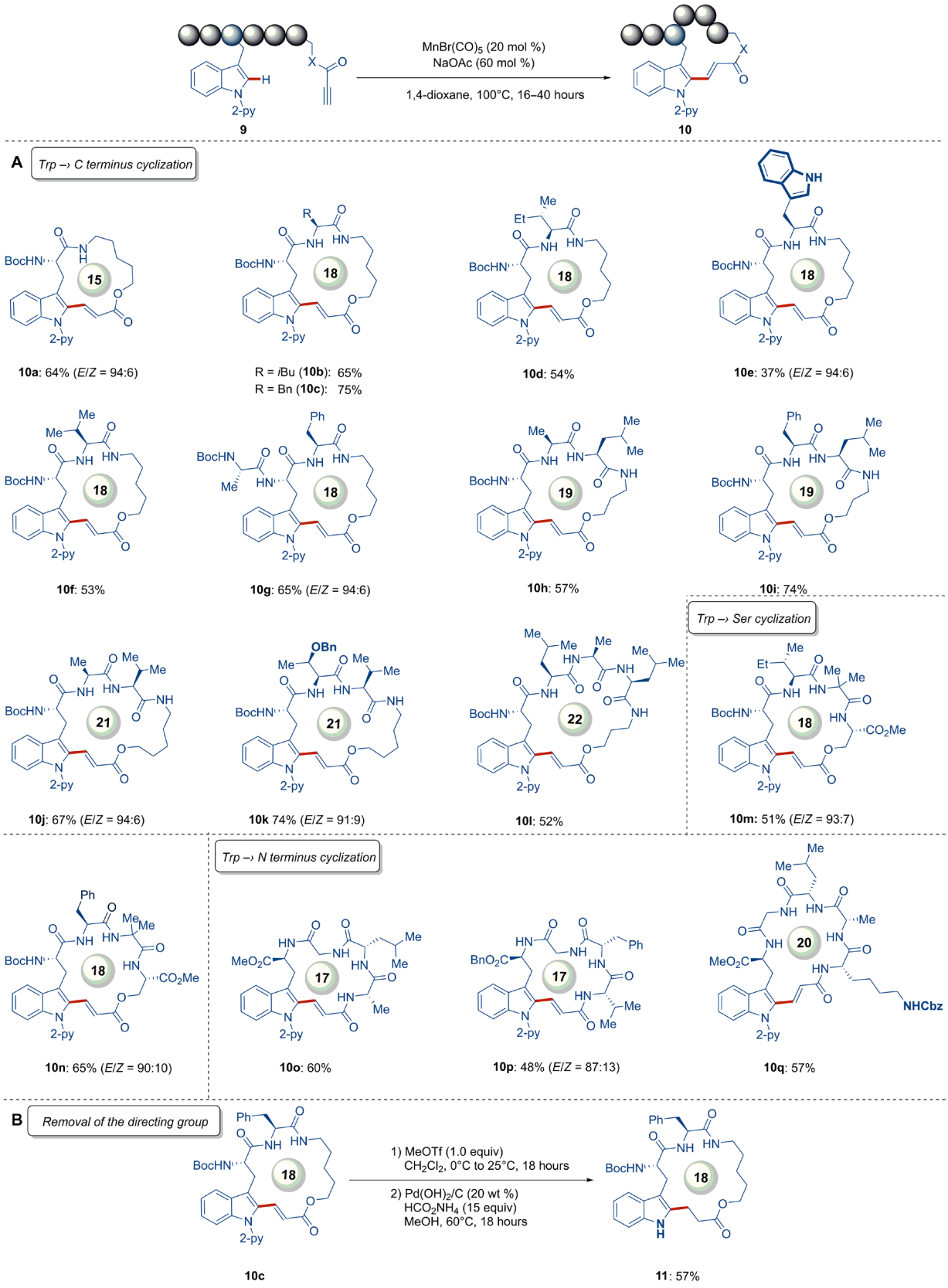


Fig. 5. Manganese-catalyzed macrocyclization and removal of the directing group. (**A**) Access to cyclic peptides of various ring sizes using the native C terminus, N terminus, and the serine side chain. (**B**) Selective methylation/hydrogenation protocol for the traceless removal of the directing group. wt %, weight %.

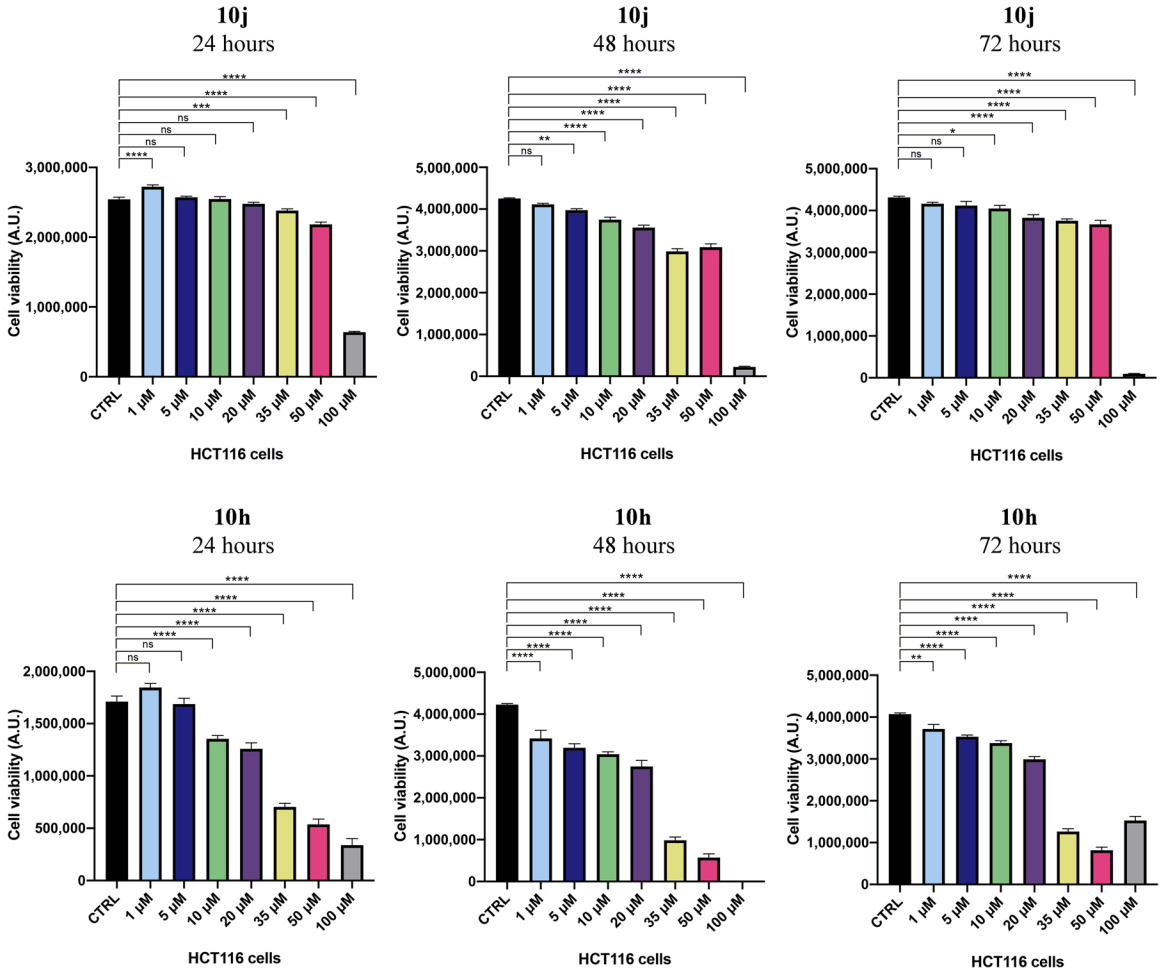


Fig. 6. Anticancer activity of cyclic peptides 10j and 10h against HCT116 cells. $^{**}P < 0.01$ and $^{***}P < 0.001$. ns, not significant. A.U., arbitrary units.

In summary, we have developed an unprecedented manganese(I)-catalyzed C—H hydroarylation with structurally complex peptides. The chemo- and position-selective peptide diversification was characterized by an excellent functional group tolerance with a plethora of sensitive groups under mild and epimerization-free reaction conditions. We also assembled hydrid multifunctional molecules by stitching peptides with natural products and sugars. This robust method paved the way for a highly efficient access to cyclic peptides via a C—H macrocyclization regime.

MATERIALS AND METHODS

General procedure A: Late-stage C—H hydroarylation on peptides

A suspension of peptide **1** or **6** (0.15 mmol, 1.0 equiv), propiolate **2** (0.23 mmol, 1.5 equiv), $MnBr(CO)_5$ [10 mole percent (mol %)], and NaOAc (30 mol %) in 1,4-dioxane (0.25 M) was stirred at 100°C for 16 hours under N_2. After cooling to ambient temperature, CH_2Cl_2 (10 ml) was added, and the mixture was concentrated in vacuo. Purification by column chromatography on silica gel afforded the desired products **3** to **8**.

General procedure B: Late-stage C—H macrocyclization of peptides

A suspension of peptide **9** (0.15 mmol, 1.0 equiv), $MnBr(CO)_5$ (20 mol %), and NaOAc (60 mol %) in 1,4-dioxane (30 ml) was stirred at 100°C for 16 hours under N_2. After cooling to ambient temperature, CH_2Cl_2 (10 ml) was added, and the mixture was concentrated in vacuo. Purification by column chromatography on silica gel afforded the desired product **10**.

CellTiter Blue assay (viability assay)

The assay was performed according to the manufacturer's protocol (Promega, Madison, USA). Briefly, 1×10^4 HCT-116 cells were seeded into 96-well, black-walled tissue culture plates (Corning, NY, USA). After 24-hour incubation at 37°C, 5% CO_2, the medium was replaced by 100 μl of medium containing the experimental compounds **10h** and **10j**. One hour before the selected time points (24 hours/48 hours/72 hours), 10 μl of resazurin was added to each well. Reduced resazurin (resorufin) was measured using the Victor Multilabel Plate Reader (PerkinElmer Inc., Waltham, USA) at 560-nm excitation/590-nm emission wavelength. Every condition was tested in triplicates, and the assay was performed three times.

The combined data of three independent experiments are shown as ±SEM.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/7/9/eabe6202/DC1

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