JOC The Journal of Organic Chemistry

pubs.acs.org/joc Article

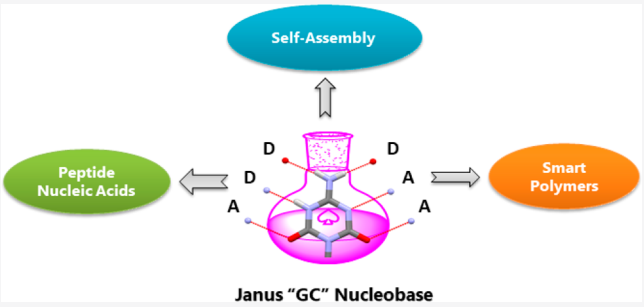
Triazine-Based Janus G–C Nucleobase as a Building Block for Self-Assembly, Peptide Nucleic Acids, and Smart Polymers

Chhuttan L. Meena, Dharmendra Singh, Bhavya Kizhakeetil, Manasa Prasad, Malini George, Srinu Tothadi, and Gangadhar J. Sanjayan*

Cite This: J. Org. Chem. 2021, 86, 3186–3195 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: This communication reports on the utility of a triazine-based self-assembling system, reminiscent of a Janus G–C nucleobase, as a building block for developing (1) supramolecular polymers, (2) peptide nucleic acids (PNAs), and (3) smart polymers. The strategically positioned self-complementary triple H-bonding arrays DDA and AAD facilitate efficient self-assembly, leading to a linear supramolecular polymer.



INTRODUCTION

Molecular self-assembly has ushered into prominence as a powerful strategy to construct complex nanostructures with innovative applications in diverse areas such as chemical synthesis,[1,2] polymer science,[3] nanotechnology,[4] smart materials,[5] and tissue engineering.[6] *De novo* designed self-assembling systems have been proven to be useful for developing functional polymers, supramolecular hydrogels, and strong adhesives[7] with a range of attractive mechanical characteristics suitable for medical applications and fabricating advanced materials.[8,9] By virtue of their structural similarity with nucleobases,[10] synthetic self-assembling systems have also found application in developing novel peptide nucleic acids (PNAs)—a class of nucleic acid mimics made of a *pseudopeptide* backbone with excellent recognition-specificity.[11] Of particular interest is the use of self-assembling motifs featuring multiple H-bonding arrays within the molecule as bifacial nucleobases (also called as "Janus bases") to target double-stranded DNA or RNA by engaging both strands at once.[12] Inspired by the nucleic acid base pairing, chemists have over the years developed a myriad of self-assembling molecules with diverse H-bonding characteristics.[13] GC hybrid molecules that are capable of forming intriguing rosette structures[14a,b] and linear supramolecular polymer networks[14c,d] have also been reported. One of the extensively studied self-assembling motifs that instantaneously forms supramolecular polymers is the melamine-cyanuric/barbituric acid complementary dual motif (MA-CA/BA) (Figure 1, top).[15]

Depending upon the substitution pattern on melamine, MA-CA/BA assembly can give rise to three different types of aggregates: the cyclic rosettes (finite), linear tapes (infinite), and crinkled tapes (infinite).[16] However, it should be

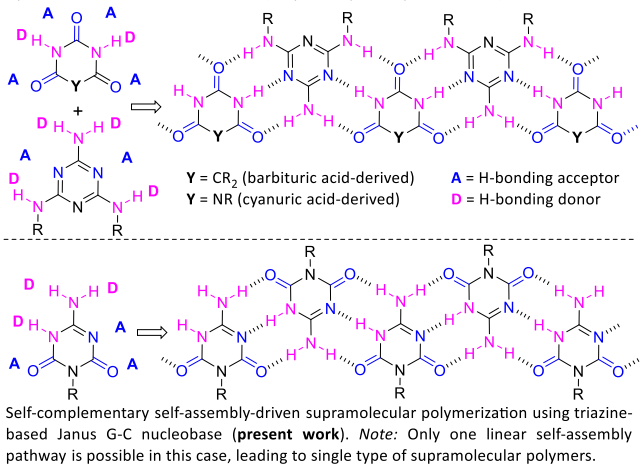


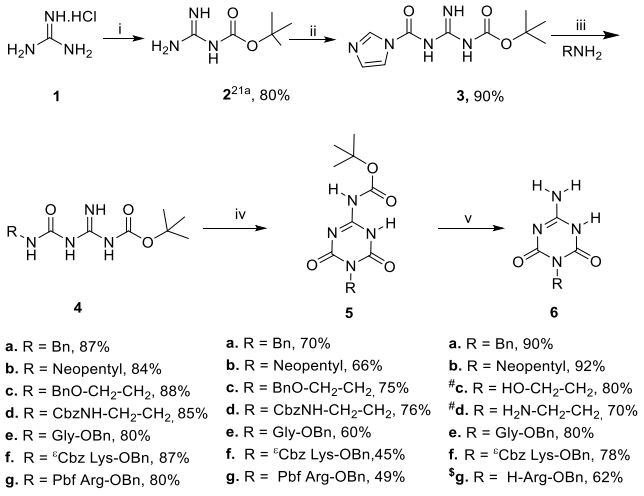
Figure 1. Complementary assembly of barbituric/cyanuric acid-melamine dual motif (top; reported[15,16]) and self-complementary self-assembly driven supramolecular polymerization of triazine-based Janus G–C nucleobase (bottom; this work).

Received: October 24, 2020
Published: February 1, 2021

emphasized that the multiple possibilities of self-assembly pathways will be detrimental to applications particularly when formation of a mixture of polymeric aggregates is not desired.[17] Furthermore, the need to maintain an equimolar proportion of the complementary self-assembling components for effecting self-assembly further adds to the practical difficulties.[18]

In this context, use of self-complementary self-assembling motifs capable of instant supramolecular polymerization but devoid of multiple assembly pathways could be of considerable advantage for practical applications. Herein, we disclose the self-assembling characteristics of a Janus G−C-base nucleic acid motif, capable of efficient self-assembly leading "exclusively" to a single set of linear supramolecular polymer—without any possibilities of multiple pathways of self-assembly and thus avoiding the formation of a mixture of supramolecular polymers (Figure 1, *vide supra*). Instantaneous supramolecular polymerization (and thus negating the prerequisite to mix individual complementary self-assembling components) coupled with its exquisite nature of following a single pathway of self-assembly (and thus avoiding a mixture of supramolecular polymers) would stand out as prominent advantages of this system which may usher its application potential particularly in the delicate areas of material and medical applications which demand structural homogeneity of the materials.[19] Owing to its structural resemblance to nucleobases, we envisaged that the self-assembling motif **6** (Scheme 1, *vide infra*), a multifaceted nucleobase featuring

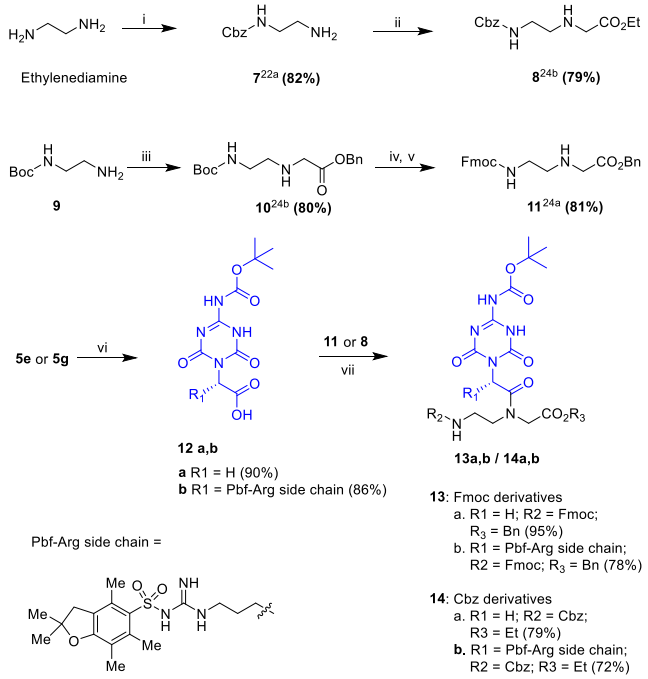
Scheme 1. Synthesis of a Triazine-Based Janus G−C Base as Potential Building Blocks for Self-Assembly[a]



[a]Reagents and conditions: (i) NaOH, $(Boc)_2O$, water, acetone; (ii) CDI, DCM, rt, 5 h; (iii) DIPEA, ACN, 50 °C, 4−5 h; (iv) CDI, $NaHCO_3$, ACN, 48 h, rt; (v) 50% TFA:DCM (1:1), 0 °C, 45 min. Notes: #, **5c** and **5d** were first debenzylated using H_2/Pd−C prior to Boc deprotection to afford **6c** and **6d**; $, **5g** was subjected to deprotection using 95% TFA in DCM to simultaneously deblock both pbf and BOC. Experimental details are in the Supporting Information.

both G and C faces within the molecule, would also find application in developing novel peptide nucleic acids (PNAs) with distinctive properties.[20a] In this regard, we synthesized novel *ready-to-be-used* peptide nucleic acid (PNA) monomer building blocks **13a,b** and **14a,b**, suitable for Fmoc-based solid/solution-phase synthesis and Cbz-based solution-phase synthesis (Scheme 2). It is noteworthy that PNAs carrying multifaceted nucleobases have found innovative applications to

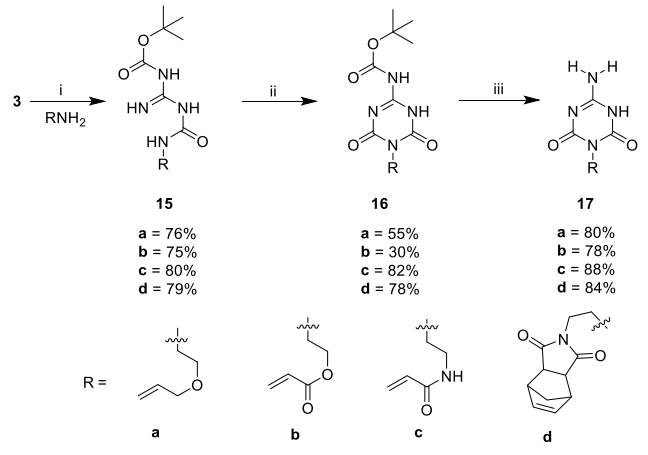
Scheme 2. Synthesis of a Triazine-Based Janus G−C Nucleobase Containing PNA Building Blocks[a]



[a]Reagents and conditions: (i) Cbz−OSu, $CHCl_3$:water (1:1); (ii) ethyl bromoacetate, ACN, K_2CO_3, 24 h, rt; (iii) DCM, TEA, benzyl bromoacetate 2 h, rt; (iv) TFA:DCM (1:1), 40 min, 0 °C; (v) Fmoc−OSu, TEA, DCM, 2 h; (vi) H_2/Pd−C, MeOH, rt; (vii) HBTU, HOBt, DIPEA, DMF, 12 h, rt.

target double-stranded nucleic acids by engaging both strands simultaneously.[20b] We also disclose the synthesis of several value-added *ready-to-be-used* monomer building blocks for polymer synthesis such as acrylamide, acrylic ester, allyl, and norbornene imide (for ROMP-ring-opening metathesis polymerization)[20c,d] derivatives **16a−d**, featuring the Janus G−C nucleobase, which may find application in developing functional polymers (Scheme 3).

Scheme 3. Synthesis of a Triazine-Based Janus G−C Nucleobase Containing Smart Polymer Building Blocks[a]



[a]Reagents and Conditions: (i) DIPEA, ACN, 45−50 °C, 4 h; (ii) CDI, $NaHCO_3$, ACN, RT, 48 h; (iii) TFA, DCM (1:1), 30 min.

https://dx.doi.org/10.1021/acs.joc.0c02530
J. Org. Chem. 2021, 86, 3186−3195

RESULTS AND DISCUSSION

We began the work with the synthesis of the triazine-based Janus G−C base **6** as potential building blocks for self-assembly (Scheme 1). Straightaway synthesis of unprotected **6** was thought to be practically imprudent primarily owing to the perceived high degree of aggregation and solubility issues which could be triggered by supramolecular polymerization aided by two sets of self-complementary H-bonding arrays within the molecule (see the crystal structure of **6d** in Figure 2,

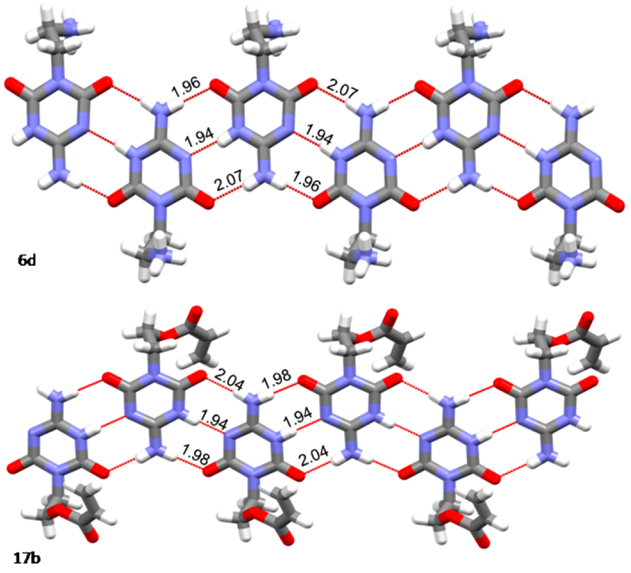


Figure 2. Single-crystal X-ray structures of triazine-based Janus G−C nucleobases **6d** and **17b** showing supramolecular self-assembly. H-bonding is highlighted in dashes (red color), above which H-bond distances (N−H···N, N···H−N, and N−H···O) are displayed in Å. This figure was made using Mercury software. Note: Crystal structure of **17d** given in the Supporting Information (p S90).

vide infra). Such a consideration was further reinforced owing to the apparent difficulties in working with insoluble value-added building blocks for application purposes. Therefore, we designed a strategy to first obtain the Janus G−C nucleobase **6** in a carbamate-protected form devoid of solubility issues—by masking the H-bonding codes, as in **5**, which can be readily converted to the free Janus G−C nucleobase **6**, as and when required (Scheme 1).

The BOC-protected Janus G−C nucleobase **5** having diverse N-substituents could be efficiently synthesized in four steps by starting from guanidine **1**, as outlined in Scheme 1. After several attempts, we found that the imidazolecarbonyl-coupled **3** could serve as a valuable reactive intermediate which could readily react with amines under mild conditions to form amidino urea **4**, which was amenable to cyclization to afford **5**. Thus, as per this plan, N-Boc-guanidine **2**, readily obtainable from guanidine by mono Boc protection,[21a] was carefully reacted with carbonyldiimidazole (CDI) to afford the reactive intermediate **3**, which was sufficiently stable to be isolated and could be preserved under ambient conditions. The novel intermediate **3** upon reaction with various primary amines furnished amidino urea **4**, which could be easily cyclized by reacting with CDI, yielding **5**. Boc deprotection of **5** finally furnished the Janus G−C nucleobase **6**, with characteristic poor solubility in common organic solvents as anticipated—obviously owing to extensive aggregation triggered by supramolecular polymerization.

PNA monomer building blocks carrying unnatural nucleobases have found application in developing PNAs for site-specific interaction with DNAs and RNAs. PNA oligomers have been shown to inhibit transcription (antigene) and translation (antisense) of genes by tight binding to DNA or mRNA.[23,24] In this context, we have synthesized a novel class of PNA building blocks **13** and **14** (Scheme 2, *vide infra*) carrying the triazine-based Janus G−C nucleobase featuring orthogonally protected backbones, which could find application in developing PNAs useful for DNA/RNA interaction/recognition studies.[25]

The PNA building blocks **13** and **14** carrying an Fmoc N-terminus protecting group which are ready for PNA synthesis on the solid phase were obtained following the synthetic routes as given in Scheme 2 (*vide infra*). **5e** (glycine-derived) and **5g** (arginine-derived) Boc-protected triazine benzyl esters were first debenzylated to afford the free carboxylic acid **12**, which were independently coupled with Fmoc-protected benzyl (2-aminoethyl)glycinate (Fmoc-aeg-OBn) **11** and Cbz-protected ethyl (2-aminoethyl) glycinate (Cbz-aeg-OEt) **8** to obtain the Fmoc analogue **13** and Cbz analogue **14**, respectively—thus furnishing the valuable triazine-based Janus G−C nucleobase-containing PNA building blocks which are useful for Fmoc-based solid-phase synthesis or Cbz-based solution-phase synthesis of PNAs.

Hydrogen-bonding plays a vital role in the designing of smart polymers and functional materials. Monomers containing self-assembling motifs offer the opportunity of developing materials featuring controlled self-assembly.[7a,26] In this context, we have synthesized a class of novel polymer building blocks **16** containing the Janus G−C nucleobase unit in a protected form (Scheme 3, *vide infra*). It is noteworthy that the Boc protecting group in the building blocks **16** would serve as a deterrent to effectively prevent self-assembly which would cause solubility issues during "covalent polymerization". Once deprotected after covalent polymerization, the Janus G−C nucleobase being self-complementary can trigger 3 + 3 type H-bonded "supramolecular polymerization", which could substantially influence the overall property of the polymers. The polymer building blocks **16** were synthesized by a synthetic route depicted in Scheme 3. The novel imidazole carbonyl-coupled reactive intermediate **3** upon reaction with various amines afforded amidino urea **15**, which could be easily cyclized by reacting with CDI, yielding **16** from which the free amino triazine **17** can be obtained quickly by Boc deprotection with TFA−DCM.

In order to provide insights into the structural details of self-assembly of the Janus G−C nucleobase, we made extensive attempts for their crystallization, even though many of them had poor solubility in common organic solvents.

However, after tenacious efforts, three molecules could be crystallized: **6d** (Scheme 1) and the polymer monomer building blocks **17b** and **17d** (Scheme 3). Whereas **6d** could be crystallized from hot aqueous methanol containing traces of HCl, **17b** and **17d** could be crystallized from DMSO (Figure 2, *vide supra*; crystal structure of **17d** given in the Supporting Information, p S90). Analysis of their single-crystal X-ray structure revealed the anticipated 3 + 3 repeating H-bonding. Each molecule is held from both sides by two sets of DDA−AAD-type triple hydrogen-bonding arrays—reminiscent of the guanine−cytosine G≡C-type triple H-bonding pattern seen in

https://dx.doi.org/10.1021/acs.joc.0c02530
J. Org. Chem. 2021, 86, 3186−3195

nucleic acids. It is noteworthy that the H-bonding parameters of **6d** and **17b** are comparable to those of native G−C base pairs.[27] Owing to self-complementarity, the DDA−AAD-type triple hydrogen-bonding leads to supramolecular polymer formation, as expected. A notable feature of this supramolecular assembly is that, unlike the CA−MA motif which often leads to the formation of mixtures of cyclic rosette/crinkled tape/linear self-assembled structures, our Janus G−C nucleobase leads to the formation of a single set of supramolecular polymer owing to the orthogonal positioning of the DDA−AAD H-bonding arrays within the molecule—increasing the hope for its application in sensitive areas wherein material homogeneity is warranted.

CONCLUSION

In conclusion, we have developed a novel class of bifacial triple hydrogen-bonding G−C motif **6**, inspired by DNA base pairing. These triazine-based Janus G−C nucleobases could serve as potential building blocks for multiple application purposes *vis-a-vis*: molecular self-assembly, nucleic acid interactions, and smart polymers. The Janus G−C base, endowed with self-complementary H-bonding codes reminiscent of guanine (G) and cytosine (C) nucleic acid bases, is capable of undergoing efficient self-assembly leading to supramolecular polymers, as is unequivocally evident from crystal structure studies. This work also describes the development of novel ready-to-use PNA monomers **13** and **14** featuring multifacial recognition sites within the molecule. The orthogonally protected PNA building blocks carrying the triazine-based Janus G−C nucleobase could find application in developing PNAs useful for DNA/RNA recognition studies. We have also developed a novel class of polymer building blocks **16** carrying multifacial H-bonding sites with potential utility in the development of smart/self-healing/functional polymers. Structural investigations unambiguously showed their G−C-like H-bonding base pairing and eventual supramolecular self-assembly owing to self-complementarity of the molecule. We are currently exploring their utility in diverse domains of areas of applications, as envisaged above, and will report the results in due course.

EXPERIMENTAL SECTION

General Information. All reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. All reactions were carried out with laboratory grade solvent, and the purity of intermediates and final compounds was checked on precoated silica gel G254 TLC plates (Merck). Visualization of spot on TLC was achieved by the use of UV light (254 nm) ninhydrin. The oil bath was used as a heating source for reactions. All synthesized compounds were purified via silica gel column chromatography by using 100−200 and 230−400 mesh silica gel. Melting points were recorded by MEL-TEMP; melting points are uncorrected; and ^{1}H, ^{13}C {^{1}H}, and DEPT-135 spectra were recorded on Bruker AV-400 and AV-500 MHz instruments, using $CDCl_3$/DMSO-d^6 as solvents and TMS as internal standard. ^{1}H NMR and ^{13}C{^{1}H} NMR chemical shifts are expressed in parts per million (ppm, δ scale) and were referenced to NMR solvent $CDCl_3$ δ 7.26, 77 ppm and DMSO-d^6 δ 2.5, 39.8 ppm, respectively. The following abbreviations were used to describe peak patterns when appropriate; bs = broad singlet, s = singlet, d = doublet, t = triplet, q = quartet, and m = multiplet. High resolution mass spectra (HRMS) were recorded on an Agilent LC/MSD TOF mass spectrometer by electrospray ionization time off light reflection (ESI-TOF) experiments. Single-crystal data were collected using a Bruker SMART APEX II single-crystal X-ray CCD.

Compound **2**. The synthesis of compound **2** was as per an earlier reported procedure.[21a] Guanidinium chloride monohydrate (23 g, 1 equiv, 240 mmol) was dissolved in water (48 mL), and NaOH was added (19.2 g, 2 equiv, 481 mmol). This was stirred at 0 °C for 15 min, followed by addition of a solution of di-*tert*-butyl dicarbonate (13.1 g, 0.25 equiv, 60 mmol) in acetone (200 mL) and stirring at room temperature for an additional 10 h. The solvent was removed under a vacuum, and the resultant residue was dissolved in water (30 mL) and extracted with ethyl acetate (3 × 100 mL). The organic layer was washed with brine solution, dried (Na_2SO_4), filtered, and concentrated *in vacuo* to dryness. The resultant residue was suspended in cold diethyl ether, and solid was filtered under a vacuum to afford compound **2** as a white powder. Yield 7.70 g (80%); R_f = 0.2 (silica gel TLC, 10% methanol in DCM); ^{1}H NMR (400 MHz, DMSO-d^6) δ 6.78 (bs, 4 H), 1.34 (s, 9 H); ^{13}C{^{1}H} NMR (100 MHz, DMSO-d^6) δ 163.9, 163.2, 76.0, 28.7, 28.7, 28.7; HRMS (ESI-TOF) *m*/*z*: [M + H]$^+$ Calcd for $C_6H_{14}N_3O_2$ 160.1081; Found 160.1079; [M + Na]$^+$ Calcd for $C_6H_{13}N_3O_2Na$ 182.0900; Found 182.0898.

Compound **3**. *N*-Boc-guanidine **2** (14 g, 87.1 equiv, 95 mmol, 1 equiv) was suspended in dichloromethane (50 mL), and 1,1-carbonyldiimidazole (CDI) was added (15.69 g, 1.1 equiv, 96.94 mmol). The solution was stirred at room temperature for 5 h. The resultant solution was washed with water (3 × 30 mL), dried (Na_2SO_4), and concentrated under a vacuum to afford **3** as a crystalline white solid, which was carried forward for the next step. Yield 20 g (90%); R_f = 0.3 tailing (silica gel TLC, 60% ethyl acetate in pet. ether); mp 155−160 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.08 (bs, 1 H), 9.27 (bs, 1 H), 8.89 (bs, 1 H), 8.54 (s, 1 H), 7.54 (s, 1 H), 6.98 (s, 1 H), 1.55 (s, 9 H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 160.8, 157.9, 153.8, 137.8, 128.8, 117.0, 83.6, 28.1, 28.1, 28.1; HRMS (ESI-TOF) *m*/*z*: [M + H]$^+$ Calcd for $C_{10}H_{16}N_5O_3$ 254.1248; Found 254.1243.

Compound **4a**. The compound **3** (1 equiv, 2.00 g, 7.9 mmol) was dissolved in acetonitrile (50 mL), and benzyl amine was added (1.2 equiv, 1.01 g, 9.52 mmol). This mixture was stirred at 50 °C in an oil bath for 4 h. The reaction mixture was cooled at room temperature and concentrated under *vacuo*. The resultant residue was dissolved in ethyl acetate (100 mL) and subsequently washed with diluted $KHSO_4$ solution (2 × 30 mL) and brine solution (30 mL) and dried (Na_2SO_4). The organic layer was concentrated under a vacuum to afford **4a** as a white solid. Yield 2.01 g (87%); mp 90−100 °C; R_f = 0.5 (silica gel TLC, 70% ethyl acetate in pet. ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.85 (bs, 3 H), 7.68 (bs, 1 H), 7.36−7.22 (m, 5 H), 4.37−4.35 (d, *J* = 5.34 Hz, 2 H), 1.48 (s, 9 H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 155.2, 154.2, 137.2, 128.8, 128.8, 128.8, 127.7, 127.6, 127.6, 86.0, 44.0, 27.9, 27.9, 27.9; HRMS (ESI-TOF) *m*/*z*: [M + H]$^+$ Calcd for $C_{14}H_{21}N_4O_3$ 293.1608; Found 293.1601; [M + Na]$^+$ Calcd for $C_{14}H_{20}N_4O_3Na$ 315.1428; Found 315.1419.

Compound **4b**. The synthetic method of **4a** was adopted to synthesize **4b**; white solid. Yield 1.81 g (84%); mp 100−110 °C; R_f = 0.5 tailing (silica gel TLC, 80% ethyl acetate in pet. ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.08 (bs, 3 H), 7.55−6.90 (bs, 1 H), 3.05 (s, 2 H), 1.53 (s, 9 H), 0.94 (s, 9 H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 159.4, 155.0, 154.0, 86.0, 51.4, 32.1, 27.8, 27.8, 27.8, 27.3, 27.2, 27.2; HRMS (ESI-TOF) *m*/*z*: [M + H]$^+$ Calcd for $C_{12}H_{25}N_4O_3$ 273.1921; Found 273.1914; [M + Na]$^+$ Calcd for $C_{12}H_{24}N_4O_3Na$ 295.1741; Found 295.1733.

Compound **4c**. *tert*-Butyl (2-(benzyloxy) ethyl) carbamate (5 g, 1 equiv, 19.89 mmol), which was synthesized as per an earlier reported procedure,[21b] was dissolved in 50% solution of TFA in DCM (50 mL) and stirred at 0 °C for 45 min. The solvent was evaporated under a vacuum and coevaporated with toluene (2 × 20 mL) to take off the residual TFA from the reaction mixture to afford TFA salt as a semisolid. The resultant TFA salt (1 equiv, 5.2 g, 1 equiv, 19.60 mmol) was dissolved in acetonitrile (40 mL), and compound **3** (1.1 equiv, 5.43 g, 21.56 mmol) and DIPEA (3 equiv, 10.22 mL, 58.81 mmol) were added. This mixture was stirred at 50 °C for 4 h. The resultant reaction mixture was cooled at room temperature and concentrated under a vacuum. The residue was dissolved in ethyl acetate (100 mL) and subsequently washed with dilute $KHSO_4$

https://dx.doi.org/10.1021/acs.joc.0c02530
J. Org. Chem. 2021, 86, 3186−3195

solution and brine solution, dried (Na_2SO_4), and concentrated under a vacuum for dryness to obtain crude **4c** as a white solid, which was purified by column chromatography using 10−90% ethyl acetate in pet. ether. The resultant white solid was triturated using cold 50% diethyl ether in pet. ether (10 mL) to afford **4c** as a pale semisolid. Yield 5.8 g (88%); R_f = 0.5 (silica gel TLC, 70% ethyl acetate in pet. ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.39 (bs, 1 H), 8.17 (bs, 1 H), 7.33−7.28 (m, 5 H), 6.33 (bs, 1 H), 4.51 (s, 2 H), 3.55−3.52 (m, 2 H), 3.41−3.37 (m, 2 H), 1.47 (s, 9 H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 158.4, 157.8, 138.0, 128.5, 128.5, 128.5, 127.9, 127.8, 127.8, 82.2, 73.2, 69.3, 39.9, 28.2. 28.2, 28.2; HRMS (ESI-TOF) m/z: [M + H]$^+$ Calcd for $C_{16}H_{25}N_4O_4$ 337.1870; Found 337.1867.

Compound ***4d***. The synthetic method of **4a** was adopted to synthesize **4d**. The benzyl (2-aminoethyl) carbamate was synthesized as per an earlier reported procedure.[22a] The resulting residue was directly purified by column chromatography using 100−200 mesh size and mobile 0−10% MeOH in dichloromethane. The solvent was removed under a vacuum, and the residue was triturated with diethyl ether to afford **4d** as an off-white solid. Yield 2.56 g (85%); mp 80−90 °C; R_f = 0.3 (silica gel, TLC, 10% methanol in DCM); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.00 (bs, 2 H), 7.32−7.28 (m, 5 H), 7.11 (bs, 1 H), 6.64 (bs, 1 H), 5.57 (bs, 1 H), 5.06 (bs, 2 H), 3.30 (m., 4 H), 1.47 (m, 9 H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 159.3, 158.8, 156.8, 156.8, 136.6, 128.6, 128.6, 128.1, 128.1, 128.1, 83.7, 66.8, 41.2, 40.0, 28.1, 28.1, 28.1; HRMS (ESI-TOF) m/z: [M + H]$^+$ Calcd for $C_{17}H_{26}N_5O_5$ 380.1928; Found 380.1924; [M + Na]$^+$ Calcd for $C_{17}H_{25}N_5O_5Na$ 402.1748; Found 402.1737.

Compound ***4e***. Compound **3** (2.00 g, 1 equiv, 7.9 mmol) was dissolved in acetonitrile (30 mL), and Gly−OBn−PTSA (1.2 equiv, 1.07 g, 9.48 mmol, which was synthesized by a previously reported procedure[21c]) and DIPEA (2.74 mL, 2 equiv 15.81 mmol) were added and then stirred at 50 °C in oil bath for 4 h. The reaction mixture was cooled at room temperature, diluted with ethyl acetate (70 mL), and subsequently washed with dilute $KHSO_4$ solution, brine solution and dried (Na_2SO_4). The resultant organic layer was concentrated under a vacuum to dryness and solid residue triturated with diethyl ether to afford **4e** as a white solid. Yield 2.23 g (80%); mp 110−115 °C; R_f = 0.4 (silica gel TLC, 80% ethyl acetate in pet. ether); ^{1}H NMR (400 MHz, DMSO-d^6) δ 10.83 (bs, 1 H), 9.37 (bs, 2 H), 8.65 (bs, 1 H), 7.35−7.32 (m, 5 H), 5.12 (s, 2 H), 3.96−3.94 (d, J = 6.1 Hz, 2 H), 1.44 (s, 9 H); ^{13}C{^{1}H} NMR (100 MHz, DMSO-d^6) δ 169.8, 154.8, 154.5, 152.6, 136.3, 129.0, 128.7, 128.7, 128.5, 126.0, 84.3, 66.7, 41.9, 28.1, 28.1, 28.1; HRMS (ESI-TOF) m/z: [M + H]$^+$ Calcd for $C_{16}H_{23}N_4O_5$ 351.1663; Found 351.1653; [M + Na]$^+$ Calcd for $C_{16}H_{22}N_4O_5Na$ 373.1482; Found 373.1470.

Compound ***4f***. Benzyl N^6-((benzyloxy)carbonyl)-L-lysinate TFA salt (2.1 g, 1 equiv, 4.33 mmol, which was synthesized by a previously reported method[21d]) was dissolved in acetonitrile (50 mL), in round-bottom flask, the compound **3** (1.20 g, 1.1 equiv, 4.76 mmol) and DIPEA (2.26 mL, 3 equiv, 13.00 mmol) were added; the mixture was stirred at 50 °C for 4−5 h. The resultant solution was cooled at room temperature and concentrated under a vacuum. The residue was diluted with ethyl acetate (100 mL) and subsequently washed with dilute $KHSO_4$ solution, brine solution and dried (Na_2SO_4). The solvent was evaporated under reduced pressure to afford **4f** as a white solid. Yield 2.3 g (87%); mp 80−90 °C; R_f = 0.4 (silica gel TLC, 80% ethyl acetate in pet. ether); ^{1}H NMR (500 MHz, DMSO-d^6) δ 9.86 (bs, 1 H), 8.23 (bs, 2 H), 7.72 (bs, 1 H), 7.36−7.32 (m, 10 H), 6.90 (t, J = 5.5 Hz, 1 H), 5.14−5.12 (m, 2 H), 5.00 (s, 2 H), 4.18−4.16 (d, J = 5.3 Hz, 1 H), 3.35 (s, 1 H), 2.96−2.95 (q, J = 6.6 Hz, 2 H), 1.68−1.65 (d, J = 8.0 Hz, 2 H), 1.43−1.38 (m, 11 H), 1.31−1.28 (m, 2 H); ^{13}C{^{1}H} NMR (125 MHz, DMSO-d^6) δ 172.7, 158.1, 156.5, 137.7, 136.4, 128.9, 128.8, 128.5, 128.3, 128.3, 128.2, 128.2, 127.8, 127.8, 127.6, 127.8, 79.1, 66.4, 65.6, 59.7, 53.1, 31.0, 29.4, 28.4, 28.4, 28.4, 23.0, 14.5; HRMS (ESI-TOF) m/z: [M + H]$^+$ Calcd for $C_{28}H_{38}N_5O_7$ 556.2771; Found 556.2766. Note: δ 4.10, 2.07, and 1.27 residual peaks of ethyl acetate.

Compound ***4g***. The BOC group deprotection of Benzyl N^2-(*tert*-butoxycarbonyl)-N^ω((2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-yl)sulfonyl)-L-argininate (5.00 g, 1 equiv, 8.11 mmol, which was synthesized by a previously reported method[21d]) by using 50% TFA in DCM (30 mL) stirred at ice-bath temperature for 45 min. The solvent was removed under a vacuum, and the remaining TFA was stripped off by coevaporating with toluene (2 × 20 mL) to afford TFA salt as a semisolid that was carried forward for the next step. The resultant TFA salt (5 g, 1 equiv, 7.92 mmol) was dissolved in acetonitrile (40 mL), and compound **3** (2.69 g, 1.1 equiv, 8.72 mmol) and DIPEA (4.13 mL, 3 equiv, 23.78 mmol) were added; the mixture was stirred at 50 °C for 4−5 h. The resultant solution was cooled at room temperature and concentrated under a vacuum. The residue was diluted with ethyl acetate (150 mL) and subsequently washed with dilute $KHSO_4$ solution and brine solution and then dried (Na_2SO_4). The solvent was evaporated under reduced pressure to afford **4g** as a white solid. Yield 5.43 g (80%); mp 90−100 °C; R_f = 0.3 (silica gel TLC, 80% ethyl acetate in pet. ether); ^{1}H NMR (400 MHz, DMSO-d^6) δ 9.01 (bs, 1 H), 8.31 (bs, 1 H), 8.13 (bs, 1 H), 7.36−7.33 (m, J = 5.4 Hz, 5 H), 6.74 (bs, 1 H), 6.45 (bs, 2 H), 5.10 (bs, 2 H), 4.23−4.21 (d, J = 5.3 Hz, 1 H), 3.05−304 (dd, J = 6.1, 10.2 Hz, 2 H), 2.92−2.69 (s, 2 H), 2.47 (s, 3 H), 2.42 (s, 3 H), 1.99 (s, 4 H), 1.97−1.90 (m, 2 H), 1.68 (bs, 1 H), 1.45 (s, 9 H), 1.39−1.38 (m, 8 H); ^{13}C{^{1}H} NMR (100 MHz, DMSO-d^6) δ 174.3, 171.3, 171.3, 157.2, 157.2, 155.8, 137.0, 135.5, 134.6, 131.2, 128.2, 128.2, 127.9, 127.7, 127.7, 124.1, 116.0, 86.1, 66.0, 52.4, 42.2, 31.3, 28.8, 28.0, 28.0, 28.0, 27.5, 27.5, 18.7, 17.4, 13.4, 12.0; HRMS (ESI-TOF) m/z: [M + H]$^+$ Calcd for $C_{33}H_{48}N_7O_8S$ 702.3280; Found 702.3274; [M + Na]$^+$ Calcd for $C_{33}H_{47}N_7O_8SNa$ 724.3099; Found 724.3082.

Compound ***5a***. Compound **4a** (2g, 5.27 mmol, 1 equiv) was dissolved in acetonitrile (25 mL), $NaHCO_3$ (0.886 g, 10.5 mmol, 1.5 equiv) and CDI (1.28 g, 7.91 mmol, 1.2 equiv) were added, and the mixture was stirred at room temperature for 48 h. The resultant precipitate was filtered under a vacuum, and solid precipitate was suspended in ethyl acetate (100 mL) and carefully acidified with dilute $KHSO_4$ solution. The organic layer was washed with brine solution, dried (Na_2SO_4), and concentrated under a vacuum to dryness. The resulting white solid was washed with diethyl ether to afford **5a** as a white solid. Yield 1.53 g (70%); mp >400 °C; R_f = 0.5 (silica gel TLC, 10% methanol in DCM); ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.24 (bs, 1 H), 10.29 (bs, 1 H), 7.48−7.46 (d, J = 6.10 Hz, 2 H), 7.30−7.28 (s, 3 H), 5.05 (s, 2 H), 1.49 (s, 9 H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 154.4, 154.1, 152.8, 149.0, 136.1, 129.1, 128.4, 128.4, 128.4, 127.9, 85.5, 44.7, 27.8, 27.8, 27.8; HRMS (ESI-TOF) m/z: [M + H]$^+$ Calcd for $C_{15}H_{19}N_4O_4$ 319.1401; Found 319.1393; [M + Na]$^+$ Calcd for $C_{15}H_{18}N_4O_4Na$ 341.1220; Found 341.1212.

Compound ***5b***. The synthetic method of **5a** was adopted to synthesize **5b**; white solid. Yield 1.45 g (66%); mp >400 °C; R_f = 0.5 (silica gel TLC, 10% methanol in DCM); ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.15 (bs, 1 H), 9.82 (bs, 1 H), 3.78 (s, 2 H), 1.51 (s, 9 H), 0.94 (s, 9 H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 155.3, 153.6, 152.8, 149.8, 85.5, 51.3, 34.0, 28.4, 28.4, 28.4, 28.0, 28.0, 28.0; HRMS (ESI-TOF) m/z: [M + H]$^+$ Calcd for $C_{13}H_{23}N_4O_4$ 299.1714; Found 299.1712; [M + Na]$^+$ Calcd for $C_{13}H_{22}N_4O_4Na$ 321.1533; Found 321.1531.

Compound ***5c***. The synthetic method of **5a** was adopted to synthesize **5c**; white solid. Yield 1.63 g (75%); mp >250 °C; R_f = 0.6 (silica gel TLC, 10% methanol in DCM); ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.08 (bs, 1 H), 9.91 (bs, 1 H), 7.31−7.30 (m, 5 H), 4.51 (s, 2 H), 4.13−4.11 (m, 2 H), 3.72−3.69 (t, J = 5.7 Hz, 2 H), 1.49 (s, 9 H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 154.2, 153.0, 153.0, 149.4, 138.1, 128.5, 128.5, 127.8, 127.7, 127.7, 85.5, 72.8, 66.5, 40.7, 27.9, 27.9, 27.9; HRMS (ESI-TOF) m/z: [M + H]$^+$ $C_{17}H_{23}N_4O_5$ 363.1663; Found 363.1656; [M + Na]$^+$ Calcd for $C_{17}H_{22}N_4O_5Na$ 385.1482; Found 385.1475.

Compound ***5d***. The synthetic method of **5a** was adopted to synthesize **5d** as a white solid. Yield 1.62 g (76%); mp >350 °C; R_f = 0.4 (silica gel TLC, 80% ethyl acetate in pet. ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.04 (bs, 1 H), 9.15 (bs, 1 H), 7.34−7.30 (m, 5 H), 5.30 (bs, 1 H), 5.15−5.07 (s, 2 H), 4.08−4.06 (m, 2 H), 3.50- 3.49 (m, 2 H), 1.53 (s, 9 H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 156.7, 155.2, 153.8, 152.6, 151.7, 149.2, 136.6, 128.5, 128.5, 128.1, 128.1, 128.1, 86.0, 66.7, 41.2, 39.9, 27.9, 27.9, 27.9; HRMS (ESI-TOF) m/z:

https://dx.doi.org/10.1021/acs.joc.0c02530
J. Org. Chem. 2021, 86, 3186−3195

$[M + H]^+$ Calcd for $C_{18}H_{24}N_5O_6$ 406.1721; Found 406.1716. Note: ^1H NMR δ 1.53 residual CH_2Cl_2, δ 1.56 residual water.

Compound ***5e***. The synthetic method of **5a** was adopted to synthesize **5e** as a white solid. Yield 1.30 g (60%); mp 280−290 °C; R_f = 0.4 (silica gel TLC, 70% ethyl acetate in pet. ether); ^1H NMR (400 MHz, DMSO-d^6) δ 11.41 (bs, 2 H), 7.37 (m, J = 3.0 Hz, 5 H), 5.18 (s, 2 H), 4.55 (s, 2 H), 1.49 (s, 9 H); $^{13}C\{^1H\}$ NMR (100 MHz, DMSO-d^6) δ 168.1, 156.8, 154.5, 153.7, 136.0, 128.9, 128.7, 128.5, 128.4, 128.3, 126.0, 84.0, 66.9, 42.4, 28.1, 28.1, 28.1; HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{17}H_{21}N_4O_6$ 377.1456; Found 377.1452; $[M + Na]^+$ Calcd for $C_{17}H_{20}N_4O_6Na$ 399.1275; Found 399.1271. Note: Residual peak of ethyl acetate at δ 1.27, 2.07, and 4.26. Note: Feebly UV active but ninhydrin active with green color on long heating.

Compound ***5f***. The synthetic method of **5a** was adopted to synthesize **5f**; white solid. Yield 0.095 g (45%); mp 80−90 °C; R_f = 0.5 (silica gel TLC, 45% ethyl acetate in pet. ether); ^1H NMR (400 MHz, DMSO-d^6) δ 11.22 (bs, 1 H), 7.36−7.30 (m, 11 H), 5.24 (t, J = 5.5 Hz, 1 H), 5.23−5.10 (m, 3 H), 5.07−4.99 (m, 2 H), 2.97−2.95 (m, 2 H), 2.08 (dd, J = 5.9, 8.9 Hz, 1 H), 1.91 (m, 1 H), 1.47 (s, 9 H), 1.41−1.23 (m, 3 H), 1.26 (d, J = 6.9 Hz, 2 H); $^{13}C\{^1H\}$ NMR (100 MHz, DMSO-d^6) δ 172.5, 172.3, 169.5, 156.6, 154.6, 153.7, 137.7, 136.3, 128.9, 128.9, 128.8, 128.8, 128.4, 128.4, 128.4, 128.2, 128.2, 128.0, 84.0, 66.7, 65.6, 54.0, 29.5, 28.1, 28.0, 28.0, 28.0, 23.2, 14.5; HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{29}H_{36}N_5O_8$ 582.2558; Found 582.2547. Note: Feebly UV active but ninhydrin active with green color on long heating.

Compound ***5g***. The synthetic method of **5a** was adopted to synthesize **5g**; white solid. Yield 1 g (49%); mp 220−230 °C; R_f = 0.4 (silica gel TLC, 10% methanol in DCM); ^1H NMR (400 MHz, $CDCl_3$) δ 11.29 (bs, 1 H), 7.89 (bs, 1 H), 7.29−7.24 (m, 5 H), 6.28−6.19 (bs, 3 H), 5.29−5.25(m, 1 H), 5.12−5.09 (m, 3 H), 5.01 (d, J = 2.3 Hz, 1 H), 3.49−3.47 (m, 1 H), 3.18−3.16 (m, 1 H), 2.92 (s, 3 H), 2.53 (s, 3 H), 2.47 (s, 3 H), 2.16 (bs, 1 H), 2.06 (s, 3 H), 1.46−1.45 (d, J = 5.3 Hz, 15 H); $^{13}C\{^1H\}$ NMR (100 MHz, $CDCl_3$) δ 169.1, 158.7, 156.3, 152.8, 152.8, 148.6, 138.4, 135.4, 133.1, 132.3, 128.6, 128.4, 128.4, 128.2, 128.2, 124.4, 117.5, 86.4, 67.4, 65.9, 54.3, 43.3, 40.7, 28.7, 28.7, 27.9, 27.9, 27.9, 25.7, 25.6, 19.3, 18.0, 15.4, 12.6; HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{34}H_{46}N_7O_9S$ 728.3072; Found 728.3071. Note: Feebly UV active but ninhydrin active with green color on long heating.

Compound ***6a***. Compound **5a** was (100 mg, 1 equiv 0.331 mmol) dissolved in 50% TFA in DCM (5 mL) and stirred at ice temperature for 45 min. The reaction mixture was concentrated in *vacuo* and coevaporated with dichloromethane (2 × 10 mL). The resulting residue was diluted with diethyl ether (5 mL), and the solid was filtered under a vacuum to afford **6a** as a white solid. Yield 0.062 g (90%); mp >400 °C; HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{10}H_{11}N_4O_2^+$ 219.0877; Found 219.0876. Note: No solubility even in hot DMSO-d^6.

Compound ***6b***. The synthetic method of **6a** was adopted to synthesize **6b** as a white solid. Yield 0.061 g (92%); mp >400 °C; the product was poorly soluble in any solvent; HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_8H_{15}N_4O_2^+$ 199.1190; Found 199.1189; $[M + Na]^+$ Calcd for $C_8H_{14}N_4O_2$ 221.1009; Found 221.1007. Note: No solubility in DMSO-d^6.

Compound ***6c***. The compound **5c** (1 g, 1 equiv, 2.47 mmol) was dissolved in methanol (5 mL) followed by addition of Pd/C (20 mol %) and stirred at room temperature for 4 h under a hydrogen (H_2) atmosphere. The reaction mixture was passed through a Celite pad (thin pad), and the pad was washed repetitively by MeOH (4 × 20 mL). The resultant filtrate was concentrated under a *vacuum* to afford benzyl free intermediate (0.6 g, 80%) as a white solid. Further, the resultant solid (0.1 g, 1 equiv) was dissolved in 50% solution of TFA in DCM and stirred at ice temperature for 45 min. The solvent was removed under a vacuum and coevaporated with DCM (2 × 5 mL). The resultant residue was diluted with diethyl ether (10 mL) and the resultant solid was washed by filtration under a vacuum to afford **6c** as a white solid; overall yield 0.051 g (80%); mp >250 °C; ^1H NMR (400 MHz, DMSO-d^6) δ 7.49 (bs, 1 H), 4.84 (bs, 3 H), 3.71−3.68 (m, 2 H), 3.44−3.41 (t, J = 6.5 Hz, 2 H); $^{13}C\{^1H\}$ NMR (400 MHz, DMSO-d^6) δ 160.9, 155.7, 152.6, 58.2, 42.9; HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_5H_9N_4O_3^+$ 173.0669; Found 173.0669. Note: Poor solubility in hot DMSO-d^6.

Compound ***6d***. The synthetic method of **6a** was adopted to synthesize **6d** as a white solid; overall yield 0.044 g (70%); mp >250 °C; ^1H NMR (400 MHz, DMSO-d^6) δ 11.49 (bs, 1 H), 7.76 (bs, 2 H), 7.42 (bs, 2 H), 3.88−3.85 (d, 2 H), 2.95 (d, J = 6.5 Hz, 2 H); $^{13}C\{^1H\}$ NMR (100 MHz, DMSO-d^6) δ 162.8, 162.7, 157.1, 69.1, 55.9; HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_5H_{10}N_5O_2^+$ 172.0829; Found 172.0829. Note: Poor solubility in DMSO-d^6, δ 3.39 (bs, residual water).

Compound ***6e***. The synthetic method of **6a** was adopted to synthesize **6e** as a white solid. Yield 0.060 g (80%); mp >250 °C; ^1H NMR (400 MHz, DMSO-d^6) δ 7.33 (m, 5 H), 7.19 (bs, 2 H), 5.12 (s, 2 H), 4.44 (s, 2 H); $^{13}C\{^1H\}$ NMR (100 MHz, DMSO-d^6) δ 168.8, 156.8, 156.8, 153.2, 136.2, 129.0, 128.7, 128.7, 128.4, 128.4, 66.7, 42.0; HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{12}H_{13}N_4O_4^+$ 277.0931; Found 277.0926; $[M + Na]^+$ Calcd for $C_{12}H_{12}N_4O_4Na$ 299.0751; Found 299.0744.

Compound ***6f***. The synthetic method of **6a** was adopted to synthesize **6f** as a white solid. Yield 0.063 g (78%); mp 270−280 °C; ^1H NMR (400 MHz, DMSO-d^6) δ 7.37−7.30 (m, 10 H), 7.23 (bs, 2H), 5.19−5.15 (dd, J = 5.0, 9.5 Hz, 1 H), 5.13−5.05 (m, 2 H), 4.99 (s, 2 H), 2.98 (q, J = 6.6 Hz, 2 H), 2.04−1.96 (m, 2 H), 1.42−1.37 (m, 2 H), 1.23−1.18 (dd, J = 6.5, 14.9 Hz, 2 H); $^{13}C\{^1H\}$ NMR (100 MHz, DMSO-d^6) δ 170.1, 159.7, 156.8, 156.6, 153.3, 137.8, 136.6, 128.9, 128.9, 128.4, 128.4, 128.2, 128.2, 128.0, 128.0, 128.0, 28.0, 66.5, 65.6, 53.4, 29.6, 28.5, 23.4, 18.3; HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{24}H_{28}N_5O_6^+$ 482.2034; Found 482.2036.

Compound ***6g***. Deprotection of **5g** using 95% TFA−DCM afforded **6g** as an insoluble material. Owing to poor solubility, it could not be satisfactorily characterized. Overall yield 0.032 g (62%); mp 175−185 °C; HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{16}H_{22}N_7O_4^+$ 376.1728; Found 376.1724.

Compound ***12a***. Compound **5e** (1 g, 2.66 mmol, 1 equiv) was dissolved in methanol (5 mL), and Pd/C was added (20 mol %); the mixture was stirred at room temperature under a H_2 atmosphere for 5 h. The resultant reaction mixture was passed through a thin Celite pad, and the filtrate was concentrated under *vacuo* to afford **12a** as a white solid. Yield 0.690 g (90%); mp >250 °C; ^1H NMR (400 MHz, DMSO-d^6) δ 11.70 (m, 3 H), 4.37 (s, 2 H), 1.48 (s, 9 H); $^{13}C\{^1H\}$ NMR (100 MHz, DMSO-d^6) δ 169.5, 169.5, 154.4, 154.4, 153.8, 83.9, 42.3, 28.1, 28.1, 28.1; HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{10}H_{15}N_4O_6$ 287.0986; Found 287.0981. Note: Residual ethyl acetate peak at δ 1.07.

Compound ***12b***. The synthetic method of **12a** (1 g, 1.37 mmol, 1 equiv) was adopted to synthesize **12b** as a white solid. Yield 0.760 g (86%); mp 220−230 °C; ^1H NMR (400 MHz, DMSO-d^6) δ 12.48 (bs, 1 H), 11.32 (bs, 2 H), 6.70 (bs, 1 H), 6.37 (bs, 1 H), 5.02 (bs, 1 H), 3.36 (m, 3 H), 3.01 (d, J = 5.3 Hz, 2 H), 2.96 (s, 2 H), 2.47 (s, 3 H), 2.42 (s, 3 H), 2.00 (m, 4 H), 1.48 (s, 9H), 1.41 (s, 6 H), 1.35 (d, J = 10.7 Hz, 2 H); $^{13}C\{^1H\}$ NMR (100 MHz, DMSO-d^6) δ 166.6, 161.8, 158.0, 156.6, 137.8, 134.6, 131.9, 124.9, 116.8, 113.1, 102.0, 86.8, 65.5, 65.5, 60.3, 43.0, 28.8, 28.8, 28.2, 28.2, 25.8, 25.8, 25.8, 18.1, 15.7, 14.6, 12.2; HRMS (ESI-TOF) m/z: $[M + H]^+$ Calcd for $C_{27}H_{40}N_7O_9S$ 638.2603; Found 638.2604.

Compound ***13a***. Compound **12a** (0.3 g, 1.05 mmol, 1 equiv) was dissolved in dry DMF (5 mL), and HBTU (0.597 g, 1.57 mmol, 1.5 equiv), HOBt (212 mg, 1.57 mmol, 1.5 equiv), DIPEA (0.364 mL, 2.09 mmol, 2 equiv) were added; the mixture was stirred at 0 °C. After stirring for 10 min at 0 °C, compound **11** (541 mg, 1.2 equiv, 1.26 mmol, which was synthesized by an earlier reported procedure[24a]) was added and the reaction mixture was stirred at room temperature for an additional 12 h. The resultant solution was diluted with ice cold water, and the solid precipitate was filtered under a vacuum. The solid residue was dissolved in ethyl acetate and subsequently washed with $KHSO_4$ solution, $NaHCO_3$ solution, and brine solution, dried (Na_2SO_4), and concentrated under *vacuo* to get a white solid product which was purified by chromatography on silica.

https://dx.doi.org/10.1021/acs.joc.0c02530
J. Org. Chem. 2021, 86, 3186−3195

The mobile phase was 50−100% EtOAc in pet. ether to afford **13a** as a white solid. Yield 0.70 g (95%); mp 180−190 °C; ^{1}H NMR (500 MHz, DMSO-d^6) δ 11.37 (bs, 1 H), 11.07 (bs, 1 H), 7.90 (d, J = 7.4 Hz, 2 H), 7.88 (d, J = 7.4 Hz, 2 H), 7.41−7.33 (m, 10 H), 5.22−5.13 (s, 2 H), 4.69 (s, 1 H), 4.55 (s, 1 H), 4.33 (bs, 1 H), 4.28 (d, J = 6.9 Hz, 1 H), 4.23 (dd, J = 6.8, 18.8 Hz, 2 H), 4.21−4.13 (s, 1 H), 3.50−3.49 (t, J = 6.3 Hz, 2 H), 3.36−3.24 (d, J = 5.9 Hz, 2 H), 1.49 (s, 9 H); ^{13}C{^{1}H} NMR (100 MHz, DMSO-d^6) δ 169.9, 169.4, 167.3, 167.2, 157.3, 156.8, 154.3, 144.4, 141.2, 136.2, 136.1, 129.4, 128.9, 128.5, 128.3, 128.1, 127.5, 125.6, 125.6, 125.6, 125.6, 120.6, 120.6, 83.9, 67.8, 67.0, 66.4, 66.0, 47.3, 47.3, 47.2, 40.6, 31.8, 28.1, 28.1, 28.1; HRMS (ESI-TOF) m/z: [M + H]$^+$ Calcd for $C_{36}H_{39}N_6O_9$ 699.2779; Found 699.2776; [M + Na]$^+$ Calcd for $C_{36}H_{38}N_6O_9Na$ 721.2598; Found 721.2592.

Compound ***13b***. The synthetic method of **13a** was adopted to synthesize **13b** as a white solid. Yield 0.387 g (78%); mp 210−220 °C; ^{1}H NMR (400 MHz, DMSO-d^6) δ 11.26 (bs, 1 H), 7.87−7.85 (d, J = 7.1 Hz, 2 H), 7.68−7.66 (d, J = 6.6 Hz, 2 H), 7.40−7.28 (m, 10 H), 7.16 (bs, 1 H), 6.72 (bs, 1 H), 6.33 (bs, 2 H), 5.22−5.17 (m, 1 H), 5.14−5.01 (m, 1 H), 4.87−4.84 (d, J = 12.1 Hz, 1 H), 4.28−4.16 (m, 4 H), 4.12−4.14 (m, 1 H), 4.10−3.91 (m, 2 H), 3.42 (d, J = 7.6 Hz, 1 H), 3.14 (bs, 3 H), 3.02 (m, 2 H), 2.94 (s, 3 H), 2.47 (s, 3 H), 2.41 (s, 2 H), 1.99 (m, 4 H), 1.44 (s, 6 H), 1.39 (m, 9 H), 1.34 (m, 2 H); ^{13}C{^{1}H} NMR (100 MHz, DMSO-d^6) δ 170.0, 157.9, 156.5, 144.4, 141.2, 137.7, 135.6, 131.9, 128.9, 128.9, 128.5, 128.5, 128.5, 128.3, 128.3, 128.3, 128.1, 128.1, 128.1, 127.5, 127.5, 125.6, 124.8, 120.5, 116.7, 86.7, 67.1, 67.1, 65.8, 60.2, 52.4, 48.9, 47.1, 42.9, 38.1, 30.6, 29.4, 28.8, 28.8, 28.1, 28.1, 28.1, 28.1, 26.4, 21.5, 21.2, 19.4, 18.0, 17.6, 14.7, 14.5, 14.4, 12.7; HRMS (ESI-TOF) m/z: [M + H]$^+$ Calcd for $C_{53}H_{64}N_9O_{12}S$ 1050.4390; Found 1050.4382.

Compound ***14a***. Compound **12a** (0.3 g, 1.05 mmol, 1 equiv) was dissolved in DMF (5 mL), and HBTU (0.597 g, 1.57 mmol, 1.5 equiv), HOBt (212 mg, 1.57 mmol, 1.5 equiv), DIPEA (0.364 mL, 2.09 mmol, 2 equiv) were added; the mixture was stirred for 5 min at 0 °C. Ethyl (2-(((benzyloxy) carbonyl) amino) ethyl) glycinate **8** (Cbz-aeg-OEt) (0.352 g, 1.26 mmol, 1.2 equiv, synthesized as per an earlier procedure)[24b] was added, and the reaction mixture was stirred at room temperature for an additional 16 h. The resultant solution was diluted with ice cold water, and solid precipitate was filtered under a vacuum. The solid residue was dissolved in ethyl acetate, subsequently washed with dilute $KHSO_4$ solution, $NaHCO_3$ solution, and brine solution, dried (Na_2SO_4), and concentrated under *vacuo* to get a white solid crude product which was purified by column chromatography silica gel (230−400). The mobile phase was EtOAc (50−90%) in pet. ether to afford **14a** as a white solid. Yield 0.449 g (79%); mp 150−160 °C; ^{1}H NMR (500 MHz, DMSO-d^6) δ 11.39 (bs, 1 H), 11.11 (bs, 1 H), 7.37−7.22 (m, 5 H), 7.31 (bs, 1 H), 5.03−5.01 (m, 2 H), 4.66 (s, 2 H), 4.51 (m, 1 H), 4.06 (m, 2 H), 4.04 (s, 2 H), 3.50−3.48 (d, J = 6.6 Hz, 2 H), 3.25 (bs, 2 H), 1.49 (s, 9 H), 1.17 (m, 3 H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 169.8, 169.1, 167.0, 162.1, 157.1, 154.2, 152.9, 136.8, 128.6, 128.4, 128.2, 128.2, 86.0, 67.1, 66.8, 62.4, 61.8, 50.7, 49.3, 49.0, 42.2, 39.5, 28.1, 28.1, 28.1, 14.3; HRMS (ESI-TOF) m/z: [M + H]$^+$ Calcd for $C_{24}H_{33}N_6O_9$ 549.2304; Found 549.2294.

Compound ***14b***. The synthetic method of **14a** was adopted to synthesize **14b** as a white solid. Yield 0.307 g (72%); mp 150−160 °C. ^{1}H NMR (400 MHz, DMSO-d^6) δ 11.47 (bs, 1 H), 11.05 (bs, 1 H), 7.35−7.32 (m, 5 H), 7.19−7.01 (m, 1 H), 6.67 (bs, 1 H), 6.36 (bs, 1 H), 5.16 (m, 1 H), 5.00−4.97 (m, 2 H), 4.08 (m, 4 H), 3.43−3.40 (dd, J = 5.7, 12.7 Hz, 2 H), 3.15−3.08 (m, 3 H), 3.02−2.96 (m, 5 H), 2.46 (s, 3 H), 2.41 (s, 3 H), 2.00−1.99 (m, 4 H), 1.47−1.46 (m, 10 H), 1.40 (s, 7 H), 1.23−1.14 (m, 3 H); ^{13}C{^{1}H} NMR (100 MHz, DMSO-d^6) δ 169.9, 168.9, 157.9, 156.6, 156.5, 137.7, 137.7, 134.6, 131.9, 128.8, 128.8, 128.3, 128.1, 128.1, 124.8, 116.7, 86.8, 84.0, 65.8, 65.7, 65.7, 61.4, 60.9, 60.2, 52.5, 47.7, 42.9, 38.8, 32.3, 31.4, 28.7, 28.7, 28.0, 28.0, 28.0, 25.9, 19.0, 18.0, 14.4, 14.2, 12.7; HRMS (ESI-TOF) m/z: [M + H]$^+$ Calcd for $C_{41}H_{58}N_9O_{12}$ 900.3920; Found 900.3925; [M + Na]$^+$ Calcd for $C_{41}H_{58}N_9O_{12}Na$ 922.3740; Found 922.3735.

Compound ***15a***. *tert*-Butyl (2-(allyloxy) ethyl) carbamate (5 g, 1 equiv, 24.87 mmol, synthesized as per an earlier reported procedure[21b]) was dissolved in 50% TFA in DCM and allowed to stir at 0 °C for 45 min. The resultant solution was stripped off and coevaporated using toluene (2 × 20 mL) to obtain the TFA salt. The resultant TFA salt (5 g, 1 equiv, 23.25 mmol) was dissolved in acetonitrile (50 mL), and compound **3** (6.44 g, 1.1 equiv, 25.58 mmol) was added. Then, DIPEA (8 mL, 2 equiv, 46.511 mmol) was added and the mixture was stirred at 50 °C in an oil bath for 5 h. The resulting reaction mixture was cooled at room temperature and concentrated under a vacuum, and the acquired residue was dissolved in ethyl acetate (150 mL) and subsequently washed with $KHSO_4$ solution and brine solution and dried (Na_2SO_4). The organic layer was concentrated under a vacuum to afford **15a** as a pale semisolid. Yield 5.05 g (76%); R_f = 0.4 (silica gel TLC, 60% ethyl acetate in pet. ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 929 (bs, 1 H), 8.08 (m, 1 H), 6.45 (m, 1 H), 5.87−5.80 (m, 1 H), 5.23−5.12 (m, 2 H), 3.95−3.94 (d, J = 5.3 Hz, 2 H), 3.49−3.48 (d, J = 5.3 Hz, 2 H), 3.46−3.33 (m, 2 H), 1.43 (s, 9 H), 1.08, bs, 1 H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 158.0, 134.5, 117.4, 82.1, 73.4, 72.2, 70.6, 69.3, 39.9, 28.2, 28.2, 28.2; HRMS (ESI-TOF) m/z: [M + H]$^+$ Calcd for $C_{12}H_{23}N_4O_4$287.1714; Found 287.1710; [M + Na]$^+$ Calcd for $C_{12}H_{22}N_4O_4Na$ 309.1533; Found 309.1526.

Compound ***15b***. 2-((*tert*-Butoxycarbonyl) amino) ethyl acrylate (10 g, 1 equiv, 46.46 mmol, synthesized as per an earlier reported procedure[24c]) was dissolved in 50% TFA in DCM (40 mL) and stirred at 0 °C for 60 min, monitored by TLC. The resultant solution was stripped off and coevaporated using toluene (2 × 20 mL) at 30 °C (to avoid undesired polymerization) to obtain TFA salt which was carried forward for the next step without purification. The resultant TFA salt (10 g, 1 equiv, 43.66 mmol) was dissolved in acetonitrile (80 mL), and compound **3** (12.15 g, 1.1 equiv, 4.80 mmol) was added. Then, DIPEA (14.96 mL, 2 equiv, 8.73 mmol) was added and the mixture was stirred at 50 °C in an oil bath for 5 h. The solution was cooled at room temperature and concentrated under a vacuum. The resultant residue was diluted with ethyl acetate (200 mL) and subsequently washed with dilute $KHSO_4$ solution and brine solution and dried (Na_2SO_4). The solvent was removed under a vacuum to afford **15b** as a pale semisolid. Yield 9.90 g (75%); R_f = 0.5 (silica gel TLC, 60% ethyl acetate in pet. ether); ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.23 (bs, 1 H), 8.39 (bs, 2 H), 6.39−6.36 (d, J = 17.5 Hz, 1 H), 6.11 (bs, 2 H), 6.07 (m, 1 H), 5.81−5.79 (d, J = 10.7 Hz, 1 H), 4.21 (bs, 2 H), 3.47 (bs, 2 H), 1.44 (d, J = 1.5 Hz, 9 H); ^{13}C{^{1}H} NMR (125 MHz,$CDCl_3$) δ 166.0, 158.1, 131.2, 128.0, 82.2, 63.5, 60.3, 49.4, 38.8, 28.1, 28.1, 28.1; HRMS (ESI-TOF) m/z: [M + H]$^+$ Calcd for $C_{12}H_{21}N_4O_5$ 301.1506; Found 301.1510.

Compound ***15c***. *tert*-Butyl (2-acrylamidoethyl) carbamate (4.28 g, 1 equiv, 20.00 mmol, synthesized as per an earlier reported procedure[24c]) was deprotected by using 50% solution of TFA in DCM (40 mL) at ice temperature for 45 min. The resultant solution was stripped off and coevaporated at 30 °C (to avoid undesired polymerization) using toluene (2 × 20 mL) to obtain the TFA salt (4.52 g, 1 equiv, 19.84 mmol) as a semisolid, which was dissolved in acetonitrile. Compound **3** (5 g, 1 equiv, 19.84 mmol) and DIPEA (3 equiv, 10.34 mL, 59.52 mmol) were added, and the solution was stirred at 50 °C in oil bath for 5 h. The resultant solution was cooled at room temperature and filtered through a cotton pad, and the filtrate was diluted with ethyl acetate (100 mL) and subsequently washed with dilute $KHSO_4$ solution and brine solution and dried (Na_2SO_4). The solvent was removed under a vacuum to afford **15c** as a white solid. Yield 4.3 g (80%); mp 95−105 °C; R_f = 0.3 (silica gel TLC, 80% ethyl acetate in pet. ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (bs, 3 H), 7.03 (bs, 2 H), 6.58 (bs, 3 H), 6.24−6.06 (m, 2 H), 5.59−5.56 (d, J = 10.7 Hz, 1 H), 3.43−3.34 (m, 4 H), 1.44 (s, 9 H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 171.3, 166.5, 157.8, 131.0, 126.4, 82.3, 60.5, 40.7, 39.5, 28.2, 28.2, 28.2; HRMS (ESI-TOF) m/z: [M + H]$^+$ Calcd for $C_{12}H_{22}N_5O_4$ 300.1666; Found 300.1662. [Note: δ 1.26, δ 2.05, and δ 4.12 are residual peaks of ethyl acetate.]

Compound ***15d***. The synthetic method of **4a** was adopted to synthesize **15d**. The mono-*endo*-amine was synthesized via an earlier

https://dx.doi.org/10.1021/acs.joc.0c02530
J. Org. Chem. 2021, 86, 3186−3195

reported procedure.[25a] Yield 3.4 g (79%); R_f = 0.4 (silica gel TLC, 60% ethyl acetate in pet. ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.28 (m, 1 H), 8.23 (m, 2 H), 6.06 (s, 2 H), 5.26 (bs, 1 H), 3.49−3.46 (m, 2 H), 3.32 (bs, 2 H), 3.28 (q, J = 6.1 Hz, 2 H), 3.26−3.21 (bs, 2 H), 1.69−1.66 (m, 1 H), 1.50−1.48 (d, J = 9.2 Hz, 1 H), 1.43 (s, 9 H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 177.9, 177.9, 161.8, 157.7, 134.5, 134.5, 134.5, 82.1, 60.4, 53.5, 52.2, 45.8, 44.9, 37.9, 37.7, 28.1, 28.1, 28.1; HRMS (ESI-TOF) m/z: [M + H]$^+$ Calcd for $C_{18}H_{26}N_5O_5$ 392.1928; Found 392.1919.

Compound ***16a***. The synthetic method of **5a** was adopted to synthesize **16a** as a white solid. Yield 1.2 g (55%); mp 130−135 °C; R_f = 0.45 (silica gel TLC, 60% ethyl acetate in pet. ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.95 (bs, 2 H), 5.83−5.79 (tdd, J = 5.6, 11.0, 16.7 Hz, 1 H), 5.23−5.11 (m, 2 H), 4.09−4.06 (t, J = 5.7 Hz, 2 H), 3.96−3.94 (d, J = 6.1 Hz, 2 H), 3.64−3.61 (t, J = 5.7 Hz, 2 H), 1.48 (s, 9 H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 154.3, 153.0, 151.4, 149.4, 134.5, 117.3, 85.5, 71.7, 66.3, 40.7, 27.9, 27.9, 27.9; HRMS (ESI-TOF) m/z: [M + H]$^+$ Calcd for $C_{13}H_{21}N_4O_5$ 313.1506; Found 313.1496; [M + Na]$^+$ Calcd for $C_{13}H_{20}N_4O_5Na$ 335.1326; Found 335.1315.

Compound ***16b***. A mixture of **15b** (2.5 g, 8.33 mmol, 1 equiv), $NaHCO_3$ (1.0 g, 12.5 mmol, 1.5 equiv), and CDI (1.62 g, 10 mmol, 1.2 equiv) in 30 mL acetonitrile was stirred at room temperature for 48 h. The resultant reaction mixture was filtered under a vacuum, and solid residue was suspended in ethyl acetate (100 mL) and acidified with dilute $KHSO_4$ solution. The organic layer was washed with brine solution, dried (Na_2SO_4), filtered, and concentrated under a vacuum at 30 °C (to avoid undesired polymerization), and the resulting semisolid was purified by column chromatography using ethyl acetate 0−80% in petroleum ether. The solvent was removed under a vacuum at 30 °C to get viscous oil which was kept in a refrigerator overnight to afford **16b** as a white solid. Yield 0.840 g (30%); mp 95−105 °C; R_f = 0.6 (silica gel TLC, 60% ethyl acetate in pet. ether); ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.14 (bs, 1 H), 9.84 (bs, 1 H), 6.40−6.35 (m, 1 H), 6.02 (m, 1 H), 5.83−5.80 (m, 1 H), 4.38−4.37 (m, 2 H), 4.21−4.18 (m, 2 H), 1.50 (s, 9 H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 166.0, 154.5, 154.2, 152.8, 149.0, 131.4, 128.1, 85.8, 61.3, 40.5, 27.9, 27.9, 27.9; HRMS (ESI-TOF) m/z: [M + H]$^+$ Calcd for $C_{13}H_{19}N_4O_6$ 327.1299; Found 327.1295; [M + Na]$^+$ Calcd for $C_{13}H_{18}N_4O_6Na$ 349.1119; Found 349.1115.

Compound ***16c***. The synthetic method of **16b** was adopted to synthesize **16c** as a white solid. Yield 2.25 g (82%); mp >250 °C; R_f = 0.3 (silica gel TLC, 10% methanol in DCM); ^{1}H NMR (400 MHz, DMSO-d^6) δ 8.18−8.17 (s, 1 H), 6.14−6.01 (m, 2 H), 5.58−5.55 (dd, J = 3.1, 9.9 Hz, 1 H), 3.82−3.79 (bs, 3 H), 3.38−3.33 (d, J = 6.1 Hz, 2 H), 1.48 (s, 9 H); ^{13}C{^{1}H} NMR (100 MHz, DMSO-d^6) δ 165.4, 165.4, 154.0, 154.0, 132.2, 132.6, 125.6, 83.3, 40.9, 36.5, 28.1, 28.1, 28.1; HRMS (ESI-TOF) m/z: [M + H]$^+$ Calcd for $C_{13}H_{20}N_5O_5$ 326.1459; Found 326.1452; [M + Na]$^+$ Calcd for $C_{13}H_{19}N_5O_5Na$ 348.1284; Found 348.1271.

Compound ***16d***. The synthetic method of **16b** was adopted to synthesize **16d** as a white solid. Yield 2.1 g (78%); mp >250 °C; R_f = 0.45 (silica gel TLC, 10% MeOH in DCM); ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.02 (bs, 1 H), 9.31 (bs, 1 H), 6.04−6.03 (m, 2 H), 4.00−3.98 (m, 2 H), 3.66−6.65 (td, J = 2.3, 4.6 Hz, 2 H), 3.31−3.24 (d, J = 19.1 Hz, 4 H), 1.69−1.67 (m, 1 H), 1.51 (m, 1 H), 1.47 (m, 9 H); ^{13}C{^{1}H} NMR (100 MHz, $CDCl_3$) δ 178.2, 178.2, 154.9, 153.8, 152.7, 149.2, 134.5, 134.5, 85.8, 52.4, 46.0, 46.0, 44.7, 44.7, 40.3, 36.1, 27.9, 27.9, 27.9; HRMS (ESI-TOF) m/z: [M + H]$^+$ Calcd for $C_{19}H_{24}N_5O_6$ 418.1721; Found 418.1716; [M + Na]$^+$ Calcd for $C_{19}H_{23}N_5O_6Na$ 440.1541; Found 440.1536.

Compound ***17a***. A solution of **16a** (0.1 g) in 50% TFA in DCM was stirred for 45 min at ice temperature. The reaction mixture was concentrated *in vacuo*; the resulting residue was dissolved in diethyl ether, and the solid was filtered in a vacuum to afford **17a** as a white solid. Yield 0.055 g (80%); mp >250; ^{1}H NMR (500 MHz, DMSO-d^6) δ 11.08 (bs, 1 H), 7.68 (bs, 1 H), 6.43 (bs, 1 H), 5.86−6.83 (tdd, J = 5.3, 10.4, 17.2 Hz, 1 H), 5.22−5.20 (m, 1 H), 5.10 (m, 2 H), 3.93−3.92 (td, J = 1.5, 5.3 Hz, 2 H), 3.84−3.82 (t, J = 6.5 Hz, 2 H), 3.50 (t, J = 6.3 Hz, 2 H); ^{13}C{^{1}H} NMR (125 MHz, DMSO-d^6) δ 156.1, 156.1, 135.0, 116.2, 70.5, 69.2, 66.1, 39.9; HRMS (ESI-TOF) m/z: [M + H]$^+$ Calcd for $C_8H_{13}N_4O_3^+$ 213.0982; Found 213.0982.

Compound ***17b***. The synthetic method of **17a** was adopted to synthesize **17b** as a white solid. Yield 0.054 g (78%); mp >300 °C; ^{1}H NMR (400 MHz, DMSO-d^6) δ 7.14 (bs, 1 H), 6.30−6.26 (dd, J = 1.6, 17.3 Hz, 1 H), 6.14−6.07 (dd, J = 10.4, 17.3 Hz, 1 H), 5.94−5.91 (dd, J = 1.5, 10.4 Hz, 1 H), 4.27−4.24 (t, J = 5.4 Hz, 2 H), 3.97−3.94 (t, J = 5.5 Hz, 2 H); ^{13}C{^{1}H} NMR (100 MHz, DMSO-d^6) δ 165.8, 156.5, 153.3, 150.2, 132.1, 128.7, 61.7, 40.6. HRMS (ESI-TOF) m/z: [M + H]$^+$ Calcd for $C_8H_{11}N_4O_4^+$ 227.0775; Found 227.0775.

Compound ***17c***. The synthetic method of **17a** was adopted to synthesize **17c** as a white solid. Yield 0.061 g (88%); mp >300 °C; note: poor solubility in DMSO-d^6; HRMS (ESI-TOF) m/z: [M + H]$^+$ Calcd for $C_8H_{12}N_5O_3^+$ 226.0935; Found 226.0932.

Compound ***17d***. The synthetic method of **17a** was adopted to synthesize **17d** as a white solid. Yield 0.064 g (84%); mp >250 °C; ^{1}H NMR (400 MHz, DMSO-d^6) δ 7.07 (bs, 3 H), 5.97−5.96 (s, 2 H), 3.71−3.69 (m, 2 H), 3.42−3.39 (d, J = 10.7 Hz, 2 H), 3.19−316 (d, J = 13.0 Hz, 4 H), 1.49 (s, 2 H); ^{13}C{^{1}H} NMR (100 MHz, DMSO-d^6) δ 177.2, 177.2, 175.5, 167.2, 155.8, 152.8, 152.8, 134.2, 134.2, 51.7, 45.2, 44.0, 38.8, 35.6; HRMS (ESI-TOF) m/z: [M + H]$^+$ Calcd for $C_{14}H_{16}N_5O_4^+$ 318.1197; Found 318.1194.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.0c02530.

Associated analytical data of all newly synthesized compounds and copies of ^{1}H, ^{13}C, DEPT-135, and HRMS spectra for compounds **2**, **3**, **4a−g**, **5a−g**, **6a−g**, **12a,b**, **13a,b**, **14a,b**, **15a−d**, **16a−d**, and **17a−d** (PDF)

Accession Codes

CCDC 2011989−2011991 and 2051830 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033.

■ AUTHOR INFORMATION

Corresponding Author

Gangadhar J. Sanjayan − *Organic Chemistry Division, Council of Scientific and Industrial Research National Chemical Laboratory (CSIR-NCL), Pune 411008, India;* orcid.org/0000-0001-7764-7329; Email: gj.sanjayan@ncl.res.in

Authors

Chhuttan L. Meena − *Organic Chemistry Division, Council of Scientific and Industrial Research National Chemical Laboratory (CSIR-NCL), Pune 411008, India*

Dharmendra Singh − *Organic Chemistry Division, Council of Scientific and Industrial Research National Chemical Laboratory (CSIR-NCL), Pune 411008, India*

Bhavya Kizhakeetil − *Organic Chemistry Division, Council of Scientific and Industrial Research National Chemical Laboratory (CSIR-NCL), Pune 411008, India*

Manasa Prasad − *Organic Chemistry Division, Council of Scientific and Industrial Research National Chemical Laboratory (CSIR-NCL), Pune 411008, India*

Malini George − *Organic Chemistry Division, Council of Scientific and Industrial Research National Chemical Laboratory (CSIR-NCL), Pune 411008, India*

Srinu Tothadi − *Organic Chemistry Division, Council of Scientific and Industrial Research National Chemical Laboratory (CSIR-NCL), Pune 411008, India;* orcid.org/0000-0001-6840-6937

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.joc.0c02530

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

C.L.M. thanks the University Grant Commission (UGC), New Delhi, Government of India, for the award of a Postdoc fellowship (Award No. F./31-1/2017/PDFSS-2017-18-RAJ-14211). The grant to G.J.S. (CSIR SSB-000726, seed grant) is also gratefully acknowledged.

■ REFERENCES

(1) (a) Steed, J. W.; Atwood, J. L. *Supramolecular Chemistry*, 2nd ed.; John Wiley & Sons, Ltd: 2009; Chapter 10, pp 591−706. DOI: 10.1002/9780470740880 (b) Whitesides, G. M.; Grzybowski, B. Self-Assembly at all Scales. *Science* **2002**, *295*, 2418−2421.

(2) Stupp, S. I.; Palmer, L. C. Supramolecular Chemistry and Self-Assembly in Organic Materials Design. *Chem. Mater.* **2014**, *26*, 507−518.

(3) (a) Lehn, J.-M. *Supramolecular Chemistry: Concepts and Perspectives*; Wiley-VCH: 1995. (b) Feng, H.; Lu, X.; Wang, W.; Kang, N.-G.; Mays, W. Block Copolymers: Synthesis, Self-Assembly, and Applications. *Polymers* **2017**, *9*, 494. (c) Gebelein, C. G. Polymers for Potential Cancer Therapy: A Brief Review. *ACS Symp. Ser.* **1982**, *186*, 193−203. (d) Lehn, J.-M. Beyond Chemical Synthesis: Self-Organization. *Isr. J. Chem.* **2018**, *58* (1−2), 136−141.

(4) (a) Whitesides, G. M.; Mathias, J. P.; Seto, C. T. Molecular Self-Assembly and Nanochemistry: A Chemical Strategy for The Synthesis of Nanostructures. *Science* **1991**, *254*, 1312−1319. (b) Hamley, I. W. Nanostructure Fabrication Using Block Copolymers. *Nanotechnology* **2003**, *14*, R39−R54.

(5) (a) Kowalski, P. S.; Bhattacharya, C.; Afewerki, S.; Langer, R. Smart Biomaterials: Recent Advances and Future Directions. *ACS Biomater. Sci. Eng.* **2018**, *4*, 3809−3817. (b) Xu, D.; Wang, W.; Gesua, D.; Kaifer, A. E. A New Series of Dendrimers Containing Two Ferrocenyl Units and a Fréchet Dendron around a Triazine Nucleus. *Org. Lett.* **2008**, *10* (20), 4517−4520.

(6) Xia, X. X.; Xu, Q.; Hu, X.; Qin, G.; Kaplan, D. L. Tunable Self-Assembly of Genetically Engineered Silk-Elastin-like Protein Polymers. *Biomacromolecules* **2011**, *12* (11), 3844−3850.

(7) (a) Das, A.; Theato, P. Activated Ester Containing Polymers: Opportunities and Challenges for the Design of Functional Macromolecules. *Chem. Rev.* **2016**, *116* (3), 1434−1445. (b) Lehn, J.-M. Supramolecular chemistry: Where from? Where to? *Chem. Soc. Rev.* **2017**, *46*, 2378−2379.

(8) (a) Hosseinkhani, H.; Hong, P. D.; Yu, D. S. Self-Assembled Proteins and Peptides for Regenerative Medicine. *Chem. Rev.* **2013**, *113* (7), 4837−4861. (b) Berda, E. B.; Foster, E. J.; Meijer, E. W. Toward Controlling Folding in Synthetic Polymers: Fabricating and Characterizing Supramolecular Single-Chain Nanoparticles. *Macromolecules* **2010**, *43* (3), 1430−1437.

(9) Xu, W.; Song, Q.; Xu, J.-F.; Serpe, M. J.; Zhang, X. Supramolecular Hydrogels Fabricated from Supramonomers: A Novel Wound Dressing Material. *ACS Appl. Mater. Interfaces* **2017**, *9* (13), 11368−11372.

(10) (a) Li, J.; Wang, Z.; Hua, C.; Tang, C. J. Supramolecular Nucleobase-Functionalized Polymers: Synthesis and Potential Biological Applications. *J. Mater. Chem. B* **2020**, *8*, 1576−7688. (b) Krishnamurthy, R. *Acc. Chem. Res.* **2012**, *45* (12), 2035−2044.

(11) Kleiner, R. E.; Brudno, Y.; Birnbaum, M. E.; Liu, D. R. DNA-Templated Polymerization of Side-Chain-Functionalized Peptide Nucleic Acid Aldehydes. *J. Am. Chem. Soc.* **2008**, *130* (14), 4646−4659.

(12) Self-assembling motifs featuring multiple H-bonding arrays within the molecule (also called "Janus bases") have been used to target double-stranded DNA or RNA by engaging both strands at once. See: (a) Branda, N.; Kurz, G.; Lehn, J.-M. Janus Wedges: A New Approach Towards Nucleobase-Pair Recognition. *Chem. Commun.* **1996**, 2443. (b) Chen, F. H.; Meena; McLaughlin, L. W. A. Janus Wedge DNA triplex with A-W_1-T and G-W_2-C Base triplets. *J. Am. Chem. Soc.* **2008**, *130*, 13190−13091. (c) Miao, S.; Liang, Y.; Marathe, I.; Mao, J.; DeSantis, C.; Bong, D. Duplex Stem Replacement with bPNA$^+$ Triplex Hybrid Stems Enables Reporting on Tertiary Interactions of Internal RNA Domains. *J. Am. Chem. Soc.* **2019**, *141* (23), 9365−9372. (d) Drożdż, W.; Walczak, A.; Bessin, Y.; Gervais, V.; Cao, X.-Y.; Lehn, L.-M.; Sébastien, U.; Stefankiewicz, A. R. Multivalent Metallosupramolecular Assemblies as Effective DNA Binding. Agents. *Chem. - Eur. J.* **2018**, *24* (42), 10802−10811.

(13) (a) Kheria, S.; Rayavarapu, S.; Kotmale, A. S.; Gonnade, R. G.; Sanjayan, G. J. Triazine-Based Highly Stable AADD-Type Self-Complementary Quadruple Hydrogen-Bonded Systems Devoid of Prototropy. *Chem. - Eur. J.* **2017**, *23*, 783−787. (b) Prabhakaran, P.; Puranik, V. G.; Sanjayan, G. J. Pre-organizing Linear (Self-Complementary) Quadruple Hydrogen-Bonding Arrays Using Intermolecular Hydrogen Bonding as the Sole Force. *J. Org. Chem.* **2005**, *70* (24), 10067−10072.

(14) (a) Fenniri, H.; Mathivanan, P.; Vidale, K. L.; Sherman, D. M.; Hallenga, K.; Wood, K. V.; Stowell, J. G. Helical Rosette Nanotubes: Design, Self-Assembly, and Characterization. *J. Am. Chem. Soc.* **2001**, *123*, 3854−3855. (b) Ma, Y.; Kolotuchin, S. V.; Zimmerman, S. C. Supramolecular Polymer Chemistry: Self-Assembling Dendrimers Using the DDA, AAD (GC-like) Hydrogen Bonding Motif. *J. Am. Chem. Soc.* **2002**, *124*, 13757−13769. (c) Yagai, S.; Iwashima, T.; Karatsu, T.; Kitamura, A. Synthesis and non-covalent polymerization of self-complementary hydrogen-bonding supramolecular synthons: N, N, A-disubstituted 4, 6-diamino-pyrimidin-2(1H)-ones. *Chem. Commun.* **2004**, 1114−1115. (d) Yagai, S.; Iwashima, T.; Kishikawa, K.; Nakahara, S.; Karatsu, T. Kitamura, A. Photoresponsive Self-Assembly and Self-Organization of Hydrogen-Bonded Supramolecular Tapes. *Chem. - Eur. J.* **2006**, *12*, 3984−3994.

(15) (a) Mathias, J. P.; Zerkowski, J. A.; Seto, C. T.; Simanek, E. E.; Whitesides, G. M. Structural Preferences of Hydrogen-Bonded Networks in Organic Solution-the Cyclic CA3*M3 "Rosette. *J. Am. Chem. Soc.* **1994**, *116*, 4316−4325. (b) Zerkowski, J. A.; Whitesides, G. M. Steric Control of Secondary, Solid-State Architecture in 1:1 Complexes of Melamines and Barbiturates That Crystallize as Crinkled Tapes. *J. Am. Chem. Soc.* **1994**, *116*, 4298−4304. (c) Mathias, J. P.; Simanek, E. E.; Whitesides, G. M. Self-Assembly Through Hydrogen Bonding: Peripheral Crowding-A New Strategy for The Preparation of Stable Supramolecular Aggregates Based on Parallel, Connected CA3.cntdot.M3 Rosettes. *J. Am. Chem. Soc.* **1994**, *116*, 4326−4340.

(16) (a) Zerkowski, J. A.; Seto, C. T.; Whitesides, G. M. Solid-State Structures of Rosette and Crinkled Tape Motifs Derived from The Cyanuric Acid Melamine Lattice. *J. Am. Chem. Soc.* **1992**, *114*, 5473−5475. (b) Zerkowski, J. A.; Macdonald, J. C.; Seto, C. T.; Wierda, D. A.; Whitesides, G. M. Design of Organic Structures in the Solid State: Molecular Tapes Based on the Network of Hydrogen Bonds Present in the Cyanuric Acid.cntdot. Melamine Complex. *J. Am. Chem. Soc.* **1994**, *116*, 2382−2391. (c) Ma, M.; Bong, D. Determinants of Cyanuric Acid and Melamine Assembly in Water. *Langmuir* **2011**, *27* (14), 8841−8853.

(17) Mathias, J. P.; Simanek, E. E.; Seto, C. T.; Whitesides, G. M. Design, Preparation, and Characterization of Hydrogen Bonded Supramolecular Aggregates Based On the Cyanuric Acid-Melamine Lattice. *Macromol. Symp.* **1994**, *77*, 157−166.

(18) (a) Seto, C. T.; Whitesides, G. M. Self-Assembly Based On the Cyanuric Acid-Melamine Lattice. *J. Am. Chem. Soc.* **1990**, *112* (17), 6409−64011. (b) Berl, V.; Huc, I.; Lehn, J.-M.; DeCian, A.; Fischer, J. Induced Fit Selection of a Barbiturate Receptor from a Dynamic

Structural and Conformational/Configurational Library. *Eur. J. Org. Chem.* **1999**, *1999*, 3089−3094.
(19) (a) Roy, D.; Cambre, J. N.; Sumerlin, B. S. Future Perspectives and Recent Advances in Stimuli-Responsive. *Prog. Polym. Sci.* **2010**, *35*, 278−301. (b) Lehn, J.-M. Perspectives in Supramolecular Chemistry-From Molecular Recognition towards Molecular Information Processing and Self-Organization. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1304−1319.
(20) (a) Nielsen, P. E. A New Molecule of Life. *Sci. Am.* **2008**, *299* (6), 64−71. (b) Nielsen, P. E.; Egholm, M.; Berg, R.; Buchardt, O. Sequence-Selective Recognition of DNA by Strand Displacement with A Thymine-Substituted Polyamide. *Science* **1991**, *254*, 1497−1500. (c) Scherman, O. A.; Ligthart, G. B. W. L.; Sijbesma, R. P.; Meijer, E. W. A Selectivity-Driven Supramolecular Polymerization of an A B Monomer. *Angew. Chem., Int. Ed.* **2006**, *45*, 2072−2076. (d) Bazzi, H. S.; Sleiman, H. F. Adenine-Containing Block Copolymers via Ring-Opening Metathesis Polymerization: Synthesis and Self-Assembly into Rod Morphologies. *Macromolecules* **2002**, *35*, 9617−9620.
(21) (a) Ando, N.; Terashima, S. A Novel Synthesis of the 2-Amino-1*H*-Imidazol-4-Carbaldehyde Derivatives and its Application to the Efficient Synthesis of 2-Aminoimidazole Alkaloids, Oroidin, Hymenidin, Dispacamide, Monobromodispacamide, and Ageladine A. *Tetrahedron* **2010**, *66*, 6224−6237. (b) Zhang, W.; Sun, T. T.; Li, Y.-X. Synthesis of the C-Terminal Penta-peptide of the Peptaibol Culicinins. *J. Pept. Sci.* **2009**, *15*, 366−368. (c) Sanz-Cervera, J. F.; Blasco, R.; Piera, J.; Cynamon, M.; Ibanez, I.; Murgu1, M.; Fustero, S. Solution versus Fluorous versus Solid-Phase Synthesis of 2, 5-Disubstituted 1, 3-Azoles, Preliminary Antibacterial Activity Studies. *J. Org. Chem.* **2009**, *74*, 8988−8996. (d) Meena, C. L.; Singh, D.; Weinmüller, M.; Reichart, F.; Dangi, A.; Marelli, U. K.; Zehler, S.; Sanjayan, G. J. Novel Cilengitide-Based Cyclic RGD Peptides as $\alpha v \beta_3$ Integrin Inhibitors. *Bioorg. Med. Chem. Lett.* **2020**, *30*, 127039.
(22) (a) Lopez, A. M.; Scarel, F.; Carrero, N. R.; Voazquez, E.; Mateo-Alonso, A.; Da Ros, T.; Prato, M. Synthesis and Characterization of Highly Water-Soluble Dendrofulleropyrrolidine bisadducts with DNA Binding Activity. *Org. Lett.* **2012**, *14*, 4450−4453. (b) Uhlmann, E.; Peyman, A.; Breipohl, G.; Will, D. W. PNA: Synthetic Polyamide Nucleic Acids with Unusual Binding Properties. *Angew. Chem., Int. Ed.* **1998**, *37*, 2796−2823.
(23) (a) Rapireddy, S.; He, G.; Roy, S.; Armitage, B. A.; Ly, D. H. Strand Invasion of Mixed-Sequence B-DNA by Acridine-Linked, γ-Peptide Nucleic Acid (γ-PNA). *J. Am. Chem. Soc.* **2007**, *129*, 15596−15600. (b) He, W.; Crawford, M. J.; Rapireddy, S.; Madrid, M.; Gil, R. R.; Ly, D. H.; Achim, C. The Structure of a γ-modified Peptide Nucleic Acid Duplex. *Mol. BioSyst.* **2010**, *6*, 1619−1629. (c) Zeng, Y.; Pratumyot, Y.; Piao, X.; Bong, D. Discrete Assembly of Synthetic Peptide-DNA Triplex Structures from Polyvalent Melamine-Thymine Bifacial Recognition. *J. Am. Chem. Soc.* **2012**, *134*, 832−835. (d) Marsh, A.; Nolen, E. G.; Gardinier, K. M.; Lehn, J. M. Janus Molecules: Synthesis of Double-Headed Heterocycles Containing Two Identical Hydrogen Bonding Arrays. *Tetrahedron Lett.* **1994**, *35* (3), 397−400.
(24) For selected recent examples of Janus/bifacial/bimodal PNAs, see: (a) Gupta, M. K.; Madhanagopal, B. R.; Datta, D.; Ganesh, K. N. Structural Design and Synthesis of Bimodal PNA that Simultaneously Binds Two Complementary DNAs to Form Fused Double Duplexes. *Org. Lett.* **2020**, *22*, 5255−5260. (b) Gupta, M. K.; Madhanagopal, B. R.; Ganesh, K. N. Peptide Nucleic Acid with Double Face: Homothymine-Homocytosine Bimodal Cα-PNA (bm-Cα-PNA) Forms a Double Duplex of the bm-PNA2: DNA Triplex. *J. Org. Chem.* **2021**, *86*, 414−428. (c) Bhingardeve, P.; Madhanagopal, B. R.; Ganesh, K. N. γ(S/R)-Bimodal Peptide Nucleic Acids (Cγ-bm-PNA) Form Coupled Double Duplexes by Synchronous Binding to Two Complementary DNAStrands. *J. Org. Chem.* **2020**, *85* (21), 13680−1369. (d) Thadke, S. A.; Hridya, V. M.; Perera, J. D. R.; Gil, R. R.; Mukherjee, A.; Ly, D. H. Shape selective bifacial recognition of double helical DNA. *Comm. Chem.* **2018**, *1*, 79. (e) Xia, X.; Piao, X.; Bong, D. Bifacial peptide nucleic acid as an allosteric switch for aptamer and ribozyme function. *J. Am. Chem. Soc.* **2014**, *136*, 7265−7268.
(25) (a) Feagin, T. A.; Shah, N. I.; Heemstra, J. M. Convenient and Scalable Synthesis of Fmoc-Protected Peptide Nucleic Acid Backbone. *J. Nucleic Acids* **2012**, *2012*, 1−6. (b) Balachandra, C.; Sharma, N. K. Instability of Amide Bond Comprising the 2-Aminotropone Moiety: Cleavable under Mild Acidic Conditions. *Org. Lett.* **2015**, *17*, 3948−3951. (c) Mitra, R.; Ganesh, K. N. Aminomethylene Peptide Nucleic Acid (am-PNA): Synthesis, Regio-/Stereospecific DNA Binding, and Differential Cell Uptake of (α/γ, R/S) am-PNA Analogues. *J. Org. Chem.* **2012**, *77* (13), 5696−5704. (d) Namivandi-Zangeneh, R.; Sadrearhami, Z.; Bagheri, A.; Sauvage-Nguyen, M.; Ho, K. K. K.; Kumar, N.; Wong, E. H. H.; Boyer, C. Nitric Oxide-Loaded Antimicrobial Polymer for the Synergistic Eradication of Bacterial Biofilm. *ACS Macro Lett.* **2018**, *7*, 592−597. (e) Sivakova, S.; Rowan, S. Nucleobases as Supramolecular Motifs. *J. Chem. Soc. Rev.* **2005**, *34*, 9−21.
(26) (a) Zhang, M.; Vedantham, P. D.; Flynn, L.; Hanson, P. R. High-Load, Soluble Oligomeric Carbodiimide: Synthesis and Application in Coupling Reactions. *J. Org. Chem.* **2004**, *69*, 8340−8344. (b) Heinzmann, C.; Salz, U.; Moszner, N.; Fiore, G. L.; Weder, C. Supramolecular Cross-Links in Poly(alkyl methacrylate) Copolymers and Their Impact on the Mechanical and Reversible Adhesive Properties. *ACS Appl. Mater. Interfaces* **2015**, *7*, 13395−13404. (c) Nadgorny, M.; Ameli, A. Functional Polymers and Nanocomposites for 3D Printing of Smart Structures and Devices. *ACS Appl. Mater. Interfaces* **2018**, *10*, 17489−17507.
(27) (a) Mo, Y. Probing The Nature of Hydrogen Bonds in DNA Base Pairs. *J. Mol. Model.* **2006**, *12*, 665−672. (b) Fonseca Guerra, C.; Bickelhaupt, F. M.; Snijders, J. G.; Baerends, E. J. Hydrogen Bonding in DNA Base Pairs: Reconciliation of Theory and Experiment. *J. Am. Chem. Soc.* **2000**, *122*, 4117−4128.

https://dx.doi.org/10.1021/acs.joc.0c02530
J. Org. Chem. 2021, 86, 3186−3195