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Synthesis of bis-1,2,3-triazolo-bridged unsymmetrical pyrrolobenzodiazepine trimers via 'click' chemistry and their DNA-binding studies

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ABSTRACT

New conceivable synthetic approach for the construction of nitrogen-rich 1,2,3-triazolo-pyrrolo[2,1-*c*][1,4]benzodiazepine (TPBD, **3a**–**c**) trimers has been developed. The first example of a bis-1,2,3-triazolo-bridged unsymmetrical PBD trimer has been successfully synthesized by employing a CuAAC type 'click' chemistry protocol. This efficient route generates tri-imine functionality in a single molecule. It has been envisaged that such tri-imine functionalities could bring in efficient interaction with DNA in a sequence-selective manner in the minor groove of duplex DNA. One of the representative analogues **3c** has shown improved DNA-binding ability (ΔT_m 23.7 °C) by thermal denaturation studies using CT-DNA and this data is also supported by molecular modeling (MD) studies.

1. Introduction

The development and construction of highly nitrogen-rich DNA-interactive new heterocyclic compounds continue to be an essential aspect for producing bioactive natural products and their structural analogues. 'Click' chemistry represents a modular approach toward the synthesis of low as well as high molecular weight compounds that access only the most practical transformations to make connections with excellent fidelity.[1] Huisgen 1,3-dipolar cycloaddition of terminal alkynes and organic azides to give five-membered 1,2,3-triazoles has emerged as a powerful linking reaction and found widespread applications ranging from combinatorial drug research[2] and material science[3] to bioconjugate chemistry.[4] Moreover, these triazoles display a broad spectrum of biological activities like antibacterial, antifungal, and antihelmintic activities,[5,6] including anticancer activity.[7]

It is well-known that DNA has long been recognized as an important cellular target in the rational design of potential chemotherapeutic and gene targeting agents.[8,9] DNA-reactive drugs exert their biological profile by inhibiting nucleic acid or protein synthesis through an interplay of non-covalent and covalent interactions in either the minor or major groove of the double helix. In addition, the DNA interstrand cross-linking (ISC) agents play an important role in cancer therapy by disrupting cell maintenance and replication. Some of the anticancer drugs (e.g., cisplatin, chlorambucil, and mitomycin C) have already been employed in clinical medicine.[10] Among them, over the years scaffolds like pyrrolo[2,1-*c*][1,4]benzodiazepines (PBDs) have gained considerable interest in the area of medicinal chemistry with respect to the development of synthetic strategies and preclinical studies, particularly as potential antitumor and gene-targeting compounds.[11] These tricyclic antitumor antibiotics are derived from *Streptomyces bacteria*,[12] members of which include DC-81, tomaymycin, and anthramycin. They exert biological activity by selective covalent binding between the imine functionality and the N_2 of guanine base in the minor groove of DNA.[13]

Thurston and co-workers have synthesized C8/C8′-linked PBD dimer (DSB-120 **2**, in Fig. 1) comprising of two DC-81 (**1**) subunits joined through their C8-positions by an inert propyldioxy spacer.[14] These have been examined for their cytotoxic effect, DNA cross-linking potency,[15] and cellular pharmacology.[13d,16–19] Investigation in our laboratory has led to a number of monomeric,[20] dimeric[21] PBD hybrids with enhanced DNA-binding affinity and promising anticancer activity. It has been observed most of these PBD dimers

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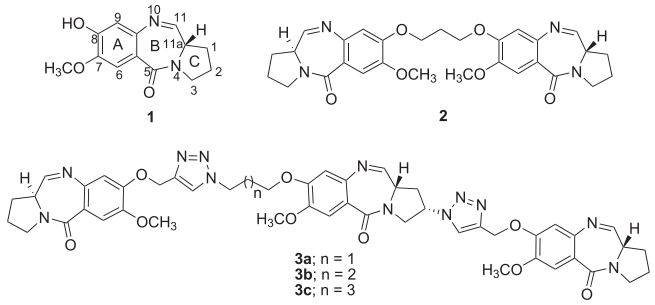


Figure 1. Representative structures of bioactive pyrrolo[2,1-*c*][1,4]benzodiazepines, DC-81 (**1**), DSB-120 (**2**), and triazolo-PBD trimer analogues (**3a**–**c**).

that the synthesis is usually problematic during the reaction work-up and chromatographic purifications by using high polar solvents. To overcome this complexity, recently we have developed a method based on 'click' chemistry protocol that has been applied for the synthesis of C8–C8/C2–C8-linked 1,2,3-triazolo-PBD dimer conjugates.[22] Based on the above findings, it is considered of interest to prepare the PBD trimers that could interact within the minor groove of DNA in a sequence-selective manner. In this context, the 'click' protocol has been applied for the synthesis of pyrrolo[2,1-*c*][1,4]benzodiazepine (PBD) trimers (**3a**–**c**) that are joined through a triazole moiety to explore their DNA-binding ability.

2. Results and discussion

The synthetic strategy reveals that the skeleton of triazole-containing PBD trimers **3a**–**c** has been assembled from the key precursors **9a**–**c** and **10**. This framework represents that one of the 1,2,3-triazole moiety is sandwiched between the two monomeric DC-81 subunits at their C8-positions of the aromatic A-rings via diether linkage by using alkane spacers and the other 1,2,3-triazole moiety is joined to the pyrrolidine C-ring through its C2-position and C8-position of the other aromatic A-ring through a monoether linkage. The earlier research relating to the synthesis and biological evaluation of PBD dimers like DSB-120 (**2**),[23] SJG-136,[24] and mixed imine–amides[25] have exhibited immense potential in the development of molecules of this type. Many attempts have been made previously in our laboratory to synthesize PBD trimers through diether linkages using simple dibromoalkane spacers in both solution-phase as well as on solid-support, but such studies have given unsatisfactory results probably owing to their insolubility problems. However, in this investigation by taking advantage of the copper-catalyzed azide–alkyne cycloaddition (CuAAC) protocol, we decided to apply such a 'click' process for the preparation of PBD trimers. This has also prompted us to explore the DNA-binding potential of such PBD trimer analogues linked through 1,2,3-triazole moieties by using the diazido functionality of **9a**–**c** and terminal alkyne group of **10**. Interestingly, it has been observed that the solubility of these PBD trimers is enhanced in most of the organic solvents and this may be attributed to the presence of two 1,2,3-triazole rings.

The synthetic approach began with the preparation of 4-(benzyloxy)-5-methoxy-2-nitrobenzoic acid (**4**), which has been synthesized from commercially available vanillin from three steps. This has been converted to its acid chloride with $SOCl_2$, and then coupled to *trans*-4-hydroxyl-L-proline methylester hydrochloride in the presence Et_3N to give the corresponding compound **5** in 84% overall yield from two steps. Mesylated product has been obtained in 88% yield by using mesylchloride and Et_3N at 0 °C to room temperature for 6 h, and then it has been successfully converted to its debenzylated product **6** (83%) employing $BF_3{\cdot}OEt_2$/EtSH. Etherification of the corresponding compound **6** by different dibromoalkane spacers in DMF with K_2CO_3 affords the desired intermediates **7a**–**c** (82–85%).

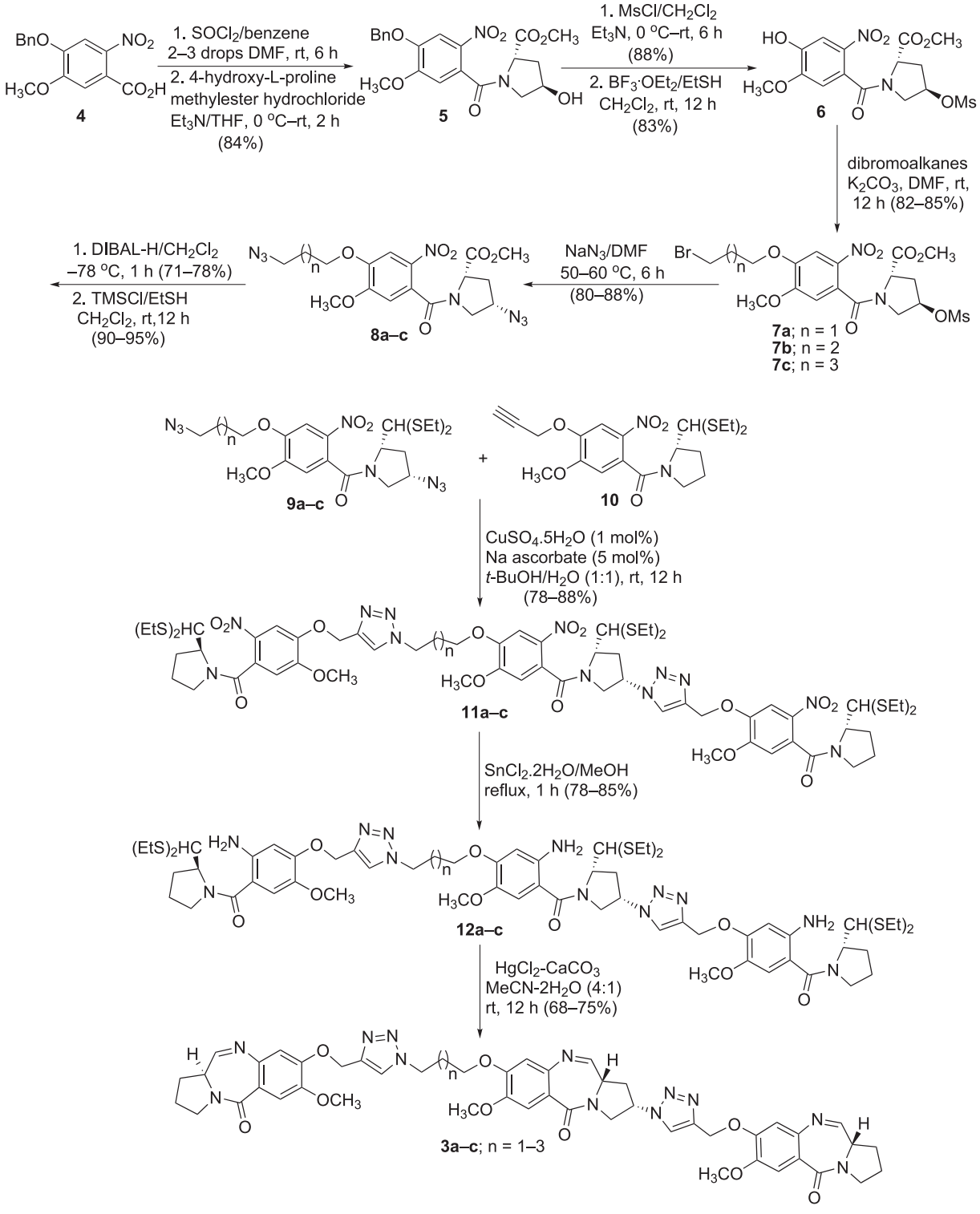
Herein, we report a new step in which C–C2/*A*–C8-diazido substituted 2-nitrobenzoyl prolinemethyl ester (**8a**–**c**) is obtained in one-pot manner by the in situ azidation of both C–C2-mesyl (bimolecular nucleophilic substitution reaction S_N2) and *A*–C8-bromo (elimination reaction) groups in presence of excess sodium azide (10 equiv) in anhydrous DMF at 60–70 °C for 6 h (80–88%). Next, the selective reduction of ester functionalities (**8a**–**c**) with DIBAL-H at −78 °C followed by aldehyde protection with ethylmercaptane has yielded the desired key diazido intermediates **9a**–**c** (90–95%). Another key intermediate **10** with terminal alkyne has been prepared by the earlier reported method.[22] Herein, the 'click' reaction has been employed for the construction of the bridged bis-1,2,3-triazolo-PBD trimer framework. Thus, trimerization reaction has been accomplished in a single step by the mixing of organic azides (**9a**–**c**, 1 mmol) and terminal alkyne (**10**, 2 mmol) in H_2O/*t*-BuOH (1:1) with 1 mol% of $CuSO_4{\cdot}5H_2O$ and 5 mol% of sodium ascorbate. The corresponding unsymmetrical bis-1,2,3-triazlolo-bridged 2-nitrobenzoyl ethylmercaptane prolinaldehyde precursors **11a**–**c** have been obtained in good yields (78–88%). Finally, the nitro groups have been reduced with excess of $SnCl_2{\cdot}2H_2O$ followed by ethylmercaptane deprotective tandem cyclization reaction using $HgCl_2/CaCO_3$ to provide the desired title 1,2,3-triazolo-PBD trimers (**3a**–**c**) in moderate to good yields (68–75%) as depicted in Scheme 1.

3. DNA-binding affinity studies

The DNA-binding affinity of these new triazolo-PBD trimers (**3a**–**c**) has been examined by thermal denaturation studies using calf thymus (CT) DNA.[15] Melting studies show that these compounds stabilize the thermal helix-coil or melting stabilization (ΔT_m) for the CT-DNA duplex at pH 7.0, incubated at 37 °C, wherein PBD/DNA molar ratio is 1:5. In this assay, the helix melting temperature changes (ΔT_m) for each compound has been studied at 0 h and after 18 h of incubation at 37 °C. Data for DC-81 (**1**) and DSB-120 (**2**) are incorporated in Table 1 for comparison. In this study, PBD trimer compounds **3a**–**c** have shown elevated melting temperatures ranging from 5.6 to 23.7 °C (Table 1). Interestingly, the ΔT_m of compound **3c** is 18.3 °C at 0 h while the melting temperature increases to 23.7 °C upon incubation for 18 h at 37 °C. Whereas for compound **3a** the ΔT_m is 10.7 °C at 0 h, which increases to 14.1 °C after incubation for 18 h. Compound **3b** elevates the helix melting temperature of CT-DNA by 5.6 °C at 0 h, however, there is not much difference in the melting temperatures after incubation for 18 h (7.9 °C) in comparison to **3a** and **3c**. In the same experiment the naturally occurring DC-81 (**1**) and the dimer DSB-120 (**2**) elevate the helix melting temperature of CT-DNA by 0.7 °C and 15.1 °C, respectively, after incubation for 18 h.

4. Molecular modeling studies

Docking of the 1,2,3-triazolo-PBD trimers (**3a**–**c**) into the minor groove of the DNA duplex was initially carried out using GOLD3.2,[26] with default settings and the binding is effectively guided by non-covalent interaction. On inspection of docked poses, all three N10-C11 imine functionalities of PBD trimer are close to the C2-amino group of three 'G' residues of 15-*mer* DNA. The 15-*mer* sequence 5′-GGGGCGAGAGAGGGG-3′ having the preferred binding site Pu-G-Pu for the PBD molecules was chosen for modeling the B-DNA duplex structure, which was prepared and minimized with OPL2005 force field using Maestro program of Schrodinger package.[27] This DNA sequence is chosen because it provided good results in the previous study and it has the Pu-G-Pu binding site for the covalent bond



Scheme 1. Synthesis of unsymmetrical bis-triazolo-PBD trimer conjugates (**3a**–**c**).

formation with the PBD trimer.[25] These calculations unambiguously revealed the extreme of covalent bonds. Thus three covalent bonds are enforced on the initial structure and the resultant complex minimized. Going by the precedence of earlier results there is a possibility of covalent bond formation. However, the current line of docking methods could not consider covalent docking, with default settings. The docking results of PBD trimer (**3a**–**c**) conjugates are presented in Table 2 along with their experimental ΔT_m values. All the compounds have fairly substantial docking fitness scores indicating that the complex formation to the DNA groove is quite facile. Compound with highest ΔT_m value also has highest GOLD score while compound with lowest ΔT_m value also has lowest GOLD score. The docked poses presented in Figure 2 showing that these conjugates bound to DNA with three covalent bonds and mainly in the minor groove region of DNA. The clearer picture of all three covalent bonding of nitrogen atom of guanine

Table 1
Thermal denaturation data for 1,2,3-triazole-PBD trimer (**3a**–**c**) conjugates with calf thymus CT-DNA

PBD compounds	[PBD]/[DNA] molar ratio[a]	ΔT_m (°C)[b] after incubation at 37 °C for	
		0 h	18 h
3a	1:5	10.7	14.1
3b	1:5	5.6	7.9
3c	1:5	18.3	23.7
DC-81 (**1**)	1:5	0.3	0.7
DSB-120 (**2**)	1:5	10.2	15.1

[a] For CT-DNA alone at pH 7.00±0.01, T_m=69.6±0.01 °C (mean value from 10 separate determinations), all T_m values are ±0.05–0.15 °C.
[b] For a 1:5 molar ratio of [PBD]/[DNA], where CT-DNA concentration=100 μM and ligand concentration=20 μM in aq sodium phosphate buffer [10 mM sodium phosphate+1 mM EDTA, pH 7.00±0.01].

Table 2
GOLD fitness score and IE of 1,2,3-triazolo-PBD trimer (**3a**–**c**) conjugates with their experimental ΔT_m values

Compounds	GOLD score[a]	IE[b]	ΔT_m (°C) at 18 h
3a	85.83	−128.03	14.1
3b	78.29	−127.45	7.9
3c	91.25	−146.51	23.7

[a] GOLD score is the best fitness score obtained in GOLD docking.
[b] 'IE' is the interaction energy calculated by using Generalized Born method after 2 ns MD simulation.

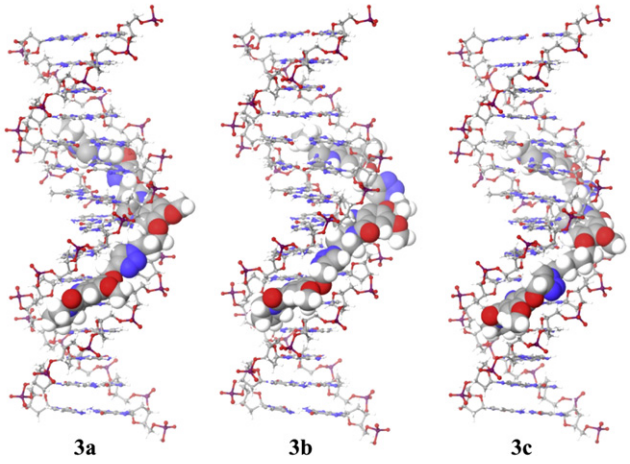


Figure 2. Docked poses of **3a**–**c** with DNA sequence 5′-GGGGCGAGAGAGGGG-3′.

and carbon atom of PBD trimer is shown in Figure 3. Compound **3c** has shown the best GOLD score probably due to five carbon chain in between the monomers, which provide the flexibility to rotate and fit in the DNA groove without unfavorable close contacts.

In order to investigate whether trimer is an optimum oligomeric unit, we resort to a docking analysis of the monomer, dimer, and tetramer along with trimer by choosing the same sequence and same region of DNA minor groove to estimate the structural and energetic compatibility of the oligomeric units of different length. This analysis brings out very clearly that the trimer is the most optimal unit with maximum binding strength and with highest structural compatibility as can be gauged from Figure 4 and Table 3. In conformity with current and earlier studies, mononer and dimer units nicely fit into the DNA minor groove region. An exhaustive study has been carried out to assess the suitability of tetramer unit to the DNA and three best among the several putative attempts were taken (Fig. 4). The results show that in none of the orientations, the tetramer is fully fitting into the DNA groove and thus it

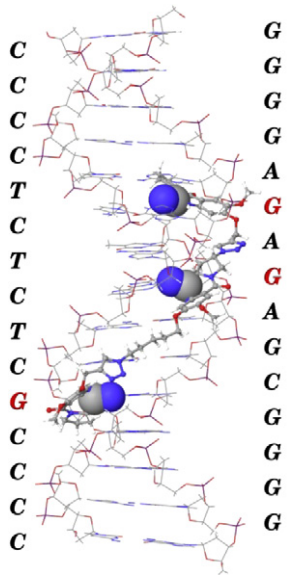


Figure 3. Covalent bonding of PBD trimer **3c** with DNA (guanine residues involved in the bonding are written in red color, C-atom of PBD and N-atom of guanine are shown in CPK).

appears to be a bit longer compared to the optimal length. The docking score also reveals that trimer binding is stronger compared to that of tetramer. It may be noted that even in tetramer the spacer chain length of five carbon units (see **3c**, where n=3) is preferred. Therefore, the current study, for the first time, reveals that trimer has the most optimal binding. These studies indicate that there is no structural variation between the double helical strands of DNA upon changing the linker length of the PBD trimer.

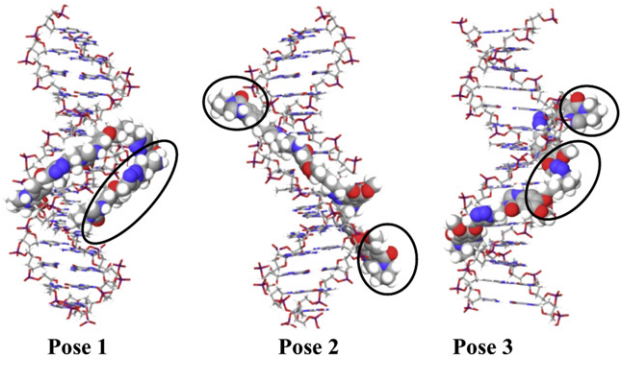


Figure 4. The three best score poses of tetramer PBD docked in the DNA groove (part of the PBD, which is not docked in the minor groove are shown in circles).

Table 3
Comparative GOLD fitness score of various PBDs

PBD	GOLD score[a]
Monomer	44.54
Dimer	73.56
Trimer (**3c**)	91.25
Tetramer (Pose 1)	84.75
Tetramer (Pose 2)	76.82
Tetramer (Pose 3)	76.73

[a] GOLD SCORE is the best fitness score obtained in GOLD docking.

After establishing the mode of binding of the novel trimer to DNA we performed more rigorous MD simulation. The MD simulation with explicit solvent, involving ~10,000 water molecules having performed up to 2 ns. Figure 5 is the snapshots of the

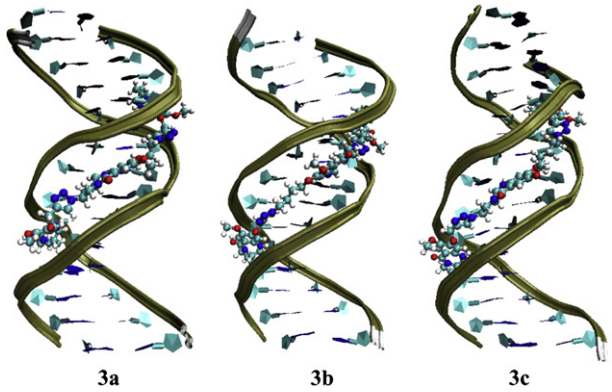


Figure 5. Snapshots of docked poses of **3a**–**c** at 2 ns MD simulation.

docked poses of compounds **3a**–**c** at 2 ns MD simulation. The simulation reveals that all the three PBD trimers (**3a**–**c**) are fairly rigid up to 2 ns and the most stable and active among them **3c** showed a very rigid structure. Thus, the MD calculations help unambiguously establish the complex formation and its stability. To see the deviation of the docked complexes during MD simulation, we plotted root-mean-square deviation (RMSD) as a function of time. RMSD of complex as well as of DNA and ligand is presented separately to show a systematic picture of deviation. It is very clear from the RMSDs that the docked poses of all the compounds are stable in 2 ns MD simulation.

Irrespective of the ligand and the simulation conditions of all the compounds remain bound to the DNA near the preferential binding position and do not experience substantial fluctuations with respect to their initial placements in the DNA groove. The interaction energy (IE), which provides a quantitative estimate of average binding energy, between the DNA and the PBDs is calculated to confirm their binding stability and presented in Table 2.[28,29] Generalized Born (GB) method, which is semi-analytical approximations to continuum electrostatics is used for the calculation of interaction energies.[30] Interaction energies of all the compounds considered, points to a stable complex formation and these trends are in excellent agreement with the experimental and docking results. Thus, the modeling studies establish that these compounds interact with the minor groove of DNA, which is not only due to strong non-covalent forces but also due to the formation of three covalent bonds.

5. Conclusion

The unsymmetrical bis-1,2,3-triazolo-PBD trimers have been designed and synthesized by employing 'click' chemistry process. Interestingly, by using this 'click' chemistry protocol the solubility aspects have been improved that facilitated the purification and isolation of the target compounds. These new PBD trimers have shown significant DNA-binding ability. Molecular modeling studies substantiate the formation of three covalent bonds with the PBD trimer and guanine. Compound **3c** appears to be the optimal binder as further increase in linker or chain length decreases the binding strength of these compounds with DNA. The structure–activity relationship and cytotoxicity studies of these new compounds are under in progress.

6. Experimental section

6.1. General methods

Purchased chemical reagents were used without further purification. Anhydrous THF, CH_2Cl_2, CH_3CN, MeOH, and DMF were prepared by distillation under nitrogen atmosphere over sodium/benzophenone, CaH_2, sodium/P_2O_5, and CaH_2/molecular sieves, respectively, and were used for reactions. Solvents for extraction and column chromatography were distilled prior to use. Sodium azide was handled with care by wearing safety glasses, facemask, gloves, and reactions were performed in a fume hood. IR spectroscopy: FT-IR Nicolet Nexus 470 equipment and KCl cell. Infrared spectra were recorded and the wave numbers are expressed in cm^{-1}. Melting points (uncorrected) were measured with an Eletrothermal apparatus. Thermal denaturation (DNA-binding) studies were evaluated by BECKMAN COULTER-DU 800 spectrophotometer. Specific rotations were recorded on SEPA-300 fixed with sodium lamp of wavelength 589 nm. ^1H and ^{13}C NMR spectra were recorded on 200, 300, 400, and 500 MHz spectrometers using tetramethyl silane (TMS) as the internal standard. Chemical shifts are reported in parts per million (ppm) downfield from tetramethyl silane. Spin multiplicities are described as s (singlet), br s (broad singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), and or m (multiplet). Coupling constants are reported in hertz (Hz). Mass spectra were recorded on a Quattro-LC (ESI). Column chromatography was performed using silica gel 60–120 and 100–200 mesh. TLC analyses were performed with silica gel plates using iodine, $KMnO_4$, and UV-lamp for visualization.

6.2. Computational details

Sybyl 6.9.2 program (Tripos Inc., St. Louis, MO) has been used for preparing the PBD conjugates. All the compounds were minimized to 0.001 kcal mol^{-1} $Å^{-1}$ root-mean-square gradients by using Gasteiger–Hückel partial atomic charges and Tripos force field. Radius of 30 Å from the middle atom of DNA was used as a central atom to scan the entire DNA sequences in molecular docking. All the PBD conjugates were subjected to a conformational search by using molecular mechanics method and minimum energy conformation was selected for final study. Previously 5′-GGGGCGAGAGAGGGG-3′ DNA sequence provide better results in the docking study of similar compounds[25] thus, we have used this sequence in the current study. Our ongoing studies indicate that the GOLD docking on DNA sequences is considered to be the best docking protocol, which reliably reproduces the crystallographic poses of DNA–ligand complex and it provided good results in our earlier studies.[31] Thus in the present study we have performed the docking calculations using GOLD3.2 program. The default parameters in GOLD3.2 (number of islands 5, population size of 100, number of operations was 100,000, a niche size of 2, and a selection pressure of 1.1, and the van der Waals and hydrogen bonding were set to 4.0 and 2.5, respectively) have been used. The 10 best conformations have been generated for each compound and the best score pose was chosen for MD simulation using AMBER 8.0.[32] The final input files were created by merging DNA and ligand in a complex as docked pose. Three covalent bonds were made in the docked poses and the structure was minimized by using impact minimization on Maestro package. The 'leaprc.gaff' (generalized amber force filed) was used to prepare the ligands while 'leaprc.ff03' was used for DNA. The 'addions' command implemented in 'xleap' of AMBER 8.0 was used to add the Na^+ ions explicitly to neutralize the system. The 'SolvateOct' command was used to solvate the complex in a 10 Å water box with TIP3P water. Equilibration of the solvated complex has been done by carrying out a short minimization (500 steps of each steepest descent and conjugate gradient method), 50 ps of heating and 50 ps of density equilibration with weak restraints on the complex followed by 500 ps of constant pressure equilibration at 300 K. The final production run is performed for the 2 ns and the coordinates are recorded in every 10 ps. Before submitting for the MM-PBSA production run we verified that the system has equilibrated. We extracted 200 snapshots from production runs by using

'extract_coords.mmpbsa' script and calculated the interaction energies by using 'binding_energy.mmpbsa' script. Both the scripts are available on AMBER web site. The final reported interaction energies are the average of all 200 snapshots.

6.3. Reaction procedures and spectral data

*6.3.1. Methyl-(2S,4R)-N-[4-benzyloxy-5-methoxy-2-nitrobenzoyl]-4-hydroxypyrrolidine-2-carboxylate (**5**).* To a stirred suspension of compound **4** (3.03 g, 10.0 mmol) and thionyl chloride (2.15 mL, 30.0 mmol) in dry benzene (40 mL), 4–5 drops of DMF was added and the stirring was continued for 6 h. The benzene was evaporated in vacuum and the resultant oil dissolved in dry THF (40 mL) and added drop wise over a period of 30 min to a stirred suspension of 4-hydroxy-L-proline methylester hydrochloride (2.14 g, 15.0 mmol), Et_3N (4.17 mL, 30 mmol), and THF/H_2O (25-5 mL) cooled in an ice bath. After the completion of addition, the temperature was allowed to rise to room temperature and stirred for an additional 1 h. THF was evaporated in vacuum and the aqueous layer was washed with ethyl acetate (50 mL). The aqueous phase was then adjusted to pH 3 using 6 N HCl and extracted with ethyl acetate, and then washed with brine dried over Na_2SO_4. The crude product was purified by column chromatography (30% ethyl acetate/hexane) afforded compound **5** as yellow oil (3.60 g, 84%). ^{1}H NMR (200 MHz, $CDCl_3$): δ=7.75 (s, 1H), 7.30–7.45 (m, 5H), 6.90 (s, 1H), 5.20 (s, 2H), 4.70–4.80 (m, 1H), 4.40 (m, 1H), 3.96 (s, 3H), 3.85 (s, 3H), 3.50–3.60 (m, 3H), 2.05–2.30 ppm (m, 2H); (ESI) MS: m/z 431 $[M]^{+\bullet}$.

*6.3.2. (2S,4R)-Methyl 1-(4-hydroxy-5-methoxy-2-nitrobenzoyl)-4-(methylsulfonyloxy)pyrrolidine-2-carboxylate (**6**).* To a stirred solution of compound **5** (4.30 g, 10.0 mmol) in CH_2Cl_2 (40 mL), Et_3N (4.17 mL, 30.0 mmol) was added at 0 °C and the reaction mixture was stirred for 30 min and mesylchloride (5.02 mL, 30.0 mmol) was added drop wisely. Then, the reaction mixture was brought to ambient temperature and stirred for an overnight to afford mesylated product. The solvent was evaporated and excess of mesylchloride was quenched with aq $NaHCO_3$ solution (40 mL). The combined reaction mixture was extracted with ethyl acetate (3×50 mL) and dried over Na_2SO_4. The organic layer was evaporated in vacuum and purified by column chromatography using ethyl acetate/hexane (6:4) to afford the methyl-(2*S*,4*R*)-*N*-(4-benzyloxy-5-methoxy-2-nitrobenzoyl)-4-[(methylsulfonyl)oxy]pyrrolidine-2-carboxylate in 88% (4.47 g) yield. ^{1}H NMR (200 MHz, $CDCl_3$): δ=7.79 (s, 1H), 7.30–7.50 (m, 5H), 6.80 (s, 1H), 5.20 (s, 2H), 4.80–4.90 (t, J=8.60 Hz, 1H), 3.80–4.10 (s, 6H), 3.42–3.60 (s, 2H), 3.09–3.20 (m, 3H), 2.60–2.80 (m, 1H), 2.20–2.40 (m, 1H), 2.20–2.25 ppm (m, 1H); ESI (MS): m/z 509 $[M]^{+\bullet}$. This above compound (4.40 g, 8.66 mmol) was taken in CH_2Cl_2 (40 mL), EtSH (12.35 mL, 173.2 mmol) and $BF_3 \cdot OEt_2$ (9.39 mL, 86.6 mmol) were added drop wise at room temperature. Stirring was continued until TLC indicated completion of the reaction. The solvent was evaporated under vacuum. The residue was quenched with aq $NaHCO_3$ solution (1×100 mL) and then extracted with ethyl acetate (3×50 mL). Later, the combined organic phase was washed with brine (1×100 mL), dried over Na_2SO_4, and the solvent was evaporated in vacuum to afford the crude product. This was further purified by column chromatography using ethyl acetate/hexane (5:5) as eluent to give (2*S*,4*R*)-methyl 1-(4-hydroxy-5-methoxy-2-nitrobenzoyl)-4-(methylsulfonyloxy)pyrrolidine-2-carboxylate (**6**, 3.0 g, 83%). ^{1}H NMR (200 MHz, $CDCl_3$+DMSO): δ=7.62 (s, 1H), 6.77 (s, 1H), 5.27 (br s, 1H), 4.69 (t, J=8.05, 8.78 Hz, 1H), 3.97 (s, 3H), 3.79 (s, 3H), 3.65 (dd, J=3.66, 8.78 Hz, 1H), 3.09 (s, 3H), 2.53–2.72 (m, 2H), 2.26–2.40 ppm (m, 2H); (ESI) MS: m/z 419 $[M]^{+\bullet}$.

*6.3.3. (2S,4R)-Methyl 1-(4-(3-bromopropoxy)-5-methoxy-2-nitrobenzoyl)-4-(methylsulfonyloxy)pyrrolidine-2-carboxylate (**7a**).* To compound **6** (1.50 g, 3.58 mmol) in dry DMF (20 mL), K_2CO_3 (2.47 g, 17.93 mmol) and 1,3-dibromopropane (0.86 g, 4.29 mmol) were added and the reaction mixture was stirred at room temperature for 12 h. This reaction mixture was poured into ice-cold water and extracted with ether and the organic layer was separated dried over anhydrous Na_2SO_4 and evaporated in vacuo. This was purified by column chromatography with ethyl acetate/hexane (6:4) as eluent to afford compound **7a** (1.60 g, 83%). ^{1}H NMR (200 MHz, $CDCl_3$): δ=7.69 (s, 1H), 6.78 (s, 1H), 5.22 (m, 1H), 4.84 (t, J=8.30 Hz, 1H), 4.24 (t, J=5.26, 6.04 Hz, 2H), 3.97 (s, 3H), 3.84 (s, 3H), 3.61 (m, 2H), 3.57 (m, 1H), 3.07 (s, 3H), 2.66–2.73 (m, 1H), 2.23–2.44 ppm (m, 4H); (ESI) MS: m/z 539 $[M]^{+\bullet}$.

*6.3.4. (2S,4R)-Methyl 1-(4-(4-bromobutoxy)-5-methoxy-2-nitrobenzoyl)-4-(methylsulfonyloxy)pyrrolidine-2-carboxylate (**7b**).* To compound **6** (1.20 g, 2.63 mmol) in dry DMF (20 mL), K_2CO_3 (1.81 g, 13.15 mmol) and 1,4-dibromopropane (0.68 g, 3.15 mmol) were added and the reaction mixture was stirred at room temperature for 12 h to afford compound **7b** (1.30 g, 82%). ^{1}H NMR (200 MHz, $CDCl_3$): δ=7.64 (s, 1H), 6.78 (s, 1H), 5.21 (m, 1H), 4.83 (t, J=7.81, 8.59 Hz, 1H), 4.12 (t, J=5.46 Hz, 2H), 3.98 (s, 3H), 3.84 (s, 3H), 3.42 (t, J=6.25, 7.03 Hz, 2H), 3.07 (s, 3H), 2.64–2.80 (m, 1H), 2.22–2.36 (m, 1H), 1.85–2.05 (m, 4H), 1.62–1.74 ppm (m, 2H); (ESI) MS: m/z 553 $[M]^{+\bullet}$.

*6.3.5. (2S,4R)-Methyl 1-(4-(5-bromopentyloxy)-5-methoxy-2-nitrobenzoyl)-4-(methylsulfonyloxy)pyrrolidine-2-carboxylate (**7c**).* To compound **6** (0.82 g, 1.96 mmol) in dry DMF (10 mL), K_2CO_3 (1.35 g, 9.80 mmol) and 1,4-dibromopropane (0.54 g, 2.35 mmol) were added and the reaction mixture was stirred at room temperature for 12 h to afford compound **7c** (0.94 g, 85%). ^{1}H NMR (200 MHz, $CDCl_3$): δ=7.64 (s, 1H), 6.78 (s, 1H), 5.21 (m, 1H), 4.83 (t, J=7.81, 8.59 Hz, 1H), 4.10 (t, J=6.25 Hz, 2H), 3.98 (s, 3H), 3.84 (s, 3H), 3.59 (m, 2H), 3.42 (t, J=6.75, 7.03 Hz, 2H), 3.07 (s, 3H), 2.64–2.76 (m, 1H), 2.22–2.36 (m, 1H), 1.85–2.05 (m, 4H), 1.62–1.74 ppm (m, 2H); (ESI) MS: m/z 567 $[M]^{+\bullet}$.

*6.3.6. (2S,4S)-Methyl 4-azido-1-(4-(3-azidopropoxy)-5-methoxy-2-nitrobenzoyl)pyrrolidine-2-carboxylate (**8a**).* To (2*S*,4*R*)-methyl 1-(4-(3-bromopropoxy)-5-methoxy-2-nitrobenzoyl)-4-(methylsulfonyloxy)pyrrolidine-2-carboxylate (**7a**, 1.50 g, 2.78 mmol) in dry DMF (15 mL), NaN_3 (1.81 g, 27.88 mmol) was added and stirred at 50–60 °C for 6 h, in this transformation both the bromo- and mesyl-functionalities can take place into azidation. Later, this reaction mixture was poured into ice-cold water and extracted with ethyl acetate (3×50 mL). The organic layer was dried over Na_2SO_4 and evaporated in vacuo, and then purified by column chromatography with ethyl acetate/hexane (7:3) to afford compound **8a** (1.28 g, 85%). ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.68 (s, 1H), 6.91 (s, 1H), 4.89 (t, J=3.77, 5.28 Hz, 1H), 4.41 (m, 1H), 4.13 (m, 2H), 3.99 (s, 3H), 3.84 (s, 3H), 3.33 (m, 2H), 2.63 (m, 1H), 2.42 (m, 1H), 2.28 (m, 1H), 1.95 ppm (m, 3H); IR (KBr): ν^- 2950, 2860, 2101, 1746, 1651, 1577, 1520, 1421, 1336, 1276, 1215, 1059, 1013, 870, 812, 756, 620, 557 cm^{-1}; (ESI) HRMS: m/z calcd for $C_{17}H_{20}N_8O_7$ 449.3386, found 449.3408 $[M+H]^{+\bullet}$.

*6.3.7. (2S,4S)-Methyl 4-azido-1-(4-(4-azidobutoxy)-5-methoxy-2-nitrobenzoyl)pyrrolidine-2-carboxylate (**8b**).* Yield 80% (1.01 g). ^{1}H NMR (300 MHz, $CDCl_3$): δ=7.56 (s, 1H), 6.81 (s, 1H), 4.75 (m, 1H), 4.33 (m, 1H), 4.12 (t, J=5.28 Hz, 2H), 3.93 (s, 3H), 3.77 (s, 3H), 3.34 (m, 2H), 3.31(dd, J=3.77, 7.55 Hz, 1H), 2.53–2.63 (m, 1H), 2.28–2.44 (m, 1H), 2.09–2.17 (m, 1H), 1.88 (m, 2H), 1.75 ppm (m, 2H); IR (KBr): ν^- 2950, 2878, 2104, 1747, 1653, 1577, 1521, 1423, 1337, 1278, 1217, 1062, 1014, 921, 872, 843, 806, 756, 622, 558 cm^{-1}; (ESI) HRMS: m/z calcd for $C_{18}H_{22}N_8O_7$ 463.1601, found 463.1633 $[M+H]^{+\bullet}$.

*6.3.8. (2S,4S)-Methyl 4-azido-1-(4-(5-azidopentyloxy)-5-methoxy-2-nitrobenzoyl)pyrrolidine-2-carboxylate (**8c**).* Yield 88% (0.73 g). ^{1}H

NMR (300 MHz, $CDCl_3$): δ=7.61 (s, 1H), 6.87 (s, 1H), 4.85 (dd, J=4.53, 4.91 Hz, 1H), 4.38 (m, 1H), 4.10 (m, 2H), 4.00 (s, 3H), 3.84 (s, 3H), 3.47 (m, 1H), 3.30 (m, 2H), 2.63 (m, 1H), 2.42 (m, 1H), 2.27 (m, 1H), 1.92 (m, 4H), 1.70 ppm (m, 2H); IR (KBr): ν^- 2944, 2869, 2100, 1744, 1651, 1575, 1518, 1421, 1334, 1275, 1213, 1058, 1011, 871, 809, 757, 619 cm^{-1}; (ESI) HRMS: m/z calcd for $C_{19}H_{24}N_8O_7$ 499.1927, found 499.1955 $[M+Na]^{+\bullet}$.

*6.3.9. ((2S,4S)-4-Azido-2-(bis(ethylthio)methyl)pyrrolidin-1-yl)(4-(3-azidopropoxy)-5-methoxy-2-nitrophenyl)methanone (**9a**).* Diisobutylaluminiumhydride solution (6 mL of 1.0 M solution in hexane) was added drop wise to a vigorously stirred solution of the (2*S*,4*S*)-methyl 4-azido-1-(4-(3-azidopropoxy)-5-methoxy-2-nitrobenzoyl) pyrrolidine-2-carboxylate (**8a**, 1.20 g, 2.67 mmol) in dry CH_2Cl_2 (20 mL) under nitrogen atmosphere at −78 °C. The reaction mixture was stirred for an additional 45 min and the excess of reagent was decomposed by careful addition of MeOH (4 mL) followed by 5% HCl (4 mL). The resulting mixture was allowed to warm to room temperature and the organic layer was removed in vacuum. The aqueous layer was extracted with ethyl acetate (4×20 mL), the organic layers were combined, and dried over Na_2SO_4. The solvent was evaporated in vacuum to afford the crude aldehyde approximately 75% (0.83 g). Without any further purifications, EtSH (0.35 mL, 4.96 mmol) was added to a stirred solution of nitroaldehyde (0.83 g, 1.98 mmol) in dry CH_2Cl_2 (15 mL) under nitrogen atmosphere. The mixture was stirred for 30 min followed by the addition of TMSCl (0.61 mL, 4.95 mmol). After a further 12 h of stirring, the reaction mixture, when TLC indicated that the reaction was completed, and then the reaction mixture was carefully neutralized with aq $NaHCO_3$ (10 mL) solution and extracted with $CHCl_3$ (2×10 mL). The combined organic phase was dried over Na_2SO_4, evaporated in vacuum, which was further purified by column chromatography (ethyl acetate) to give compound **9a** (0.98 g, 95%). ^1H NMR (300 MHz, $CDCl_3$): δ=7.69 (s, 1H), 6.81 (s, 1H), 4.78 (m, 2H), 4.73–4.83 (m, 2H), 4.19 (t, J=6.04 Hz, 2H), 3.95 (s, 3H), 3.57 (t, J=6.79, 6.04 Hz, 2H), 3.47 (dd, J=7.55, 3.02 Hz, 1H), 3.21 (t, J=10.57, 9.82 Hz, 1H), 2.65–2.85 (m, 5H), 2.10–2.18 (m, 2H), 1.34 ppm (t, J=7.55 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=166.6, 154.7, 148.4, 137.1, 127.5, 109.1, 108.5, 66.2, 59.7, 57.5, 56.4, 53.8, 52.7, 48.0, 32.8, 28.3, 26.3, 15.1 ppm; IR (KBr): ν^- 2952, 2929, 2873, 2102, 1645, 1577, 1520, 1421, 1337, 1276, 1217, 1065, 868, 812, 755 cm^{-1}; (ESI) HRMS: m/z calcd for $C_{20}H_{28}N_8NaO_5$ 547.1516, found 547.1540 $[M+Na]^{+\bullet}$.

*6.3.10. ((2S,4S)-4-Azido-2-(bis(ethylthio)methyl)pyrrolidin-1-yl)(4-(4-azidobutoxy)-5-methoxy-2-nitrophenyl)methanone (**9b**).* Yield 90% (0.53 g). ^1H NMR (300 MHz, $CDCl_3$): δ=7.61 (s, 1H), 6.75 (s, 1H), 4.77 (m, 2H), 4.08–4.15 (m, 2H), 3.95 (s, 3H), 3.41 (t, J=6.79, 6.04 Hz, 2H), 3.16 (m, 2H), 2.66–2.81 (m, 5H), 1.93–2.02 (m, 2H), 1.79–1.87 (m, 4H), 1.35 ppm (t, J=7.55 Hz, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=167.2, 155.3, 148.7, 137.3, 128.7, 109.1, 108.6, 68.8, 59.2, 58.8, 56.1, 53.6, 52.9, 52.1, 32.8, 28.6, 26.0, 22.8, 18.2, 14.9 ppm; IR (KBr): ν^- 2929, 2871, 2102, 1646, 1577, 1520, 1421, 1337, 1275, 1217, 1064, 869, 803, 753 cm^{-1}; (ESI) HRMS: m/z calcd for $C_{21}H_{30}N_8NaO_5$ 561.1673, found 561.1697 $[M+Na]^{+\bullet}$.

*6.3.11. ((2S,4S)-4-Azido-2-(bis(ethylthio)methyl)pyrrolidin-1-yl)(4-(5-azidopentyloxy)-5-methoxy-2-nitrophenyl)methanone (**9c**).* Yield 93% (0.67 g). ^1H NMR (300 MHz, $CDCl_3$): δ=7.60 (s, 1H), 6.74 (s, 1H), 4.66–4.79 (m, 2H), 4.09 (t, J=5.14, 6.61 Hz, 2H), 3.94 (s, 3H), 3.40–3.49 (m, 1H), 3.32 (t, J=6.42, 6.61 Hz, 2H), 3.17 (t, J=10.0 Hz, 1H), 2.62–2.85 (m, 5H), 1.85–1.95 (m, 2H), 1.50–1.74 (m, 6H), 1.35 ppm (t, J=7.34 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=166.6, 154.4, 148.6, 137.1, 127.1, 109.0, 108.1, 69.2, 59.6, 57.4, 56.4, 53.6, 52.6, 51.1, 32.8, 28.5, 26.3, 23.2, 15.1, 14.8 ppm; IR (KBr): ν^- 2931, 2870, 2102, 1645, 1576, 1520, 1421, 1337, 1276, 1218, 1065, 869, 810, 753 cm^{-1}; (ESI) HRMS: m/z calcd for $C_{22}H_{32}N_8O_5$ 553.2010, found 553.2026 $[M+H]^{+\bullet}$.

*6.3.12. (4-((1-((3S,5S)-5-(Bis(ethylthio)methyl)-1-(4-(3-(4-((4-((S)-2-(bis(ethylthio)methyl)pyrrolidine-1-carbonyl)-2-methoxy-5-nitrophenoxy)methyl)-1H-1,2,3-triazol-1-yl)propoxy)-5-methoxy-2-nitrobenzoyl)pyrrolidin-3-yl)-1H-1,2,3-triazol-4-yl)methoxy)-5-methoxy-2-nitrophenyl)((S)-2-(bis(ethylthio)methyl)pyrrolidin-1-yl) methanone (**11a**).* (2*S*)-*N*-[(4-propargyloxy)-5-methoxy-2-nitrobenzoyl]pyrrolidine-2-carboxaldehyde diethyl thioacetal (**9a**, 0.50 g, 0.95 mmol) and (2*S*,4*S*)-*N*-[4-(3-azido-1-propoxy)-5-methoxy-2-nitrobenzoyl]-4-azidopyrrolidine-2-carboxaldehyde diethyl thioacetal (**10**, 0.92 g, 2.10 mmol) were suspended in a 1:1 mixture of H_2O and *t*-BuOH (30 mL). Freshly prepared sodium ascorbate solution (19.0 mg, 5 mol %) was added followed by $CuSO_4 \cdot 5H_2O$ (4.84 mg, 1 mol %). The heterogeneous mixture was stirred vigorously for 12 h, at which point it cleared and TLC analysis indicated complete consumption of the reactants. To this reaction mixture, 2 mL of 3% ammonia solution was added for quenching of excess $CuSO_4 \cdot 5H_2O$ and stirred for further 10 min. *t*-BuOH was rotavaporated under reduced pressure, the aqueous layer was diluted with $CHCl_3$ (50 mL), stirred for another 10–15 min, and then filtered through a Celite bed. The combined reaction mixture was extracted with $CHCl_3$ (3×50 mL), washed with brine, and dried over anhydrous Na_2SO_4. This was further purified by column chromatography using ethyl acetate (100%) afforded **11a** in 1.08 g (82%) of pure product as an off-white powder. Mp: 125–127 °C. ^1H NMR (300 MHz, $CDCl_3$): δ=7.88 (s, 1H), 7.87 (s, 1H), 7.78 (s, 1H), 7.73 (s, 1H), 7.64 (s, 1H), 6.84 (s, 3H), 5.33 (s, 2H), 5.30 (s, 2H), 4.91 (m, 1H), 4.86–5.09 (m, 5H), 4.62–4.73 (m, 4H), 4.13 (m, 2H), 3.96 (s, 3H), 3.93 (s, 6H), 3.19–3.32 (m, 4H), 2.97–3.07 (m, 1H), 2.65–2.87 (m, 11H), 2.48–2.57 (m, 2H), 2.21–2.32 (m, 2H), 2.04–2.16 (m, 2H), 1.90–2.01 (m, 1H), 1.75–1.84 (m, 1H), 1.61 (m, 5H), 1.35 ppm (m, 18H); ^{13}C NMR (125 MHz, $CDCl_3$): δ=166.5, 166.1, 154.4, 154.2, 148.4, 147.4, 137.2, 128.7, 127.1, 109.5, 109.4, 109.1, 108.4, 96.1, 68.8, 63.0, 62.7, 60.9, 59.7, 56.9, 56.2, 52.9, 52.7, 50.0, 49.7, 49.2, 27.2, 26.4, 26.1, 25.4, 24.6, 15.0, 14.9 ppm; (ESI) HRMS: m/z calcd for $C_{60}H_{80}N_{12}O_{15}S_6$ 1401.4263, found 1401.4277 $[M+H]^{+\bullet}$.

*6.3.13. (4-((1-((3S,5S)-5-(Bis(ethylthio)methyl)-1-(4-(4-(4-((4-((S)-2-(bis(ethylthio)methyl)pyrrolidine-1-carbonyl)-2-methoxy-5-nitrophenoxy)methyl)-1H-1,2,3-triazol-1-yl)butoxy)-5-methoxy-2-nitrobenzoyl)pyrrolidin-3-yl)-1H-1,2,3-triazol-4-yl)methoxy)-5-methoxy-2-nitrophenyl)((S)-2-(bis(ethylthio)methyl)pyrrolidin-1-yl) methanone (**11b**).* Yield 78% (0.61 g). Mp: 120–122 °C. ^1H NMR (300 MHz, $CDCl_3$): δ=7.76–7.82 (m, 4H), 7.55 (s, 1H), 6.78 (s, 3H), 5.29 (s, 2H), 5.21 (s, 2H), 4.79–5.04 (m, 4H), 4.86–5.09 (m, 5H), 4.64 (m, 2H), 4.49 (m, 2H), 4.10 (m, 2H), 3.95 (s, 3H), 3.92 (s, 3H), 3.90 (s, 3H), 3.37 (t, J=6.79, 7.55 Hz, 1H), 3.23 (m, 4H), 2.66–2.86 (m, 11H), 1.92–2.36 (m, 7H), 1.74–1.86 (m, 3H), 1.50 (m, 2H), 1.35 ppm (m, 18H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=167.1, 166.7, 155.1, 148.5, 147.9, 146.4, 138.4, 137.1, 128.7, 127.8, 126.2, 111.7, 109.8, 108.1, 67.7, 63.5, 62.8, 61.2, 58.8, 57.1, 56.2, 55.6, 52.8, 50.4, 49.8, 34.5, 28.3, 27.2, 26.2, 25.4, 24.6, 22.0, 18.4, 15.2, 14.8 ppm; (ESI) HRMS: m/z calcd for $C_{61}H_{82}N_{12}O_{15}S_6$ 1415.4420, found 1415.4407 $[M+H]^{+\bullet}$.

*6.3.14. (4-((1-((3S,5S)-5-(Bis(ethylthio)methyl)-1-(4-(5-(4-((4-((S)-2-(bis(ethylthio)methyl)pyrrolidine-1-carbonyl)-2-methoxy-5-nitrophenoxy)methyl)-1H-1,2,3-triazol-1-yl)pentyloxy)-5-methoxy-2-nitrobenzoyl)pyrrolidin-3-yl)-1H-1,2,3-triazol-4-yl)methoxy)-5-methoxy-2-nitrophenyl)((S)-2-(bis(ethylthio)methyl)pyrrolidin-1-yl) methanone (**11c**).* Yield 88% (0.77 g). Mp: 113–115 °C. ^1H NMR (300 MHz, $CDCl_3$): δ=7.79 (m, 2H), 7.66 (s, 2H), 7.50 (s, 1H), 6.76 (m, 3H), 5.16–5.30 (m, 5H), 4.77–4.83 (m, 5H), 4.62 (m, 2H), 4.37 (m, 3H), 4.26 (m, 2H), 3.94 (s, 3H), 3.90 (s, 3H), 3.88 (s, 3H), 3.21 (m, 5H), 2.64–2.84 (m, 11H), 2.20–2.34 (m, 4H), 1.66–2.09 (m, 10H), 1.59 (m,

2H), 1.33 ppm (m, 18H); ^{13}C NMR (75 MHz, $CDCl_3$): δ=166.7, 166.3, 154.5, 148.6, 147.3, 142.6, 142.3, 138.2, 137.0, 128.8, 127.1, 123.0, 110.6, 109.3, 108.9, 69.0, 63.0, 60.9, 59.6, 56.4, 53.9, 52.7, 50.1, 29.8, 28.0, 27.1, 26.4, 26.1, 25.4, 24.5, 23.0, 22.7, 18.3, 14.9 ppm; (ESI) HRMS: *m*/*z* calcd for $C_{62}H_{84}N_{12}O_{15}S_6$ 1429.4576, found 1429.4593 $[M+H]^{+\bullet}$.

*6.3.15. (2S,11aS)-7-Methoxy-2-(4-(((S)-7-methoxy-5-oxo-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yloxy)methyl)-1H-1,2,3-triazol-1-yl)-8-(3-(4-(((S)-7-methoxy-5-oxo-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yloxy)methyl)-1H-1,2,3-triazol-1-yl)propoxy)-2,3-dihydro-1H-benzo[e]pyrrolo [1,2-a][1,4]diazepin-5(11aH)-one (**3a**).* Compound **11a** (100 mg, 0.071 mmol) was dissolved in MeOH (20 mL) by sonication at 25 °C for 10 min, $SnCl_2{\cdot}2H_2O$ (237 mg, 1.07 mmol) was added and refluxed for 1.0 h or until the TLC indicated that reaction was complete. The methanol was evaporated by vacuum and the aqueous layer was then carefully adjusted to pH 8 with 10% $NaHCO_3$ (20 mL) solution and separated the tin salts through Celite which was extracted with ethyl acetate (3×30 mL). The combined organic phase was dried over anhydrous Na_2SO_4 and evaporated under vacuum to afford the crude amino diethyl thioacetal **12a** (74 mg, 85%). Next step, the diethanthiol deprotective-cyclization reaction was performed without any further purification. A solution of **12a** (70 mg, 0.53 mmol), $HgCl_2$ (108 mg, 0.40 mmol), and $CaCO_3$ (40 mg, 0.40 mmol) in CH_3CN/H_2O (4:1, 15 mL) was stirred at room temperature for 12 h until TLC (ethyl acetate) indicates complete loss of starting material. The reaction mixture was diluted with $CHCl_3$ (20 mL), stirred for another 10 min, and filtered through a Celite bed. The clear yellow organic supernatant was extracted with saturated 5% $NaHCO_3$ (10 mL) and the combined organic phase was dried in anhydrous Na_2SO_4. The organic layer was evaporated in vacuum and the final crude product was purified by preparative TLC, $CHCl_3$/MeOH (95:5) as eluent affords the desired target compound **3a** (38 mg, 72%). $[\alpha]_D^{33}$ +307 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.65–7.82 (m, 5H), 7.49–7.51 (m, 3H), 6.98 (s, 1H), 6.80 (m, 2H), 5.22–5.34 (m, 4H), 4.94 (m, 2H), 4.68 (m, 1H), 4.58 (m, 3H), 3.95 (s, 3H), 3.92 (s, 6H), 3.72–3.84 (m, 4H), 3.52–3.60 (m, 2H), 2.62–2.92 (m, 4H), 2.29–2.43 (m, 4H), 1.85–2.11 ppm (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=167.2, 163.2, 158.4, 154.2, 152.0, 150.2, 149.1, 148.9, 146.9, 144.2, 139.3, 134.1, 133.9, 128.2, 118.3, 114.0, 110.9, 108.2, 106.3, 104.1, 68.3, 57.0, 56.4, 55.5, 52.9, 52.1, 50.2, 46.6, 33.0, 32.4, 29.6, 26.9, 22.5, 18.4 ppm; (ESI) HRMS: *m*/*z* calcd for $C_{48}H_{50}N_{12}O_9$ 971.4079, found 971.4122 $[M+CH_3OH+H]^{+\bullet}$.

*6.3.16. (2S,11aS)-7-Methoxy-2-(4-(((S)-7-methoxy-5-oxo-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yloxy)methyl)-1H-1,2,3-triazol-1-yl)-8-(4-(4-(((S)-7-methoxy-5-oxo-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yloxy)methyl)-1H-1,2,3-triazol-1-yl)butoxy)-2,3-dihydro-1H-benzo[e]pyrrolo[1,2-a] [1,4]diazepin-5(11aH)-one (**3b**).* Yield (35 mg, 68%). $[\alpha]_D^{33}$ +291 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.73–7.92 (m, 5H), 7.65 (s, 2H), 7.49 (s, 1H), 6.97 (m, 1H), 6.80 (s, 1H), 6.73 (s, 1H), 5.21–5.32 (m, 4H), 4.93 (m, 2H), 4.67 (m, 2H), 4.46 (m, 2H), 3.94 (s, 3H), 3.91 (s, 6H), 3.70–3.83 (m, 6H), 3.51–3.59 (m, 2H), 2.63–2.90 (m, 4H), 2.29 (t, *J*=5.12, 7.32 Hz, 2H), 2.01–2.11 (m, 6H), 1.80 ppm (m, 2H); (ESI) HRMS: *m*/*z* calcd for $C_{49}H_{52}N_{12}O_9$ 985.4248, found 985.4307 $[M+CH_3OH+H]^{+\bullet}$.

*6.3.17. (2S,11aS)-7-Methoxy-2-(4-(((S)-7-methoxy-5-oxo-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yloxy)methyl)-1H-1,2,3-triazol-1-yl)-8-(5-(4-(((S)-7-methoxy-5-oxo-2,3,5,11a-tetrahydro-1H-benzo[e]pyrrolo[1,2-a][1,4]diazepin-8-yloxy)methyl)-1H-1,2,3-triazol-1-yl)pentyloxy)-2,3-dihydro-1H-benzo[e]pyrrolo [1,2-a][1,4]diazepin-5(11aH)-one (**3c**).* Yield (42 mg, 75%). $[\alpha]_D^{33}$ +312 (*c* 1.0, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.27 (m, 2H), 8.17 (s, 1H), 8.01 (s, 1H), 7.96 (s, 1H), 7.46 (m, 3H), 7.05 (s, 1H), 6.96 (s, 1H), 6.89 (s, 1H), 4.53 (m, 6H), 4.23 (m, 1H), 4.10 (t, *J*=6.59, 7.32 Hz, 1H), 3.95–4.02 (m, 2H), 3.90 (s, 3H), 3.87 (s, 6H), 3.69–3.73 (m, 4H), 3.33 (m, 2H), 3.07 (t, *J*=7.32, 8.05 Hz, 2H), 2.47–2.54 (m, 2H), 2.32–2.36 (m, 2H), 2.06 (m, 2H), 1.79–1.91 (m, 6H), 1.40–1.44 ppm (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=167.0, 160.2, 159.2, 158.6, 153.9, 151.3, 149.3, 148.5, 147.4, 144.2, 139.6, 133.8, 129.6, 118.7, 114.8, 111.4, 110.0, 107.3, 105.5, 104.9, 68.1, 56.6, 56.1, 55.9, 53.2, 52.4, 50.1, 46.6, 33.0, 31.9, 31.6, 29.5, 27.8, 22.7, 22.3, 14.0 ppm; (ESI) HRMS: *m*/*z* calcd for $C_{50}H_{56}N_{12}O_9$ 999.4477, found 999.4500 $[M+CH_3OH+H]^{+\bullet}$.

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