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Synthesis and properties of a novel nucleoside derivative possessing a 2,3,5,6-tetraazabenzo[*cd*]azulene skeleton

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ABSTRACT

We describe herein the synthesis and properties of the novel nucleoside derivative, 4,7-diamino-2-(2-deoxy-β-D-*erythro*-pentofuranosyl)-2,6-dihydro-7*H*-2,3,5,6-tetraazabenzo[*cd*]azulene (**1**). The palladium catalyzed cross-coupling reaction of 2,4-diamino-5-iodo-7-(2-deoxy-β-D-*erythro*-pentofuranosyl) pyrrolo[2,3-*d*]pyrimidine (**9**) with acrylonitrile afforded 2,4-diamino-5-[(*E*)-1-cyano-2-ethenyl]-7-(2-deoxy-β-D-*erythro*-pentofuranosyl)pyrrolo[2,3-*d*]pyrimidine (**10**) in 77% yield, which was treated with NaOMe in MeOH in the presence of NaSPh to give the desired **1** in 64% yield. Whereas **1** was stable in concentrated ammonia at room temperature, it was gradually hydrolyzed in water to give 4-amino-2-(2-deoxy-β-D-*erythro*-pentofuranosyl)-2,6-dihydro-7*H*-2,3,5,6-tetraazabenzo[*cd*]azulen-7-one (**12**). Density functional calculations indicated that **12** was 20 kcal/mol more thermodynamically stable than **1** in a model study.

1. Introduction

In the field of nucleic acid chemistry, the development of artificial base-pairing motifs beyond the Watson–Crick base pairs is an area of active research. Thus far, many non-canonical base-pairing motifs consist of alternative hydrogen bonding patterns;[1–3] however, hydrophobic interactions[4] and metal coordination[5] also have been designed for application in bioorganic chemistry, biotechnology, and medicinal chemistry.

As is well known, the natural A:T and G:C pairs involve two and three hydrogen bonds (H-bonds), respectively, and the number of H-bonds plays an essential role in the thermal stability of DNA duplexes. Accordingly, DNA duplexes containing larger numbers of G:C pairs are more thermally stable than those containing the same numbers of A:T pairs. We therefore assumed that the base pairs containing four H-bonds would thermally stabilize DNA duplexes significantly. We have already reported the synthesis of nucleoside derivatives possessing an imidazo[5′,4′:4,5]pyrido[2,3-*d*]pyrimidine (Im) and a 1,8-naphthyridine (Na) moiety,[6–8] which were expected to form base pair with four H-bonds when they were introduced into oligodeoxynucleotides (ODNs). The resulting Im:Na pairs, such as ImO^N:NaN^O (Fig. 1a) were specific and thermally stabilized DNA duplexes markedly independent of the sequence context.[7] In addition, we recently succeeded in demonstrating the selective recognition of the unnatural Im:Na pair by DNA polymerases.[9,10] These successful results prompted us to develop a new skeleton to form an alternative base pair with four H-bonds. As shown in Fig. 1b, the Im base can be considered as a ring-expanded analog of purine (pu) toward the minor groove, while the Na base would be a ring-expanded analog of pyrimidine (py) toward the major groove. Therefore, a shift of the intrastrand C1′–C1′ position may occur in this base pair compared with a natural pu:py base pair (Fig. 1c). To avoid this possible distortion, we envisioned a new base-pairing motif in which both bases are expanded toward the major groove (Fig. 1d), whose C1′–C1′ position is expected to similar to the natural pu:py base pair. In order to form such a base pair with a series of Na bases, we designed a nucleoside derivative possessing a 2,3,5,6-tetraazabenzo[*cd*]azulene (Ba) base.

In this work, we investigated the synthesis and chemical properties of the novel nucleoside derivative **1** (BaN^N) possessing a 4,7-diamino-2,3,5,6-tetraazabenzo[*cd*]azulene skeleton as a new nucleobase (Fig. 1e). Our synthetic approach involved a palladium catalyzed cross-coupling reaction of the 7-deaza-7-iodo-2,6-diaminopurine derivative **9** with acrylonitrile, followed by cyclization. We found that the desired 4,7-diamino-2,3,5,6-tetraazabenzo[*cd*]azulene derivative **1** was unexpectedly converted into the corresponding 7-oxo derivative **12** (BaO^N) gradually in aqueous media. Herein, we present the experimental details of the results of this research.

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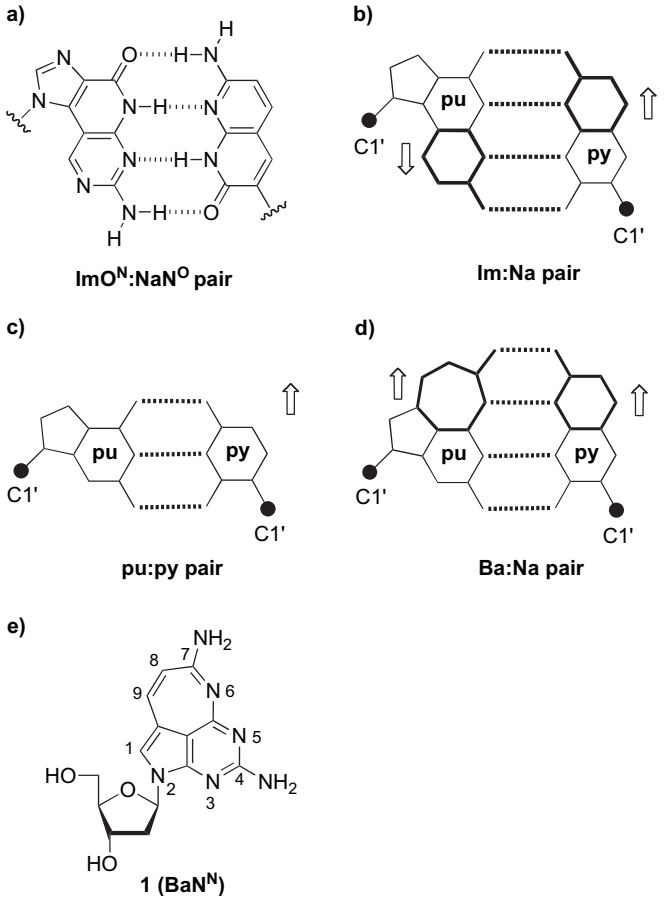
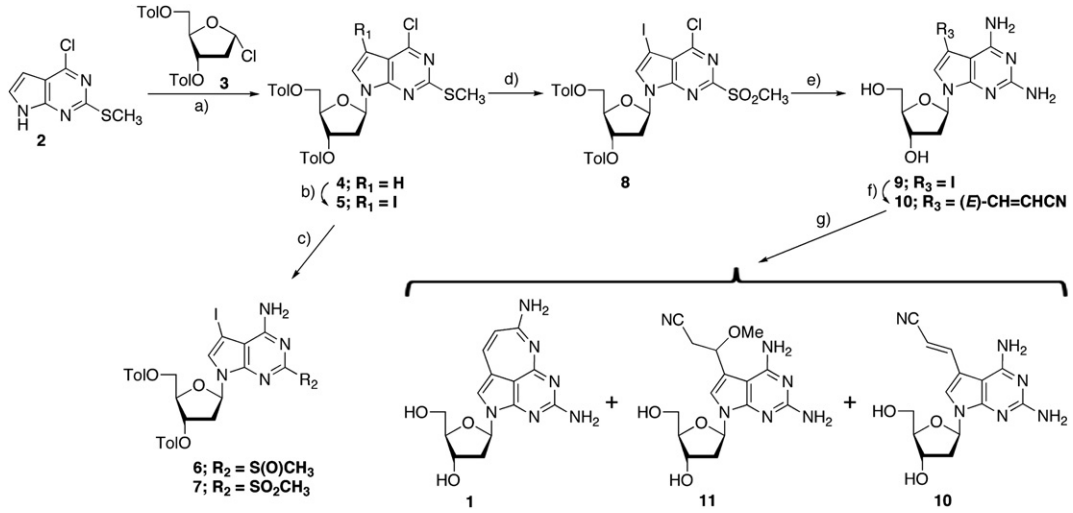


Fig. 1. (a) Structure of ImO^N:NaN^O base pair, (b) schematic representation of Im/Na pair, (c) schematic representation of natural pu:py pair, (d) schematic representation of Ba:Na pair, (e) structure of target compound, BaN^N (**1**).

2. Results and discussion

Thus far, few studies have been reported for the synthesis of nucleoside derivatives possessing a 2,3,5,6-tetraazabenzo[*cd*]azulene skeleton as the nucleobase.[11,12] These types of compounds were originally prepared from 7-iodo-7-deazapurine derivatives via the palladium catalyzed cross-coupling reaction, followed by cyclization. Based on this strategy, the synthesis of the desired compound was examined. As illustrated in Scheme 1, the 7-deazapurine derivative **4** was first prepared by the sodium salt glycosylation[13] of 4-chloro-2-(methylthio)pyrrolo[2,3-*d*]pyrimidine (**2**)[14] with 1-chloro-2-deoxy-3,5-di-*O*-*p*-toluoyl-α-D-*erythro*-pentofuranose (**3**).[15] Iodination of **4** with *N*-iodosuccinimide (NIS) gave the desired 7-iodo derivative **5**.[16] Booth et al. have already reported that the 2-SMe group in **5** was first oxidized with *m*-chloroperbenzoic acid (*m*-CPBA) to the sulfone, which was then treated with saturated ammonia in 1,4-dioxane at 140 °C in a sealed tube to afford the 2,6-diamino derivative **9**.[17] In our hands, however, no nucleophilic substitution at the 2-position took place and we obtained a mixture of **6** and **7** in 28% and 50% yield, respectively. To promote the desired reaction, compound **5** was treated with *m*-CPBA (2.5 equiv). The resulting **8** then was heated in liquid ammonia at 120 °C for 24 h in a sealed tube. The desired diamino derivative **9** thus was obtained in 58% yield, and no 2-methylsulfonyl compound like **7** was detected. Increasing the reaction temperature to 150 °C resulted in reduction of the isolated yield, although the starting material was consumed within 12 h. In contrast, the reaction was not complete even after heating the mixture at 100 °C for 4 days. Since the 2,6-diamino derivative **9** was obtained in good yield, the palladium catalyzed cross-coupling reaction of **9** with acrylonitrile was next investigated (Table 1). According to the conditions reported by Seela and Zulauf,[18] **9** was treated with acrylonitrile in the presence of $Pd(PPh_3)_4$ and CuI as the catalysts to afford **10** in 55% yield (entry 1). The *E*-geometry of the olefin moiety of **10** was confirmed by the coupling constant in the ^1H NMR spectrum (J=16.1 Hz). When the reaction was carried out in the absence of CuI, the chemical yield of **10** was decreased to 41% yield (entry 2). The best result was obtained in the presence of 20 mol % each of $(PhCN)_2PdCl_2$ and CuI (entry 5). With the desired **10** in hand, we next examined the cyclization toward the 2,3,5,6-tetraazabenzo[*cd*]azulene nucleoside **1** under basic conditions. The results are shown in Table 2. When **10** was treated with NaOMe in MeOH under reflux, the target compound **1** was obtained in 36% yield along with **11** (19%) and the starting material **10** (22%) (entry 1). Since **11** was considered as an intermediate of the desired cyclization, the separated **11** was again treated with NaOMe in MeOH. As a result, formation of **1** along with **10** was observed by TLC analysis. In order to push the cyclization, NaSPh, a more nucleophilic base, was used instead of NaOMe (entry 2). In this case, however formation of the intermediate **11** was increased to 42%, while that of **1** was decreased to 11%. Based on these results, **10** was



Scheme 1. Reagents: (a) Ref. 13; (b) Ref. 16; (c) Ref. 17; (d) *m*-CPBA, CH_2Cl_2; (e) liq. NH_3, 120 °C; (f) acrylonitrile, $(PhCN)_2PdCl_2$, CuI, Et_3N, DMF, 70 °C; (g) NaSPh, NaOMe, MeOH, 70 °C.

treated with a mixture of NaSPh and NaOMe in MeOH under reflux to afford **1** in 64% yield (entry 3). The structure of **1** was confirmed as follows. In the ^{1}H NMR spectrum, the two amino proton signals of **1** were shifted compared with those of **10** (from 6.52 to 5.80 ppm to 7.58 and 5.78 ppm), and the olefinic proton signals were observed at 6.76 and 5.61 ppm as doublets (J=11.5 Hz). In addition, the disappearance of the absorption around 2100 cm^{-1} in the IR spectrum, which would indicate the existence of a cyano group also supported the structure of **1**.

Table 1
Palladium catalyzed cross-coupling reaction of **9** with acrylonitrile[a]

Entry	Catalysts	Time (h)	**10** (%)	**9** (%)
1	$Pd(PPh_3)_4$ (10 mol %), CuI (20 mol %)	5	55	0
2	$Pd(PPh_3)_4$ (10 mol %)	8	41	0
3	$(PPh_3)_2PdCl_2$ (10 mol %), CuI (20 mol %)	8	38	15
4	$(PhCN)_2PdCl_2$ (10 mol %), CuI (20 mol %)	24	63	20
5	$(PhCN)_2PdCl_2$ (20 mol %), CuI (20 mol %)	18	77	0

[a] All reactions were carried out in DMF in the presence of Et_3N at 70 °C.

Table 2
Cyclization of **10** under basic conditions to give the desired **1**[a]

Entry	Base	Time (h)	**1** (%)	**11** (%)	Recover of **10** (%)
1	NaOMe	24	36	19	22
2	NaSPh[b]	36	11	42	27
3	NaOMe+NaSPh[c]	24	64	5	9

[a] All reactions were carried out in MeOH under reflux.
[b] 2 equiv of NaSPh was used.
[c] See Experimental section.

As described above, the new nucleoside derivative **1** possessing a 4,7-diamino-2,3,5,6-tetraazabenzo[*cd*]azulene skeleton has been successfully prepared. Prior to the incorporation of **1** into the ODNs, we investigated whether compound **1** would be stable under the basic conditions used for ODN synthesis. In general, an ODN loaded on CPG support prepared on a DNA synthesizer is treated with concentrated ammonia at 55 °C or room temperature to remove the protecting groups and detach the ODN from the CPG support. Thus, **1** was treated with concentrated ammonia, and its stability was analyzed by HPLC. As a result, decomposition of **1** was observed upon treatment with concentrated ammonia at 55 °C ($t_{1/2}$ was estimated to be 10 h) (Fig. 2a).[19] On the other hand, **1** was fairly stable in concentrated ammonia at room temperature (Fig. 2b). These results suggest that incorporation of **1** into ODN can likely be carried out. However, an unexpected reaction of **1** occurred in water. Thus, crystallization of **1** from water was examined to isolate an analytically pure sample. After two weeks, the resulting crystals were collected and analyzed by ^{1}H NMR. Surprisingly, the ^{1}H NMR spectrum of the crystals was not identical with **1**. The ^{1}H NMR spectrum in DMSO-d_6 showed the olefinic proton signals at 6.93 and 5.58 ppm as doublets (J=12.0 Hz), and proton signals exchangeable with D_2O were observed at 10.08 ppm (1H) and 6.25 ppm (2H), respectively. From these data, the isolated compound was assigned as the 4-amino-2,3,5,6-tetraazabenzo[*cd*]azulen-7-one derivative **12**, the structure of which was also supported by a molecular ion peak at m/z 317, one mass heavier than **1**, in its mass spectrum. In order to estimate the rate of this hydrolytic conversion, an aqueous solution of **1** was incubated at 25 °C, and an aliquot of the reaction mixture at the appropriate time was analyzed by HPLC. As seen in Fig. 3, the formation of **12** increased as **1** decreased, and the $t_{1/2}$ of this hydrolysis was estimated to be 5 days. This result indicates that the 4,7-diamino-2,3,5,6-tetraazabenzo[*cd*]azulene skeleton in ODN will be gradually converted into the 4-amino-2,3,5,6-tetraazabenzo[*cd*]azulen-7-one skeleton in an aqueous media, even if the incorporation of **1** into ODN succeeded.

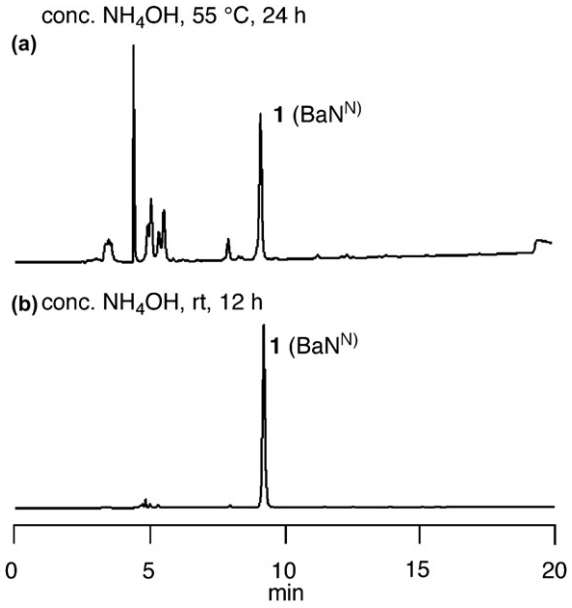


Fig. 2. HPLC profiles of treatment of **1** with concentrated ammonia.

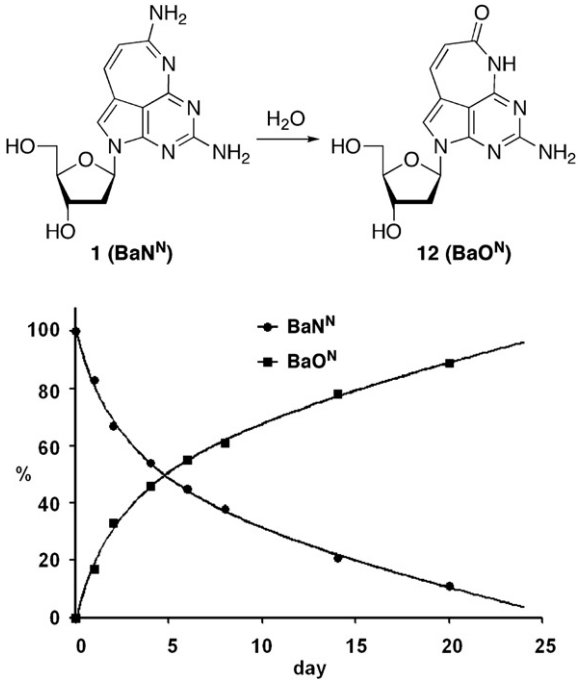


Fig. 3. Hydrolysis of **1** in H_2O at 25 °C.

Doering and Odum have reported a similar hydrolysis of 2-amino-3*H*-azepine to 1,2-dihydro-2-keto-3*H*-azepine in boiling water.[20] In our study, the amidine moiety in the seven membered ring was also hydrolyzed to the lactam. In order to understand whether such a hydrolysis is a thermodynamically favored pathway, theoretical calculations were carried out to estimate relative energies. Since the hydrolysis of **1** into **12** is thought to occur via a nucleophilic attack of water to form a tetrahedral intermediate, the energy of the intermediate was also calculated for comparison. To make the calculations easier, a series of model compounds (**13**, **14**, and **15**) were designed for the calculations in which a methyl group was substituted for the 2-deoxyribose moiety. The structures were optimized by and the absolute energies were calculated with

B3LYP/6-31G*. Existence of the intermediate **14** was confirmed by frequency calculations, which gave a positive frequency of vibration. As can be seen in Fig. 4, the tetrahedral intermediate **14** was more stable than **13** (6.99 kcal/mol). The 2,3,5,6-tetraazabenzo[*cd*]azulen-7-one derivative **15** was 14.8 kcal/mol more stable than **14**, and 21.79 kcal/mol more stable than **13**. Thus, the theoretical calculations support the hypothesis that the reaction pathway leading from **1** to **12** via a tetrahedral intermediate resulting from the attack of water was thermodynamically favored, which is in agreement with our experimental results. As described above, compound **1** was stable in concentrated ammonia at room temperature, and no hydrolyzed product **12** was observed by HPLC analysis. Under these conditions, the nucleophile is ammonia, and not water. Therefore, the resulting tetrahedral intermediate will return to **1** if ammonia attacks the 7-position of **1**.

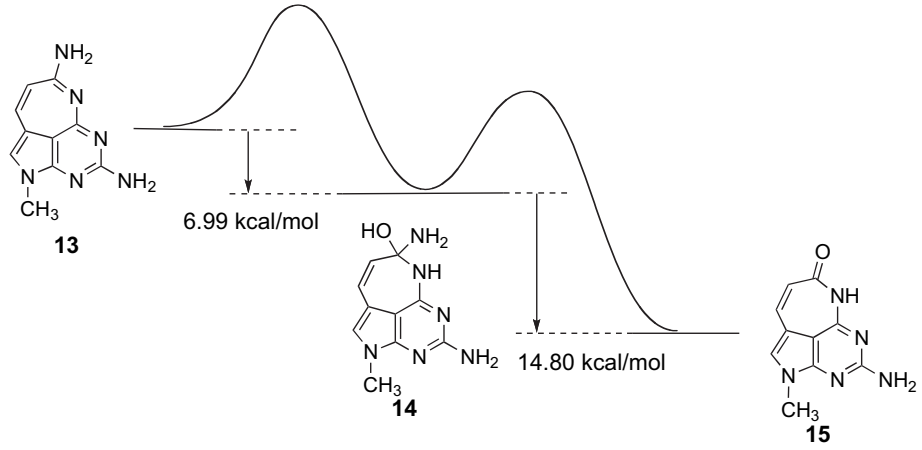


Fig. 4. Relative energies of **13**, **14**, and **15** calculated with B3LYP/6-31G*.

In conclusion, we have designed the novel nucleoside derivative **1** possessing a 4,7-diamino-2,3,5,6-tetraazabenzo[*cd*]azulene skeleton. The desired compound was prepared via the palladium catalyzed cross-coupling reaction of **9** with acrylonitrile, followed by cyclization under basic conditions. The resulting 4,7-diamino-2,3,5,6-tetraazabenzo[*cd*]azulene derivative **1** was stable in concentrated ammonia at room temperature, whereas it was gradually hydrolyzed to the corresponding 7-oxo derivative **12** (BaO^N) in water. Transformation of the structure proceeds via a thermodynamically favored pathway, which is 20 kcal/mol more favorable from **1** to **12** as calculated by B3LYP/6-31G*. Incorporation of **12**, which is expected to form a complementary base pair with NaN^O, is now under investigation. The results will be reported in due course.

3. Experimental section

3.1. General methods

Physical data were measured as follows: Melting points are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded at 270 or 500 MHz and 67.5 or 125 MHz instruments in $CDCl_3$ or DMSO-d_6 as the solvent with tetramethylsilane as an internal standard. Chemical shifts are reported in parts per million (δ), and signals are expressed as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad). All exchangeable protons were detected by addition of D_2O. TLC was done on Merck Kieselgel F_{254} precoated plates. Silica gel used for column chromatography was YMC gel 60A (70–230 mesh). Iatrobeads used for column chromatography was 6RS-8090 (Mitsubishi Chemical Medience Co.). Aminosilica gel was Chromatorex NH-DM1020 (Fuji Silysia Chemical LTD.).

*3.1.1. 2,4-Diamino-5-iodo-7-(2-deoxy-β-D-erythro-pentofuranosyl)-pyrrolo[2,3-d]pyrimidine (**9**)*[21]. To a solution of **5**[16] (6.1 g, 9.0 mmol) in CH_2Cl_2 (90 mL) was added *m*-CPBA (60% purity, 6.5 g, 23 mmol) at 0 °C, and the reaction mixture was stirred for 1 h at room temperature. The reaction mixture was diluted with $CHCl_3$, and the organic layer was washed with saturated aqueous Na_2CO_3, followed by brine. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by a short silica gel column, eluted with $CHCl_3$:AcOEt (49:1), to give **8**. The resulting **8** was then heated in liquid ammonia (ca. 100 mL) at 120 °C for 24 h in a steel container. After being removed ammonia, the resulting solid was suspended in MeOH and corrected. The corrected solid containing ammonium salts was dissolved in hot DMF (ca. 10 mL), which was purified by an Iatrobeads column, eluted with MeOH in $CHCl_3$ (2–50%), to give **9** (1.8 g, 53%) as a pale brown solid. Since the desired **9** was detected in the filtrate, the solvent was removed and the residue was purified by an aminosilica gel column, eluted with MeOH in $CHCl_3$ (2–4%), to give additional **9** (190 mg, 5%): ^{1}H NMR (DMSO-d_6) δ 7.18 (s, 1H), 6.31 (dd, 1H, J=5.6 and 8.6 Hz), 6.16 (br s, 2H, exchangeable with D_2O), 5.81 (br s, 2H, exchangeable with D_2O), 5.18 (d, 1H, J=3.6 Hz, exchangeable with D_2O), 4.98 (t, 1H, J=5.4 Hz, exchangeable with D_2O), 4.26 (m, 1H), 3.74 (m, 1H), 3.48 (m, 2H), 2.33 (m, 1H), 2.03 (m, 1H).

*3.1.2. 2,4-Diamino-5-[(E)-1-cyano-2-ethenyl]-7-(2-deoxy-β-D-erythro-pentofuranosyl)pyrrolo[2,3-d]pyrimidine (**10**).* To a solution of 9 (100 mg, 0.26 mmol) in DMF (3 mL) including Et_3N (74 μL, 0.52 mmol), CuI (10 mg, 0.052 mmol), and $(PhCN)_2PdCl_2$ (25 mg, 0.052 mmol) was added acrylonitrile (0.2 mL, 5.2 mmol), and the reaction mixture was heated at 70 °C for 24 h. The solvent was removed in vacuo, and the residue was purified by an aminosilica gel column, eluted with MeOH in $CHCl_3$ (4%), to give **10** (63 mg, 77%) as an yellow solid. An analytical sample was crystallized from H_2O/MeOH to give pale yellow crystals: mp 272–274 °C (colored); IR (2214 cm^{-1}); FAB-LRMS *m*/*z* 317 [(M+H)$^+$]; ^{1}H NMR (DMSO-d_6) δ 7.93 (d, 1H, J=15.8 Hz), 7.71 (s, 1H), 6.52 (br s, 2H, exchangeable with D_2O), 6.34 (dd, 1H, J=5.9 and 8.6 Hz), 5.98 (d, 1H, J=15.8 Hz), 5.80 (br s, 2H, exchangeable with D_2O), 5.24 (d, 1H, J=4.1 Hz, exchangeable with D_2O), 5.00 (t, 1H, J=5.7 Hz, exchangeable with D_2O), 4.29 (m, 1H), 3.76 (m, 1H), 3.49 (m, 2H), 2.34 (ddd, 1H, J=8.6, 5.4, and 13.1 Hz), 2.10 (ddd, 1H, J=5.9, 2.3, and 13.1 Hz); ^{13}C NMR

(DMSO-d_6) δ 160.1, 157.9, 154.2, 143.8, 119.8, 119.4, 111.8, 93.7, 91.2, 87.2, 82.2, 70.9, 62.0, 49.4. Anal. Calcd for $C_{14}H_{16}N_6O_3 \cdot 0.25H_2O$: C, 52.41; H, 5.18; N, 26.20. Found: C, 52.68; H, 5.30; N, 26.03.

*3.1.3. 4,7-Diamino-2-(2-deoxy-β-D-erythro-pentofuranosyl)-2,6-dihydro-7H-2,3,5,6-tetraazabenzo[cd]azulene (**1**) and 2,4-diamino-5-(1-cyano-2-methoxy-2-ethyl)-7-(2-deoxy-β-D-erythro-pentofuranosyl) pyrrolo[2,3-d]pyrimidine (**11**).* A solution of **10** (385 mg, 1.22 mmol) in 0.1 M NaOMe in MeOH (122 mL) containing NaSPh (81 mg, 0.61 mmol) was heated at 70 °C for 24 h. The solvent was removed in vacuo, and the residue was purified by an aminosilica gel column, eluted with MeOH in $CHCl_3$ (4–10%), to give **1** (245 mg, 64%) as an orange solid, **11** (22 mg, 5%) as a brown solid, and starting material **10** (30 mg, 9%), respectively.

Physical data for **1**: EI-LRMS: *m*/*z* 316 (M^+); EI-HRMS: Calcd for $C_{14}H_{16}N_6O_3$ 316.1284, found 316.1265 (M^+); UV λ_{max} (H_2O) 430 nm (ε=530), 296 nm (ε=13,500), 261 nm (ε=21,500), 247 nm (ε=22,100); ^{1}H NMR (DMSO-d_6) δ 7.58 (br s, 2H, exchangeable with D_2O), 7.05 (s, 1H), 6.76 (d, 1H, *J*=11.5 Hz), 6.25 (dd, 1H, *J*=5.7 and 8.3 Hz), 5.78 (br s, 2H, exchangeable with D_2O), 5.61 (d, 1H, *J*=11.5 Hz), 5.21 (br s, 1H, exchangeable with D_2O), 5.04 (br s, 1H, exchangeable with D_2O), 4.27 (m, 1H), 3.75 (m, 1H), 3.45 (m, 1H), 2.33 (ddd, 1H, *J*=8.3, 5.5, and 13.2 Hz), 2.07 (ddd, 1H,=5.7, 2.3, and 13.2 Hz); ^{13}C NMR (CD_3OD) δ 166.5, 163.2, 163.0, 154.5, 137.4, 119.7, 117.5, 116.1, 105.1, 89.2, 87.0, 73.2, 63.8, 41.0.

Physical data for **11** (obtained as 1:1 diastereomeric mixtures): FAB-LRMS *m*/*z* 349 [$(M+H)^+$]; FAB-HRMS Calcd for $C_{15}H_{20}N_6O_4$ 348.1546, found 349.1635 [$(M+H)^+$]; ^{1}H NMR (DMSO-d_6) δ 7.03 and 7.03 (each s, each 1H), 6.32 (m, 1H), 6.30 (br s, 2H, exchangeable with D_2O), 5.69 (br s, 2H, exchangeable with D_2O), 5.17 (m, 1H, exchangeable with D_2O), 5.00 (m, 1H, exchangeable with D_2O), 4.70 (m, 1H), 4.28 (m, 1H), 3.76 (m, 1H), 3.50 (m, 2H), 3.24 and 3.22 (each s, each 3H), 2.94 and 2.83 (each m, each, 1H), 2.33 (m, 1H), 2.05 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 160.0 and 160.0, 157.5 and 157.5, 153.8 and 153.8, 118.1 and 118.0, 117.5 and 117.5, 112.9 and 112.9, 93.8 and 93.8, 86.9 and 86.9, 82.3 and 82.2, 72.8 and 72.7, 71.1 and 71.0, 62.2 and 62.1, 55.6 and 55.3, 39.4 and 39.2, 25.4 and 25.4.

*3.1.4. Physical data for 4-amino-2-(2-deoxy-β-D-erythro-pentofuranosyl)-2,6-dihydro-7H-2,3,5,6-tetraazabenzo[cd]azulen-7-one (**12**).* Mp 210 °C (colored), 247 °C (decomp.); UV λ_{max} (H_2O) 392 nm (ε=1170), 301 nm (ε=8830), 242 nm (ε=33,300); λ_{max} (0.5 M HCl) 311 nm (ε=6820), 279 nm (ε=10,600), 243 nm (ε=33,000); λ_{max} (0.5 M NaOH) 243 nm (ε=22,100); EI-LRMS *m*/*z* 317 (M^+); EI-HRMS Calcd for $C_{14}H_{15}N_5O_4$ 317.1124, found 317.1130 (M^+); ^{1}H NMR (DMSO-d_6) δ 10.08 (d, 1H, *J*=1.2 Hz, exchangeable with D_2O), 7.34 (s, 1H), 6.93 (d, 1H, *J*=12.0 Hz), 6.29 (dd, 1H, *J*=5.9 and 7.9 Hz), 6.25 (br s, 2 H, exchangeable with D_2O), 5.58 (d, 1H, *J*=11.6 Hz), 5.26 (d, 1H, *J*=3.6 Hz, exchangeable with D_2O), 5.02 (t, 1H, *J*=5.8 Hz, exchangeable with D_2O), 4.30 (m, 1H), 3.77 (m, 1H), 3.49 (m, 2H), 2.49 (m, 1H), 2.12 (ddd, 1H, *J*=6.1, 2.3, and 12.8 Hz); ^{13}C NMR (DMSO-d_6) δ 166.8, 161.6, 154.7, 153.1, 133.8, 120.0, 119.9, 113.1, 97.7, 87.3, 82.4, 70.9, 61.9, 39.6. Anal. Calcd for $C_{14}H_{15}N_5O_4 \cdot 0.5H_2O$: C, 51.53; H, 4.94; N, 21.46. Found: C, 51.52; H, 4.68; N, 21.44.

3.1.5. Theoretical calculation. All DFT calculations were performed using the Gaussian 03W. Structures were initially optimized by PM3. The resulted conformations were optimized at B3LYP/6-31G*. Finally, single point energies were calculated at B3LYP/6-31G*. Absolute energies are; **15**: −735.5011668 a.u.; **14**: −792.0254966 a.u.; **13**: −792.0143577 a.u.

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Supplementary data

Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2010.08.063.

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