Multicopper Clusters Catalyze the Oxidative Phenol Macrocyclization (OxPM) of Linear Peptides

Authors:

Anna Libman,[1] Mor Ben-Lulu,[1] Eden Gaster,[1] Ratnadeep Bera,[1] Alexander I. Shames,[2] Omer Shaashua,[1] Vlada Vershinin,[1] Yury Torubaev,[1] and Doron Pappo[1,*]

Affiliations:

[1]Department of Chemistry, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel

[2]Department of Physics, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel

Abstract:

The biosynthesis of glycopeptide antibiotics such as vancomycin and other biologically active biaryl-bridged and diaryl ether-linked macrocyclic peptides includes key enzymatic oxidative phenol macrocyclization(s) of linear precursors. However, a simple and step-economical biomimetic version of this transformation remains underdeveloped. Here, we report highly efficient conditions for preparing biaryl-bridged and diaryl ether-linked macrocyclic peptides based on multicopper(II) catalysts. The selective syntheses of ring models of vancomycin and the arylomycin cyclic core illustrate the potential of this technology to facilitate the assembly of complex antibiotic macrocyclic peptides whose syntheses are considered highly challenging. The unprecedented ability of multicopper clusters to chelate tethered diphenols and promote intramolecular over intermolecular coupling reactions demonstrates that copper clusters can catalyze redox transformations that are not accessible by smaller metal catalysts.

Introduction:

In the past two decades, a growing number of bioactive macrocyclic peptides with appropriate physicochemical and pharmacokinetic properties have been approved as drugs by the FDA.[1] The potent glycopeptide antibiotics (GPAs) vancomycin (Figure 1A) and teicoplanin and other semisynthetic analogs (such as telavancin), which consist of biaryl-bridged and diaryl ether cross-linked multicyclic heptapeptides, are essential drugs for treating life-threatening infections of resistant Gram-positive bacteria.[2] Nevertheless, a consistent rise in mortality from resistant infections remains a major threat to global health, and new antibacterial agents are urgently needed.[3] In an effort to launch the next generation of antibiotics, Genentech introduced the biaryl-bridged macrocyclic peptides G0775 and GDC-5338 (Figure 1C), two synthetic analogs of arylomycin natural products that display unique and promising activity against contemporary multidrug-resistant Gram-negative bacteria.[4] Despite the proven therapeutic potential of biaryl-bridged and diaryl ether-linked macrocyclic peptides, their structural complexity makes their preparation challenging. As a result, these cyclopeptides have been under-explored as targets for drug discovery.[5]

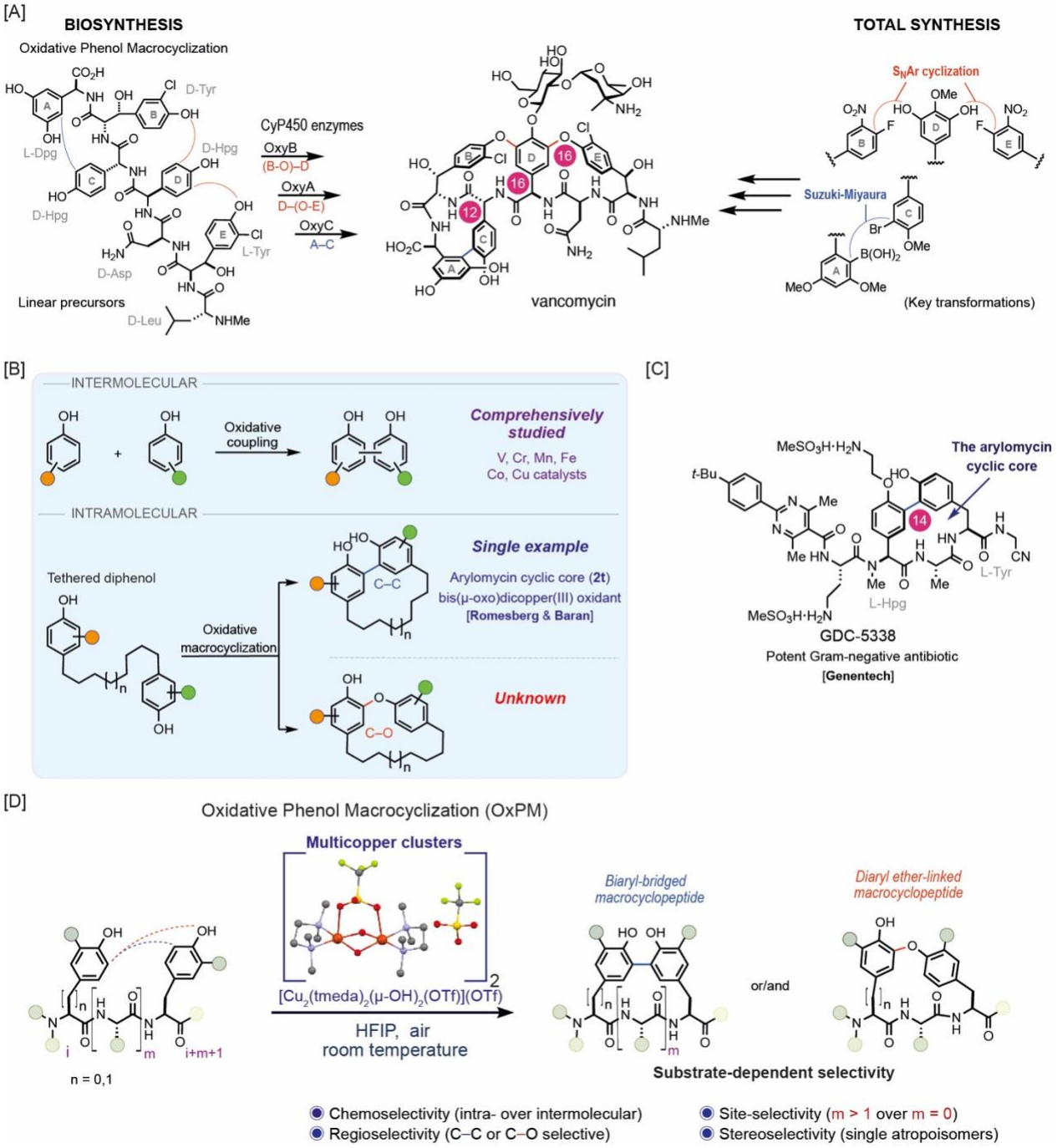


Figure 1. Approach overview. [A] Biosynthesis of vancomycin through consecutive oxidative phenol macrocyclization (OxPM) steps catalyzed by CyP450 enzymes (OxyA, OxyC, and OxyB) and the key biaryl- and diaryl ether-forming steps in Boger's total synthesis of vancomycin;[6] [B] Comparison between the intermolecular oxidative coupling of phenols and the intramolecular oxidative macrocyclization of tethered diphenols; [C] The potent Gram-negative antibiotic GDC-5338[7] that conserved the macrocyclic core of the arylomycin natural products; [D] This study: General OxPM technology for preparing biaryl-bridged and diaryl ether-linked macrocyclopeptides based on multicopper clusters.

The biosynthesis of these macrocyclic peptides involves selective metalloenzymatic oxidative phenol macrocyclization (OxPM) step(s) of linear precursors (Figure 1A).[2,8] Native and engineered cytochrome P450 enzymes have been successfully applied to the synthesis of vancomycin fragments and the arylomycin

cyclic core from their parent linear peptides.[7a,9] However, the substrate specificity of biocatalysts limits their utility for preparing the structurally diverse macrocyclopeptides that are needed in biomedical research.[9b] Instead, the synthesis of bioactive biaryl-bridged and diaryl ether-linked macrocyclic peptides starts from unnatural amino acid building blocks and still relies on reactions that are not fully customized to peptide chemistry, such as palladium-catalyzed Suzuki-Miyaura reaction and S_NAr reaction for preparing the biaryl and diaryl ether bonds, respectively (Figure 1A).[2a,6,7b,10] As a result, the total syntheses of these macrocyclic peptides require extensive synthetic manipulation before and after the key coupling step(s), thereby limiting the synthetic freedom for derivatization. Thus, metal-catalyzed OxPM of linear peptides stands as the most promising technique for addressing the synthetic challenges of preparing GPAs and related cyclopeptides in terms of simplicity and step economy. Nonetheless, while intermolecular metal-catalyzed oxidative coupling between two phenols is an established transformation[11] that can be applied in the preparation of biaryl-bridged macrocyclopeptides,[12] the metal-catalyzed OxPM of tethered diphenols is still underdeveloped (Figure 1B).[11a,13]

The development of a suitable metal-catalyzed OxPM methodology for the synthesis of GPA antibiotics is not straightforward, as it requires several selectivity challenges to be addressed: (a) the reaction should favor intramolecular oxidative macrocyclization of tethered diphenol peptides over intermolecular oxidative phenol coupling (Figure 1B), which produces dimers and oligomers; (b) the method should be site-selective with a preference for coupling two remote phenol-based amino acids instead of neighboring phenolic amino acids (e.g., i+2 over i+1 selectivity, where i = the position of the first phenolic-based amino acid in the formed macrocycle); (c) the reaction should support the selective formation of both biaryl-bridged (C–C) and diaryl ether-linked (C–O) macrocyclopeptides; (d) undesired over-oxidation of the products should be avoided to enable access to more complex multicyclic systems; and (e) the stereochemistry around the newly formed biaryl bond (axial chirality) or diaryl ether bond (atroposelectivity) should be controlled. In the only successful example of an OxPM reaction, Baran and Romesberg synthesized arylomycin cyclic core **2t** (Figure 3B) from its linear precursor in a moderate yield of 60% by using a bis(μ-oxo)dicopper(III) oxidant.[14] This important example demonstrated the potential of copper complexes to promote such a transformation. However, the generality of their conditions was not proven (only a single biaryl-bridged macrocycle was prepared), the mechanism remains unknown, and essential aspects regarding the coupling selectivity were overlooked.

We proposed that copper clusters may be suitable catalysts for the OxPM reaction, as they have a vacancy for chelating tethered diphenols and can accept two electrons during the coupling reaction. To this end, we describe the general and highly selective aerobic OxPM of tethered diphenols catalyzed by hydroxo tetracopper(II) clusters $[Cu^{II}_4(\mathbf{N_2})_4(\mu3\text{-}OH)_4](Y)_4$ ($\mathbf{N_2}$ is a diamine ligand, and Y is a weak counter-anion). The practical reaction can be adjusted to address most of the above selectivity requirements (a)-(e) and has been successfully applied to the preparation of structurally diverse biaryl-bridged and diaryl ether-linked mono- and bicyclopeptides that are inaccessible with current methods (Figure 1D). While multicopper

clusters have been identified in the active sites of oxidases, such as laccase,[15] their potential to act as redox catalysts in organic synthesis remains unexplored.[16] In this study, we demonstrate the application of tetracopper clusters as operative catalysts in transformations that are not accessible with smaller metal catalysts.

Results and Discussion

The study commenced with the exploration of conditions for the selective oxidative macrocyclization of tripeptides using different redox catalysts under aerobic conditions (Supplementary Materials). To distinguish between the two phenolic units of the tripeptide, we chose BocNH-(*t*-Bu)Tyr-Ala-Tyr-OMe (**1a**) with a labile *t*-Bu group at the phenolic *ortho* position of one of the tyrosine units as the substrate.[12]

In general, Cu^{II}(**N2**)X and Cu^{II}(**N2**)X_2 complexes with diamine ligands (**N2**) and strongly coordinating anions (X = Cl, Br, or I) form monomeric and dimeric complexes with structural resemblance to biological dicopper oxidases (e.g., tyrosinase)[17] and promote intermolecular oxidative phenol coupling between two [Cu^{I}(**N2**)(ArO·)Cl] intermediates [e.g., **N2** = N,N,N',N'-tetramethyl ethylenediamine (tmeda)].[18] Room temperature EPR analysis of [Cu^{II}(**N2**)(μ-OH)]$_2Cl_2$ complexes (^{N2}**Cu$_2^{Cl}$** [**N2** = tmeda, 2,2'-bipyridine (bpy) and 1,10-phenanthroline (phen)] in HFIP (10 mM) showed complete disassembly of these complexes to a detectible monomeric [Cu^{II}(**N2**)Cl]$^+$ species (Figure 2A, red X marks, and supplementary materials). These monomeric copper complexes promoted the non-selective oxidative coupling of tripeptide **1a**, resulting in the formation of a mixture of undefined oligomers. We proposed that the inability of the [Cu^{II}(**N2**)Cl]$^+$ species to simultaneously chelate both tyrosine units in linear peptide **1a** leads to intermolecular coupling, making the OxPM reaction less favorable (Figure 2D).

To solve this problem, we turned to hydroxo tetracopper clusters [Cu^{II}(N2)(μ3-OH)]$_4Y_4$ (^{N2}**Cu$_4^{Y}$**, Y = semi-coordinated and hydrogen bond-accepting anions) that form highly ordered tetracopper structures, including cubane-type crystals (Figure 2D). [15d,19] Although preparing these clusters is straightforward, their application as catalysts in organic reactions has remained scarce. Room temperature EPR measurements of ^{tmeda}**Cu$_4^{OTf}$**, ^{tmeda}**Cu$_4^{ClO_4}$**, ^{bpy}**Cu$_4^{OTf}$**, ^{bpy}**Cu$_4^{ClO_4}$**, ^{phen}**Cu$_4^{Otf}$**, and ^{phen}**Cu$_4^{ClO_4}$** clusters in HFIP (0.05 M) revealed that less than 20% of the total Cu(II) ions could be assigned to monomeric [Cu^{II}(**N2**)OR]$^+$ (R = H or $CH(CF_3)_2$) species, indicating that the majority of the copper ions establish multicopper structures with strong antiferromagnetic coupling (Figure 2B). The ability of the axial triflate and perchlorate ions to bring two copper atoms into close proximity provides the requisite structural stabilization of hydroxo multicopper(II) clusters, as previously demonstrated.[19c,20] Indeed, HRMS-ES (negative mode) analysis supports the presence of tetracopper clusters in polar solvents (Supplementary materials). Fortunately, mixing linear peptide **1a** with 0.25 equiv of all the above ^{N2}**Cu$_4^{Y}$** clusters (N2 = tmeda, bpy, or phen; Y = OTf or ClO_4) in HFIP (air atmosphere, rt, 24h) resulted in highly chemoselective OxPM reactions, affording the desired macrocyclic peptide **2a** in high HPLC yields as a single isomeric product (Figure 2A). Under these conditions, the dimeric and oligomeric peptides expected from intermolecular oxidative coupling processes were almost undetectable.

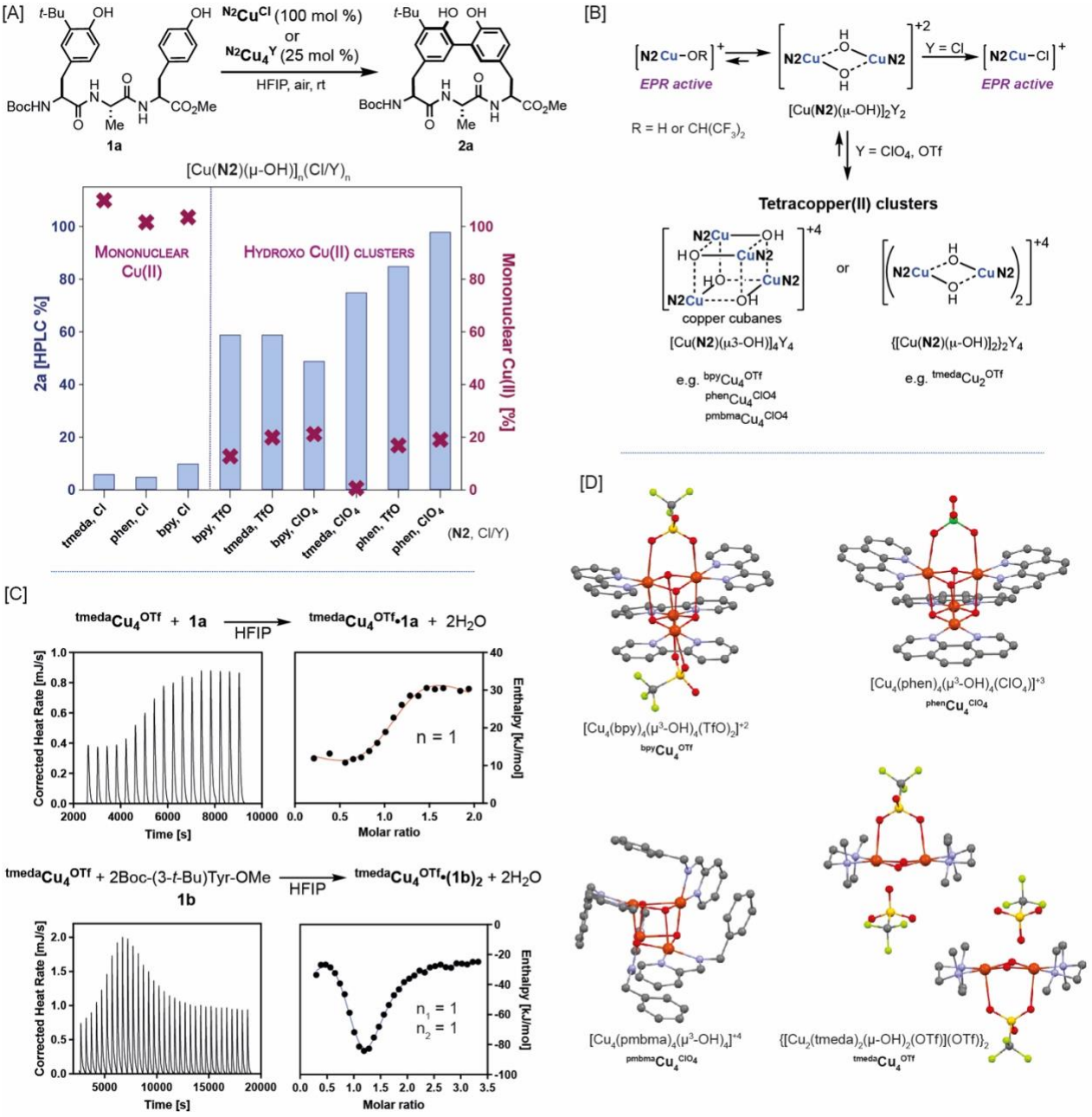
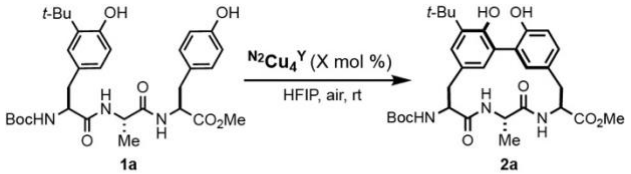


Figure 2. Involvement of copper clusters in inducing the unique oxidative macrocyclization selectivity. [A] Intramolecular oxidative macrocyclization reaction facilitated by $^{N2}\mathbf{Cu_2}^{Cl}$ and $^{N2}\mathbf{Cu_4}^{Y}$ complexes (N2 = tmeda, bpy, and phen; Y= OTf, ClO_4). In the associated plot, the red Xs mark the percentage of monomeric Cu^{II} species in solutions of $^{N2}\mathbf{Cu_2}^{Cl}$ and $^{N2}\mathbf{Cu_2}^{X}$ in HFIP (0.01 M) determined by time-dependent EPR analysis; the blue columns indicate the yield of **2a** determined by HPLC using mesitylene as an internal standard. Reaction conditions: **1a** (1 mmol), $^{N2}\mathbf{Cu_2}^{Cl}$ (50 mol %) or $^{N2}\mathbf{Cu_4}^{Y}$ (25 mol %), HFIP (1 mL), air atmosphere, room temperature, 24 h. [B] Structures of the main hydroxo copper(II) species in an HFIP solution of the $^{N2}\mathbf{Cu_4}^{Y}$ clusters; [C] Isothermal titration calorimetry (ITC) of $^{tmeda}\mathbf{Cu_4}^{OTf}$ (0.06 mM) binding. Data from automatic injections of 5 μL portions of **1a** (0.5 mM) or BocNH-(*t*-Bu)Tyr-OMe **1b** into the $^{tmeda}\mathbf{Cu_4}^{OTf}$-containing cell (*left*). The plot of the total heat released as a function of ligand concentration for the titration (circles, *right*). The red and blue lines denote the best fit for a single site model. [D] ORTEP representations of $^{bpy}\mathbf{Cu_4}^{OTf}$ (CCDC 2004836), $^{phen}\mathbf{Cu_4}^{ClO4}$ (CCDC 1426042), $^{pmbma}\mathbf{Cu_4}^{ClO4}$ (CCDC 915281), and $^{tmeda}\mathbf{Cu_4}^{OTf}$ (CCDC 1936795). Hydrogen atoms and counterions were removed for clarity. Abbreviations: tmeda = *N*,*N*,*N'*,*N'*-tetramethylethylenediamine, bpy = bipyridine, phen = 1,10-phenanthroline, pmbma = *N*-(2-pyridinylmethylene)benzenemethanamine.

Further evidence for the involvement of tetracopper clusters in the reaction was obtained from isothermal titration calorimetry (ITC) experiments (Figure 2C). The binding isotherm between $^{tmeda}\mathbf{Cu_4}^{OTf}$ and **1a** displays sigmoidal curvature with a binding stoichiometry corresponding to an interaction between one peptide and one $^{tmeda}\mathbf{Cu_4}^{OTf}$ molecule (N = ~1). Similarly, the binding isotherm between $^{tmeda}\mathbf{Cu_4}^{OTf}$ and tyrosine **1b** involves the stepwise binding of two tyrosine molecules with one $^{tmeda}\mathbf{Cu_4}^{OTf}$ molecule ($N_1 = N_2$ = ~1). The *N* values in the two ITC experiments provide evidence for the binding stoichiometry of two phenols with the $^{tmeda}\mathbf{Cu_4}^{OTf}$ cluster. Furthermore, the single binding constant for **1a** ($K_a = 3.57x10^6\ M^{-1}$) is larger than the binding constants of the individual tyrosine **1b** molecules [$K_{a1} = 0.86x10^6\ M^{-1}$ and $K_{a2} = 0.56x10^6\ M^{-1}$], indicating that chelation of two tethered phenols to $\mathbf{Cu_4}^{OTf}$ is energetically preferred over the binding of two distinct tyrosine molecules. These results are in agreement with crossover experiments, which showed that the $^{tmeda}\mathbf{Cu_4}^{OTf}$ complex favors OxPM over oxidative coupling reactions (see supplementary materials). This desirable OxPM selectivity can be attributed to the unique ability of multicopper clusters to simultaneously chelate the two tethered phenols in the linear peptide, thereby enforcing their spatial proximity during the coupling reaction.

The OxPM reaction can be performed under catalytic conditions. Our studies revealed that under sub-stoichiometric conditions, $^{tmeda}\mathbf{Cu_4}^{OTf}$ and $^{phen}\mathbf{Cu_4}^{ClO_4}$ (25 mol %) promotes the aerobic OxPM of linear peptide **1a**, affording **2a** in 85% and 89% isolated yield, respectively (Table 1, entry 1 and 2); however, a lower loading of the former clusters had a negative impact on the reaction efficiency (entry 3). To overcome this limitation, we identified a hydroxo multicopper(II) cluster, namely $^{pmbma}\mathbf{Cu_4}^{ClO_4}$ (pmbma = *N*-(2-pyridinylmethylene)benzenemethanamine) that mediate the reaction under catalytic conditions (5 mol % loading, HFIP, air, rt) with similar intramolecular coupling selectivity, affording the desired macrocyclopeptide **2a** in 88% isolated yield after only 24 h (entry 4). The reaction is concentration-independent and can be performed even at 0.1 M. However, the reaction is restricted to HFIP or HFIP-containing solvent mixtures, such as HFIP:DCM (Supplementary materials). Indeed, comprehensive mechanistic studies support the premise that HFIP is not an innocent solvent in this reaction.[21]



1a 2a

catalyst	X [mol %]	time [h]	isolated yield [%]
$^{tmeda}\mathbf{Cu_4}^{OTf}$	25	24	85
$^{phen}\mathbf{Cu_4}^{ClO_4}$	25	24	89
$^{phen}\mathbf{Cu_4}^{ClO_4}$	5	168	53
$^{pmbma}\mathbf{Cu_4}^{ClO_4}$	5	24	88

Table 1. Aerobic multicopper-catalyzed OxPM of linear peptide **1a**.

We then examined the generality of the reaction by applying the method to the synthesis of structurally diverse biaryl-bridged macrocyclic peptides. This part of the study was pursued with the $^{tmeda}Cu_4^{OTf}$ cluster, as it displayed high reactivity and oxidative macrocyclization selectivity for a long list of linear peptides. The optimized conditions suitable for a wide variety of linear peptide derivatives involved the mixing of linear precursor **1** with $^{tmeda}Cu_4^{OTf}$ (0.4-1.2 equiv) and TMEDA (0.4-1.2 equiv), which serves as a base, in a vial containing HFIP (0.05 M) under air atmosphere at room temperature. High C–C coupling selectivity was observed for linear peptides with two tyrosine residues having the general formula (3-R^1)Tyr–Ax–(3-R^2)Tyr (Ax = a variable amino acid residue), affording 15-membered biaryl-bridged macrocyclopeptides **2a-2l** as single atropoisomers in moderate to good yields (Figure 3A). In general, the efficiency of the macrocyclization reaction depends on the stereoelectronic nature of the two phenolic units. While the presence of a single 3-*t*-Bu group on one of the tyrosine units had no effect on the reaction efficiency, a significant drop in the yield was observed when both tyrosine units were substituted with the bulky group [compare **2c** (R^1 = R^2 = H, 88% yield) and **2d** (R^1 = H, R^2 = *t*-Bu, 85%)] with **2e** (R^1 = R^2 = *t*-Bu, 40%)]. A similar trend was observed when an electron-withdrawing halide atom was introduced [compare **2d** (90%) with **2f** (R^1 = Cl, R^2 = *t*-Bu, 64%) and **2c** (88%) with **2g** (R^1 = H, R^2 = Br, 35%]. The identity of the side chain in the backbone amino acid also influenced the OxPM efficiency [compare **2a** (Ax = Ala, 90%) with **2j** (Ax = Val, 59%) and **2e** (Ax = Ala, 40%) with **2i** (Ax = Pro, 68%)]. The absolute configuration of the biaryl bond in compound **2h** was assigned as R_A based on 2D-NMR experiments and X-ray crystallography (Figure 3A and supplementary materials).

The OxPM of linear peptides with two tyrosine units located at positions i+3 (Tyr–Ax–Ay–Tyr) and i+4 (Tyr–Ax–Ay–Az–Tyr) was also possible, affording 18- and 21-membered macrocyclic peptides **2m** and **2n** in 50% and 61% yield, respectively (Figure 3A). On the other hand, the neighboring phenolic-based amino acids (i+1) in (L,L)- or (L,D)-Boc-Tyr–Tyr-OMe (**1p**) failed to cyclize to the medium-sized 12-membered ring (Figure 3B). Instead, a mixture of the starting material and oligomeric products was observed. The ability of the $^{tmeda}Cu_4^{OTf}$ cluster to distinguish between neighboring (i+1) and remote (i+2) phenolic-based amino acids provides the site selectivity required for preparing GPA analogs based on the OxPM strategy. Indeed, the reactions of Ac-Tyr–Tyr–Tyr-OMe (**1q**) and Boc-Tyr–Hpg–Tyr-OMe (**1r**) showed complete site selectivity, affording macrocyclopeptides **2q** and **2r** in 53% and 74% yield, respectively (Figure 3B).

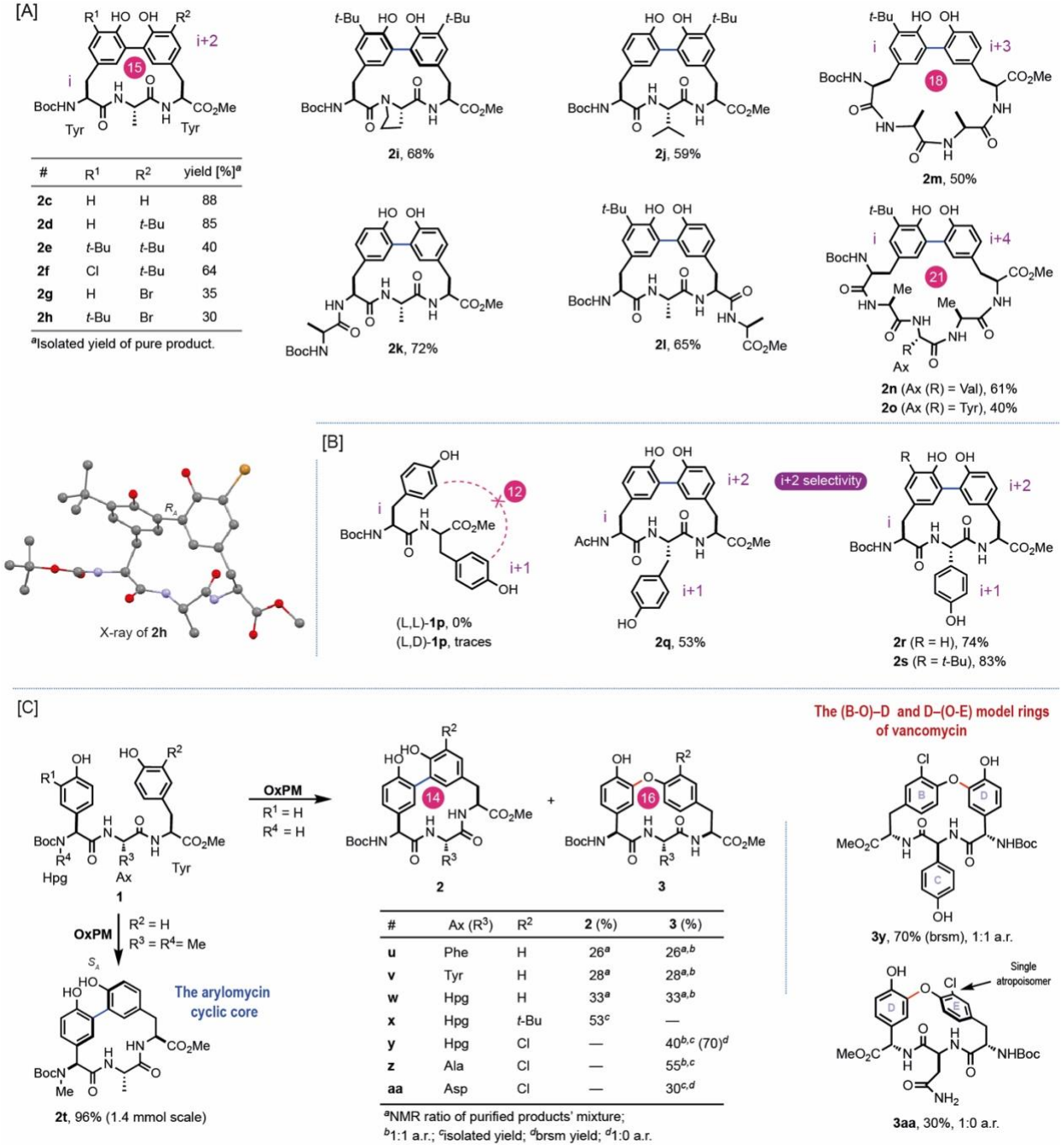
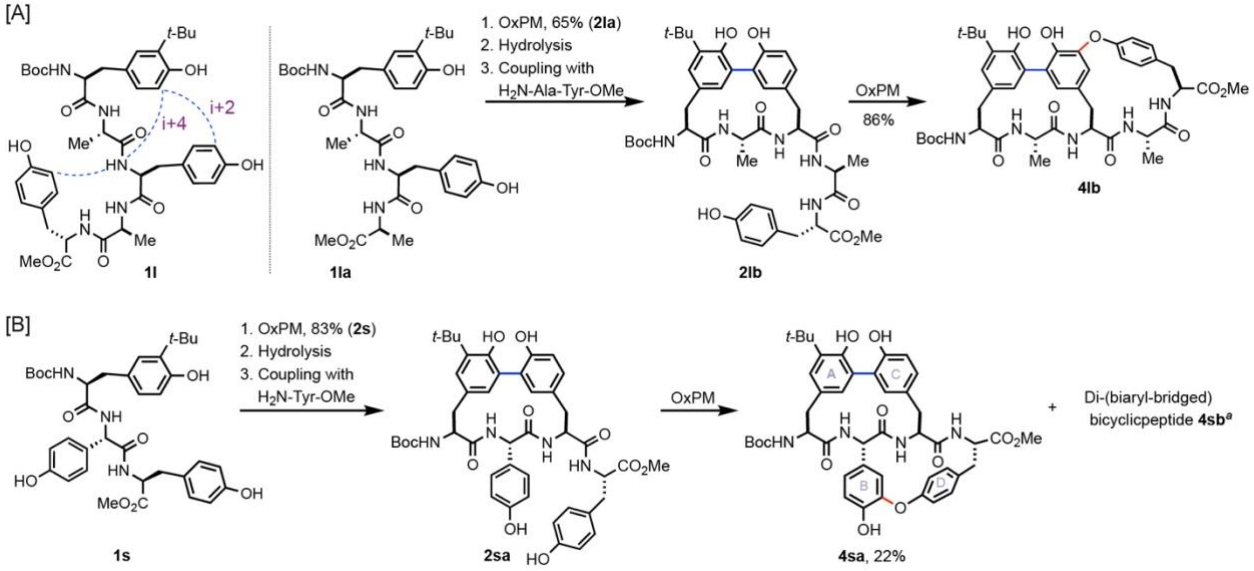


Figure 3. Reaction scope- synthesis of biaryl-bridged macrocyclic peptides by $^{tmeda}Cu_4^{OTf}$-catalyzed aerobic OxPM. [A] Examples of dityrosine-bridged macrocyclic peptides prepared under our general conditions. X-ray structure of **2h** (recrystallized from MeOH/diethyl ether, CCDC: 2210849). [B] Site selectivity (i+2 over i+1) demonstrated by the OxPM reaction to form Boc-Tyr-Hpg-Tyr-OMe (**2r**) and Boc-Tyr-Tyr-Tyr-OMe (**2q**). [C] Regioselectivity (C–C vs C–O) in the OxPM of linear peptides with the general formula Boc-(3-R^1)Hpg-Ax-(3-R^2)Tyr-OMe. Synthesis of the arylomycin cyclic core **2t** and the vancomycin model macrocycles **3y** and **3aa**. General conditions: linear peptide **1** (1 equiv), $^{tmeda}Cu_4^{OTf}$ (0.4 to 1.2 equiv, added in batches until the starting material was consumed), TMEDA (0.4-1.2 equiv), HFIP (0.01 M), air atmosphere, room temperature. See supplementary materials for full experimental details.

Next, we elucidated the structure-dependent regioselectivity (C–C vs. C–O coupling) of the reaction using tripeptides with the general formula Hpg–Ax–Tyr; these tripeptides form the peptide sequence of the

arylomycin antibiotics and the GPA aglycones and therefore serve as their model ring compounds (Figure 3C). The OxPM of arylomycin linear precursor **1t** showed excellent C–C coupling selectivity, affording arylomycin cyclic core **2t** in 96% isolated yield, even on a 1.4 mmol scale.[14] In contrast, linear peptides in which the central amino acid (Ax) corresponds to bulkier residues, such as Phe (**1u**), Tyr (**1v**), and Hpg (**1w**), did not undergo regioselective OxPM (Figure 3C). Instead, we isolated equal amounts of 14-membered biphenol-bridged macrocyclic peptides [**2u** (26%), **2v** (28%), or **2w** (33%)] and 16-membered diaryl ether cross-linked macrocyclic peptides [**3u** (26%), **3v** (28%), or **3w** (33%)]. However, the regioselectivity could be successfully controlled by introducing a discrete substituent at the C3-position of the tyrosine unit (R^2). For example, the inclusion of a bulky *tert*-butyl group in Boc-Hpg–Hpg–(3-*t*-Bu)Tyr-OMe (**1x**) gave rise to complete C–C coupling selectivity (**2x,** 53% yield). Replacing the *tert*-butyl group with a chloride atom resulted in C–O coupling selectivity, as in the GPAs biosynthesis. The OxPM reactions of Boc-Hpg–Ax–(3-Cl)Tyr-OMe tripeptides [Ax = Hpg (**1y**), Ala (**1z**) or Asn (**1aa**)], which required excess $^{tmeda}Cu_4^{OTf}$ (2.4 equiv) and TMEDA (1.2 equiv) to ensure satisfactory conversions, afforded solely the diaryl ether-linked macrocyclic peptides **3y** (40% yield, 70% bsmr, 1:1 a.r.), **3z** (55% yield, 1:1 a.r.), and **3aa** (30% yield, 1:0 a.r.), respectively.



Scheme 1. Synthesis of bicyclopeptides by the $^{tmeda}Cu_4^{OTf}$-catalyzed OxPM-by-OxPM approach. A] Synthesis of bicyclopeptide **4lb** by the OxPM-by-OxPM approach; B] Application of the OxPM-by-OxPM approach to the preparation of vancomycin's A–C/(B-O)–D model bicyclic compound **4sa**. See supplementary materials for full experimental details. Isolated yields, atroposelectivity ratio (a.r.). [a]bicyclicpeptide **4sb** was not isolated in pure form.

The ordered OxPM steps in the biosynthesis of GPAs are controlled by a family of highly specific oxidases (Figure 1A). Mimicking this strategy to prepare multi-macrocyclic peptides is expected to be challenging and lead to a complex mixture of products when using highly general multicopper catalysts. For example, the OxPM reaction of pentapeptide Boc-(3-*t*-Bu)Tyr–Ala–Tyr–Ala–Tyr-OMe **1l** displayed poor site selectivity, as the excess of $^{tmeda}Cu_4^{OTf}$ afforded both the 21-membered macrocyclopeptide **2o** (30%,

i+4/C–C selectivity, see the structure in Figure 3A) and 15-membered macrocyclopeptide **2la** (i+2/C–C selectivity), which upon a second OxPM event (i+2/C–O selectivity) yielded bicyclopeptide **4la** in 40% isolated yield (Scheme 1A). To overcome the site-selectivity problem, an OxPM-by-OxPM approach based on discrete assembly of the rings was adopted. This method enabled the selective synthesis of bicyclopeptide **4la** from tetrapeptide **1la** via two highly selective OxPM events (65% and 88% yields, respectively, Scheme 1A). Finally, the OxPM-by-OxPM approach was applied to the synthesis of bicyclopeptides **4sa** and **5sa** in four steps from Boc-(3-*t*-Bu)Tyr–Hpg–Tyr-OMe **1s** (Scheme 1B). In agreement with our selectivity studies, the first cyclization displayed complete C–C coupling selectivity, affording biaryl-bridged macrocyclopeptide **2s** (see the structure in Figure 3B) in 83% yield, while the second macrocyclization was nonselective, affording a 1:1 mixture of **4sa** (i+2/C–O selectivity, 22% isolated yield) and bicyclopeptide **4sb** (i+2/C–C selectivity). Notably, cyclopeptides **3y**, **3aa** and bicyclocpeptide **4sa** serve as (B-O)–D, (D-O)–E, and A–C/(B-O)–D model rings of vancomycin, respectively. For the first time, a suitable technology that can provide direct synthetic pathways to novel antibiotic GPA analogs from readily available natural amino acids is available.

Conclusions

The biosynthesis of antibiotic macrocyclic peptides and many other phenolic-based macrocyclic natural products involves the key OxPM of their linear precursors, a transformation that had been missing from the toolbox of chemists. In this study, we successfully developed a general and efficient biomimetic method for the oxidative macrocyclization of simple linear diphenolic peptides based on μ-hydroxo tetracopper catalysts. The multicopper cluster $^{tmeda}Cu_4^{OTf}$ favors intramolecular oxidative coupling between two remote phenolic-based amino acids (i+m, m≥2) while leaving the more oxidizable biphenol products untouched. It imposes substrate-dependent regioselective (C–C or C–O coupling) and stereoselective (axial atroposelectivity) reactions and can be applied to the assembly of multiring systems. The novel method was applied with high efficiency and selectivity to prepare an extensive library of biaryl-bridged and diaryl ether-linked mono- and bimacrocyclic peptides, including synthetic analogs of the GPAs and the cyclic core of the arylomycin-based antibiotic drugs. Therefore, we expect this novel OxPM reaction to be a game-changing technology in developing next-generation cyclic and multicyclic peptide antibiotic drugs and other biaryl-bridged and diaryl ether-linked macrocycles. Finally, the potential of synthetic copper clusters to promote organic reactions that are not accessible with smaller metal catalysts is demonstrated.

AUTHOR INFORMATION

Corresponding Author

Doron Pappo – Department of Chemistry, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel
ORCID.org/0000-0002-8363-8709

Authors

Anna Libman – Department of Chemistry, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel
ORCID.org/0000-0003-4332-629X

Mor Ben-Lulu – Department of Chemistry, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel
Eden Gaster – Department of Chemistry, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel
ORCID.org/0009-0008-0789-5149
Ratnadeep Bera – Department of Chemistry, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel
Alexander I. Shames – Department of Physics, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel
ORCID.org/0000-0002-0574-1911
Omer Shaashua – Department of Chemistry, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel
Vlada Vershinin – Department of Chemistry, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel
Yuri Torubaev – Department of Chemistry, Ben-Gurion University of the Negev, Beer-Sheva 84105, Israel
ORCID.org/0000-0002-7835-0591

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