Seven Post-synthetic Covalent Reactions in Tandem Leading to Enzyme-like Complexity within Metal–Organic Framework Crystals

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ABSTRACT: The design of enzyme-like complexity within metal–organic frameworks (MOFs) requires multiple reactions to be performed on a MOF crystal without losing access to its interior. Here, we show that seven post-synthetic reactions can be successfully achieved within the pores of a multivariate MOF, MTV-IRMOF-74-III, to covalently incorporate tripeptides that resemble the active sites of enzymes in their spatial arrangement and compositional heterogeneity. These reactions build up H₂N-Pro-Gly-Ala-CONHL and H₂N-Cys-His-Asp-CONHL (where L = organic struts) amino acid sequences by covalently attaching them to the organic struts in the MOFs, without losing porosity or crystallinity. An enabling feature of this chemistry is that the primary amine functionality (−CH₂NH₂) of the original MOF is more reactive than the commonly examined aromatic amines (−NH₂), and this allowed for the multi-step reactions to be carried out in tandem within the MOF. Preliminary findings indicate that the complexity thus achieved can affect reactions that were previously accomplished only in the presence of enzymes.

Specifically, we chose a functionalized IRMOF-74-III (Scheme 1), constructed from magnesium oxide rods joined by terphenylene organic struts [Mg₂(L), where H₄L = 3,3′-dihydroxy-(1,1′:4′,1″-terphenyl)-4,4″-dicarboxylic acid], to make an extended structure based on the parent structure of MOF-74, but having 25 Å one-dimensional channels. The highly porous MTV-74-III allows for facile diffusion of reactants into the pore interiors and the targeting of specific sites within the MOF.

We prepared a series of MTV-IRMOF-74-III materials using two types of organic struts, H₂L-CH₃ and H₂L-CH₂NHBoc (hereafter, MTV-(CH₃)(1-x)(CH₂NHBoc)x, x = 0.2–0.8; Boc = tert-butyloxycarbonyl), to study the optimal concentration of reactive sites for carrying out tandem post-synthetic reactions. We found that among these, MTV-(CH₃)₀.₆(CH₂NHBoc)₀.₄ led to efficient reactant diffusion into the MOF pores and, concomitantly, better overall yield. Additionally, we compared the reactivity of MTV-(CH₃)₁.₀(CH₂NHBoc)₀, functionalized with primary amines [MTV-(CH₃)₀.₆(CH₂NH₂)₀.₄], to that of the analogous material functionalized with aromatic amines [MTV-(CH₃)₀.₆(CH₂NH₂)₀.₄] toward peptide bond formation starting with alanine (Ala). We show that, under the same reaction conditions, primary amine nucleophiles lead to 97% greater reaction yield compared to their aromatic amine counterparts. We further demonstrate that MTV-IRMOF-74-III-(CH₃)₀.₆(CH₂NHBoc)₀.₄ can be used as a reactant for the sequential loading of tripeptides (H₂N-Pro-Gly-Ala-CONHL and H₂N-Cys-His-Asp-CONHL, with Gly = glycine, Pro = proline, Asp = aspartic acid, His = histidine, Cys = cysteine, and L = organic struts) through seven tandem reactions. Preliminary results showed that the MTV functionality in the pores of MTV-IRMOF-74-III-tripeptides can catalyze stereoselective chlorinations (H₂N-Pro-Gly-Ala-CONHL), and even sequence-specific peptide bond cleavage (H₂N-Cys-His-Asp-CONHL), as the enzyme tobacco etch virus (TEV) endopeptidase.

For clarity, the post-synthetic strategy and its implementation, results, and catalytic activity are covered in several sections below to show that (i) a linear relationship exists between input.
Unlike MTV-MOF-5 and MTV-MOF-177,6,7 the output ratio present in the resulting structure (output ratio) was estimated. In order to overcome this challenge, we utilized the MTV approach, whereby the unfunctionalized strut is used to dilute the struts having the reactive groups in the MOF, and investigate its effect on the reaction yield. Hence, we prepared a series of MTV-(CH3)x(CH2NH2)y as starting material to carry out consecutive post-synthetic reactions, in order to mitigate potential guest fusion problems and steric repulsion between functionalities in the pores.

Synthesis of MTV-IRMOF-74-III Series and Control of Functionality Ratio. The organic struts of MTV-IRMOF-74-III were functionalized with Boc-protected primary amines and methyl groups, according to reported methodology.1 It is known that reactions performed in fully functionalized MOFs lead to drastic loss of their accessible pore volume, making it impossible to perform further tandem transformations.3 In order to overcome this challenge, we utilized the MTV approach, whereby the unfunctionalized strut is used to dilute the struts having the reactive groups in the MOF, and investigate its effect on the reaction yield. Hence, we prepared a series of MTV-(CH3)x(CH2NH2)y as starting material to carry out consecutive post-synthetic reactions, in order to mitigate potential guest fusion problems and steric repulsion between functionalities in the pores.

Aromatic amines are by far the most studied nucleophiles in post-synthetic functionalization of MOFs.8 Therefore, the reactivity of aromatic amines was compared with that of the primary amines. We prepared the analogous compound MTV-(CH3)x(CH2NH2)y and performed the same post-synthetic peptide bond formation reaction with Boc-protected Ala.1H NMR analysis after the reaction indicates no Ala loading, as evidenced by the absence of the signal corresponding to the singlet of the Boc group (δ = 1.35, Figure S32, SI). This observation highlights the importance of having highly reactive functionalities (i.e., primary amines) in the pores of MOFs to perform efficient post-synthetic reactions.

Post-synthetic Reactions Steps (3)—(7). Quantitative loading of Ala in MTV-(CH3)x(CH2NH2)y prompted us to proceed to the third reaction [(3), Scheme 1] by cleaving the Boc-protecting groups at the N-terminal of Ala by microwave heating. From 1H NMR analysis of the microwave-treated material, we confirmed a 76% yield in the deprotection reaction, as indicated by the downshift and integral of the δ = 1.31 singlet of the Boc group (Figure S5, SI). Deprotected MTV-(CH3)x(CH2NH2)y was then used to covalently bind Ala [step (2), Scheme 1; Section S2.9, SI]. The obtained crystalline powder sample was analyzed by powder X-ray diffraction (PXRD), 1H NMR spectroscopy, FT-IR, and HPLC (Sections S3, S4, S5, and S8, respectively, SI). We found that the yield of peptide bond formation with Ala was nearly quantitative (97%) when the ratio of CH2NH2Boc was below 0.6 (Figure S2, SI). It is presumed that higher concentration of primary amines in IRMOF-74-III might hamper the fast diffusion of reagents to the pore interiors. Thus, we chose MTV-(CH3)x(CH2NH2)y as starting material to carry out consecutive post-synthetic reactions, in order to mitigate potential guest diffusion problems and steric repulsion between functionalities in the pores.

Reactivity and Control of Specific Functionality in MTV-IRMOF-74-III. The MTV-(CH3)x(CH2NH2)y series was employed toward post-synthetic peptide bond formation using protected Ala (Scheme 1).1 In a typical example, microwave-treated MTV-(CH3)x(CH2NH2)y sample showed quantitative cleavage of the Boc group, which was confirmed by the disappearance of its representative singlet (δ = 1.31 for CH3 group) in the 1H NMR spectrum of the digested sample (Section S4, SI). Deprotected MTV-(CH3)x(CH2NH2)y was then used to covalently bind Ala [step (2), Scheme 1; Section S2.9, SI]. The obtained crystalline powder sample was analyzed by powder X-ray diffraction (PXRD), 1H NMR spectroscopy, FT-IR, and HPLC (Sections S3, S4, S5, and S8, respectively, SI). We found that the yield of peptide bond formation with Ala was nearly quantitative (97%) when the ratio of CH2NH2Boc was below 0.6 (Figure S2, SI). It is presumed that higher concentration of primary amines in IRMOF-74-III might hamper the fast diffusion of reagents to the pore interiors. Thus, we chose MTV-(CH3)x(CH2NH2)y as starting material to carry out consecutive post-synthetic reactions, in order to mitigate potential guest diffusion problems and steric repulsion between functionalities in the pores.

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Ala methyl group doublet (from $\delta = 1.19$ to $1.33$; Figure S34, SI). Similar to the reactions shown in steps (1) and (2), Boc-deprotections [(3), (5), and (7)] and amino acid loadings [(4) and (6)] were performed to incorporate $\text{H}_2\text{N-Pro-Gly-Ala-CONHL}$ peptide into the pores of MTV-IRMOF-74-III (Scheme 1). It is worth noting that the maximum number of post-synthetic modifications in tandem previously reported in MOFs is four.2

PXRD analyses confirmed that the crystallinity and structure remain intact throughout the seven reactions by the coincidence of their profile with the pattern calculated for unfunctionalized IRMOF-74-III (Figure 1a). The ratio of integrals for the methyl group in L-CH$_3$ and for each of the loaded amino acids in the acid-digested $^1$H NMR spectra, was used to quantify the yield of each performed reaction. The tripeptide $\text{H}_2\text{N-Pro-Gly-Ala-CONHL}$ was covalently bound to the MOF with an overall yield of 57% (average of 93% yield per step, Section 2, SI).

After the removal of guest molecules from the pores (Section S1,4, SI), the porosity of the product of the seven post-synthetic reactions was evaluated by measurement of the $N_2$ adsorption isotherm at 77 K. A steep $N_2$ uptake was observed below $P/P_0 = 0.1$, which is indicative of the presence of permanent porosity even after the seven reactions (Figure 1b). The observed Brunauer–Emmett–Teller (BET) surface area of the tripeptide-loaded sample (1760 m$^2$ g$^{-1}$) is lower than that of the starting material MTV-(CH$_3$)$_{0.6}$-(CH$_2$NH-Boc)$_{0.4}$ (2330 m$^2$ g$^{-1}$). This is because of the covalently attached tripeptide, which occupies a significant amount of the pore space.

Building Complexity in the Pores. Encouraged by the successful sequential loading of tripeptide in the MOF pores, we proceeded to covalently bind another peptide sequence of the starting material MTV-(CH$_3$)$_{0.6}$-(CH$_2$NHBoc)$_{0.4}$ (blue), and product after seven post-synthetic reactions, [MTV-(CH$_3$)$_{0.6}$-(CH$_2$NH-Ala-Gly-Pro-NH$_2$)$_{0.2}$] (red). (a) $N_2$ isotherms at 77 K for MTV-(CH$_3$)$_{0.6}$-(CH$_2$NH-Asp-His-Cys-NH$_2$)$_{0.1}$ was studied as a synthetically modifiable IRMOF-74-III (Figure 1a). The ratio of integrals for the methyl group in L-CH$_3$ and for each of the loaded amino acids in the acid-digested $^1$H NMR spectra, was used to quantify the yield of each performed reaction. The tripeptide $\text{H}_2\text{N-Pro-Gly-Ala-CONHL}$ was covalently bound to the MOF with an overall yield of 57% (average of 93% yield per step, Section 2, SI).

Figure 1. (a) PXRD patterns for simulated IRMOF-74-III (black), starting material MTV-(CH$_3$)$_{0.6}$-(CH$_2$NHBoc)$_{0.4}$ (blue), and product after seven post-synthetic reactions, [MTV-(CH$_3$)$_{0.6}$-(CH$_2$NH-Ala-Gly-Pro-NH$_2$)$_{0.2}$] (red). (b) $N_2$ isotherms at 77 K for MTV-(CH$_3$)$_{0.6}$-(CH$_2$NHBoc)$_{0.4}$ (blue) and MTV-(CH$_3$)$_{0.6}$-(CH$_2$NH-Ala-Gly-Pro-NH$_2$)$_{0.2}$ (red).

Selective Catalytic Activity of the MTV-MOF. Amino acids and short peptides are known to be active organocatalysts.11 We chose to evaluate our MTV-IRMOF-III tripeptides in two different catalytic reactions that strongly depend on the complexity of the pores.

The presence of H$_2$N-Cys-His-Asp-CONHL tripeptide in the pores of IRMOF-74-III was confirmed by the mass of the parent fragment found by ESI-MS analysis of the digested sample (calcd m/z 734.2 Da, found 734.4 Da). PXRD analysis and $N_2$ isotherms at 77 K showed that MTV-(CH$_3$)$_{0.6}$-(CH$_2$NH-Asp-His-Cys-NH$_2$)$_{0.1}$ retained crystallinity and porosity (BET = 1920 m$^2$ g$^{-1}$) after seven post-synthetic covalent reactions (Figures S17 and S41, SI).

Under the same conditions, Cat. A was found to provide 69% more of the desired product compared to Cat. B (94% vs 25%, Figure S48, SI). This is expected since there is less available pore volume in Cat. B.

Notably, a significant increase in the ee of this $\alpha$-chlorination reaction was observed when functionalized MOFs were used as catalysts compared to the reported homogeneous-phase proline catalysis (20% ee vs 2% ee). This increased asymmetric induction, which is likely due to the increased stereochemical constraints within the congested MOF framework,13 is especially striking, given that the catalytic sites are not entirely uniform (Scheme 1). Ultimately, these results demonstrate the exciting potential of imparting control of catalyst reactivity and selectivity by the molecular tuning of the MOF architecture and the active site.

Next, we turned our attention to recreating the high selectivity exhibited by the enzyme TEV endopeptidase within a synthetically modified MOF framework. Specifically, MTV-(CH$_3$)$_{0.6}$-(CH$_2$NH-Asp-His-Cys-NH$_2$)$_{0.1}$ was studied as a heterogeneous catalyst in sequence-specific peptide cleavage due to its TEV-like peptide sequence (Figure 2a).

TEV protease is known to recognize selectively the amino acid serine (Ser) in a peptide substrate and cleave the amide bond in which Ser is involved. In order to achieve such precision, three amino acids in its active site, Asp, His, and Cys/Ser, have been proposed to participate cooperatively (Figure 2b). It is believed that the acid polarizes His, which simultaneously activates the nucleophile, Cys, for attack on

were used to protect the amino acid side chains (in Asp, His, and Cys, respectively). After the Asp and His loadings [steps (2) and (4), Scheme S2, SI], the MOFs were washed with DMF, and the Fmoc groups were removed under basic conditions (Section S2, SI). MTV-(CH$_3$)$_{0.6}$-(CH$_2$NH-Asp-His-Cys-NH$_2$)$_{0.1}$ was obtained with an overall yield of 20% (Section S2, SI). $^1$H NMR and HPLC of the digested samples were used to follow the reactions (Figures S35 and S44, SI).
Ala-Ser-Ala-CONH₂ (Cys-NH₂)₀.₁ (Cat. C) was investigated for selective cleavage of preservation of MTV-(CH₃)₀.₆(CH₂NH-Ala-Gly-Pro-NH₂)₀.₂ catalytic reactions (chlorinations or peptide cleavage) was observed that the PXRD pattern of the sample after these complex, spatially induced catalytic transformations. We further in the MTV functionalized MOF allows for the recreation of is ongoing to improve conversion; however, this preliminary ized MOF showed the formation of the cleavage product. Work

***Figure 2. (a) Catalytic cleavage of pentapeptide 1 by Cat. C [MTV-IRMOF-74-III-(CH₃)₀.₆(CH₂NH-Asp-His-Cys-NH₂)₀.₁] in the specific sequence containing serine. (b) Cartoon representation of the enzyme TEV endoprotease, highlighting the three amino acids that participate the peptide bond of serine in the pentapeptide H₂N-Ala-Tyr-Ala-CO₂H(323.2 Da, found 323.9 Da) by ESI-MS analysis of the reaction supernatant. The conversion was found to be approximately 5% by HPLC analysis (Figures S47 and S51, SI). Under the same conditions, neither of the performed control reactions using the molecular analogue of the tripeptide, H₂N-Cys-His-Asp-CONH₂, nor the unfunctionalized MOF showed the formation of the cleavage product. Work is ongoing to improve conversion; however, this preliminary result shows that molecular control of the sequence of peptides in the MTV functionalized MOF allows for the recreation of complex, spatially induced catalytic transformations. We further observed that the PXRD pattern of the sample after these catalytic reactions (chlorinations or peptide cleavage) was identical to that of the activated sample, thus indicating full preservation of MTV-(CH₃)₀.₆(CH₂NH-Ala-Gly-Pro-NH₂)₀.₂ and MTV-(CH₃)₀.₆(CH₂NH-Asp-His-Cys-NH₂)₀.₁ structures (Figures S18 and S19, SI).

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**Notes**

The authors declare no competing financial interest.

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