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carba-Nucleopeptides (*c*NPs): A Biopharmaceutical Modality Formed through Aqueous Rhodamine B Photoredox Catalysis

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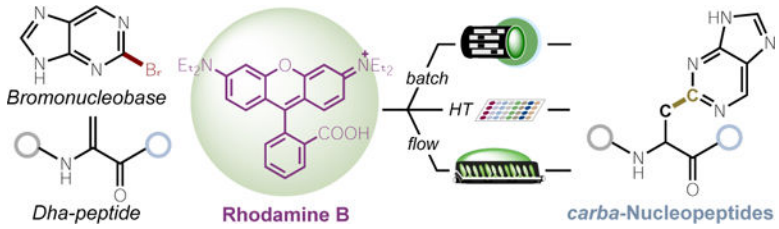
Abstract

Exchanging the ribose backbone of an oligonucleotide for a peptide can enhance its physiologic stability and nucleic acid binding affinity. Ordinarily, the eneamino nitrogen atom of a nucleobase is fused to the side chain of a polypeptide through a new C–N bond. The discovery of C–C linked nucleobases in the human transcriptome reveals new opportunities for engineering nucleopeptides that replace the traditional C–N bond with a non-classical C–C bond, liberating a captive nitrogen atom and promoting new hydrogen bonding and π-stacking interactions. We report the first late-stage synthesis of C–C linked *carba*-Nucleopeptides (*c*NPs) using aqueous Rhodamine B photoredox catalysis. We prepare brand-new *c*NPs in batch, in parallel, and in flow using three long-wavelength photochemical setups. We detail the mechanism of our reaction by experimental and computational studies and highlight the essential role of diisopropylethylamine as a bifurcated two-electron reductant.

Graphical Abstract



Exchanging the amino acid side chains of a polypeptide with C–N linked nucleobases forms oligonucleotide-like structures (*aza*-Nucleopeptides) with favorable physiochemical properties. Connecting the nucleobase to the peptide through a C–C bond can promote new, beneficial noncovalent interactions. A general method to make C–C linked *carba*-Nucleopeptides in batch, parallel, and flow using aqueous Rhodamine B photoredox catalysis is reported.

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Supporting information for this article is given via a link at the end of the document

Keywords

Nucleopeptides; Photoredox catalysis; Flow Synthesis; Parallel Synthesis; Rhodamine B

Introduction

Artificial DNA/RNA-mimetics can act as viable drugs, vehicles for understanding disease pathology, and as building blocks for new biomaterials and nanostructures.[1] One way to imitate endogenous oligonucleotides is to tether nucleobases to the side chains of a polypeptide via a C–N linkage (peptide nucleic acids).[2–4] These form stable hybrids with naturally occurring nucleic acid sequences and targets (e.g., duplexes, triplexes, G-quadraplexes, i-motifs, and aptamers)[2,5–7], and unlike classical oligonucleotides, cannot be degraded by nucleases.[8] In recent years, other strategies for connecting nucleobases to peptides have emerged, such as uniting the N1 (pyrimidine) or N9 (purine) nitrogen atoms of one or more nucleobases with alanine-based amino acids through sp^3C–N bonds, referred to as *aza*-Nucleopeptides (*a*NPs).[9] These structures conserve the amide backbone of peptides enabling them to self-assemble and to recognize target oligonucleotides in ways akin to standard peptides. The discovery of the 'fifth nucleotide', pseudouridine,[10] unlocks new opportunities for nucleopeptides. Pseudouridine is linked to its ribose ring through an unusual C–C bond. This ubiquitous yet enigmatic feature allows pseudouridine to form an extra hydrogen bond and to enhance its π-stacking capabilities, which enables the proper folding of tRNAs and rRNAs and the efficient decoding and processing of mRNAs in the human ribosome, essential for protein synthesis. Pseudouridine in small nuclear RNAs also enhances spliceosomal RNA-pre-mRNA interactions, heavily regulating gene expression.[11,12] Hence, connecting a polypeptide to the carbon atom of even a *single* nucleobase, herein termed *carba*-Nucleopeptide (*c*NP), has the capacity to transform the function and chemical versatility of nucleopeptides, but no general method for assembling *c*NPs exists (Figure 1). To explore the untapped potential of *carba*-Nucleopeptides, a synthetic platform to readily access libraries of *c*NPs is needed.

The C–C linkage of *c*NPs could be made via a fragment-based approach, combining a C-centered nucleobase radical with a dehydroalanine (Dha) side chain, derived from a single cysteine reside, in a peptide. This type of approach has been successfully used by our lab (with boronic acids) and others to incorporate unnatural amino acids and non-ribosomal side chains into peptides and proteins through photoredox catalysis.[13–14] Unfortunately, *borono*-nucleobases are unstable[15] and, therefore, have low commercial availability and challenging syntheses. Their high oxidation potential also makes them less apt to form radicals by photocatalyzed oxidative deborylation.[16] Thus, to leverage our established radical-to-Dha approach for *c*NP synthesis, we needed to identify a different radical precursor and a new photocatalyst. *bromo*-Nucleobases are a readily accessible class of nucleobase analogs for chemical synthesis. We imagined that single-electron transfer (SET) reduction of a bromonucleobase by an aqueous photocatalyst could cleave the C–Br bond and unveil a free C-centered nucleobase radical. To this end, fluorescent organic dyes are water soluble and strongly reducing in their photoexcited state. Their ability to absorb longer wavelengths (>500 nm) of light also enables them to mediate photochemical transformations

at wavelengths more amenable to biomolecules.[17] (Our lab and others have found that some peptides are readily degraded with higher energy <450 nm light).[18] By operating through SET reduction, organic dyes are also less likely to cause undesirable side reactions on peptides, which generally have amino acids that can be oxidatively modified, including Tyr[19], Trp[20], Met[21,22], His[23,24], N-termini[25], and C-termini[26]. The discovery of an organic dye that can facilitate *c*NP synthesis would not only provide ready access to a novel biopharmaceutical modality but bolster the utility of fluorescent organic dyes for biomolecular synthesis.

Results and Discussion

To identify a suitable photocatalyst for *c*NP synthesis, we surveyed several organic dyes and metal-based photocatalysts (10 mol%) using 2-bromopyridine (1 equiv.) as a surrogate for a bromonucleobase and water as solvent. (The single-electron reduction potential of 2-bromopyridine is similar to a bromonucleobase, and the 2-pyridyl radical has been shown to engage Dha residues by open-shell conjugate addition.[13a]) We also included 1-benzyl-3-carbamoylpyridinium bromide (reduced in situ) as a sacrificial reductant. Ac-Gly-Pro-**Dha**-Phe-$CONH_2$ (2.3 μmol) was used as a test peptide and irradiation was provided by two 525 nm Kessil lamps (40 W each). We found Rhodamine B (Rh B), a standard dye for many biotechnology applications,[27–29] to be the best photocatalyst, delivering the desired *c*NP in 21% conversion. Using diisopropylethylamine (DIPEA) as reductant and decreasing photocatalyst loading to 2 mol% increased the conversion to 44%. These conditions translated well when the bromonucleobase, 2-bromopyrimidine, was used in place of 2-bromopyridine in our standard reaction (52% conversion). Including 2,2,2-trifluoroethanol (TFE) as a cosolvent (5% v/v) enhanced the efficiency of the reaction to 80% conversion, presumably by decreasing the reduction potential of 2-bromopyrimidine through hydrogen bonding.[30] Finally, adding a small amount of acetonitrile (5% v/v) increased the homogeneity of the reaction and provided an optimal 87% conversion to the desired *c*NP product. Common iridium- and ruthenium-based photocatalysts performed less admirably in our reaction with two 440 nm Kessil lamps (Table 1). Additionally, we found that our optimized reaction conditions with 2-bromopyrimidine could be successfully applied to the unprotected variant of our standard peptide, NH_2-Gly-Pro-**Dha**-Phe-CO_2H (44% conversion to *c*NP). See Supporting Information for full optimization details.

We assessed the scope of bromopyrimidines that can be used for *c*NP synthesis (Scheme 1). (**Note:** The reaction was performed on a 23 μmol scale for ease of *c*NP product isolation. Interestingly, the reaction performed slightly better on scale and with lower catalyst loading (0.5 mol%). For example, 2-bromopyrimidine achieved >99% conversion to the desired *c*NP product with a 77% isolated yield, as a mixture of two epimers.) We found that our reaction tolerated a diverse array of substituents on the pyrimidine ring including several functional groups that are prone to reduction, such as exogenous halogen atoms[31] (compounds **3**, **4**, **5**) and a nitrile[32] (compound **8**). Unprotected aryl amines (prone to oxidation)[33] were also tolerated (compounds **7** and **8)**. For a tosyl protected alcohol, our reaction conditions not only afforded the desired *c*NP product **9**, but also removed the tosyl protecting group in the same reaction. The ability to accomplish both transformations (alkylation and deprotection) on the same substrate is remarkable considering that only 0.5 mol% catalyst is used in the

reaction. Finally, we found that our reaction could furnish *c*NP **10** having a decarbonyluracil side chain, an established group with herbicidal activity.[34] While uracil has traditionally been employed as a radical 'acceptor',[35] our reaction shows its competence as a radical 'donor'. To our knowledge, this reactivity has not been demonstrated previously for uracil.

We explored whether the position of the bromine atom in our standard pyrimidine affected the efficiency of *c*NP formation. 5-Bromopyrimidine failed to react to any significant extent (<1% conversion to *c*NP), and most of the bromide was recovered after the reaction. This suggests that reduction of the bromonucleobase at position-C5 of the pyrimidine ring is not favored. In contrast, bromides adjacent to electron withdrawing sp^2-nitrogen atoms in the pyrimidine ring readily formed radicals. Taking advantage of this innate difference in reactivity, we performed our coupling reaction using 2,5-dibromopyrimidine. As expected, we obtained primarily the C2-alkylated *c*NP product (13:1 selectivity) determined by ^1H NMR analysis (Figure S6). Our finding demonstrates that nucleobase radical formation is governed primarily by electronics, with more electron deficient C–Br sites being more susceptible to SET reduction. With this understanding, we strongly suspect nucleobases that are inherently electron rich (such as pseudouridine) and/or have activated C–Br sites could be problematic due to their higher reduction potentials. The inability to generate radicals at every conceivable position in a standard nucleobase poses a limitation to our methodology.

We next examined bromopurines and purine-like nucleobases. To our delight, a wide array of bromopurines underwent successful fragment coupling with our standard Dha-peptide to afford new *c*NP products. In general, bromopurines where the bromide is adjacent to a sp^2-nitrogen atom worked best in our reaction. Of significance, we found that non-coding purine mimetics, deaminoadenine (**11**) and decarbonylguanine (**12**) were coupled in good yields, replacing the endogenous amine and carbonyl groups of the parent nucleobases with a new C–C linkage, respectively. These two nucleobase mimetics are ideal substrates to probe the importance of hydrogen bonding in guanine and adenine nucleotides for base recognition and oligonucleotide folding, as they eliminate one hydrogen bond from each nucleobase that is normally used for base-pairing.[36,37] Aside non-coding nucleobases **11** and **12**, we prepared the deaminoadenine analog (**18**) wherein N1 of the purine ring is transposed to the C6 position. Five additional non-coding nucleobase analogs were also successfully prepared, each having three nitrogen atoms scattered over the purine ring. As suspected, nucleobases **16** and **17** were less effective due to higher electron density at their C–Br sites. The corresponding bromide that makes *c*NP **18** is a highly colored material and likely presents its own challenges for photodriven Dha-diversification. The chemical/biological consequence of retaining the [5:6]-ring fusion of purines and redistributing the nitrogen atoms is largely unexplored, but we expect this will enable the purine to participate in unprecedented non-covalent interactions (e.g., new hydrogen bonding and π-stacking interactions), which could be useful for any number of applications, *inter alia*, medicine, chemical biology, and biomaterials. To conclude our survey, we examined simple bromoarenes. Without the nitrogen atom, we questioned whether our methodology would work at all. Gratifyingly, bromoarenes bearing a ketone **19** and an aldehyde **20** were viable substrates in our reaction. Thus, our method also has the potential to install non-heteroatom-containing arylbromides into Dha-peptides, including those with biorthogonal handles.[38]

We examined different peptide sequences for *c*NP synthesis. We prepared three medicinally relevant cysteine-containing peptides (caspase active site peptide,[39] antioxidant peptide A,[40] and ATN-161 integrin inhibitor[41]), converted their cysteine amino acids to Dha residues, and performed our standard coupling reaction with 2-bromopyrimidine. In each case, *c*NP products were isolated in good yields (compounds **21**, **22**, and **23**). Considering the peptides we used, our reaction appears amenable to peptides having an unprotected C-termini (compounds **21** and **22**), a free N-terminal proline and basic amino acid side chains (Arg and Lys, compound **22**), and oxidatively sensitive amino acids (His[24] and Met[22], compounds **22** and **23**). We also examined the hypothalamic decapeptide Gonadorelin, sequence: Pyr-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-$CONH_2$. Residues His-2, Tyr-5, and Arg-8 of Gonadorelin form a tight triad that stabilizes the peptide in a conformation necessary for its biological activity.[42] Replacing the **π**-rich phenol ring of Tyr-5 with a more electron deficient imidazole ring increases affinity for the Gonadorelin receptor.[43] This suggested to us that substituting Tyr-5 with a dinitrogen-containing heteroaromatic ring that contains a bioisostere for a hydroxyl group might further improve activity. Accordingly, we used our newly minted *c*NP chemistry to exchange the phenol ring of Tyr-5 with an unprecedented 5-fluoropyrimidine side chain (compound **24**). Its synthesis demonstrates clearly the unique opportunities of our side chain addition strategy (and reducing organophotocatalysts) for *c*NP synthesis in medicinal chemistry. Furthermore, the innate chemoselectivty of our reaction across four different Dha-containing peptides suggest that our synthetic approach could well translate to work on more complex peptides, including those with longer amino acid sequences, and potentially on proteins.

To detail the important biological applications of *c*NPs calls for strategies to simultaneously generate many numbers of disparate *c*NPs (for hit-to-lead peptide optimization) and to access larger quantities of the best peptide analogs for completing more advanced studies.[9b,44–46] In this vein, we sought to adopt our protocol for *c*NP synthesis to parallel and to flow technologies. The Lumidox® Generation II 527 nm reactor from Analytical Sales and Services Inc. is a two-in-one system that enables the user to transition between high-throughput and preparative (flow) synthesis. For parallel synthesis, a 96-well vial rack (containing unreacted samples) is placed atop an LED array with an integrated cooling base. All 96 samples are irradiated at once. For flow synthesis, the 96-well rack is exchanged for a pre-assembled unit containing flow-tubing of desired path length and transparency. An exogenous syringe pump is attached to the tubing inlet and controls the sample flow rate. We first optimized our reaction for parallel synthesis. We performed our reaction on a scale of 2.3 μmol (1 mg peptide) at 200 mW *per* well with Ac-Gly-Pro-**Dha**-Phe-$CONH_2$ and 16 different bromides (9 pyrimidines, 5 purines, and 2 arenes) shown to work in batch at 23 μmol at 80 W *per* reaction. After 15 hours of irradiation, the light source was removed, and polymer-bound 2-mercaptoethylamine resin was added to each reaction (stirred for 5 hours at 40 °C). This resin sequesters any unreacted Ac-Gly- Pro-**Dha**-Phe-$CONH_2$ and allows it to be conveniently filtered away. Unreacted bromide was removed by diethyl ether extraction. Finally, all products were purified in tandem by solid-phase extraction (SPE) with a Waters Oasis HLB sorbent plate (60 mg sorbent per well) using 5% aq. NaOH followed by 50% TFE/H_2O as eluent. ~50% of our *c*NP products were obtained asa mixture of two diastereomers (epimers) in purities of ≥ 65%, with respect to all other components

found in the final purified LC spectrum and amounts of ≥ 0.05 mg (Figure 2A). Our lab has shown that peptide mixtures meeting these two criteria are suitable for direct biochemical testing, with peptide mixtures giving comparable results to single diastereomer products.[14] For complete experimental details see Table S6.

Next, we optimized our reaction in flow. In many cases, performing a reaction in flow requires that entirely new reaction conditions be identified so that the reaction is homogeneous, avoids the formation of precipitates, and can be concentrated. It is also highly desirable to use comparable (and low) stoichiometries between different reactants. Fortunately, our reaction already satisfies many of these criteria, and this allowed us to start our optimization using 1.0 equiv. of peptide (58 μmol), 1.5 equiv. of 2-bromopyrimidine, 1.5 equiv. of DIPEA, and 0.5 mol% Rh B, at 0.1 M. In flow (flow rate: 14 μL/min; residence time: 1 hour and 55 minutes), we achieved a 38% isolated yield of our desired *c*NP product. More importantly, we generated the same amount of product in this 2.6 h reaction that would normally take us 15 h to make in batch: ~ a 6-fold improvement in time (Figure 2B). This is remarkable if you consider that our flow system has ~ 4 x less wattage (80 W for batch *vs.* 19.2 W for flow). While S–S,[47,48] S–O,[49] S–C,[50,51] and C–N[52] bond forming reactions have been achieved on peptides in flow by photoredox catalysis, our work represents the first example of C–C bond formation.

As a final point of interest, we probed the mechanism of our reaction. SET reduction of 2-bromopyrimidine by photoexcited Rhodamine B could generate a C-centered nucleobase radical, which can be captured by radical trap TEMPO•. Indeed, adding TEMPO• to our standard reaction without Ac-Gly-Pro-**Dha**-Phe-$CONH_2$ yielded a pyrimidine-TEMPO adduct in 3% conversion by LC-MS analysis (Figure 3A). While this observation is consistent with SET reduction by Rhodamine B, it does not indicate whether Rhodamine B reduces our bromonucleobase from its photoexcited (*RhB) or reduced (RhB•-) state, as our reaction contains the sacrificial amine reductant DIPEA (**i**). SET oxidation of DIPEA (0.68 V *vs.* SCE)[53] by photoexcited Rhodamine B (0.92 V *vs.* SCE)[54,55] is exergonic. Direct SET reduction of 2-bromopyrimidine (−1.86 *vs.* SCE)[56] by photoexcited Rhodamine B (−1.3 V *vs.* SCE)[54,55] is endergonic. Thus, reductive quenching of Rhodamine B to yield highly reducing RhB•- (more negative potential than −1.3 V *vs.* SCE) and DIPEA• (**ii**) appears most probable, and RhB•- converts our bromonucleobase to a C-centered radical (**iii**) via SET reduction. The nucleobase radical is free to engage our Dha-peptide (**iv**) by open-shell conjugate addition, producing a captodative α-carbonyl radical (**v**; ΔE = −45 kcalmol^{-1} at B3LYP/6–311+G**). DIPEA• reduces the α-carbonyl radical to an α-carbonyl anion by SET. The resulting iminium (**vi**) is hydrolyzed to diisopropylamine and acetaldehyde,[57] and the α-carbonyl anion is protonated by solvent to give the final *c*NP product (**vii**; ΔE = −53 kcalmol^{-1} at B3LYP/6–311+G**). It is critical to point out that the reduction of the α-carbonyl radical (**v**) by oxidized DIPEA (**ii**) is highly endergonic (+107 kcalmol^{-1}). However, this SET process becomes favorable when the resulting iminium ion is hydrolyzed to diisopropylamine and acetaldehyde giving a net −53 kcalmol^{-1} driving force (Figure 3B). We find this to be an important result considering emerging reports on the promiscuity of α-amino radicals as halogen-atom transfer reagents for photoredox catalysis and as bifurcated SET reductants for photolytic reactions.[13b, 58–59]

We now show that in addition to being modest reducing agents, α-amino radicals can help drive endergonic steps in photoredox catalyzed reactions via oxidative fragmentation. This mechanistic paradigm could find general applications for the design of other photocatalyzed transformations, especially those performed in aqueous solvent. In support of our proposed mechanistic steps: (1) HRMS analysis of our crude reaction mixture revealed the formation of diisopropylamine (102.13 *m/z*, Figure S5), (2) using D_2O as solvent furnished the α-deuterated *c*NP product (>95% *d*-content, Figure 3A), and (3) when triphenylamine (1.09 *vs.* SCE)[60]–similar oxidation potential to DIPEA but lacks α-hydrogen atoms–was used as a sacrificial reductant, only a small amount of *c*NP was formed, highlighting the importance of an α-amino radical to *c*NP formation (Table 1). Our experimental results are consistent with the mechanism depicted in Figure 3B.

Conclusion

In conclusion, we have developed a general method to access *carba*-Nucleopeptides having new pyrimidine and purine side chains. We demonstrate the compatibility of our system for parallel synthesis and for on-scale synthesis in flow using a brand-new photochemical setup. Finally, we detail the mechanism of our reaction, demonstrating the feasibility of organic dyes (such as Rhodamine B) to perform reductive transformations under biocompatible conditions, and for α-amino radicals to offset endergonic reactions through oxidative fragmentation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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References

[1]. a) Veedu RN, Wengel J, Chem. Biodivers 2010, 7, 536–542; [PubMed: 20232325] b) Ziach K, Chollet C, Parissi V, Prabhakaran P, Marchivie M, Corvaglia V, Bose PP, Laxmi-Reddy K, Godde F, Schmitter J-M, Chaignepain S, Pourquier P, Huc I, Nat. Chem 2018, 10, 511–518; [PubMed: 29610464] c) Yang H, Xi W, Polymers 2017, 9, 666;d) Swenson CS, Velusamy A, Argueta-Gonzalez HS, Heemstra JM, J. Am. Chem. Soc 2019, 141, 19038–19047; [PubMed: 31711285] e) Del Prado A, González-Rodríguez D, Wu Y-L, ChemistryOpen 2020, 9, 409–430; [PubMed: 32257750] f) Wang F, Liu B, Huang P-JJ, Liu J, Anal. Chem 2013, 85, 12144–12151; [PubMed: 24237266] g) Baumann V, Winkler J, Future Med. Chem 2014, 6, 1967–1984; [PubMed: 25495987] h) Kulkarni JA, Witzigmann D, Thomson SB, Chen S, Leavitt BR, Cullis PR, van der Meel R, Nat. Nanotechnol 2021, 16, 630–643. [PubMed: 34059811]

[2]. Pellestor F, Paulasova P, Eur. J. Hum. Genet 2004, 12, 694–700. [PubMed: 15213706]

[3]. Singh KR, Sridevi P, Singh RP, Eng. Rep 2020, 2, e12238. [PubMed: 32838227]

[4]. Das A, Pradhan B, Chem. Biol. Drug Des 2021, 97, 865–892. [PubMed: 33314595]

[5]. Goux E, Lespinasse Q, Guieu V, Perrier S, Ravelet C, Fiore E, Peyrin E, Methods 2016, 97, 69–74. [PubMed: 26455538]

[6]. Modi S, Wani AH, Krishnan Y, Nucleic Acids Res 2006, 34, 4354–4363. [PubMed: 16936319]

[7]. Panyutin IG, Onyshchenko MI, Englund EA, Appella DH, Neumann RD, Curr. Pharm. Des 2012, 18, 1984–1991. [PubMed: 22376112]

[8]. Patel R, Sarma S, Shukla A, Parmar P, Goswami D, Saraf M, Mol. Biol. Rep 2020, 47, 8113–8131. [PubMed: 32990905]

[9]. a) Takahashi T, Hamasaki K, Kumagai I, Ueno A, Mihara H, Chem. Commun 2000, 349–350;b) Pomplun S, Gates ZP, Zhang G, Quartararo AJ, Pentelute BL, J. Am. Chem. Soc 2020, 142, 19642–19651; [PubMed: 33166454] c) Chaltin P, Lescrinier E, Lescrinier T, Rozenski J, Hendrix C, Rosemeyer H, Busson RHC, van Aerschot A, Herdewijn P, Helv. Chim. Acta 2002, 85, 2258–2283;d) Miyanishi H, Takahashi T, Mihara H, Bioconjug. Chem 2004, 15, 694–698; [PubMed: 15264855] e) Takahashi T, Hamasaki K, Ueno A, Mihara H, Bioorg. Med. Chem 2001, 9, 991–1000; [PubMed: 11354682] f) Du X, Zhou J, Li X, Xu B, Interface Focus 2017, 7, 20160116; [PubMed: 29147550] g) Tomassi S, Ieranò C, Mercurio ME, Nigro E, Daniele A, Russo R, Chambery A, Baglivo I, Pedone PV, Rea G, Napolitano M, Scala S, Cosconati S, Marinelli L, Novellino E, Messere A, Di Maro S, Bioorg. Med. Chem 2018, 26, 2539–2550; [PubMed: 29656988] h) Yuan D, Du X, Shi J, Zhou N, Zhou J, Xu B, Angew. Chem. Int. Ed 2015, 54, 5705–5708;i) Li X, Kuang Y, Lin H-C, Gao Y, Shi J, Xu B, Angew. Chem. Int. Ed 2011, 50, 9365–9369;j) Roviello GN, Musumeci D, Bucci EM, Pedone C, Int. J. Pharm 2011, 415, 206–210; [PubMed: 21689737] k) Roviello GN, Musumeci D, RSC Adv 2016, 6, 63578–63585; [PubMed: 28496969] l) Murai K, Inagaki K, Hiraoka C, Minoshima S, Kinoshita T, Nagata K, Higuchi M, CrystEngComm 2019, 21, 3557–3567;m) Musumeci D, Mokhir A, Roviello GN, Bioorganic Chem 2020, 100, 103862;n) Scognamiglio PL, Platella C, Napolitano E, Musumeci D, Roviello GN, Molecules 2021, 26, 3558; [PubMed: 34200901] o) Boback K, Bacchi K, O'Neill S, Brown S, Dorsainvil J, Smith-Carpenter JE, Molecules 2020, 25, 5493;p) Kumar Bandela Anil, Nathaniel Wagner, Hava Sadihov, Sara Morales-Reina, Agata Chotera-Ouda, Kingshuk Basu, Rivka Cohen-Luria, Andrés de la Escosura, Gonen Ashkenasy, Proc. Natl. Acad. Sci 2021, 118, e2015285118; [PubMed: 33622789] q) Roviello GN, Musumeci D, Bucci EM, Pedone C, Mol. Biosyst 2011, 7, 1073–1080; [PubMed: 21203614] r) Roviello G, Vicidomini C, Di Gaetano S, Capasso D, Musumeci D, Roviello V, RSC Adv 2016, 6, 14140–14148; [PubMed: 29057071] s) Musumeci D, Roviello V, Roviello GN, Int. J. Nanomedicine 2018, 13, 2613–2629; [PubMed: 29750033] t) Tomassi S, Montalban FF, Russo R, Novellino E, Messere A, Di Maro S, Symmetry 2019, 11, 567.

[10]. Li X, Ma S, Yi C, Curr. Opin. Chem. Biol 2016, 33, 108–116. [PubMed: 27348156]

[11]. Zhao Y, Dunker W, Yu Y-T, Karijolich J, Front. Bioeng. Bio.technol 2018, 6, 8.

[12]. Charette M, Gray MW, IUBMB Life 2000, 49, 341–351. [PubMed: 10902565]

[13]. a) Aycock RA, Vogt DB, Jui NT, Chem. Sci 2017, 8, 7998–8003; [PubMed: 29619169] b) Constantin T, Zanini M, Regni A, Sheikh NS, Juliá F, Leonori D, Science 2020, 367, 1021–1026; [PubMed: 32108109] c) de Bruijn AD, Roelfes G, Chem. Eur. J 2018, 24, 11314–11318; [PubMed: 29939448] d) Bottecchia C, Noël T, Chem. Eur. J 2019, 25, 26–42; [PubMed: 30063101] e) Faraggi TM, Rouget-Virbel C, Rincón JA, Barberis M, Mateos C, García-Cerrada S, Agejas J, de Frutos O, MacMillan DWC, Org. Process Res. Dev 2021, 25, 1966–1973; [PubMed: 35663840] f) Zhang O, Schubert JW, J. Org. Chem 2020, 85, 6225–6232; [PubMed: 32268730] g) Wang J, Shao Z, Tan K, Tang R, Zhou Q, Xu M, Li YM, Shen Y, J. Org. Chem 2020, 85, 9944–9954; [PubMed: 32646216] h) Seo H, Katcher MH, Jamison TF, Nat. Chem 2017, 9, 453–456; [PubMed: 28430203] i) Shatskiy A, Axelsson A, Stepanova EV, Liu J-Q, Temerdashev AZ, Kore BP, Blomkvist B, Gardner JM, Dinér P, Kärkäs MD, Chem. Sci 2021, 12, 5430–5437; [PubMed: 34168785] j) Yin H, Zheng M, Chen H, Wang S, Zhou Q, Zhang Q, Wang P, J. Am. Chem. Soc 2020, 142, 14201–14209; [PubMed: 32787248] k) Ji P, Zhang Y, Dong Y, Huang H, Wei Y, Wang W, Org. Lett 2020, 22, 1557–1562; [PubMed: 32045253] l) Shah AA, Kelly MJ, Perkins JJ, Org. Lett 2020, 22, 2196–2200; [PubMed: 32109071] m) Reich D, Trowbridge A, Gaunt MJ, Angew. Chem. Int. Ed 2020, 59, 2256–2261;n) Trowbridge A, Reich D, Gaunt MJ, Nature 2018, 561, 522–527; [PubMed: 30258135] o) Merkens K, Aguilar Troyano FJ, Djossou J, Gómez-Suárez A, Adv. Synth. Catal 2020, 362, 2354–2359;p) Sim J, Campbell MW, Molander GA, ACS Catal 2019, 9, 1558–1563; [PubMed: 31588366] q) Rossolini T, Ferko B, Dixon DJ, Org. Lett 2019, 21, 6668–6673; [PubMed: 31397159] r) Rossolini T, Leitch JA, Grainger R, Dixon DJ, Org. Lett 2018, 20, 6794–6798; [PubMed: 30350662] s) Aycock RA,

Pratt CJ, Jui NT, ACS Catal 2018, 8, 9115–9119;t) van Lier RCW, de Bruijn AD, Roelfes G, Chem. Eur. J 2021, 27, 1430–1437; [PubMed: 32896943] u) Josephson B, Fehl C, Isenegger PG, Nadal S, Wright TH, Poh AWJ, Bower BJ, Giltrap AM, Chen L, Batchelor-McAuley C, Roper G, Arisa O, Sap JBI, Kawamura A, Baldwin AJ, Mohammed S, Compton RG, Gouverneur V, Davis BG, Nature 2020, 585, 530–537; [PubMed: 32968259] v) Brandhofer T, Mancheño OG, ChemCatChem 2019, 11, 3797–3801;w) Yamamoto H, Wu A, Synfacts 2020, 16, 1372.

[14]. Immel JR, Chilamari M, Bloom S, Chem. Sci 2021, 12, 10083–10091. [PubMed: 34377401]

[15]. Knapp DM, Gillis EP, Burke MD, J. Am. Chem. Soc 2009, 131, 6961–6963. [PubMed: 19405470]

[16]. Chilamari M, Immel JR, Bloom S, ACS Catal 2020, 10, 12727–12737.

[17]. Wang H, Li W-G, Zeng K, Wu Y-J, Zhang Y, Xu T-L, Chen Y, Angew. Chem. Int. Ed 2019, 58, 561–565.

[18]. Zhang L, Floyd BM, Chilamari M, Mapes J, Swaminathan J, Bloom S, Marcotte EM, Anslyn EV, ACS Chem. Biol 2021, 16, 2595–2603. [PubMed: 34734691]

[19]. Li BX, Kim DK, Bloom S, Huang RY-C, Qiao JX, Ewing WR, Oblinsky DG, Scholes GD, MacMillan DWC, Nat. Chem 2021, 13, 902–908. [PubMed: 34183819]

[20]. Tower SJ, Hetcher WJ, Myers TE, Kuehl NJ, Taylor MT, J. Am. Chem. Soc 2020, 142, 9112–9118. [PubMed: 32348670]

[21]. Taylor MT, Nelson JE, Suero MG, Gaunt MJ, Nature 2018, 562, 563–568. [PubMed: 30323287]

[22]. Kim J, Li BX, Huang RY-C, Qiao JX, Ewing WR, MacMillan DWC, J. Am. Chem. Soc 2020, 142, 21260–21266. [PubMed: 33290649]

[23]. Chen X, Ye F, Luo X, Liu X, Zhao J, Wang S, Zhou Q, Chen G, Wang P, J. Am. Chem. Soc 2019, 141, 18230–18237. [PubMed: 31635455]

[24]. Huvaere K, Skibsted LH, J. Am. Chem. Soc 2009, 131, 8049–8060. [PubMed: 19459626]

[25]. Wang S, Zhou Q, Zhang X, Wang P, Angew. Chem. Int. Ed 2022, 61, e202111388.

[26]. Bloom S, Liu C, Kölmel DK, Qiao JX, Zhang Y, Poss MA, Ewing WR, MacMillan DWC, Nat. Chem 2018, 10, 205–211. [PubMed: 29359756]

[27]. Forster S, Thumser AE, Hood SR, Plant N, PLOS ONE 2012, 7, e33253. [PubMed: 22470447]

[28]. Chen YY, Wood AW, Bioelectromagnetics 2009, 30, 583–590. [PubMed: 19507188]

[29]. Oyekanmi AA, Ahmad A, Hossain K, Rafatullah M, J. Mol. Liq 2019, 281, 48–58.

[30]. Seath CP, Vogt DB, Xu Z, Boyington AJ, Jui NT, J. Am. Chem. Soc 2018, 140, 15525–15534. [PubMed: 30354095]

[31]. Zeman CJ, Kim S, Zhang F, Schanze KS, J. Am. Chem. Soc 2020, 142, 2204–2207. [PubMed: 31927964]

[32]. Szostak M, Sautier B, Spain M, Procter DJ, Org. Lett 2014, 16, 1092–1095. [PubMed: 24494855]

[33]. Capperucci A, Tanini D, Chemistry 2022, 4, 77–97.

[34]. Kozak A; Shapiro I Novel non-coding heterocyclic amino acids (nchaa) and their use as herbicides, 2020, WO/2020/250223.

[35]. Zhang W, Xiang X-X, Chen J, Yang C, Pan Y-L, Cheng J-P, Meng Q, Li X, Nat. Commun 2020, 11, 638. [PubMed: 32005825]

[36]. Reha-Krantz LJ, Hariharan C, Subuddhi U, Xia S, Zhao C, Beckman J, Christian T, Konigsberg W, Biochemistry 2011, 50, 10136–10149. [PubMed: 22023103]

[37]. Leconte AM, Hwang GT, Matsuda S, Capek P, Hari Y, Romesberg FE, J. Am. Chem. Soc 2008, 130, 2336–2343. [PubMed: 18217762]

[38]. Xu Y, Wang Y, Liu P, Chu G-C, Xu H, Li Y-M, Wang J, Shi J, Org. Biomol. Chem 2018, 16, 7036–7040. [PubMed: 30238118]

[39]. Troy CM, Stefanis L, Prochiantz A, Greene LA, Shelanski ML, Proc. Natl. Acad. Sci 1996, 93, 5635–5640. [PubMed: 8643629]

[40]. Kalmodia S, Vandhana S, Tejaswini Rama BR, Jayashree B, Sreenivasan Seethalakshmi T, Umashankar V, Yang W, Barrow CJ, Krishnakumar S, Elchuri SV, Cancer Nanotechnol 2016, 7, 1. [PubMed: 26900409]

[41]. Stoeltzing O, Liu W, Reinmuth N, Fan F, Parry GC, Parikh AA, McCarty MF, Bucana CD, Mazar AP, Ellis LM, Int. J. Cancer 2003, 104, 496–503. [PubMed: 12584749]

[42]. Shinitzky M, Fridkin M, Biochim. Biophys. Acta 1976, 434, 137–143. [PubMed: 7307]

[43]. Millar RP, Flanagan CA, Milton RC, King JA, J. Biol. Chem 1989, 264, 21007–21013. [PubMed: 2556391]

[44]. Porta R, Benaglia M, Puglisi A, Org. Process Res. Dev 2016, 20, 2–25.

[45]. Pastre JC, Browne DL, Ley SV, Chem. Soc. Rev 2013, 42, 8849–8869. [PubMed: 23999700]

[46]. Quartararo AJ, Gates ZP, Somsen BA, Hartrampf N, Ye X, Shimada A, Kajihara Y, Ottmann C, Pentelute BL, Nat. Commun 2020, 11, 3183. [PubMed: 32576815]

[47]. Bottecchia C, Erdmann N, Tijssen PMA, Milroy L-G, Brunsveld L, Hessel V, Noël T, ChemSusChem 2016, 9, 1781–1785. [PubMed: 27329945]

[48]. Talla A, Driessen B, Straathof NJW, Milroy L-G, Brunsveld L, Hessel V, Noël T, Adv. Synth. Catal 2015, 357, 2180–2186.

[49]. Emmanuel N, Mendoza C, Winter M, Horn CR, Vizza A, Dreesen L, Heinrichs B, Monbaliu J-CM, Org. Process Res. Dev 2017, 21, 1435–1438.

[50]. Bottecchia C, Rubens M, Gunnoo SB, Hessel V, Madder A, Noël T, Angew. Chem. Int. Ed 2017, 56, 12702–12707.

[51]. Vara BA, Li X, Berritt S, Walters CR, Petersson EJ, Molander GA, Chem. Sci 2018, 9, 336–344. [PubMed: 29629102]

[52]. Su J, Mo J-N, Chen X, Umanzor A, Zhang Z, Houk KN, Zhao J, Angew. Chem. Int. Ed 2022, 61, e202112668.

[53]. Pischel U, Zhang X, Hellrung B, Haselbach E, Muller P-A, Nau WM, J. Am. Chem. Soc 2000, 122, 2027–2034.

[54]. Yoshioka E, Kohtani S, Jichu T, Fukazawa T, Nagai T, Kawashima A, Takemoto Y, Miyabe H, J. Org. Chem 2016, 81, 7217–7229. [PubMed: 27314306]

[55]. Montalti M, Credi A, Prodi L, & Gandolfi MT Handbook of Photochemistry (3rd ed.). 2006, CRC Press.

[56]. O'Reilly JE, Elving PJ, J. Electroanal. Chem 1977, 75, 507–522.

[57]. DeLaive PJ, Foreman TK, Giannotti C, Whitten DG, J. Am. Chem. Soc 1980, 102, 5627–5631.

[58]. Nicastri MC, Lehnherr D, Lam Y, DiRocco DA, Rovis T, J. Am. Chem. Soc 2020, 142, 987–998. [PubMed: 31904228]

[59]. Wang M, Wang C, Huo Y, Dang X, Xue H, Liu L, Chai H, Xie X, Li Z, Lu D, Xu Z, Nat. Commun 2021, 12, 6873. [PubMed: 34824205]

[60]. Yurchenko O, Freytag D, zur Borg L, Zentel R, Heinze J, Ludwigs S, J. Phys. Chem. B 2012, 116, 30–39. [PubMed: 22148470]

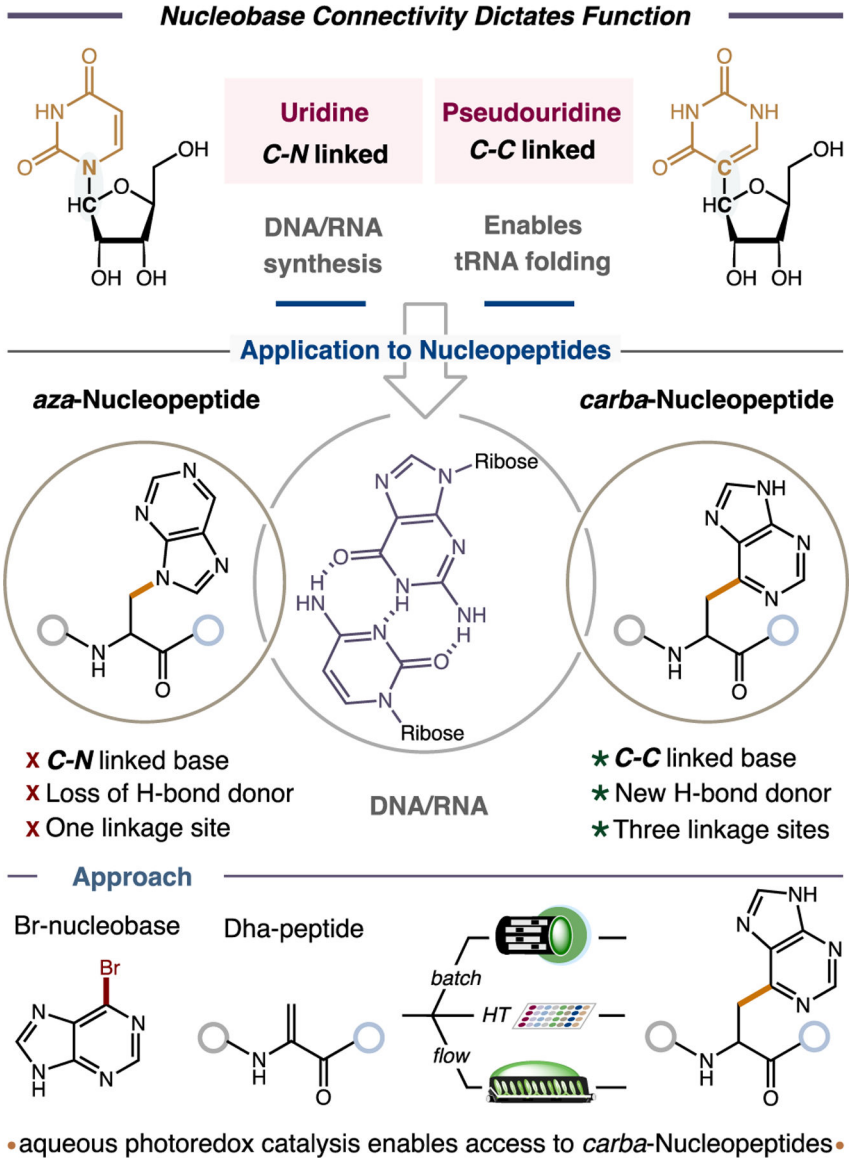


Figure 1.

The connectivity of a nucleobase can dictate its role in biological systems as demonstrated by the endogenous nucleobase pseudouridine. Applying this concept to Nucleopeptides creates a brand new class of molecules called carba-Nucleopeptides with untapped functions that could be synthesized through radical-Dha coupling.

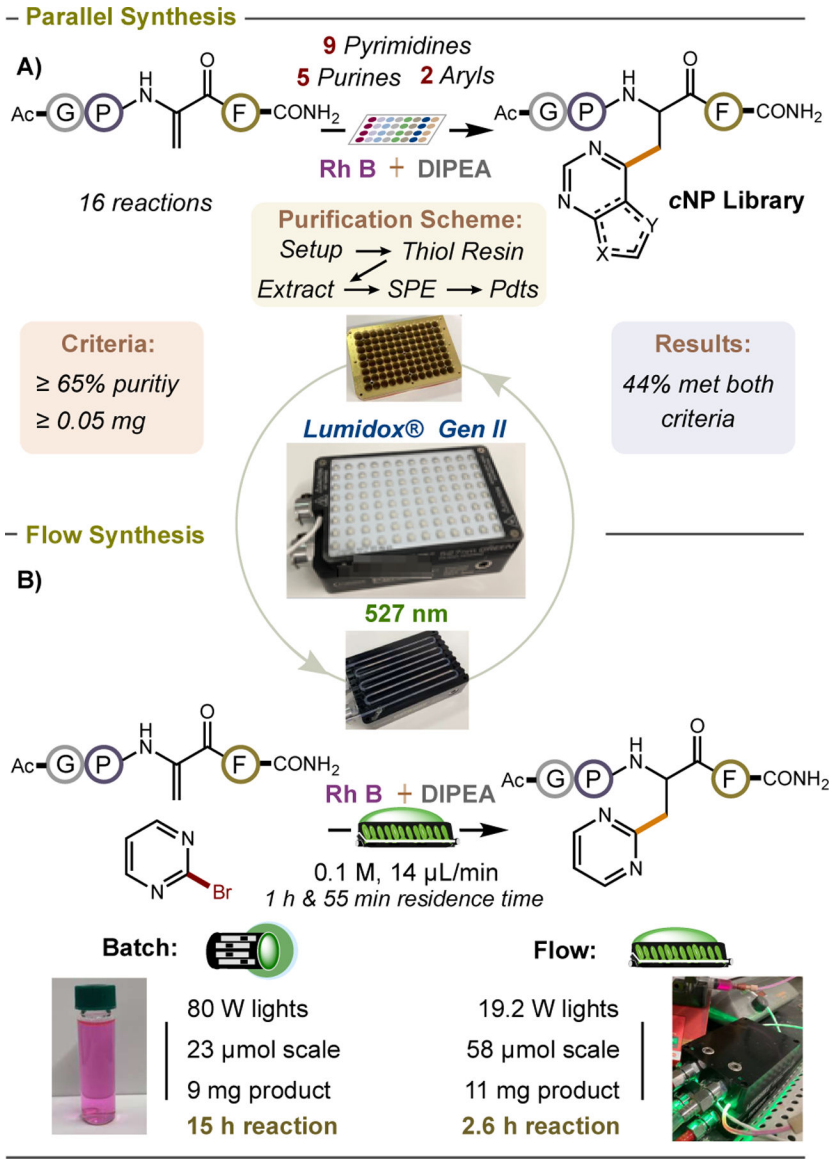


Figure 2.

Merging cNP synthesis with the Lumidox® Gen II system for applications in A) parallel synthesis of cNP libraries and B) flow synthesis of cNPs.

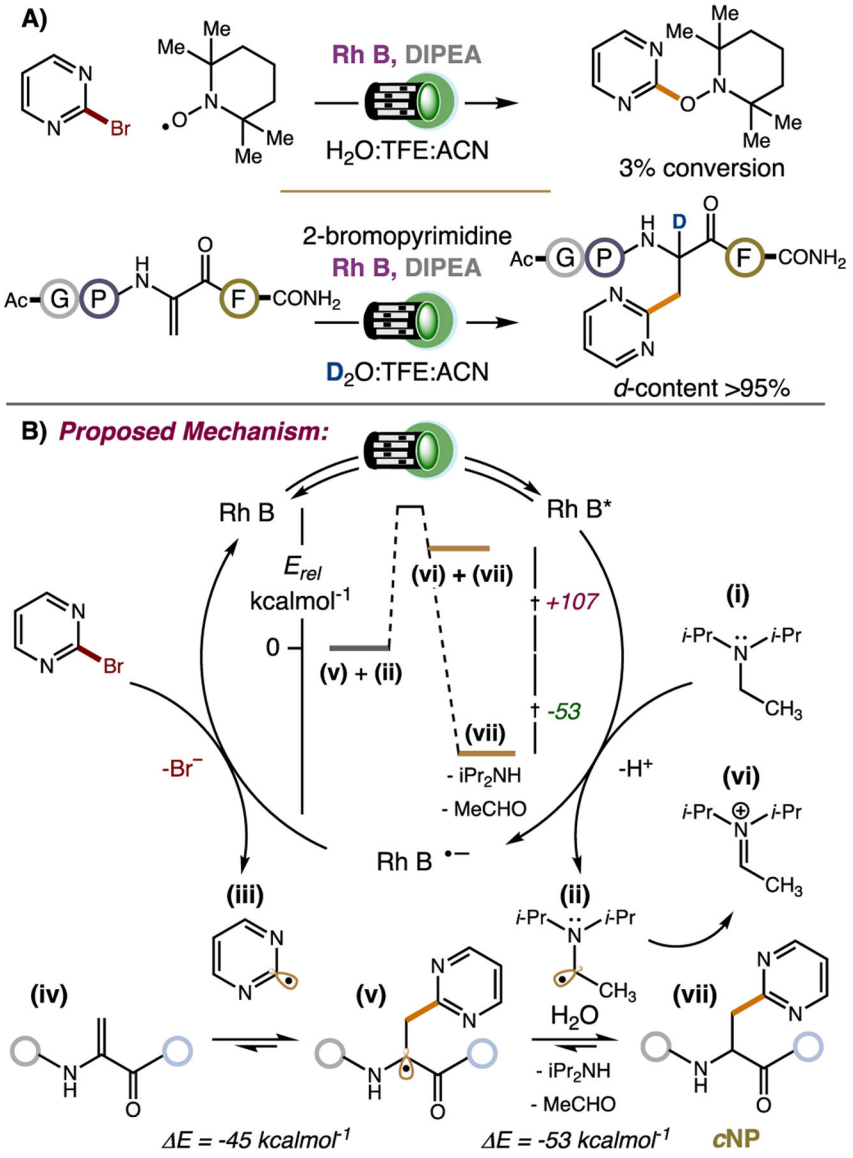
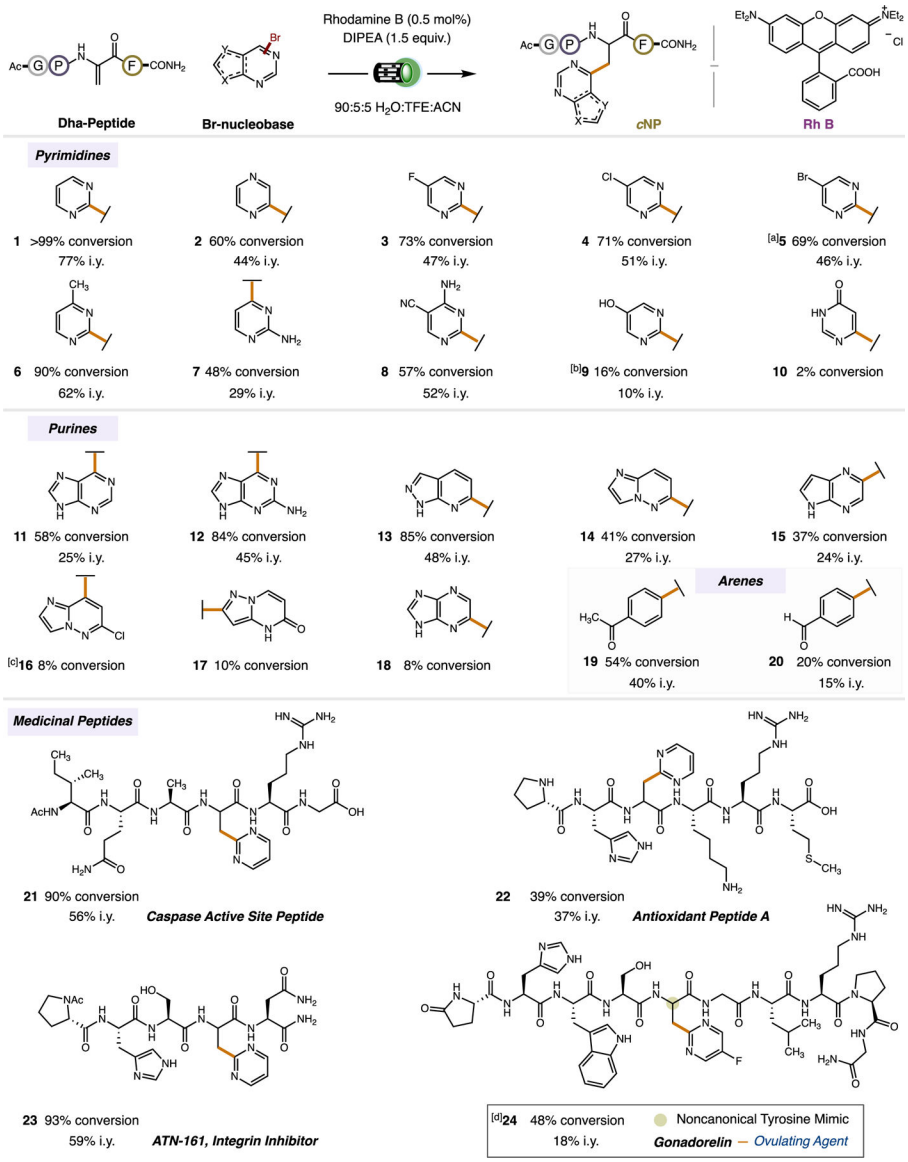


Figure 3.

A) TEMPO trapping and a deuterium labeling experiment. B) Proposed mechansim for cNP synthesis.

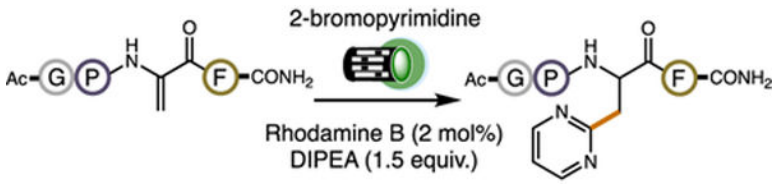


Scheme 1.

Scope of nucleobase bromides and medicinal peptides. All reactions were performed using 23 pmol of Ac-GPDhaF-$CONH_2$ or other Dhapeptide, 5 equiv. of bromonucleobase, 0.5 mol% of Rhodamine B, and 1.5 equiv. of diisopropylethylamine in a solution of 90:5:5 H_20:ACN:TFE (3 mM overall concentration) with 15 h of Green light (525 nm) irridation. [a] **5** yielded two cNP products giving a 13:1 selectivity for the C2-alkylated cNP *vs.* C5-alkylated cNP. [b] Reaction was performed with 5-OTs-2-bromopyrimidine. The Tosyl group deprotected under the reaction conditions to yield cNP **9.** [c] Reaction performed with 4 mol% Rhodamine B. [d] Reaction performed on a 10 pmol scale. Conversion [%] is defined as the % area of the product peaks at 214 nm as compared to all other peptide peaks in the LC chromatQ4ram. i.y. = isolated yield.

Table 1.

Reaction optimization parameters.



Entry	Deviation from standard conditions[a]	Conversion [%][b]
1	none	87
2	no Rhodamine B	0
3	no light	0
4	no DIPEA	<5
5	triphenylamine as amine	<5
6[c]	$Ir(ppy)_2(dtbby)PF_6$ as photocatalyst	21
7[c]	$Ru(bpy)_3Cl_2$ as photocatalyst	14
8	23 μmol scale (0.5 mol% photocatalyst)	>99

[a]Reactions performed using 2.3 μmol of Ac-GPDhaF-$CONH_2$, 5 equiv. of 2-bromopyrimidine, 2 mol% of Rhodamine B, and 1.5 equiv. of amine in a solution of 90:5:5 H_20:ACN:TFE (3 mM overall concentration) with 15 h of Green light (525 nm) irridation.

[b]Conversion [%] defined as the % area of the product peaks at 214 nm as compared to all other peptide peaks in the LC chromatogram.

[c]Reaction performed with two Blue lights (440 nm). ppy — 2-phenylpyridine, dtbbpy — 4,4’-di-tert-butyl-2,2’-dipyridyl, bpy — 2,2’-bipyridine