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Synthesis of *C*-Glycosyl Pyrrolo[3,4-*c*]carbazole-1,3(2*H*,6*H*)-diones as a Scaffold for Check Point Kinase 1 Inhibitors

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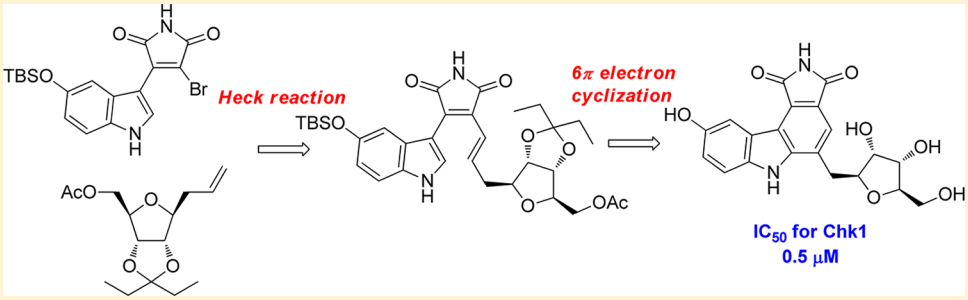
Supporting Information



ABSTRACT: Indolocarbazole natural products are known to possess a variety of biological activities that hold promise as cancer chemotherapeutic agents. We newly designed *C*-glycosyl pyrrolo[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione derivatives **7** and **8**, which are natural-product-like scaffolds. Compounds **7** and **8** were stereoselectively and efficiently synthesized using *β*-selective *C*-allylation, Heck reaction, and thermal 6*π*-electron cyclization/oxidative aromatization. Their potential as Chk1 inhibitors was investigated, and **7** and **8** exhibited an inhibitory activity with IC_{50} values of 0.5−9.5 *μ*M, which is good activity for scaffolds. The key intermediate **23** was obtained by five steps from D-ribose in 33% overall yield by this synthetic route, which would enable us to prepare a range of analogues in order to investigate further structure−activity relationship studies in the optimization process.

INTRODUCTION

Indolocarbazole natural products are known to possess a variety of biological activities that hold promise as cancer chemotherapeutic agents. For examples, staurosporine[1−3](Figure 1, **1**), UCN-01[4−7] (**2**) and K252a[8−10] (**3**) are potent inhibitors of protein kinases including PKC,[11] and rebeccamycin[12−14] (**4**) is a topoisomerase inhibitor, and all of them exhibited antitumor activity. Among them, UCN-01 (Figure 1, **2**) is a potent inhibitor of check point kinase 1 (Chk1), which plays a crucial role in the G2/M checkpoint in cell cycle. The activation of cell cycle checkpoints provides an opportunity for cancer cells to repair DNA damage by reducing the antitumor effect of DNA-damaging agents.[15] Premature mitotic entry of cells with unrepaired DNA leads to mitotic catastrophe and/or apoptosis. Therefore drugs that abrogate the DNA damage-induced G2/M checkpoints should selectively sensitize p53-deficient cancer cells to anticancer therapies.[15−18] UCN-01 has been shown to enhance the therapeutic activity of DNA-damaging agents in animal models[19] and is currently in phase II clinical trials[20] in the United States. Several other derivatives of this class of natural products shown in Figure 1 have entered clinical trials as anticancer drugs. Therefore, this class of natural products is a good lead to pursue in the development of anticancer agents in many aspects. In an effort to develop new Chk1 inhibitors, we designed a pyrrolo[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione scaffold **5** (IC_{50} = 9.0 *μ*M) through a structural simplification of UCN-01 (Figure 2).[21] The further structure−activity relationship (SAR) of the scaffold has been investigated with guidance from structure-based drug design (SBDD), and the compound **6** (IC_{50} = 31 nM) was found to have an inhibitory activity similar to that of UNC-01 (IC_{50} = 5.6 nM). However, a shortcoming

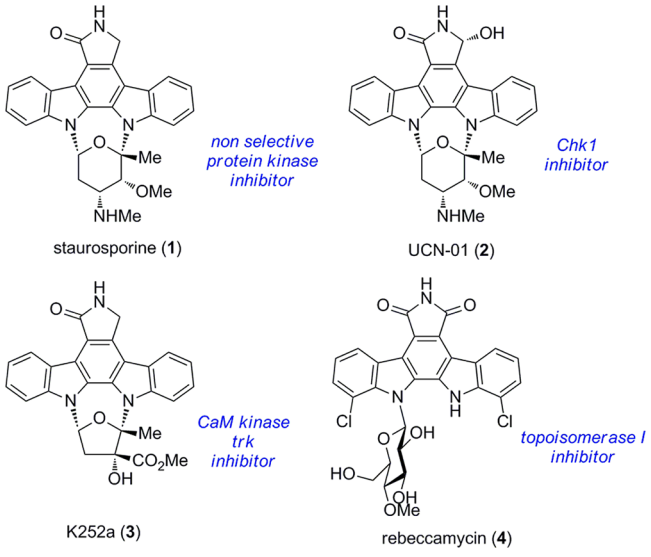


Figure 1. Structures of indolocarbazole natural product.

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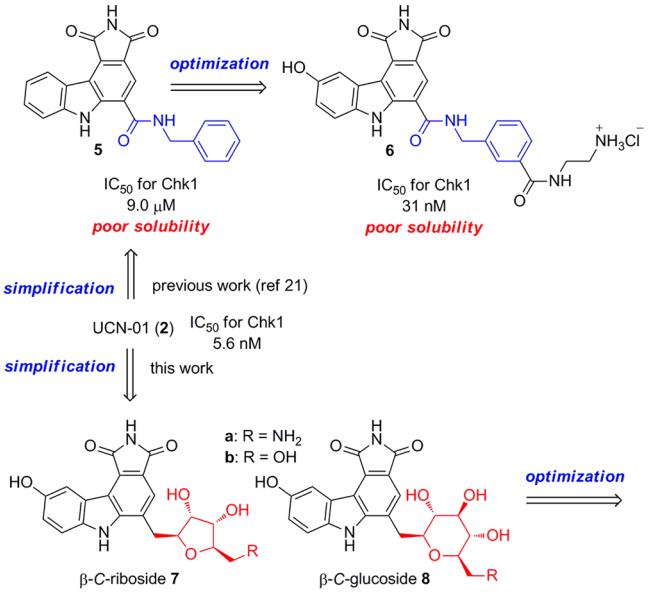


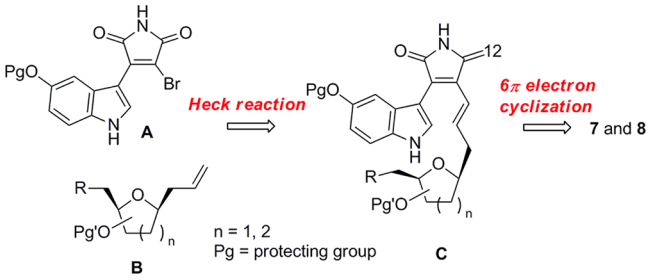
Figure 2. Design of Chk1 inhibitors.

of **5** and **6** was poor aqueous solubility, and precipitation occurred during the biological evaluation. The chemical structure of natural products shown in Figure 1 surrogates a sugar moiety, introduction of which to hydrophobic molecules improves an aqueous solubilty in many case. Therefore, we newly designed *C*-glycosyl pyrrolo[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione derivatives **7** and **8**, which are more natural-product-like scaffolds. Different from natural products, the sugar moiety was attached to the pyrrolo[3,4-*c*]carbazole chromophore by a chemically and biologically stable *C*−*C* bond. Here we describe a stereoselective and efficient synthesis of *C*-glycosyl pyrrolo-[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione derivatives as potential Chk1 inhibitors.

RESULTS AND DISCUSSION

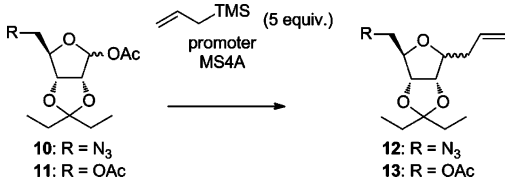
The synthetic strategy to the analogues is outlined in Scheme 1. Suitably protected bromoindolylmaleimide **A** was first coupled

Scheme 1



to *C*-allyl-D-glycoside derivatives **B** by Heck reaction to give **C**. Either photochemical or thermal 6π-electron cyclization of **C** followed by oxidative aromatization would afford the pyrrolo-[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione linking to the glycoside **7** or **8**. As for the synthesis of **7**, it is quite difficult to synthesize β-*C*-ribosides by a Lewis acid-promoted direct alkylation of ribosyl donors[23−25] since a neighboring group participation[26] is not usually effective in *C*-ribosylation reactions.[27,28] We recently developed the highly β-selective *O*- and *C*-ribosylation via unusual outside attack of the nucleophile with the use of a 3-pentylidene protecting group at the 2,3-hydroxyl groups.[28−30] In these studies, only the ribosyl fluoride was examined to use as a glycosyl donor. In this study, the *C*-allylation with 1-*O*-acetates **10** and **11**, which are more stable and more easily prepared than the corresponding fluorides, was first investigated. The 1-*O*-acetates **10** and **11** were prepared by acetylation of the known 5-azide-2,3-(3-pentylidene)-D-ribofuranose and 2,3-(3-pentylidene)-D-ribofuranose, respectively.[29] The results of *C*-allylation are summarized in Table 1. First, **10**

Table 1. C-Allylation of 1-*O*-Acetyl-3-pentylidene Ribofuranose



entry	donor	promoter (equiv)	solvent	temp (°C)	yield (%)[a]	ratio (β/α)[b]
1	**10**	BF_3·OEt_2 (3.5)	CH_2Cl_2	0	20	20/1
2	**10**	$SnCl_4$ (0.5)	CH_2Cl_2	0	60	>25/1
3	**11**	BF_3·OEt_2 (3.5)	CH_2Cl_2	0	g	1.5/1
4	**11**	$SnCl_4$ (0.5)	CH_2Cl_2	0	70	5/1
5	**11**	TMSOTf (0.5)	MeCN	0	59	>25/1
6	**11**	TMSOTf (0.5)	EtCN	−50	75	>25/1

[a]Combined isolated yields after column chromatography. [b]Anomeric ratio determined from ^{1}H NMR integration values of selected protons.

was treated with allyltrimethysilane (5.0 equiv) and BF_3·OEt_2 (3.5 equiv) and molecular sieves 4 Å (MS4 Å) in CH_2Cl_2 at 0 °C. The reaction proceeded slowly compared to that with the ribosyl fluoride, and the desired **12** was obtained only in 20% yield with a large amount of unreacted **10** remaining in the reaction mixture (entry 1). ^{1}H NMR analysis of the product revealed a β/α ratio of 20/1, and good β-selectivity was achieved as expected. The use of $SnCl_4$, a stronger promoter than BF_3·OEt_2, improved the yield of **12** with increased stereoselectivity (60%, β/α >25/1). On the other hand, the *C*-allylation of **11**, which possesses the acetoxy substituent at the 5-position, resulted in reduced stereoselectivity (entries 3 and 4). A neighboring group participation of the acetoxy group to the oxocarbenium intermediate at the β-face would decrease the stereoselectivity, as was indicated by our previous study.[29] The β-selectivity was improved with the conditions where TMSOTf was used as a promoter in MeCN to give the desired β-*C*-allyl riboside **13** as a major product (>25/1, entry 5). Lowering the reaction temperature (−50 °C) in EtCN suppressed the undesired hydrolysis of the 3-pentylidene group and gave **13** in 75% yield (entry 6).

With the β-selective ribosylation with acetate donors established, the synthesis of **7** was conducted (Scheme 2). Staudinger reduction of the azide group of **12** followed by Boc-protection of the liberated amine afforded **14** in 99% yield over two steps. Next, the Heck reaction linking **14** to bromo-5-methoxyindolylmaleimide[31] (**15**) was investigated (Table 2). The *C*-allylriboside **14** was treated with **15** under the conditions used in the synthesis of **5** in our previous study ($Pd(OAc)_2$, Bu_3P, DMF, 80 °C, 2 h).[21] However, the desired

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Scheme 2

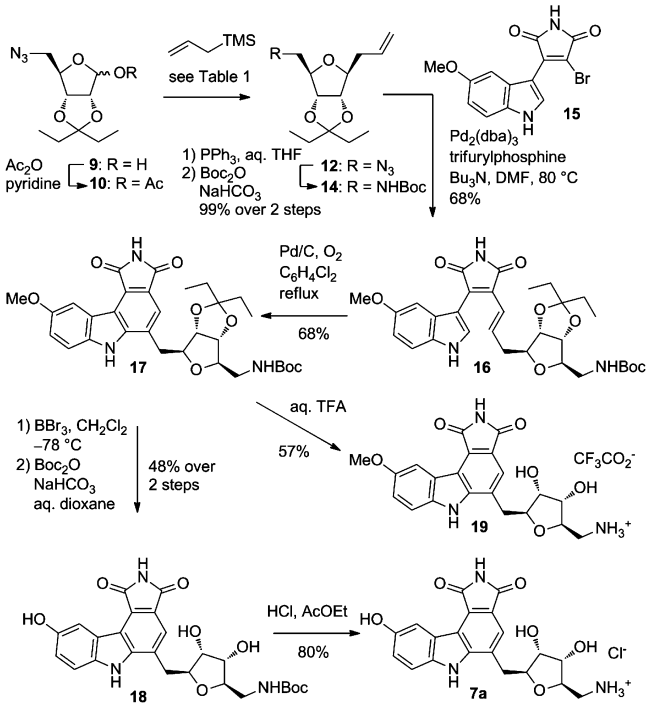


Table 2. Optimization of Heck Reaction[a]

entry	catalyst	ligand	yield of **16** (%)
1	$Pd(OAc)_2$	PBu_3	0
2	$Pd(OAc)_2$	trifurylphosphine	10
3	$Pd(OAc)_2$	bis(diphenylphosphino)propane	0
4	$Pd_2(dba)_3$	trifurylphosphine	68
5	$Pd_2(dba)_3$	bis(diphenylphosphino)propane	0
6	$(Ph_3P)_2PdCl_2$	trifurylphosphine	0
7	$(Ph_3P)_2PdCl_2$	bis(diphenylphosphino)propane	0

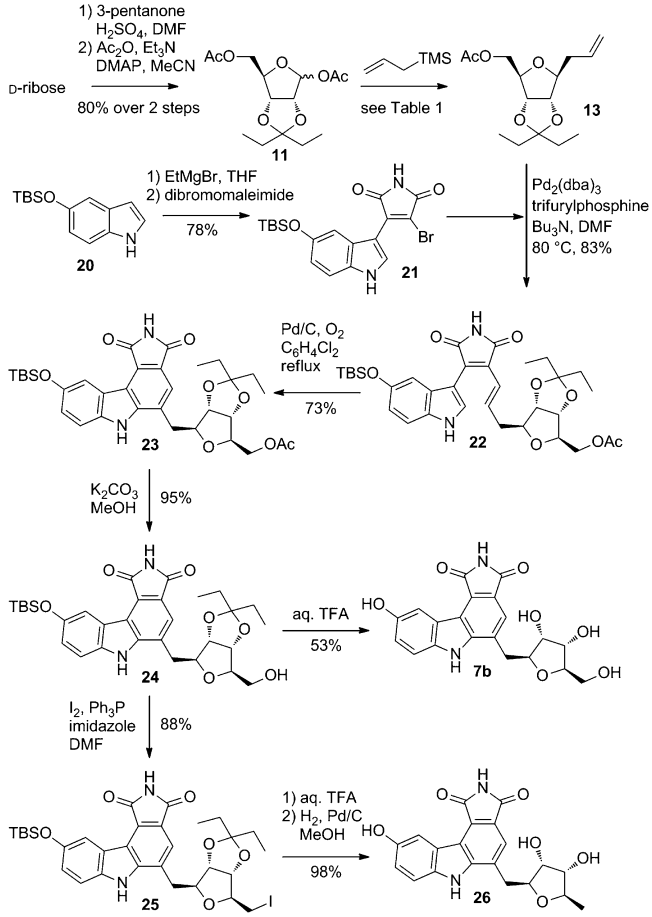
[a]Reaction was carried out with 10 mol % catalyst, 40 mol % ligands, 1.1 equiv of Bu_3N in DMF at 80 °C for 2 h.

16 was not obtained at all (entry 1). Optimization of the reaction conditions was then pursued to find the conditions to give **16** by changing a ligand. The use of tri(2-furyl)phosphine gave a small amount of **16** (entry 2). Use of a bidentate phosphine ligand, bis(diphenylphosphino)propane, gave no improvement (entry 3).

When the catalyst was changed to $Pd_2(dba)_3$ in the presence of tri(2-furyl)phosphine, much improvement of the yield of **16** was achieved (68%, entry 4). The use of the bidentate phosphine ligand gave no product in this case, either (entry 5), and $(Ph_3P)_2PdCl_2$ was not effective at all (entries 6 and 7). Photochemical 6π-electron cyclization followed by oxidative aromatization using a medium-pressure mercury lamp (400 W) in THF under oxygen atmosphere gave a trace amount of the desired pyrrolo[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione **17** with extensive decomposition of **16**. The yield of **17** was improved by thermal 6π-electron cyclization conditions. Namely, heating of **16** in 1,3-dichlorobenzene under reflux in the presence of Pd/C gave **17** in 68% yield. Thermal 6π-electron cyclization was much more scalable than that of the photochemical reaction. Deprotection of the 3-pentylidene, the methyl, and the Boc groups of **17** (BBr_3, CH_2Cl_2, −78 °C) followed by Boc protection of the liberated amine gave **18** in 48% yield over two steps. Finally deprotection of the Boc group successfully afforded the target compound **7a** as a hydrochloride salt. *O*-Methyl derivative **19** was obtained by treatment of **17** with aq TFA in 57% yield.

With the synthetic strategy established, other analogues were prepared as shown in Scheme 3. In this synthetic scheme, the

Scheme 3



methyl group used as protection of the phenol was changed to a TBS group. A magnesium salt of the resulting **20** was then reacted with the dibromomaleimide to afford **21** in 78% yield. In a manner similar to the synthesis of **17**, **23** was prepared by the Heck reaction of **13** with **21** ($Pd_2(dba)_3$, tri(2-furyl)-phosphine, Bu_3N, DMF, 80 °C, 2 h, 83%) and thermal 6π-electron cyclization followed by oxidative aromatization (Pd/C, 1,3-dichlorobenzene, 170 °C, 3 h, 73%). Removal of the acetyl group of **23** gave **24** (K_2CO_3, MeOH, 95%), which was treated with aq TFA to give **7b** in 53% yield. Deoxy analogue **26** was obtained by conversion of the hydroxyl group of **24** to the iodo group (I_2, PPh_3, imidazole, DMF, 88%), deprotection (aq TFA) and hydrogenolysis (H_2, Pd/C, MeOH, 98% over two steps).

Glucopyranose-type analogues **8a** and **8b** were also prepared as shown in Scheme 4. Basically the synthetic strategy was the same as in Scheme 3 except for the use of known *β*-*C*-allylglucoside **27**,[32] from which the key intermediate **30** was obtained over three steps. Deprotection of the TBS group of **30** provided **8b**. On the other hand, after the primary hydroxyl group of **30** was converted to the azide group to give **31** (TsCl, pyridine, then NaN_3, DMF, 73% over two steps), the last target compound **8a** was obtained by reduction of the azide group

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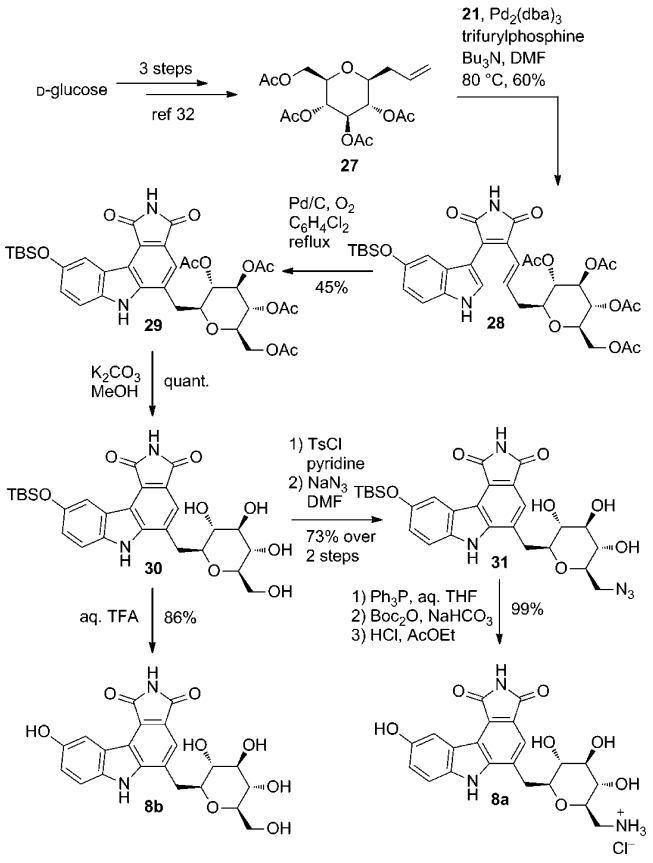
Scheme 4



(Ph_3P, H_2O, THF), protection of the liberated amine (Boc_2O, $NaHCO_3$) and deprotection of **31** (HCl, AcOEt, 99% over three steps).

The Chk1 inhibitory activity of the *C*-glycosyl pyrrolo[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione derivatives was then evaluated (Table 3). All the analogues have greater aqueous solubility

Table 3. Chk1 Inhibitory Activity of C-Glycosylpyrrolo[3,4-*c*]carbazole-1,3-(2*H*,6*H*)-diones

	compound					
	7a	**7b**	**19**	**26**	**8a**	**8b**
IC_{50} (μM)[a]	0.9	0.5	9.5	6.0	2.3	1.1

[a]IC_{50}: concentration of drug (μM) to inhibit the phosphorylation of a Cdc25 substrate peptide by Chkl. Values are an average of three separate determinations; variation was generally +15%.

compared to **5** and **6**, and no precipitation was observed at all in the assay. All analogues exhibited a moderate Chk1 inhibitory activity with IC_{50} values of 0.5−9.5 μM. The ribofuranose-type analogues are better inhibitors than the glucopyranose-type analogues (**7a**,**b** vs **8a**,**b**), and the analogues possessing the hydroxyl group at the methylene carbon show 2-fold better activity than those with the amino group (**7b** vs **7a** and **8b** vs **8a**). Decreased inhibitory activity found in the deoxy analogue **26** (**7b** vs **26**) indicates that these polar functional groups of the sugar moiety are contributory to the activity. The phenolic hydroxyl group at the pyrrolo[3,4-*c*]carbazole-1,3-(2*H*,6*H*)-dione is also important for the activity, and its methylation decreased the inhibitory activity by a factor of 10 (**7a** vs **19**). In order to predict the mode of binding to Chk1, analogues **7b** and **8b** were docked to the active site of the public domain crystal structure published for Chk1 (PDB accession code 1nvq)[33] using the Glide program,[34−36] and the results were compared to that of **5** (Figure 3). This model suggested that **5**, **7b**, and **8b** share a very similar binding mode with matching occupancy of the binding pocket and key hydrogen bonds to the hinge domain (Glu85 and the hydrogen atom at the NH of maleimide moiety, and Cys87 and the carbonyl oxygen of the maleimide). The sugar moieties of **7b** and **8b** are superimposable onto the benzyl group of **5** with additional contact between the primary hydroxyl group and the Glu91 residue. The prediction is in accordance with the decreased activity of deoxy analogue **26** compared to those of **7b** and **8b**. These docking models with Chk1 indicated that there is a space, where additional functional groups can be introduced around the introduced sugar moiety of **7b** or **8b**. This allows us to conduct structure-based drug design based on **7b** or **8b** to optimize the Chk1 inhibitory activity as in the discovery of **6**, and further optimization would lead to development of a novel Chk1 inhibitor.

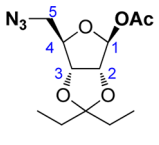
CONCLUSION

Here *C*-glycosyl pyrrolo[3,4-*c*]carbazole-1,3(2*H*,6*H*)-dione scaffolds **7** and **8** were stereoselectively and efficiently synthesized using β-selective *C*-allylation, followed by Heck reaction and thermal 6π-electron cyclization/oxidative aromatization. Their potential as Chk1 inhibitors was investigated to find that **7** and **8** exhibited an inhibitory activity with IC_{50} values of 0.5−9.5 μM, which were good activity as scaffolds. The key intermediate **23** was obtained by five steps from D-ribose in 33% overall yield. This synthetic strategy makes it easier to prepare a range of analogues in order to investigate further SAR study in optimization process.

EXPERIMENTAL SECTION

General Experimental Methods. ^1H and ^{13}C NMR chemical shifts were reported in parts per million (δ) relative to tetramethylsilane (0.00 ppm) as internal standard otherwise noted. Coupling constant (J) was reported in herz (Hz). Abbreviations of multiplicity were as follows; s: singlet, d: doublet, t: triplet, q: quartet, m: multiplet, br: broad. Data were presented as follows; chemical shift (multiplicity, integration, coupling constant). Assignment was based on $^1H-^1H$ COSY, HMBC and HMQC NMR spectra. The mass analyzer type used for the HRMS measurements was TOF. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60F254 plates. Normal-phase column chromatography was performed on Merck silica gel 5715 or Kanto Chemical silica gel 60N (neutral). Flash column chromatography was performed on Merck silica gel 60.

1-*O*-Acetyl-5-azido-5-deoxy-2,3-*O*-(3-pentylidene)-β-D-ribofuranose (10). A mixture of 5-azido-5-deoxy-2,3-*O*-(3-pentylidene)-



β-D-ribofuranose (832 mg, 3.4 mmol), Et_3N (0.52 mL, 3.7 mmol), DMAP (42 mg, 0.34 mmol), and Ac_2O (0.35 mL, 3.7 mmol) in MeCN (34 mL) was stirred at room temperature for 40 min. The reaction was quenched with MeOH, and the mixture was concentrated. The residue was partitioned between AcOEt (100 mL) and H_2O (50 mL). The organic layer was washed with 0.2 M aqueous HCl (50 mL), saturated aqueous $NaHCO_3$ (50 mL), and brine (50 mL); then it was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column

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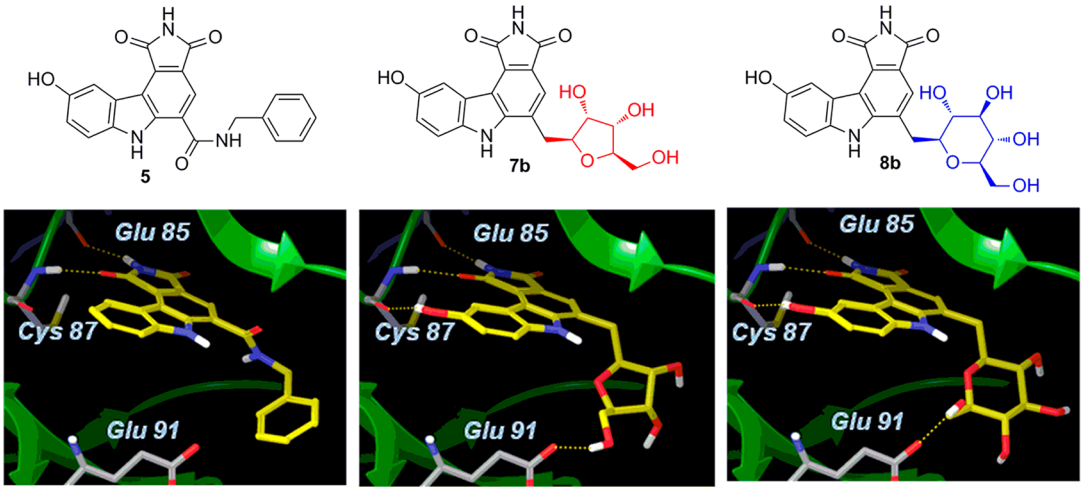
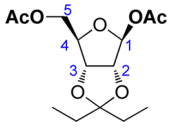


Figure 3. Predicted binding mode of *C*-glycoside Chk1 inhibitors.

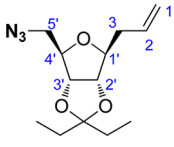
chromatography (φ 7.5 cm × 17 cm, $CHCl_3$) to give **10** (867 mg, 89%) as a colorless syrup. ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.28 (s, 1H, H-1), 4.75 (d, 1H, H-2, J = 6.0 Hz), 4.67 (d, 1H, H-3, J = 5.9 Hz), 4.44 (dd, 1H, H-4, J = 6.6, 6.6 Hz), 3.46 (dd, 1H, H-5, J = 7.6, 13.3 Hz), 3.26 (dd, 1H, H-5, J = 6.2, 13.3 Hz), 1.72 (q, 2H, CH_2CH_3, J = 7.8 Hz), 1.59 (q, 2H, CH_2CH_3, J = 7.8 Hz), 0.93 (t, 3H, CH_2CH_3, J = 7.8 Hz), 0.88 (t, 3H, CH_2CH_3, J = 7.8 Hz); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.3, 117.8, 102.6, 86.9, 85.6, 82.1, 53.3, 29.6, 29.0, 21.3, 8.43, 7.48; FABMS-LR m/z = 308 (MNa^+); FABMS-HR calcd for $C_{12}H_{19}N_3O_5Na$ 308.1222, found 308.1211 (MNa^+).

1,5-*O*-Diacetyl-2,3-*O*-(3-pentylidene)-β-D-ribofuranose (11). A mixture of 2,3-*O*-(3-pentylidene)-β-D-ribofuranose (2.84 g, 13



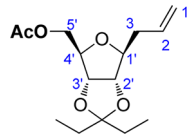
mmol), Et_3N (4 mL, 28.6 mmol), DMAP (0.16 g, 1.3 mmol), Ac_2O (2.7 mL, 28.6 mmol) in MeCN (130 mL) was stirred at room temperature for 3.5 h. The reaction was quenched with MeOH, and the mixture was concentrated. The residue was partitioned between AcOEt (150 mL) and H_2O (50 mL). The organic layer was washed with 0.2 M aqueous HCl (50 mL), saturated aqueous $NaHCO_3$ (50 mL), and brine (50 mL), dried over Na_2SO_4, filtered, and concentrated to give **11** (3.59 g, 92%) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.23 (d, 1H, H-1, J = 1.7 Hz), 4.73 (m, 2H, H-2, H-3), 4.49 (dt, 1H, H-4, J = 1.7, 6.8 Hz), 4.15 (ddd, 1H, H-5a, J = 2.3, 6.9, 11.5 Hz), 4.10 (ddd, 1H, H-5b, J = 1.7, 6.9, 11.5 Hz), 2.10 (s, 3H, OAc), 2.06 (s, 3H, OAc), 1.72 (dq, 2H, pentylidene-CH_2, J = 1.7, 7.4 Hz), 1.59 (dq, 2H, pentylidene-CH_2, J = 1.7, 7.4 Hz), 0.92 (dt, 3H, pentylidene-CH_3, J = 1.7, 7.4 Hz), 0.88 (dt, 3H, pentylidene-CH_3, J = 1.7, 7.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.6, 169.5, 117.7, 102.3, 85.7, 85.6, 82.0, 64.3, 29.6, 29.1, 21.3, 21.0, 8.5, 7.5; FABMS-LR m/z = 325 (MNa^+); FABMS-HR calcd for $C_{14}H_{22}O_7Na$ 325.1267, found 325.1247 (MNa^+).

3-[5-Azido-5-deoxy-2,3-*O*-(3-pentylidene)-β-D-ribofuranosyl]-1-propene (12). A mixture of **10** (4.2 g, 14.7 mmol),



allyltrimethylsilane (11.7 mL, 73.5 mmol) and molecular sieve 4 A (17 g) in CH_2Cl_2 (170 mL) was stirred at room temperature for 20 min. Then, the mixture was cooled to 0 °C and $SnCl_4$ (0.86 mL, 7.4 mmol) was added. The reaction mixture was stirred for 30 min and quenched with Et_3N. The insolubles were filtered off through Celite pad, the filtrate was partitioned between AcOEt (150 mL) and H_2O (150 mL). The organic layer was washed with brine (150 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (φ 7.5 cm × 17 cm, hexane/AcOEt = 19/1−9/1) to give **12** (2.4 g, 60%) as a yellow syrup. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.84 (m, 1H, H-2), 5.17 (ddd, 1H, H-1a, J = 1.8, 3.5, 17.2 Hz), 5.13 (dd, 1H, H-1b, J = 1.8, 10.8 Hz), 4.53 (dd, 1H, H-3′, J = 4.6, 6.9 Hz), 4.40 (dd, 1H, H-2′, J = 4.6, 6.9 Hz), 4.05 (dd, 1H, H-4′, J = 4.6, 8.6 Hz), 3.98 (dd, 1H, H-1′, J = 6.3, 11.5 Hz), 3.56 (dd, 1H, H-5′a, J = 3.5, 13.2 Hz), 3.33 (dd, 1H, H-5′b, J = 4.6, 13.2 Hz), 2.42 (m, 2H, H-3), 1.74 (q, 2H, pentylidene-CH_2, J = 7.5 Hz,), 1.60 (q, 2H, pentylidene-CH_2, J = 7.5 Hz), 0.95 (t, 3H, pentylidene-CH_3, J = 7.5 Hz), 0.87 (t, 3H, pentylidene-CH_3, J = 7.5 Hz); $[\alpha]^{25}_D$ +54.8 (c 0.87, $CHCl_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ 133.5, 119.6, 118.0, 84.5, 83.9, 82.9, 82.3, 52.4, 37.9, 29.8, 29.4, 8.56, 8.01; FABMS-LR m/z = 268 (MH^+); FABMS-HR calcd for $C_{13}H_{22}N_3O_3$ 268.1661, found 268.1653 (MH^+).

3-[5-*O*-Acetyl-2,3-*O*-(3-pentylidene)-β-D-ribofuranosyl]-1-propene (13). A mixture of **11** (61.2 mg, 0.2 mmol),



allyltrimethylsilane (160 μL, 1.0 mmol) and molecular sieve 4 A (200 mg) in EtCN (2 mL) was stirred at room temperature for 20 min then cooled down to −50 °C. Trimethylsilyl trifluoromethanesulfonate (18 μL, 0.1 mmol) was added to the solution, which was stirred for 2 h. The reaction was quenched with Et_3N, and molecular sieves were filtered to remove. The filtrate was partitioned between AcOEt (30 mL) and H_2O (20 mL). The organic layer was washed with brine (20 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (φ 1.5 cm × 10.5 cm, hexane/AcOEt = 10/1) to give **13** (43 mg, 75%) as a syrup. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.80 (m, 1H, H-2), 5.12 (m, 2H, H-1), 4.47 (dd, 1H, H-3′, J = 4.0, 6.9 Hz), 4.38 (dd, 1H, H-2′, J = 4.6, 7.5 Hz), 4.26 (dt, 1H, H-5′a, J = 6.3, 13.8 Hz), 4.08 (m, 2H, H-4′ and H-5′b), 4.08 (m, 1H, H-1′, J = 4.6, 6.3 Hz), 2.36 (dt, 2H, H-3, J = 1.8, 6.3 Hz), 2.07 (s, 3H, OAc), 1.72 (q, 2H, pentylidene-CH_2, J = 7.5 Hz), 1.58 (q, 2H, pentylidene-CH_2, J = 7.5 Hz), 0.94 (t, 3H, pentylidene-CH_3, J = 7.5 Hz), 0.85 (t, 3H, pentylidene-CH_3, J = 7.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.9, 133.4, 119.3, 118.0, 84.3, 83.9, 82.2, 81.8, 64.6, 37.9, 29.8, 29.4, 21.0, 8.5, 8.0; FABMS-LR m/z = 285 (MH^+); FABMS-HR calcd for $C_{15}H_{25}O_5$ 285.1697, found 285.1695 (MH^+).

3-[5-*tert*-Butoxycarbonylamino-5-deoxy-2,3-*O*-(3-pentylidene)-β-D-ribofuranosyl]-1-propene (14). A mixture of **12** (2.2 g,

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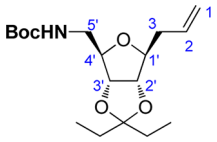
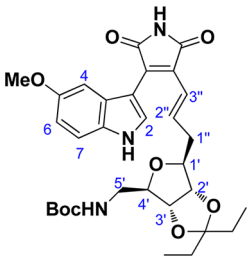
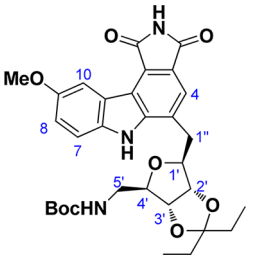


8.2 mmol), triphenylphosphine (6.45 g, 24.6 mmol) and H_2O (7.1 mL, 400 mmol) in THF (82 mL) was stirred at 50 °C for 30 min. Then the solution was treated with $NaHCO_3$ (1.38 g, 16.4 mmol) and Boc_2O (3.8 mL, 16.4 mmol) at room temperature for 1 h. The reaction mixture was concentrated, and the residue was partitioned between AcOEt (200 mL) and H_2O (100 mL × 2). The organic layer was washed with brine (100 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (φ 3.5 cm × 14 cm, hexane/AcOEt = 10/1−5/1) to give **14** (2.78 g, 99%) as a yellow syrup. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.82 (m, 1H, H-2), 5.16 (dd, 1H, H-1a, J = 1.8, 17.2 Hz), 5.13 (dd, 1H, H-1b, J = 1.1, 10.3 Hz), 4.76 (s, 1H, NH), 4.44 (dd, 1H, H-3′, J = 4.6, 7.4 Hz), 4.35 (dd, 1H, H-2′, J = 4.5, 7.4 Hz), 3.94 (m, 2H, H-1′, H-4′), 3.45 (m, 1H, H-5′a), 3.28 (m, 1H, H-5′b), 2.37 (m, 2H, H-3), 1.73 (q, 2H, pentylidene-CH_2, J = 7.4 Hz), 1.58 (q, 2H, pentylidene-CH_2, J = 7.5 Hz), 1.45 (s, 9H, *t*-butyl), 0.95 (t, 3H, pentylidene-CH_3, J = 7.5 Hz), 0.86 (t, 3H, pentylidene-CH_3, J = 7.5 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 156.1, 133.6, 119.2, 118.0, 84.5, 83.6, 83.0, 82.7, 42.7, 37.9, 29.8, 29.4, 28.5, 8.5, 8.0; ESIMS-LR m/z = 364 (MNa^+); ESIMS-HR calcd for $C_{18}H_{31}NO_5Na$ 364.2100, found 364.2091 (MNa^+).

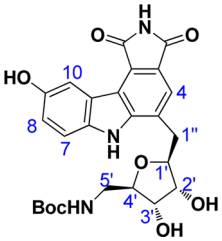
4-{1*E*-3-[5-*tert*-Butoxycarbonylamino-5-deoxy-2,3-*O*-(3-pentylidene)-β-D-ribofuranosyl]-1-propenyl}-3,4-dihydro-3-(5-methoxy-1*H*-indol-3-yl)-1*H*-pyrrole-2,5-dione (16). A mixture of **15** (284 mg, 0.88 mmol), **14** (300 mg, 0.88 mmol), $Pd_2(dba)_3$ (91 mg, 0.088 mmol), Bu_3N (240 μL, 0.99 mmol) and tri(2-furyl)phosphine (80 mg, 0.35 mmol) in DMF (9 mL) was stirred at 80 °C for 2 h. The mixture was partitioned between AcOEt (100 mL) and H_2O (100 mL × 2). The organic layer was washed with brine (100 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (φ 2.5 cm × 11.5 cm, hexane/AcOEt = 2/1) to give **16** (350 mg, 68%) as a red foam. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.98 (s, 1H, indole-NH), 7.67 (s, 1H, H-2), 7.53 (s, 1H, H-4), 7.32 (d, 1H, H-7, J = 9.1 Hz), 7.11 (s, 1H, maleimide-NH), 7.00 (dt, 1H, H-2″, J = 7.2, 15.8 Hz), 6.92 (dd, 1H, H-6, J = 2.2, 9.0 Hz), 6.59 (d, 1H, H-3″, J = 16.3 Hz), 4.87 (s, 1H, Boc-NH), 4.42 (d, 1H, H-3′, J = 3.6 Hz), 4.33 (dd, 1H, H-2′, J = 5.0, 6.8 Hz), 3.97 (m, 1H, H-1′), 3.92 (m, 1H, H-4′), 3.82 (s, 3H, OMe), 3.41 (dd, 1H, H-5′a, J = 4.6, 13.6 Hz), 3.29 (dd, 1H, H-5′b, J = 5.4, 13.1 Hz), 2.65 (m, 1H, H-1″a), 2.48 (dd, 1H, H-1″b, J = 6.8, 14.1 Hz), 1.70 (q, 2H, pentylidene-CH_2, J = 7.7 Hz), 1.56 (q, 2H, pentylidene-CH_2, J = 7.7 Hz), 1.24 (s, 9H, *t*-butyl), 0.92 (t, 3H, pentylidene-CH_3, J = 7.3 Hz), 0.84 (t, 3H, pentylidene-CH_3, J = 7.7 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.2, 156.3, 155.2, 137.1, 131.4, 129.4, 126.2, 123.0, 119.6, 113.6, 112.6, 105.9, 103.3, 84.3, 83.2, 83.1, 82.8, 79.8, 75.2, 55.9, 42.8, 37.9, 29.9, 29.7, 29.4, 28.5, 25.0, 8.6, 8.1; ESIMS-LR m/z = 604 (MNa^+); ESIMS-HR calcd for $C_{31}H_{39}N_3O_8Na$ 604.2635, found 604.2637 (MNa^+).



5-{[5-*tert*-Butoxycarbonylamino-5-deoxy-2,3-*O*-(3-pentylidene)-β-D-ribofuranosyl]methyl}-9-methoxypyrrolo-[3,4-*c*]-carbazole-1,3-dione(17). A mixture of **16** (10 mg, 0.017 mmol) and 10%Pd/C (1.2 mg) in 1,3-dichlorobenzene (5 mL) was heated under reflux for 5.5 h. The insolubles were filtered off through Celite pad, and the filtrate was concentrated. The residue was purified by silica gel column chromatography (φ 0.7 cm × 8 cm, hexane/AcOEt = 2/1) to give **17** (4.7 mg, 48%) as a red foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.22 (s, 1H, indole-NH), 8.50 (s, 1H, H-10), 7.72 (d, 1H, H-7, J = 8.6 Hz), 7.67 (s, 1H, H-4), 7.40 (s, 1H, maleimide-NH), 7.20 (dd, 1H, H-8, J = 2.3, 9.1 Hz), 4.80 (s, 1H, Boc-NH), 4.43 (m, 2H, H-2′, H-3′), 4.10 (m, 1H, H-1′), 3.99 (s, 4H, OMe, H-4′), 3.68 (m, 1H, H-5′a), 3.42 (d, 1H, H-1″a, J = 14.3 Hz), 3.21 (m, 1H, H-5′b), 3.16 (d, 1H, H-1″b, J = 14.3 Hz), 1.74 (q, 2H, pentylidene-CH_2, J = 7.5 Hz), 1.61 (q, 2H, pentylidene-CH_2, J = 7.4 Hz), 1.47 (s, 9H, *t*-butyl), 0.93 (t, 3H, pentylidene-CH_3, J = 7.5 Hz), 0.73 (t, 3H, pentylidene-CH_3, J = 7.4 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.6, 169.9, 156.8, 154.5, 144.4, 136.8, 126.4, 125.6, 123.8, 121.5, 120.8, 120.1, 118.4, 112.7, 106.8, 84.9, 84.3, 83.7, 81.9, 80.2, 55.9, 55.8, 42.3, 37.2, 29.7, 29.3, 28.5, 8.5, 8.0; ESIMS-LR m/z = 602 (MNa^+); ESIMS-HR calcd for $C_{31}H_{37}N_3O_8Na$ 602.2473, found 602.2490 (MNa^+).

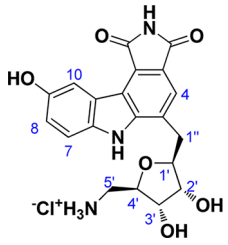


5-{[5-*tert*-Butoxycarbonylamino-5-deoxy-β-D-ribofuranosyl]-methyl}-9-hydroxypyrrolo-[3,4-*c*]carbazole-1,3-dione (18). A solution of **17** (92 mg, 0.16 mmol) in CH_2Cl_2 (1.6 mL) was treated with BBr_3 (1.0 M solution in CH_2Cl_2, 0.64 mL, 0.64 mmol) at 0 °C for 2 h. The reaction mixture was concentrated, and the residue was dissolved in dioxane (2.1 mL). The solution was treated with $NaHCO_3$ (36 mg, 0.43 mmol) and Boc_2O (0.096 mL, 0.42 mmol) at room temperature for 3 h. The reaction mixture was partitioned between AcOEt and H_2O. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated to give **18** (38 mg, 0.076 mmol, 48%) as a red solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.42 (s, 1H, indole-NH), 10.97 (s, 1H, maleimide-NH), 9.22 (s, 1H, phenol-OH), 8.22 (d, 1H, H-10, J = 2.3 Hz), 7.62 (s, 1H, H-4), 7.46 (d, 1H, H-7, J = 8.6 Hz), 7.04 (dd, 1H, H-8, J = 2.3, 8.6 Hz), 6.88 (t, 1H, Boc-NH, J = 6.3 Hz), 4.85 (m, 2H, 2′-OH, 3′-OH), 3.97 (m, 1H, H-1′), 3.73 (m, 1H, H-3′), 3.64 (m, 2H, H-2′, H-4′), 3.29 (dd, 1H, H-1″a, J = 4.0, 14.3 Hz), 3.10 (dd, 1H, H-1″b, J = 8.0, 14.3 Hz), 3.01 (m, 1H, H-5′a), 2.91 (m, 1H, H-5′b), 1.37 (s, 9H, *t*-butyl); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.6, 169.9, 156.8, 154.5, 144.4, 136.8, 126.4, 125.6, 123.8, 121.5, 120.8, 120.1, 118.4, 112.7, 106.8, 84.9, 84.3, 83.7, 81.94, 80.2, 55.9, 55.8, 42.3, 37.2, 29.7, 29.3, 28.5, 8.5, 8.0; ESIMS-LR m/z = 520 (MNa^+); ESIMS-HR calcd for $C_{25}H_{27}N_3O_8Na$ 520.16900, found 520.1686 (MNa^+).



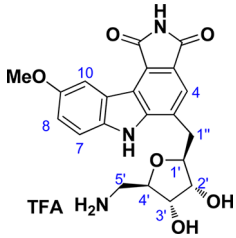
5-[(5-Amino-5-deoxy-β-D-ribofuranosyl)methyl]-9-hydroxypyrrolo-[3,4-*c*]carbazole-1,3-dione Hydrochloride Salt (7a). Compound **18** (25 mg, 0.050 mmol) was treated with 4 M HCl/AcOEt (1 mL) at room temperature for 45 min. The reaction was concentrated, and the resulting residue was triturated from diethylether to give **7a** (16 mg, 0.040 mmol, 80%) as an yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.80 (s, 1H, indole-NH), 10.99 (s, 1H, maleimide-NH), 9.24 (s, 1H, phenol-OH), 8.22 (d, 1H, H-10, J = 2.3 Hz), 7.99 (s, 2H, NH_2), 7.67 (s, 1H, H-4), 7.46 (d, 1H, H-7, J = 8.6 Hz), 7.05 (dd, 1H, H-8, J = 2.3, 8.6 Hz), 5.09 (s, 2H, 2′-OH, 3′-OH), 4.17 (m, 1H, H-1′), 3.80 (m, 3H, H-2′, H-3′, H-4′), 3.28

dx.doi.org/10.1021/jo4020672 | J. Org. Chem. 2013, 78, 12065−12075



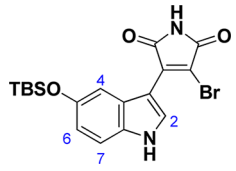
(m, 2H, H-1″), 3.01 (m, 1H, H-5′a), 2.81 (m, 1H, H-5′b); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.6, 170.6, 151.4, 143.7, 135.6, 127.0, 124.9, 123.4, 121.0, 119.4, 118.0, 117.8, 112.3, 108.9, 83.2, 79.1, 74.0, 71.9, 41.4, 35.1; ESIMS-LR m/z = 398 (M^+-HCl); ESIMS-HR calcd for $C_{20}H_{20}N_3O_6$ 398.1347, found 398.1352 (M^+-HCl).

5-[(5-Amino-5-deoxy-β-D-ribofuranosyl)methyl]-9-methoxypyrrolo-[3,4-*c*]carbazole-1,3-dione Trifluoroacetic Acid Salt

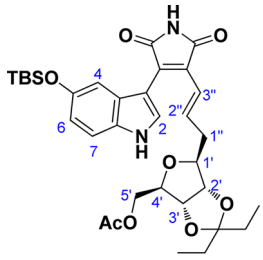


(19). Compound **17** (450 mg, 0.78 mmol) was treated with 80% aqueous TFA (10 mL) at room temperature for 3 h. The reaction was concentrated and the residue was triturated with diethylether to give **18** as an yellow solid (199 mg, 57%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.90 (s, 1H, indole-NH), 11.05 (s, 1H, maleimide-NH), 8.37 (d, 1H, H-10, J = 2.3 Hz), 7.91 (s, 2H, NH_2), 7.71 (s, 1H, H-4), 7.56 (d, 1H, H-7, J = 8.6 Hz), 7.21 (dd, 1H, H-8, J = 2.3, 8.6 Hz), 5.13 (s, 1H, OH), 5.02 (d, 1H, OH, J = 4.5 Hz), 4.18 (m, 1H, H-1′), 3.86 (s, 3H, OMe), 3.80 (m, 3H, H-2′, H-3′, H-4′), 3.25 (m, 2H, H-1″), 3.01 (dd, 1H, H-5′a, J = 2.8, 12.7 Hz), 2.81 (dd, 1H, H-5′b, J = 7.7, 13.2 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.6, 153.7, 143.6, 136.4, 127.3, 125.0, 123.8, 120.8, 119.7, 118.1, 117.5, 112.7, 106.6, 83.1, 79.2, 74.0, 72.0, 55.5, 41.6, 35.1; ESIMS-LR m/z = 412 (MH^+); ESIMS-HR calcd for $C_{21}H_{22}N_3O_6$ 412.1503, found 412.1512 (MH^+).

4-Bromo-2,5-dihydro-3-(5-*tert*-butyldimethylsilyloxy-1*H*-indol-3-yl)-1*H*-pyrrole-2,5-dione (21). A solution of **20** (495 mg,

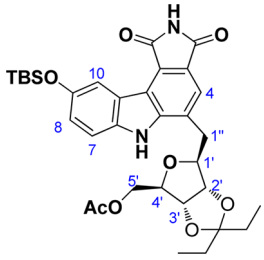


2.0 mmol) in THF (25 mL) was treated with EtMgBr (1.0 M solution in THF, 2.0 mL, 2.0 mmol) at 0 °C for 1 h. The solution was added dropwise to a solution of dibromomaleimide (127 mg, 0.5 mmol) in THF (5 mL) at 0 °C, which was stirred for 22 h. The reaction was quenched with saturated aqueous NH_4Cl, and the whole mixture was concentrated in vacuo. The residue was partitioned between AcOEt (50 mL) and H_2O (30 mL). The organic layer was washed with brine (30 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (φ 1.5 cm × 17.5 cm, hexane/AcOEt = 6/1−3/1) to give **21** (164 mg, 78%) as a red solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.97 (br s, 1H, indole-NH), 11.29 (s, 1H, maleimide-NH), 8.00 (d, 1H, H-2, J = 2.3 Hz), 7.37 (d, 1H, H-4, J = 2.3 Hz), 7.35 (d, 1H, H-7, J = 8.6 Hz), 6.75 (dd, 1H, H-6, J = 2.3, 8.6 Hz), 0.96 (s, 9H, *t*-butyl), 0.18 (s, 6H, methyl ×2); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.3, 167.5, 149.3, 138.1, 132.0, 125.4, 116.5, 113.6, 112.8, 111.8, 103.5, 25.7, 18.0; ESIMS-LR m/z = 419 (MH^-); ESIMS-HR calcd for $C_{18}H_{20}$ Br N_2O_3Si 419.0432, found 419.0440 (MH^-).



4-{1*E*-3-[5-*O*-Acetyl-2,3-*O*-(3-pentylidene)-β-D-ribofuranosyl]-1-propenyl}-3-(5-*tert*-butyldimethylsilyloxy-1*H*-indol-3-yl)-2,5-dihydro-1*H* -pyrrole-2,5-dione (22). A mixture of **21** (1.7 g, 4.0 mmol), **13** (2.3 g, 8.1 mmol), $Pd_2(dba)_3$ (414 mg, 0.4 mmol), Bu_3N (2.1 mL, 8.8 mmol) and tri(2-furyl)phosphine (371 mg, 1.6 mmol) in DMF (40 mL) was stirred at 80 °C for 3.5 h. The reaction mixture was concentrated, and the residue was partitioned between AcOEt (300 mL) and H_2O (200 mL). The organic layer was washed with brine (200 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (φ 3.5 cm × 18 cm, hexane/AcOEt = 3/1−2/1) to give **22** (2.03 g, 83%) as a red foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.15 (br s, 1H, indole-NH), 7.56 (d, 1H, H-2, J = 2.3 Hz), 7.23 (d, 1H, H-7, J = 8.6 Hz), 7.07 (d, 1H, H-4, J = 1.8 Hz), 7.03 (m, 1H, H-2″), 6.79 (dd, 1H, H-6, J = 2.3, 8.6 Hz), 6.55 (d, 1H, H-3″, J = 16.0 Hz), 4.48 (dd, 1H, H-3′, J = 4.0, 6.9 Hz), 4.39 (dd, 1H, H-2′, J = 4.0, 6.9 Hz), 4.27 (dd, 1H, H-5′a, J = 5.8, 13.8 Hz), 4.10 (m, 3H, H-1′, H-4′, H-5′b), 2.60 (m, 1H, H-1″a), 2.49 (m, 1H, H-1″b), 2.05 (s, 3H, OAc), 1.71 (q, 2H, pentylidene-CH_2, J = 7.5 Hz), 1.57 (q, 2H, pentylidene-CH_2, J = 7.5 Hz), 0.98 (s, 9H, *t*-butyl), 0.93 (t, 3H, pentylidene-CH_3, J = 7.5 Hz), 0.84 (t, 3H, pentylidene-CH_3, J = 7.5 Hz), 0.18 (s, 6H, methyl ×2); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.6, 171.5, 171.1, 150.5, 136.7, 131.9, 131.8, 129.6, 128.4, 126.4, 123.2, 119.5, 117.4, 112.3, 110.9, 105.4, 84.3, 83.6, 82.1, 81.9, 64.5, 38.4, 29.7, 29.4, 25.9, 21.0, 18.3, 8.5, 8.0, −4.2; ESIMS-LR m/z = 647 (MNa^+); ESIMS-HR calcd for $C_{33}H_{44}N_2O_8NaSi$ 647.2759, found 647.2766 (MNa^+).

5-{[5-*O*-Acetyl-2,3-*O*-(3-pentylidene)-β-D-ribofuranosyl]-methyl}-9-*tert*-butyldimethylsilyloxypyrrolo-[3,4-*c*]carbazole-



1,3-dione (23). A mixture of **22** (2.0 g, 3.3 mmol) and 10%Pd/C (150 mg) in 1,3-dichlorobenzene (100 mL) was heated under reflux for 10 h. The insolubles were filtered off through Celite pad, and the filtrate was concentrated. The residue was purified by silica gel column chromatography (φ 3.5 cm × 18 cm, hexane/AcOEt = 3/1) to give **23** (1.47 g, 73%) as a red foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.56 (s, 1H, indole-NH), 8.48 (d, 1H, H-10, J = 2.3 Hz), 7.84 (br s, 1H, maleimide-NH), 7.67 (s, 1H, H-4), 7.50 (d, 1H, H-7, J = 8.6 Hz), 7.12 (dd, 1H, H-8, J = 2.3, 8.6 Hz), 4.51 (dd, 1H, H-2′, J = 2.3, 12.0 Hz), 4.47 (dd, 1H, H-4′, J = 4.6, 7.5 Hz), 4.43 (dd, 1H, H-3′, J = 5.8, 7.5 Hz), 4.21 (m, 2H, H-1′ and H-5′a), 4.11 (m, 1H, H-5′b), 3.48 (dd, 1H, H-1″a, J = 2.3, 14.9 Hz,), 3.22 (dd, 1H, H-1″b, J = 8.0, 14.9 Hz), 1.93 (s, 3H, OAc), 1.75 (q, 2H, pentylidene-CH_2, J = 7.5 Hz), 1.60 (q, 2H, pentylidene-CH_2, J = 7.5 Hz), 1.04 (s, 9H, *t*-butyl), 0.95 (t, 3H, pentylidene-CH_3, J = 7.5 Hz), 0.87 (t, 3H, pentylidene-CH_3, J = 7.5 Hz), 0.30 (s, 6H, methyl ×2); ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.2, 170.1, 169.6, 150.2, 144.9, 136.8, 126.0, 125.9, 124.1, 122.4, 121.9, 121.4, 120.4, 120.2, 114.9, 112.2, 85.1, 83.5, 82.6, 81.1, 63.5, 36.9, 29.7, 29.3, 16.0, 20.8, 18.5, 8.5, 8.0, −4.3; ESIMS-LR m/z = 645 (MNa^+); ESIMS-HR calcd for $C_{33}H_{42}N_2O_8NaSi$ 645.2603, found 645.2594 (MNa^+).

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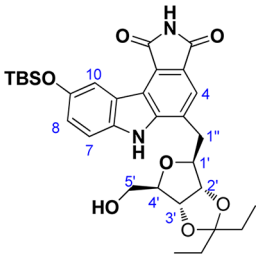
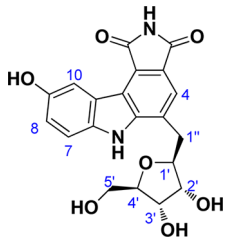


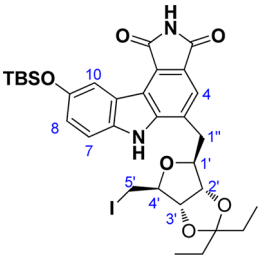
9-*tert*-Butyldimethylsilyloxy-5-{[2,3-*O*-(3-pentylidene)-β-D-ribofuranosyl]methyl}-pyrrolo-[3,4-*c*]carbazole-1,3-dione (24). A mixture of **23** (26.3 mg, 0.042 mmol) and K_2CO_3 (7 mg, 0.051 mmol) in MeOH (1 mL) was stirred at room temperature for 1 h. The reaction mixture was neutralized with AcOH and partitioned between AcOEt (20 mL) and H_2O (20 mL). The organic layer was washed with brine (20 mL), dried over Na_2SO_4, filtered, and concentrated to give **24** (23.2 mg, 95%) as a red form. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.85 (s, 1H, indole-NH), 10.20 (s, 1H, maleimide-NH), 8.35 (d, 1H, H-10, J = 2.3 Hz), 7.70 (s, 1H, H-4), 7.48 (d, 1H, H-7, J = 8.6 Hz), 7.09 (dd, 1H, H-8, J = 2.3, 8.6 Hz), 5.08 (t, 1H, OH), 4.68 (dd, 1H, H-3′, J = 3.5, 6.9 Hz), 4.59 (dd, 1H, H-2′, J = 4.1, 6.9 Hz), 4.27 (ddd, 1H, H-1′, J = 4.0, 6.3, 8.1 Hz), 3.91 (dt, 1H, H-4′, J = 4.6, 8.0 Hz), 3.53 (t, 2H, H-5′, J = 5.2 Hz), 3.28 (m, 2H, H-1″), 1.58 (q, 2H, pentylidene-CH_2, J = 7.5 Hz), 1.50 (q, 2H, pentylidene-CH_2, J = 7.5 Hz), 0.98 (s, 9H, *t*-butyl), 0.78 (t, 3H, pentylidene-CH_3, J = 7.5 Hz), 0.75 (t, 3H, pentylidene-CH_3, J = 7.5 Hz), 0.24 (s, 6H, methyl ×2); ^{13}C NMR (100 MHz, DMSO-d_6) δ 170.5, 170.5, 148.8, 143.6, 136.7, 126.6, 125.1, 123.7, 121.5, 121.0, 120.0, 118.0, 117.0, 113.8, 112.3, 85.1, 84.6, 83.4, 82.2, 61.9, 35.6, 29.0, 28.7, 25.7, 18.0, 8.4, 7.6, −4.4; ESIMS-LR m/z = 579 (MH^-); ESIMS-HR calcd for $C_{31}H_{39}N_2O_7Si$ 579.2532, found 579.2536 (MH^-).

9-Hydroxy-5-[(β-D-ribofuranosyl)methyl]-pyrrolo-[3,4-*c*]-carbazole-1,3-dione (7b). Compound **24** (10 mg, 0.017 mmol) was



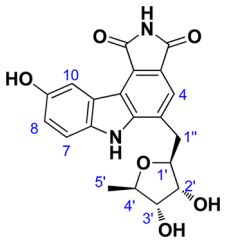
treated with 80% aqueous TFA (1 mL) at room temperature for 1 h. The reaction was concentrated, and the residue was purified by silica gel column chromatography (φ 0.7 cm × 15 cm, chloroform/MeOH = 9/1−0/1)) to give **7b** (3.6 mg, 53%) as a red solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.59 (s, 1H), 10.59 (s, 1H), 9.22 (s, 1H), 8.22 (d, 1H, H-10, J = 2.3 Hz), 7.64 (s, 1H, H-4), 7.42 (d, 1H, H-7, J = 8.6 Hz), 7.04 (dd, 1H, H-8, J = 2.3, 8.6 Hz), 4.90 (t, 1H, 5′-OH, J = 5.2 Hz), 4.84 (d, 1H, 2′-OH, J = 5.7 Hz, exchanged with D_2O), 4.81 (d, 1H, 3′-OH, J = 5.8 Hz, exchanged with D_2O), 4.05 (m, 1H, H-1′), 3.88 (dt, 1H, H-3′, J = 5.2, 5.7 Hz), 3.74 (dt, 1H, H-2′, J = 5.2, 5.8 Hz), 3.67 (dt, 1H, H-4′, J = 4.0, 4.6 Hz), 3.42 (m, 2H, H-5′), 3.26 (dd, 1H, H-1″a, J = 4.1, 14.4 Hz), 3.12 (dd, 1H, H-1″b, J = 8.6, 14.9 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.6, 170.6, 151.3, 143.9, 135.5, 127.4, 124.8, 123.4, 121.1, 119.4, 117.9, 117.8, 112.2, 108.9, 84.2, 82.0, 74.6, 70.9, 61.6, 35.5; ESIMS-LR m/z = 397 (MH^-); ESIMS-HR calcd for $C_{20}H_{17}O_7N_2$ 397.1041, found 397.1042 (MH^-)

9-*tert*-Butyldimethylsilyloxy-5-{[5-deoxy-5-iodo-2,3-*O*-(3-pentylidene)-β-D-ribofuranosyl]methyl}-pyrrolo-[3,4-*c*]-carbazole-1,3-dione (25). A mixture of **24** (375 mg, 0.65 mmol), PPh_3 (530 mg, 2.0 mmol), imidazole (157 mg, 2.3 mmol) and iodine (508 mg, 2.0 mmol) in DMF (10 mL) was stirred at room temperature for 40 min. The mixture was partitioned between AcOEt (150 mL) and saturated aqueous $Na_2S_2O_3$ (100 mL). The organic layer was washed with brine (100 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel



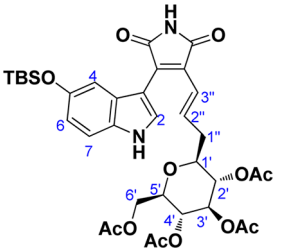
column chromatography (φ 2.5 cm × 13 cm, hexane/AcOEt = 4/1) to give **25** (393 mg, 88%) as a red foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.51 (s, 1H, indole-NH), 8.48 (d, 1H, H-10, J = 2.3 Hz), 7.77 (s, 1H, maleimide-NH), 7.69 (s, 1H, H-4), 7.48 (d, 1H, H-7, J = 8.6 Hz), 7.11 (dd, 1H, H-8, J = 2.3, 8.6 Hz), 4.57 (dd, 1H, H-2′, J = 5.8, 7.5 Hz), 4.39 (dd, 1H, H-3′, J = 4.6, 7.5 Hz), 4.27 (ddd, 1H, H-1′, J = 2.3, 5.8, 8.6 Hz), 3.80 (dd, 1H, H-4′ J = 4.6, 8.6 Hz), 3.48 (m, 2H, H-1″a, H-5′a), 3.32 (m, 2H, H-1″b, H-5′b), 1.75(q, 2H, pentylidene-CH_2, J = 7.5 Hz), 1.63 (q, 2H, pentylidene-CH_2, J = 7.5 Hz), 1.04 (s, 9H, *t*-butyl), 0.94 (t, 3H, pentylidene-CH_3, J = 7.5 Hz), 0.90 (t, 3H, pentylidene-CH_3, J = 7.5 Hz), 0.31 (s, 3H, methyl), 0.31 (s, 3H, methyl); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.7, 169.2, 150.4, 145.1, 136.7, 126.3, 126.0, 124.3, 122.5, 122.1, 121.2, 120.7, 120.4, 115.0, 112.1, 85.5, 84.8, 84.7, 82.5, 37.1, 29.8, 29.3, 26.0, 18.5, 8.6, 8.0, −4.2, −4.2; ESIMS-LR m/z = 713 (MNa^+); ESIMS-HR calcd for $C_{31}H_{39}IN_2O_6NaSi$ 713.1514, found 713.1528 (MNa^+).

5-[(5-Deoxy-β-D-ribofuranosyl)methyl]-9-hydroxypyrrolo-[3,4-*c*]carbazole-1,3-dione (26). A solution of **25** (69 mg, 0.1



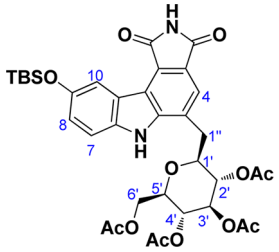
mmol) in 80% aqueous TFA (1 mL) was stirred at room temperature for 15 min. The reaction mixture was concentrated to give a triol as a red solid. A mixture of the triol and 10%Pd/C (2 mg) in EtOH (1 mL) was vigorously stirred under H_2 atmosphere at room temperature for 5 days. The insolubles were filtered off through Celite pad, and the filtrate was concentrated. The residue was purified by preparative TLC (chloroform/MeOH = 4/1) to give **26** (12 mg, 98%) as a yellow solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.32 (d, 1H, H-10, J = 2.3 Hz), 7.67 (s, 1H, H-4), 7.40 (d, 1H, H-7, J = 9.2 Hz), 7.07 (dd, 1H, H-8, J = 2.3, 9.2 Hz), 4.18 (m, 1H, H-1′), 3.88 (t, 1H, H-2′, J = 5.5 Hz), 3.80 (dd, 1H, H-4′, J = 6.0, 6.4 Hz), 3.49 (t, 1H, H-3′, J = 6.0 Hz), 3.38 (dd, 1H, H-1″a, J = 4.6, 15.1 Hz), 3.21 (dd, 1H, H-1″b, J = 7.8, 15.1 Hz), 1.16 (d, 3H, Me, J = 6.4 Hz); ^{13}C NMR (125 MHz, CD_3OD) δ 172.9, 171.9, 152.5, 145.7, 137.5, 127.8, 126.6, 125.0, 123.0, 121.1, 118.8, 112.8, 110.7, 84.0, 80.4, 77.4, 75.6, 36.7, 30.8, 19.2; ESIMS-LR m/z = 381 (MH^-); ESIMS-HR calcd for $C_{20}H_{17}O_6N_2$ 381.1092, found 381.1094 (MH^-).

3-(5-*tert*-Butyldimethylsilyloxy-1*H*-indol-3-yl)-2,5-dihydro-4-{1*E*-3-(2,3,4,6-*O*-tetraacetyl-β-D-glucopyranosyl)-1-propen-



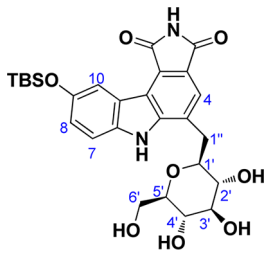
yl}-1*H* -pyrrole-2,5-dione (28). A mixture of **21** (15 mg, 0.037 mmol), **27**[32] (27 mg, 0.073 mmol), $Pd_2(dba)_3$ (4 mg, 0.0037 mmol), Bu_3N (20 μL, 0.08 mmol) and tri(2-furyl)phosphine (3.5 mg, 0.015 mmol) in DMF (1 mL) was stirred at 80 °C for 3 h. The residue was partitioned between AcOEt (30 mL) and H_2O (20 mL × 3). The organic layer was washed with brine (20 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (φ 0.7 cm × 9 cm, hexane/AcOEt = 2/1) to give **28** (16 mg, 60%) as a red foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.33 (s, 1H, indole-NH), 7.63 (d, 1H, H-2, *J* = 2.3 Hz), 7.26 (s, 1H, maleimide-NH), 7.22 (d, 1H, H-7, *J* = 8.6 Hz), 6.99 (d, 1H, H-4, *J* = 2.3 Hz), 6.86 (dt, 1H, H-2″, *J* = 6.9, 16.0 Hz), 6.77 (dd, 1H, H-6, *J* = 2.3, 9.2 Hz), 6.54 (d, 1H, H-3″, *J* = 16.0 Hz), 5.17 (t, 1H, H-3′, *J* = 9.8 Hz), 5.04 (t, 1H, H-4′, *J* = 9.7 Hz), 4.89 (t, 1H, H-2′, *J* = 9.8 Hz), 4.15 (dd, 1H, H-6′a, *J* = 4.6, 12.6 Hz), 3.81 (dd, 1H, H-6′b, *J* = 1.7, 12.6 Hz), 3.58 (m, 2H, H-5′ and H-1′), 2.40 (m, 2H, H-1″), 2.01 (s, 3H, OAc), 2.00 (s, 3H, OAc), 1.99 (s, 3H, OAc), 1.98 (s, 3H, OAc), 0.99 (s, 9H, *t*-butyl), 0.17 (s, 3H, methyl), 0.17 (s, 3H, methyl); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.9, 171.8, 171.1, 170.5, 169.8, 169.7, 150.2, 136.7, 131.9, 131.5, 130.0, 127.9, 126.0, 122.5, 117.1, 112.4, 111.4, 105.2, 75.3, 74.4, 72.0, 68.5, 62.1, 36.5, 25.8, 20.8, 20.7, 20.7, 18.2, 14.2, 14.0, −4.3; ESIMS-LR *m*/*z* = 735 (MNa^+); ESIMS-HR calcd for $C_{35}H_{44}N_2O_{12}NaSi$ 735.2556, found 735.2570 (MNa^+).

9-*tert*-Butyldimethylsilyloxy-5-{(2,3,4,6-*O*-tetraacetyl-β-D-glucopyranosyl)methyl}-pyrrolo-[3,4-*c*]carbazole-1,3-dione



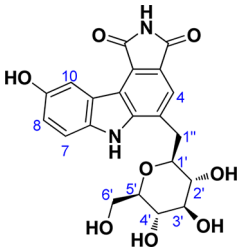
(29). A mixture of **28** (16 mg, 0.022 mmol) and 10%Pd/C (3 mg) in 1,3-dichlorobenzene (2 mL) was heated under reflux for 1.5 h. The insolubles were filtered off through Celite pad, and the filtrate was concentrated. The residue was purified by silica gel column chromatography (φ 0.7 cm × 10 cm, hexane/AcOEt = 3/1) to give **29** (7 mg, 45%) as a red foam. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.48 (s, 1H, indole-NH), 8.49 (d, 1H, H-10, *J* = 2.3 Hz), 7.64 (s, 1H, H-4), 7.53 (s, 1H, maleimide-NH), 7.50 (d, 1H, H-7, *J* = 8.6 Hz), 7.12 (dd, 1H, H-8, *J* = 2.3, 8.6 Hz), 5.20 (t, 1H, H-3′, *J* = 9.7 Hz), 5.08 (t, 1H, H-4′, *J* = 9.7 Hz), 4.95 (t, 1H, H-2′, *J* = 9.7 Hz), 4.36 (dd, 1H, H-6′a, *J* = 1.8, 12.6 Hz), 4.09 (dd, 1H, H-6′b, *J* = 5.2, 12.6 Hz), 3.81 (m, 1H, H-1′), 3.63 (ddd, 1H, H-5′, *J* = 1.8, 5.2, 10.3 Hz), 3.21 (dd, 1H, H-1″a, *J* = 8.6, 14.9 Hz), 3.14 (dd, 1H, H-1″b *J* = 1.2, 14.9 Hz), 2.18 (s, 3H, OAc), 2.03 (s, 3H, OAc), 2.00 (s, 3H, OAc), 1.90 (s, 3H, OAc), 1.04 (s, 9H, *t*-butyl), 0.30 (s, 3H, methyl), 0.30 (s, 3H, methyl); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.0, 170.4, 170.2, 169.8, 169.6, 169.4, 150.1, 144.7, 136.6, 125.9, 125.1, 124.0, 122.3, 121.7, 120.8, 119.9, 79/08. 76.1, 74.0, 71.2, 68.1, 61.9, 35.1, 20.7, 20.5, 20.5, 20.4, 18.3, −4.4, −4.4; ESIMS-LR *m*/*z* = (MNa^+); ESIMS-HR calcd for $C_{35}H_{42}N_2O_{12}NaSi$ 733.2399, found 733.2407 (MNa^+).

9-*tert*-Butyldimethylsilyloxy-5-{(β-D-glucopyranosyl)-methyl}-pyrrolo-[3,4-*c*]carbazole-1,3-dione (30). A mixture of **29**



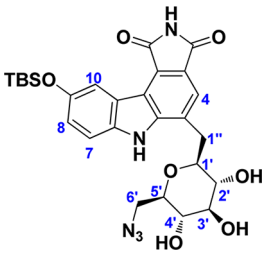
(7 mg, 0.0098 mmol) and K_2CO_3 (1 mg, 0.003 mmol) in MeOH (1 mL) was stirred at room temperature for 1 h. The reaction mixture was neutralized with AcOH and partitioned between AcOEt (30 mL) and H_2O (20 mL). The organic layer was washed with brine (20 mL), dried over Na_2SO_4, filtered, and concentrated to give **30** (5.3 mg, quant.) as a red form. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.36 (s, 1H, indole-NH), 10.98 (s, 1H, maleimide-NH), 8.36 (d, 1H, H-10, *J* = 2.3 Hz), 7.62 (s, 1H, H-4), 7.51 (d, 1H, H-7, *J* = 8.6 Hz), 7.08 (dd, 1H, H-8, *J* = 2.9, 8.6 Hz), 5.22 (d, 1H, OH, *J* = 5.8 Hz), 4.98 (d, 1H, OH, *J* = 4.0 Hz), 4.94 (d, 1H, OH, *J* = 5.8 Hz), 4.68 (t, 1H, OH, *J* = 5.8 Hz), 3.64 (ddd, 1H, H-6′a, *J* = 1.8, 5.2, 11.5 Hz), 3.52 (m, 1H, H-1′), 3.48 (m, 1H, H-1″a), 3.39 (m, 1H, H-6′b), 3.19 (dt, 1H, H-4′, *J* = 3.4, 8.6 Hz), 3.13 (m, 2H, H-1″b and H-5′), 3.01 (m, 2H, H-2′ and H-3′), 0.99 (s, 9H, *t*-butyl), 0.24 (s, 6H, dimethyl); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.7, 170.6, 148.9, 144.3, 136.8, 127.9, 125.1, 123.8, 121.5, 121.1, 120.2, 118.2, 113.8, 112.8, 80.6, 79.3, 78.1, 73.1, 70.5, 61.6, 34.5, 25.8, 18.1, −4.4; ESIMS-LR *m*/*z* = (MH^-); ESIMS-HR calcd for $C_{27}H_{33}N_2O_8Si$ 541.2012, found 541.2023 (MH^-).

5-{(β-D-Glucopyranosyl)methyl}-9-hydroxypyrrolo-[3,4-*c*]-carbazole-1,3-dione (8b). A solution of **30** (5.3 mg, 0.0098 mmol)



in 80% aqueous TFA (1 mL) was stirred at room temperature for 15 min. The reaction mixture was concentrated. The residue was purified by preparative TLC (chloroform/MeOH = 4/1) to give **8b** (3.6 mg, 86%) as a yellow solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.27 (s, 1H, indole-NH), 10.95 (s, 1H, maleimide-NH), 9.23 (s, 1H, phenol-OH), 8.22 (d, 1H, H-10, *J* = 2.3 Hz), 7.59 (s, 1H, H-4), 7.45 (d, 1H, H-7, *J* = 8.6 Hz), 7.02 (dd, 1H, H-8, *J* = 2.3, 8.6 Hz), 5.29 (s, 1H, OH), 5.04 (s, 1H, OH), 4.97 (s, 1H, OH), 4.66 (s, 1H, OH), 3.63 (d, 1H, H-6′a, *J* = 11.5 Hz), 3.52 (m, 1H, H-1′), 3.48 (d, 1H, H-1″a, *J* = 17.2 Hz), 3.39 (m, 1H, H-6′b), 3.19 (t, 1H, H-4′, *J* = 9.2 Hz), 3.12 (m, 2H, H-1″b and H-5′), 3.01 (m, 2H, H-2′ and H-3′); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.6, 151.4, 144.1, 135.5, 127.6, 124.9, 123.4, 121.1, 119.7, 118.1, 117.7, 112.6, 108.8, 80.5, 79.1, 78.0, 73.1, 70.3, 61.4, 34.3; ESIMS-LR *m*/*z* = 451 (MNa^+); ESIMS-HR calcd for $C_{32}H_{40}N_2O_8NaSi$ 451.1117, found 451.1111 (MNa^+).

5-{(6-Azido-6-deoxy-β-D-glucopyranosyl)methyl}-9-*tert*-butyldimethylsilyloxypyrrolo-[3,4-*c*]carbazole-1,3-dione (31). A



mixture of **30** (355 mg, 0.65 mmol) in pyridine (5 mL) was treated with TsCl (148 mg, 0.77 mmol) at 0 °C, and the resulting mixture was stirred at room temperature for 12 h. Water was added to the mixture, which was stirred for additional 2 h and partitioned between AcOEt (50 mL) and H_2O (30 mL × 3). The organic layer was washed with brine (30 mL), dried over Na_2SO_4, filtered, and concentrated to give a tosylate. A mixture of the tosylate and sodium azide (65 mg, 1.0 mmol) in DMF (5 mL) was stirred at 50 °C for 10 h. The reaction was partitioned between AcOEt (50 mL) and H_2O (30 mL × 3). The organic layer was washed with brine (30 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel

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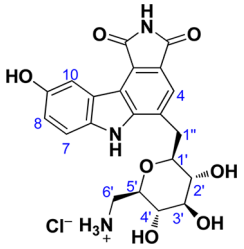
column chromatography (φ 1.5 cm × 17 cm, chloroform/MeOH = 30/1) to give **31** (269 mg, 73%) as a red solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.50 (s, 1H, indole-NH), 10.94 (s, 1H, maleimide-NH), 8.35 (d, 1H, H-10, J = 2.3 Hz), 7.68 (s, 1H, H-4), 7.48 (d, 1H, H-7, J = 8.6 Hz), 7.07 (dd, 1H, H-8, J = 2.3, 8.6 Hz), 5.34 (d, 1H, OH, J = 5.8 Hz), 5.18 (d, 1H, OH, J = 5.8 Hz), 5.13 (d, 1H, OH, J = 5.2 Hz), 3.70 (dt, 1H, H-1′, J = 2.3, 9.2 Hz), 3.55 (dd, 1H, H-1″a, J = 1.8, 15.5 Hz), 3.29 (m, 3H, H-4′ and H-5′- and H-6′a), 3.10 (m, 4H, H-1″b and H-2′ and H-3′ and H-6′b), 0.97 (s, 9H, *t*-butyl), 0.23 (s, 6H, dimethyl); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.6, 148.8, 144.1, 136.8, 127.4,124.8, 123.9, 121.4, 121.1, 119.5, 117.9, 113.3, 112.5, 78.9, 78.7, 77.9, 73.7, 71.1, 51.7, 33.3, 25.7, 28.1, −4.5; ESIMS-LR m/z = 566 (MH^-); ESIMS-HR calcd for $C_{27}H_{32}N_5O_7Si$ 566.2077, found 566.2084 (MH^-).

5-[(6-Amino-6-deoxy-β-D-ribofuranosyl)methyl]-9-hydroxypyrrolo-[3,4-*c*]carbazole-1,3-dione hydrochloride salt (8a). A



mixture of **31** (269 mg, 0.47 mmol), triphenylphosphine (370 mg, 1.41 mmol) and H_2O (0.42 mL, 23.5 mmol) in THF (5 mL) was stirred at 50 °C for 1 h. Then the solution was treated with $NaHCO_3$ (160 mg, 1.9 mmol) and Boc_2O (0.44 mL, 1.9 mmol) at room temperature for 30 min. The reaction mixture was partitioned between AcOEt (50 mL) and H_2O (30 mL). The organic layer was washed with brine (30 mL), dried over Na_2SO_4, filtered, and concentrated. The residue was purified by silica gel column chromatography (φ 1.5 cm × 16 cm, chloroform/MeOH = 50/1−30/1) to give a Boc-protected amine. This material was treated with 4 M HCl/AcOEt (1 mL) at room temperature for 45 min. The reaction was concentrated and filtrated, and the residue was triturated from AcOEt to give **8a** as a yellow solid (221 mg, 99%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 11.50 (s, 1H, indole-NH), 10.94 (s, 1H, maleimide-NH), 8.35 (d, 1H, H-10, J = 2.3 Hz), 7.68 (s, 1H, H-4), 7.48 (d, 1H, H-7, J = 8.6 Hz), 7.07 (dd, 1H, H-8, J = 2.3, 8.6 Hz), 5.34 (d, 1H, OH, J = 5.8 Hz), 5.18 (d, 1H, OH, J = 5.8 Hz), 5.13 (d, 1H, OH, J = 5.2 Hz), 3.70 (dt, 1H, H-1′, J = 2.3, 9.2 Hz), 3.55 (dd, 1H, H-1″a, J = 1.8, 15.5 Hz), 3.29 (m, 3H, H-4′, H-5′ and H-6′a), 3.10 (m, 4H, H-1″b, H-2′, H-3′ and H-6′b), 0.97 (s, 9H, *t*-butyl), 0.23 (s, 6H, dimethyl); ^{13}C NMR (125 MHz, DMSO-d_6) δ 170.7, 170.6, 151.3, 144.2, 135.6, 129.8, 126.4, 124.9, 123.3, 121.0, 120.1, 117.9, 117.7, 112.4, 108.8, 79.1, 77.6, 76.0, 72.3, 71.3, 33.1; ESIMS-LR m/z = 428 (MH^+); ESIMS-HR calcd for $C_{21}H_{22}N_3O_7$ 428.1452, found 428.1454 (MH^+).

Molecular Docking. UCN-01/the active site of Chk1 complex structure (PDB code: 1nvq)[33] was used for the docking. The docking calculations were performed using the Schrödinger software suite with default settings if not indicated otherwise. For protein preparation, all the crystallographic water molecules were removed, hydrogens were added, and bond orders were assigned using Maestro's Protein Preparation Wizard (Maestro ver. 8.6). The added hydrogen atoms were minimized with all heavy atoms fixed using the OPLS2001 force field. For each of the three structures, energy grids were built using the default value of protein atom scaling. This box was centered around the centroid of the UCN-01. Docking was performed using Schrödinger Glide.[34−36] Calculation was conducted using the standard-precision (SP) mode with default settings. As for global energy-minimized conformation search, key binding site side residues were refined in the presence of inhibitor by a 100,000-step MCMM conformational search in MacroModel 9 (Schrodinger, LLC, New York, NY).

Chk1 Kinase Assay. Recombinant human Chk1 was obtained from Cyclex. The test compounds and were dissolved in DMSO, and diluted in four different concentrations, respectively. The assay was performed using a Cyclex Checkpoint Activity Kit according to the manufacturer's instructions. Chk1 inhibitory activity was calculated based on Chk1 activity in the presence and absence of compounds. Kinase activity was determined by reading the absorbance at dual wavelengths 450/540 nm on a microplate reader.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Preparation of ^{1}H, ^{13}C NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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