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Nucleosides and nucleotides. Part 212: Practical large-scale synthesis of 1-(3-*C*-ethynyl-β-D-*ribo*-pentofuranosyl)cytosine (ECyd), a potent antitumor nucleoside. Isobutyryloxy group as an efficient anomeric leaving group in the Vorbrüggen glycosylation reaction☆

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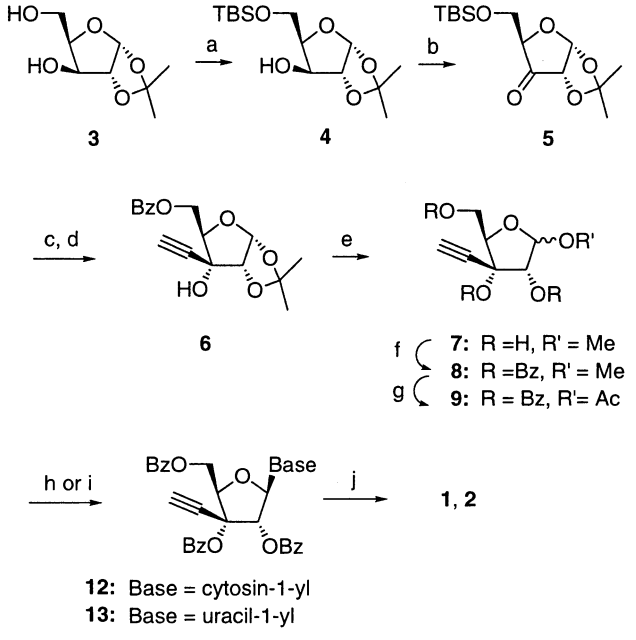
Abstract—A practical synthetic route to the antitumor nucleoside, 1-(3-*C*-ethynyl-β-D-*ribo*-pentofuranosyl)cytosine (ECyd, **1**) from 1,2-*O*-isopropylidene-D-xylofuranose (**3**) has been developed. Since most of the compounds were obtained as crystals, the target ECyd was prepared without any chromatographic purification in 31% overall yield from compound **3**. The isobutyryloxy group was found to be an effective leaving group at the anomeric position of the 3-β-*C*-ethynyl glycosyl donors in the key Vorbrüggen glycosylation reaction. Using a similar procedure without chromatographic purification, the uracil congener EUrd [1-(3-*C*-ethynyl-β-D-*ribo*-pentofuranosyl)uracil (**2**), which also has a potent antitumor effect, was synthesized from **3** in 39% overall yield.

1. Introduction

We previously designed and synthesized the potent antitumor nucleosides 1-(3-*C*-ethynyl-β-D-*ribo*-pentofuranosyl)cytosine (ECyd, **1**) and its uracil congener (EUrd, **2**).[1–3] They were converted, via phosphorylation by cellular uridine/cytidine kinase followed by nucleotide kinases into the corresponding 5′-*O*-triphosphates, which inhibited RNA synthesis and demonstrated a potent antitumor effect both in vitro and in vivo.[4]

To develop ECyd and/or EUrd as antitumor drugs, we planned to investigate their pharmacological, toxicological, and physicochemical properties. Since a few hundred grams of the compounds were required, we needed a practical method for the large-scale preparation of ECyd and EUrd. The previous route for synthesizing ECyd and EUrd shown in Scheme 1[1,2] would be reasonable because: (1) the 1,2-*O*-isopropylidene-D-xylofuranose (**3**) is readily available; (2) the ethynyl group could be introduced highly stereoselectively from the β-face at the 3-position of the keto sugar **5**;[5] and (3) the nucleobase could be stereoselectively introduced



Scheme 1. Conditions: (a) TBSCl, pyridine; (b) CrO_3, pyridine, Ac_2O, CH_2Cl_2; (c) TMSC≡CH, BuLi, THF, −78°C; (d) (1) Bu_4NF, THF, rt, (2) BzCl, pyridine, rt; (e) aqueous 20% HCl, MeOH, rt; (f) BzCl, DMAP, pyridine, 100°C; (g) concentrated H_2SO_4, Ac_2O, AcOH, rt; (h) **10**, $SnCl_4$, CH_3CN; (i) **11**, TMSOTf, CH_3CH; (j) NH_3/MeOH.

☆ See Ref. 19.

Keywords: antitumor compounds; glycosylation; nucleosides; pyrimidines.
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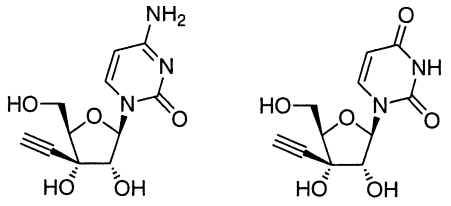


Figure 1. Structures of ECyd and EUrd.

via the neighboring 2-*O*-acetyl group participation by the Vorbrüggen glycosylation procedure.[6] However, it seems to be inefficient for the large scale preparation of these compounds since: (1) each step needs chromatographic purification because of the non-crystalline compounds, (2) the toxic CrO_3 used for the oxidation of the xylose derivative **4**, and (3) the very expensive Lewis acid TMSOTf used as the promoter for the glycosylation (Fig. 1).

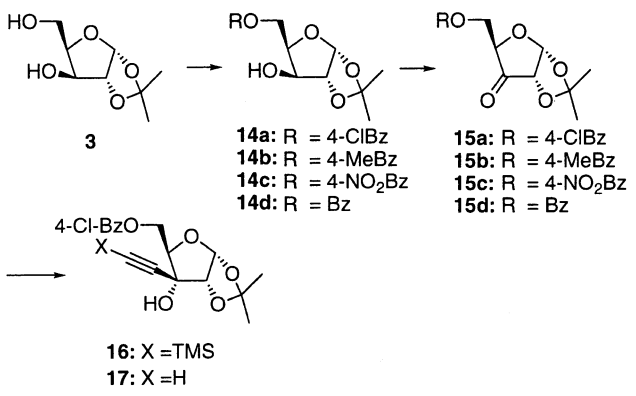
Considering these facts, we decided to modify the previous route to develop a practical method for the large-scale preparation of ECyd and EUrd.

2. Results and discussion

2.1. Synthesis of the 3β-*C*-ethynyl sugars

In a large-scale synthesis, crystalline compounds which can be easily purified without chromatography in each step are highly desirable. Therefore, we tried to develop a crystalline branched sugar having an ethynyl group at the 3β-position as the sugar unit for synthesizing ECyd and EUrd (Scheme 2).

A TBS group was used for the protection of the 5-hydroxyl in the previous route as shown in Scheme 1. However, *O*-acyl sugars are often crystallized more easily than the corresponding *O*-silyl sugars. To find an effective protecting group giving crystalline compounds, we first examined several acyl groups, i.e. 4-chlorobenzoyl (4-ClBz), 4-toluoyl (4-MeBz), 4-nitrobenzoyl (4-NO_2Bz), and benzoyl (Bz) groups, as the protecting group at the 5-hydroxyl of 1,2-*O*-isopropylidene-D-xylofuranose (**3**). When **3** was treated with an acyl halide and Et_3N in CH_2Cl_2 at 0°C, it gave the corresponding 5-*O*-acyl derivatives **14a–d** in 73–80% yield. Although the 4-MeBz (**14b**) and the Bz (**14d**) derivatives were oils, the 4-ClBz (**14a**) and the 4-NO_2Bz (**14c**) derivatives were obtained as crystals. Thus, crystalline **14a** and **14c** were subjected to the next steps. However, oxidation of **14c** with CrO_3 proved to be unsuccessful, and the desired 3-keto derivative **15c** was not obtained in pure form. On the other hand, following the previous method,[2] the 4-ClBz derivative **14a** was successfully converted into the corresponding 3-keto derivative **15a** and the 3-*C*-TMS-ethynyl and 3-*C*-ethynyl derivatives, **16** and **17**, all of which were obtained as stable crystalline compounds. Therefore, the 4-ClBz group was selected as the protecting group at the 5-hydroxyl of the sugar moiety.



Scheme 2.

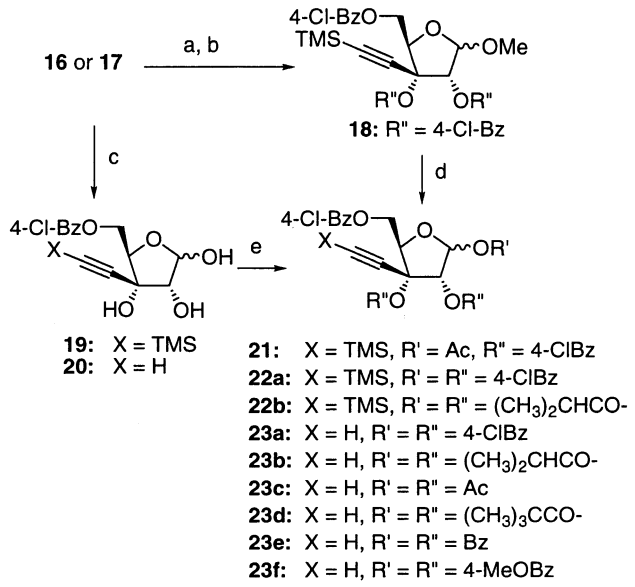
Table 1. Oxidation of **14a**

Entry	Conditions	Isolated yield (%)[a]
1	DMSO, DCC, H_3PO_4, rt, 2 h	64
2	DMSO, DCC, CF_3CO_2H, pyridine, rt, 30 min	74
3	DMSO, Ac_2O, rt, 10 h	37
4	NaOCl, AcOH, rt, 8 h	0
5	$Ca(OCl)_2$, AcOH–CH_3CN, 0°C, 8 h	0
6	NBS, Et_2O, MeOH, H_2O, rt, 8 h	0
7	NBS, pyridine, MeOH, H_2O, rt, 8 h	0
8	NCS, Et_2O, MeOH, H_2O, rt, 8 h	0
9	TEMPO (cat.), NaOCl, CH_2Cl_2–aq. $NaHCO_3$, 0°C, 15 min	77
10	TEMPO (cat.), $Ca(OCl)_2$, CH_2Cl_2–aq. $NaHCO_3$, 0°C, 2.5 h	43

Reactions were carried out in 3 mmol scale.
[a] Compound **15a** was isolated without chromatographical separation.

Next, reaction conditions for each step in the large-scale conversion of **3** to **16** or **17** were investigated. The large-scale synthesis of **14a** was achieved with 1.1 equiv. of 4-ClBzCl and 3 equiv. of Et_3N in CH_2Cl_2. About 200 g (74%) of the pure **14a** was obtained by crystallization without chromatography. Oxidation of the secondary alcohol at the 3-position of **14a** was next examined on a 3 mmol scale (Table 1). Whereas in the original procedure a CrO_3/Ac_2O/pyridine system was used,[1–3] here an alternative method without toxic CrO_3 was needed. Therefore, various oxidations of the hydroxyl group were examined. Oxidations using DMSO,[7–11] such as the Moffatt oxidation, gave the desired compound **15a** in good yield (Table 1, entries 1 and 2). However, the reaction produced crystalline dicyclohexylurea, removal of which required troublesome work-up. Although oxidation with hypochlorite salt,[12–14] NBS,[15,16] or NCS did not give **15a** (entries 4–8), catalytic TEMPO-oxidation[17,18] successfully furnished the desired compound. When NaOCl was used as a co-oxidant (entry 9), the oxidation was complete within 15 min and gave **15a** in excellent yield (3 mmol scale, 77% yield). A similar reaction using $Ca(OCl)_2$, required a longer reaction time, and resulted in a lower yield (entry 10). Thus, the reaction conditions for entry 9 were selected, and 171 g (88% yield) of **15a** was obtained by crystallization without chromatographic purification in the large scale reaction.

Introduction of an ethynyl or a TMS-ethynyl group was next examined. When **15a** (200 g, 600 mmol) was treated with TMSC≡CMgBr (1.3 equiv.), prepared from EtMgBr and TMSC≡CH in situ, in THF at 4°C, a TMS-ethynyl group



Scheme 3. (a) HCl/H_2O–MeOH; (b) 4-ClBzCl, Et_3N, DMAP, CH_2Cl_2; (c) HCO_2H–H_2O; (d) concentrated H_2SO_4, Ac_2O, AcOH, rt; (e) acyl-chloride, Et_3N, DMAP, CH_2Cl_2.

was stereoselectively introduced from the β-side[1–3,5] to afford **16** in 91% yield (238 g), which was obtained as crystals without column chromatography. Similarly, the 3-*C*-ethynyl derivative **17** was also prepared from **15a** by treatment with HC≡CMgBr in 89% yield.

Thus, a practical synthesis of the 3-*C*-TMS-ethynyl and 3-*C*-ethynyl sugars **16** and **17** was developed, which was applied to the following glycosylation reactions.

2.2. Preparation of glycosyl donors

The potency of the glycosyl donors is probably influenced by the acyl protecting groups at the hydroxyls. However, it may be difficult to predict the potency of the O-acyl-protected donors in the glycosylation reaction, since the effect of the acyl groups is complicated by: (1) an electron withdrawing acyl group at the 1-O-position making the donor reactive but also possibly making the donor unstable; (2) a less electron withdrawing acyl group at the 2-O-position, which is desirable for the effective neighboring group participation to form the β-product highly selectively; and (3) electron withdrawing protecting groups, e.g. acyl groups, at the 2, 3, and 5-hydroxyls, which are undesirable in reducing the reactivity of the donor. Accordingly, glycosyl donors having various acyl groups on the hydroxyls, i.e. **21**, **22a**,**b**, and **23a**–**f**, were prepared from **16** and **17**, as shown in Scheme 3, to experimentally identify an efficient donor.

The 3-*C*-TMS-ethynyl sugar **16** was heated under reflux with HCl in aqueous MeOH and gave the corresponding 1-*O*-methyl sugar, which was acylated with 4-ClBzCl to afford the tri-*O*-(4-ClBz)-1-*O*-methyl sugar **18** (87%). Treatment of **18** with $AcOH/Ac_2O/H_2SO_4$ afforded 1-*O*-acetyl-2,3,5-tri-*O*-(4-ClBz)-3-*C*-[2-(trimethylsilyl)ethynyl]-D-*ribo*-pentofuranose (**21**) in 77% yield.

Removal of the 1,2-*O*-isopropylidene group of **16** was achieved by heating in 50% aqueous HCO_2H under reflux to give **19**. Using 213 g (500 mmol) of **16**, 187 g (97%) of **19** was obtained after crystallization. The same treatment of **17** with aqueous HCO_2H also readily gave crystalline **20**. A similar reaction of **16** using AcOH instead of HCO_2H proceeded rather slowly, and the yield of **19** was lower. Treatment of **19** or **20** with various acyl chlorides in the presence of Et_3N and DMAP in CH_2Cl_2 provided the glycosyl donors **22a**, **22b**, and **23a**–**23f**, which contained the same acyl groups at the 1-, 2-, and 3-O-positions, respectively (Scheme 3).

2.3. The Vorbrüggen glycosidation reaction with the 3-*C*-TMS-ethynyl and 3-*C*-ethynyl glycosyl donors

The donors were applied to the Vorbrüggen glycosylation reaction[6] with 2-*O*,4-*N*-bis-TMS-cytosine ($(TMS)_2$Cyt, **10**) or bis-TMS-uracil ($(TMS)_2$Ura, **11**), which were prepared by refluxing the nucleobase in hexamethyldisilazane (HMDS) in the presence of $(NH_4)_2SO_4$ followed by evaporation. The reactions were performed with 0.5 mmol of the glycosyl donor in MeCN, which is an efficient solvent for the Vorbrüggen glycosylation reaction. The results are summarized in Table 2 (Scheme 4).

Although TMSOTf was used effectively as the promoter of the glycosylation step in the previous synthetic procedure for ECyd and EUrd,[2] it is very expensive. We examined various cheaper Lewis acids as the promoter for the glycosylation using $(TMS)_2$Ura and the 1-*O*-acetyl-3-*C*-TMS-ethynyl donor **21** in MeCN. As a result, the rather cheap Lewis acid, $SnCl_4$, was identified as an effective alternative promoter for the glycosylation, while other Lewis acids, such as $BF_3{\cdot}OEt_2$, $TiCl_4$, $ZnCl_2$, or $AlCl_3$, proved ineffective (data not shown). As seen in entry 1, treatment of the donor **21** with 4 equiv. of $(TMS)_2$Ura (**11**) in the presence of 5 equiv. of $SnCl_4$ at room temperature produced the desired 3-*C*-TMS-ethynyluridine derivative **25a** in 83% yield, a result comparable with that using TMSOTf as the promoter (entry 2). Therefore, using $SnCl_4$ as the promoter, reactions with the various donors were next investigated, with the reaction temperature fixed at 30°C (entries 3–18).

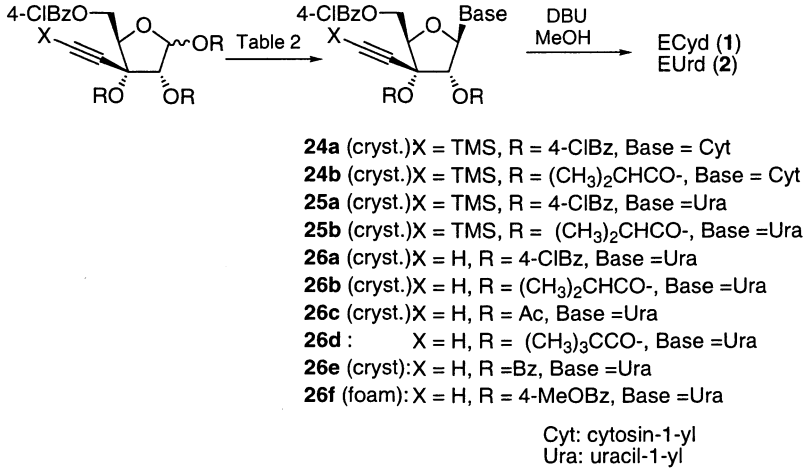
When the 3-*C*-TMS-ethynyl donor **21** was treated with 2 equiv. of $(TMS)_2$Ura and 3 equiv. of $SnCl_4$ for 3 h, compound **25a** was obtained in 82%-isolated yield (entry 3). The reactions using the other 3-*C*-TMS-ethynyl donors **22a** and **22b** were carried out under the same conditions as for entries 3 and 4. Although the 1,2,3,5-tetra-*O*-(4-ClBz) donor **22a** was ineffective (entry 4, 50%), the reaction of the 1,2,3-tri-*O*-isobutyryl-5-*O*-(4-ClBz) donor **22b** gave the desired nucleoside **25b** in 89% yield. Similarly, **21** and **22b** were effective donors in the reactions with $(TMS)_2$Cyt (**10**) as the acceptor to give the corresponding cytosine nucleosides **24a** and **24b** in excellent yields (entries 6 and 8), while the 1,2,3,5-tetra-*O*-(4-ClBz) donor **22a** was again ineffective (entry 7).

Next, the 3-*C*-ethynyl donors **23a**–**f**, having various acyloxy leaving groups at the 1-position, were examined. They were treated with 2 equiv. of $(TMS)_2$Ura and 3 equiv. of $SnCl_4$ for 20 h (entries 9–14). The reaction with the 1,2,3-tri-*O*-acetyl

Table 2. Glycosylation reactions of persilylated nucleobase with various acylated sugars in CH_3CN

Entry	Substrate				$(TMS)_2$ base	Lewis acid (equiv.)	Time (h)	Temperature (°C)	Product	Yield[a] (%)	Recovery[a] (%)
	Number	X	R′	R″	Base (eq).						
1	**21**	TMS	Ac	4-ClBz	Ura (4)	$SnCl_4$ (5)	15	rt	**25a**	83	0
2	**21**	TMS	Ac	4-ClBz	Ura (4)	TMSOTf (5)	17	rt	**25a**	91	0
3	**21**	TMS	Ac	4-ClBz	Ura (2)	$SnCl_4$ (3)	3	30	**25a**	82	4
4	**22a**	TMS	4-ClBz	4-ClBz	Ura (2)	$SnCl_4$ (3)	3	30	**25a**	50	27
5	**22b**	TMS	Isobutyryl	Isobutyryl	Ura (2)	$SnCl_4$ (3)	3	30	**25b**	89	<1
6	**21**	TMS	Ac	4-ClBz	Cyt (2)	$SnCl_4$ (3)	3	30	**24a**	73	6
7	**22a**	TMS	4-ClBz	4-ClBz	Cyt (2)	$SnCl_4$ (3)	3	30	**24a**	42	48
8	**22b**	TMS	Isobutyryl	Isobutyryl	Cyt (2)	$SnCl_4$ (3)	3	30	**24b**	81	8
9	**23a**	H	4-ClBz	4-ClBz	Ura (2)	$SnCl_4$ (3)	20	30	**26a**	62	9
10	**23b**	H	Isobutyryl	Isobutyryl	Ura (2)	$SnCl_4$ (3)	20	30	**26b**	78	0
11	**23c**	H	Ac	Ac	Ura (2)	$SnCl_4$ (3)	20	30	**26c**	10	0
12	**23d**	H	Pivaloyl	Pivaloyl	Ura (2)	$SnCl_4$ (3)	20	30	**26d**	0	Quant.
13	**23e**	H	Bz	Bz	Ura (2)	$SnCl_4$ (3)	20	30	**26e**	78	0
14	**23f**	H	4-MeOBz	4-MeOBz	Ura (2)	$SnCl_4$ (3)	20	30	**26f**	76	0
15	**23a**	H	4-ClBz	4-ClBz	Ura (2)	$SnCl_4$ (3)	3	30	**26a**	17	39
16	**23b**	H	Isobutyryl	Isobutyryl	Ura (2)	$SnCl_4$ (3)	3	30	**26b**	62	3
17	**23e**	H	Bz	Bz	Ura (2)	$SnCl_4$ (3)	3	30	**26e**	54	24
18	**23f**	H	4-MeOBz	4-MeOBz	Ura (2)	$SnCl_4$ (3)	3	30	**26f**	65	10

[a] Entries 1, 2: yield by HPLC analysis. The other entries: isolated yields of the product and the recovered donor.



Scheme 4.

donor **23c** gave the desired nucleoside product **26c** in poor yield (entry 11) and the 1,2,3-tri-*O*-pyvaloyl donor **23d** was virtually inactive under the same conditions. However, glycosylation reactions of the 1,2,3-tri-*O*-isobutyryl, 1,2,3-tri-*O*-Bz, and 1,2,3-tri-*O*-(4-MeOBz) donors, **23b**, **23e**, and **23f**, respectively, proceeded smoothly to give the corresponding 3-*C*-TMS-ethynyl nucleosides in excellent yields (entries 10, 13, and 14, 76–78%). The reaction of the 1,2,3-tri-*O*-(4-ClBz) donor **23a** proceeded rather slowly to give the nucleoside product **26a** in 62% yield (entry 9) but when the reaction was stopped after 3 h, compound **26a** was produced in only 17% yield (entry 15). When the reactions of the donors **23b**, **23e**, and **23f**, which had effectively produced the desired nucleosides after 20 h, were stopped after 3 h, their yields decreased somewhat (entries 16–18).

As described above, the potency of the donor is influenced by the acyl groups on the hydroxyls. Based on the yield and the reaction rate, the 1-*O*-acetyl-2,3,5-tri-*O*-(4-ClBz) donor **21**, the 1,2,3-tri-*O*-isobutyryl donors **22b** and **23b**, the 1,2,3-tri-*O*-Bz donor **23e**, and the 1,2,3-tri-*O*-(4-MeOBz) donor **23f** appeared to be efficient donors in the glycosylation reaction. In choose the best donor for large-scale synthesis, we considered many points. The steps for synthesizing **21** from **16** are longer than those for the preparation of other donors. Among the glycosylated nucleoside products derived from the candidate donors, the product **26f** (prepared from **23f**) was not obtained as crystals but as an amorphous solid, while the other products **25a**, **25b**, **24b**, **26b** and **26e** were crystalline. From the viewpoint of safety, especially in a large-scale synthesis, the 3-*C*-TMS-ethynyl sugar **16** is preferable to the 3-*C*-ethynyl sugar **17**, since an excess of the toxic and explosive acetylene is needed for the preparation of **17**. Based on these considerations and our experimental results, we selected the 1,2,3-tri-*O*-isobutyryl-5-*O*-(4-ClBz) donor **22b** for the glycosylation reaction. To our knowledge, this is the first glycosylation reaction that utilizes the isobutyryloxy group as a leaving group at the anomeric position.

2.4. Large scale synthesis of ECyd

Treatment of 347 g (900 mmol) of the 3-*C*-TMS-ethynyl sugar **19** with isobutyryl chloride, Et_3N, and DMAP in toluene at 25–35°C for 6 h gave 544 g of the donor **21b** after the usual water work-up and evaporation. The donor, without purification, was treated with $(TMS)_2$Cyt **10**, prepared from 120 g (1.08 mol, 1.2 equiv. to **19**) of cytosine, and 359 g (1.35 mol, 1.5 equiv. to **19**) of $SnCl_4$ in toluene at 50°C for 3 h. The reaction successfully produced 434 g of the cytosine nucleoside **24b** in 78% yield from **19**, after crystallization from isopropyl ether (IPE) and heptane. We found that removal of the TMS and the acyl groups of **24b** proceeded more smoothly using DBU/MeOH, compared with the previous NH_3/MeOH system.[2] Compound **24b** (430 g, 700 mmol) was deprotected with DBU (41.8 g, 270 mmol) in MeOH at 40°C for 3 h to furnish 128 g (68%) of ECyd (**1**) after crystallization from aqueous MeOH. Thus, an efficient large-scale synthesis of ECyd without any column chromatography has been achieved. EUrd (**2**) was also synthesized by a similar non-chromatographic procedure.

3. Conclusion

A practical procedure for the large-scale synthesis of ECyd and EUrd from 1,2-*O*-isopropylidene-D-xylofuranose **3** (7 steps, 31 and 39% yield, respectively), without any column chromatography, was developed. In this study, we found that the isobutyryloxy group was an efficient leaving group for the glycosylation reaction with 3-*C*-ethynyl and 3-*C*-TMS-ethynyl donors.

4. Experimental

4.1. General methods

Solvents and reagents are obtained from commercial sources and are not purified unless specified. Melting points were measured on a Yanagimoto MP-3 micromelting point apparatus (Yanagimoto, Japan) and were not corrected. The ^{1}H NMR spectra were recorded on a JEOL JNM-LA400 (400 MHz) or JEOL JNM-EX270 (270 MHz) spectrometer with tetramethylsilane (0.00 ppm) as an internal standard. Chemical shifts were reported in parts per million (δ), and signals were expressed as a s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad). Coupling constants (J) were reported in Hz. Fast atom bombardment mass spectrometry (FAB-MS) was done on a JEOL JMS-SX102A instrument at an ionizing voltage of 70 eV. TLC was done on Merck Silica gel 60 F_{254} pre-coated plates. The silica gel used for column chromatography was Merck Silica gel 60 (70–230 mesh).

4.1.1. 5-*O*-(4-Chlorobenzoyl)-1,2-*O*-isopropylidene-D-xylofuranose (14a). 4-ClBzCl (113 mL, 891 mmol) was added dropwise to a solution of **3** (154 g, 810 mmol) and Et_3N (339 mL, 2.43 mol) in CH_2Cl_2 (1.5 L) at 0°C, and the mixture was stirred at the same temperature for 2 h. Saturated aqueous $NaHCO_3$ (500 mL) was added and partitioned. The CH_2Cl_2 layer was washed with H_2O (×2) and brine, dried over $MgSO_4$, and filtrated. The filtrate was evaporated under reduced pressure to give a residue, which was crystallized from hexane–$CHCl_3$ to give **14a** (196 g, 74% as pale yellow powder): mp 101–102°C; FAB-MS (NaI), m/z 351 $(M+Na)^+$; ^{1}H NMR ($CDCl_3$) δ 7.99 (d, 2H, J=8.8 Hz), 7.44 (d, 2H, J=8.8 Hz), 5.96 (d, 1H, J=3.7 Hz), 4.79 (dd, 1H, J=9.3, 13.1 Hz), 4.60 (d, 1H, J=3.7 Hz), 4.37–4.41 (m, 2H), 4.18 (br, 1H), 3.05 (d, 1H, J=3.9 Hz, exchanged with D_2O), 1.51 (s, 3H), 1.33 (s, 3H); Anal. calcd for $C_{15}H_{17}ClO_6$: C, 54.80; H, 5.21. Found: C, 54.80; H, 5.16.

4.1.2. 1,2-*O*-Isopropylidene-5-*O*-(4-toluoyl)-D-xylofuranose (14b). 4-CH_3BzCl (727 μL, 5.5 mmol) was added dropwise to a solution of **3** (950 mg, 5.0 mmol) and Et_3N (2.09 mL, 15 mmol) in CH_2Cl_2 (9.5 mL) at 0°C, and the mixture was stirred at room temperature for 2 h. Saturated aqueous $NaHCO_3$ (10 mL) was added and partitioned. The CH_2Cl_2 layer was washed with H_2O (×2) and brine, dried over $mgSO_4$, and filtrated. The filtrate was purified by silica gel column chromatography to give **14b** (1.23 g, 80% as a colorless hygroscopic oil): FAB-MS, m/z 309 $(M+H)^+$; ^{1}H NMR ($CDCl_3$) δ 7.94 (d, 2H, J=8.2 Hz), 7.26 (d, 2H, J=8.2 Hz), 5.96 (d, 1H, J=3.6 Hz), 4.80 (dd, 1H, J=9.6, 12e.5 Hz), 4.60 (d, 1H, J=3.6 Hz), 4.34–4.39 (m, 2H), 4.16 (dd, 1H, J=3.0, 3.3 Hz), 3.38 (d, 1H, J=3.9 Hz, exchanged with D_2O), 2.42 (s, 3H), 1.51 (s, 3H), 1.32 (s, 3H); Anal. calcd for $C_{16}H_{20}O_6 \cdot H_2O$: C, 58.89; H, 6.79. Found: C, 59.20; H, 6.76.

4.1.3. 1,2-*O*-Isopropylidene-5-*O*-(4-nitrobenzoyl)-D-xylofuranose (14c). 4-NO_2BzCl (1.02 g, 5.5 mmol) was added to a solution of **3** (950 mg, 5.0 mmol) and Et_3N (2.09 mL, 15 mmol) in CH_2Cl_2 (9.5 mL) at 0°C, and the mixture was stirred at room temperature for 2 h. Saturated aqueous $NaHCO_3$ (10 mL) was added and partitioned. The CH_2Cl_2 layer was washed with H_2O (×2) and brine, dried over $mgSO_4$, and filtrated. The filtrate was purified by silica gel column chromatography to give a residue, which was crystallized from Et_2O to give **14c** (1.23 g, 73% as white crystals): mp 104–105°C; FAB-MS, m/z 340 $(M+H)^+$; ^{1}H NMR ($CDCl_3$) δ 8.31 (d, 2H, J=9.2 Hz), 8.24 (d, 2H, J=9.2 Hz), 5.99 (d, 1H, J=3.6 Hz), 4.81 (dd, 1H, J=6.6, 11.0 Hz), 4.59 (d, 1H, J=3.6 Hz), 4.40–4.53 (m, 2H), 4.24 (dd, 1H, J=2.3, 4.6 Hz), 2.64 (d, 1H, J=4.9 Hz, exchanged with D_2O), 1.52 (s, 3H), 1.34 (s, 3H); Anal. calcd for $C_{15}H_{17}NO_8$: C, 53.10; H, 5.05; N, 4.13. Found: C, 53.08; H, 5.06; N, 4.27.

4.1.4. 5-*O*-Benzoyl-1,2-*O*-isopropylidene-D-xylofuranose (14d). BzCl (638 μL, 5.5 mmol) was added dropwise to a solution of **3** (950 mg, 5.0 mmol) and Et_3N (2.09 mL, 15 mmol) in CH_2Cl_2 (9.5 mL) at 0°C, and the mixture was stirred at room temperature for 2 h. Saturated aqueous $NaHCO_3$ (10 mL) was added and partitioned. The CH_2Cl_2 layer was washed with H_2O (×2) and brine, dried over $MgSO_4$, and filtrated. The filtrate was purified by silica gel column chromatography to give **14d** (1.17 g, 79% as a colorless oil): FAB-MS, m/z 295 $(M+H)^+$; ^{1}H NMR ($CDCl_3$) δ 8.06 (d, 2H, J=7.3 Hz), 7.42–7.63 (m, 3H), 5.96 (d, 1H, J=3.6 Hz), 4.80 (dd, 1H, J=9.2, 12.9 Hz), 4.60 (d, 1H, J=3.6 Hz), 4.35–4.43 (m, 2H), 4.19 (dd, 1H, J=2.3, 4.0 Hz), 3.32 (br, 1H, exchanged with D_2O), 1.51 (s,

3H), 1.32 (s, 3H); Anal. calcd for $C_{15}H_{18}O_6 \cdot 1/4H_2O$: C, 60.30; H, 6.24. Found: C, 60.31; H, 6.29.

4.1.5. 5-*O*-(4-Chlorobenzoyl)-1,2-*O*-isopropylidene-D-*erythro*-pentofuranose-3-urose (15a). To a solution of **14a** (195 g, 593 mmol) and 2,2,6,6-tetramethylpiperidin-1-oxy (937 mg, 5.93 mmol) in CH_2Cl_2 (990 mL) was added a mixture of aqueous sodium hypochlorite solution (336 mL, 8.5–13.5% active chlorine), $NaHCO_3$ (112 g), and H_2O (1.9 L) at 0°C, and the mixture was stirred at the same temperature for 30 min. After addition of 2-propanol (19.5 mL), the resulting mixture was stirred for 10 min at room temperature and then partitioned. The CH_2Cl_2 layer was washed with H_2O (×2), dried over $MgSO_4$, and filtrated. The filtrate was evaporated to give a residue, which was crystallized from hexane–$CHCl_3$ to give **15a** (171 g, 88% as white powder): mp 111–112°C; FAB-MS *m/z* 327 $(M+H)^+$; ^1H NMR ($CDCl_3$) δ 7.89 (d, 2H, *J*=8.5 Hz), 7.42 (d, 2H, *J*=8.5 Hz), 6.12 (d, 1H, *J*=4.4 Hz), 4.68–4.73 (m, 2H), 4.46 (dd, 1H, *J*=5.1, 13.1 Hz), 4.41 (d, 1H, *J*=4.4 Hz), 1.52 (s, 3H), 1.44 (s, 3H); Anal. calcd for $C_{15}H_{15}ClO_6$: C, 55.14; H, 4.63. Found: C, 55.12; H, 4.60.

4.1.6. 5-*O*-(4-Chlorobenzoyl)-1,2-*O*-isopropylidene-3-*C*-[2-(trimethylsilyl)ethynyl]-D-*ribo*-pentofuranose (16). Tri-methylsilylacetylene (92.0 g, 900 mmol) was added dropwise to a solution of EtMgBr (0.96 M, 768 g, 770 mmol in THF) at 0°C. The mixture was added dropwise to a solution of **15a** (200 g, 600 mmol) in THF (1.2 L) at 4°C, and the mixture was stirred for 30 min at 4°C. Aqueous NH_4Cl solution (15%, 360 mL) was added to the reaction mixture, which was then warmed to room temperature. Following phase separation, the THF layer was washed with aqueous NaCl solution (25%, 160 mL×2), and then evaporated. The residue was crystallized from MeOH–H_2O to give **16** (238 g, 91% as white powder): mp 130–132°C; EI-MS *m/z* 425 $(M)^+$; ^1H NMR ($CDCl_3$) δ 8.02 (d, 2H, *J*= 8.5 Hz), 7.41 (d, 2H, *J*=8.5 Hz), 5.95 (d, 1H, *J*=3.9 Hz), 4.74 (dd, 1H, *J*=3.6, 12.0 Hz), 4.17 (d, 1H, *J*=3.9 Hz), 4.53 (dd, 1H, *J*=7.8, 12.0 Hz), 4.17 (dd, 1H, *J*=3.6, 7.8 Hz), 2.93 (s, 1H, exchanged with D_2O), 1.60 (s, 3H), 1.39 (s, 3H), 0.19 (s, 9H); Anal. calcd for $C_{20}H_{25}ClO_6Si$: C, 56.53; H, 5.93. Found: C, 56.58; H, 5.96.

4.1.7. 5-*O*-(4-Chlorobenzoyl)-3-*C*-ethynyl-1,2-*O*-isopropylidene-D-*ribo*-pentofuranose (17). To a solution of **15a** (6.52 g, 20.0 mmol) in THF (26 mL) was added HC≡CMgBr (0.5 M, 50 mL, 25 mmol in THF) dropwise at 0°C under an argon atmosphere, and the mixture was stirred at 0°C for 40 min. After addition of aqueous NH_4Cl solution (15%, 16 mL), the resulting mixture was partitioned. The organic layer was washed with aqueous NaCl solution (25%, 16 mL), and evaporated. A solution of the residue in 2-propanol (15 mL) was added dropwise to H_2O (15 mL), and the resulting precipitated crystals were collected by filtration to give **17** (6.28 g, 89% as white crystals): mp 136–137°C; FAB-MS (KI), *m/z* 391 $(M+K)^+$; ^1H NMR ($CDCl_3$) δ 8.01 (d, 2H, *J*=8.6 Hz), 7.41 (d, 2H, *J*=8.6 Hz), 5.95 (d, 1H, *J*=3.6 Hz), 4.55–4.76 (m, 3H), 4.15–4.20 (m, 1H), 3.01 (br s, 1H), 2.65 (s, 1H), 1.61 (s, 3H), 1.46 (s, 3H); Anal. calcd for $C_{17}H_{17}ClO_6$: C, 57.88; H, 4.86. Found: C, 57.95; H, 4.82.

4.1.8. Methyl 2,3,5-tri-*O*-(4-chlorobenzoyl)-3-*C*-[2-(trimethylsilyl)ethynyl]-D-*ribo*-pentofuranoside (18). To a solution of **16** (30.0 g, 70.6 mmol) in a mixture of MeOH (760 mL) and H_2O (35 mL) was added conc HCl (190 mL), and the resulting mixture was heated at 55°C for 30 min. After cooling the mixture, Et_3N (300 mL) was added and evaporated, and the residue was partitioned between H_2O (450 mL) and EtOAc (500 mL). The organic layer was washed with H_2O (300 mL) and dried over $MgSO_4$, and evaporated to dryness. To a solution of the residue in CH_2Cl_2 (270 mL), Et_3N (31.9 mL, 229 mmol) and DMAP (254 mg, 2.0 mmol), and 4-ClBzCl (35.6 g, 208 mmol) was added at 0°C. After stirring the mixture at room temperature for 3 h, H_2O (200 mL) was added, and the resulting mixture was partitioned. The organic layer was washed with saturated $NaHCO_3$ solution and H_2O, dried over $MgSO_4$, and evaporated to a half volume to give precipitates. After filtration of the precipitates, the filtrate was evaporated and the residue was purified by silica gel column chromatography (20% EtOAc in hexane) to give **18** (40.6 g, 87% as colorless foam): FAB-MS (KI) *m/z* 713 $(M+K)^+$; ^1H NMR (DMSO-d_6) δ 7.84 (m, 6H), 7.31–7.46 (m, 6H), 5.97 (d, 0.6H, *J*= 1.5 Hz), 5.74 (d, 0.4H, *J*=4.4 Hz), 5.42 (d, 0.4H, *J*=4.4 Hz), 5.12 (d, 0.6H, *J*=1.5 Hz), 4.69–4.95 (m, 3H), 3.45 (s, 1.8H), 3.42 (s, 1.2H), 0.14 (s, 5.4H), 0.01 (s, 3.6H); α-anomer/β-anomer=4:6; Anal. calcd for $C_{32}H_{29}Cl_3O_8Si$: C, 56.85; H, 4.32. Found: C, 56.87; H, 4.25.

4.1.9. 5-*O*-(4-Chlorobenzoyl)-3-*C*-[2-(trimethylsilyl)-ethynyl]-D-*ribo*-pentofuranose (19). A suspension of **16** (213 g, 500 mmol) in a mixture of 50% aqueous HCO_2H (2 L) was heated under reflux for 45 min, and then cooled. After addition of H_2O (1 L), the resulting mixture was stirred for 1 h to give precipitates. The resulting precipitates were collected by filtration and washed with H_2O (500 mL×2), $NaHCO_3$ solution (3%, 250 mL×2), and H_2O (500 mL×2) to give **19** (187 g, 97% as a white powder): mp 130–132°C; FAB-MS *m/z* 385 $(M+H)^+$; ^1H NMR ($CDCl_3$) δ 7.98 (d, 2H, *J*=8.5 Hz), 7.43 (d, 2H, *J*=8.5 Hz), 5.47–5.51 (dd, 1H, *J*=4.4, 8.7 Hz), 4.46–4.63 (m, 3H), 4.24 (dd, 1H, *J*=4.4, 6.8 Hz), 3.58 (d, 1H, *J*=6.8 Hz), 3.13 (s, 1H), 3.07 (d, 1H, *J*=8.7 Hz), 0.15 (s, 9H); Anal. calcd for $C_{17}H_{21}ClO_6Si$: C, 53.05; H, 5.50. Found: C, 53.09; H, 5.52.

4.1.10. 5-*O*-(4-Chlorobenzoyl)-3-*C*-ethynyl-D-*ribo*-pentofuranose (20). Compound **20** (52.2 g, 81% as white powder) was obtained from **17** (73 g, 10.2 mmol), according to the above procedure for preparing **19** from **16**: mp 116–117.5°C; FAB-MS (KI), *m/z* 351 $(M+K)^+$; ^1H NMR ($CDCl_3$) δ 7.98 (d, 2H, *J*=8.8 Hz), 7.43 (d, 2H, *J*= 8.8 Hz), 5.47–5.51 (m, 1H), 4.50–4.68 (m, 3H), 4.26 (t, 1H, *J*=4.9 Hz), 3.69 (d, 1H, *J*=8.3 Hz), 3.34 (s, 1H), 3.19 (d, 1H, *J*=4.9 Hz), 2.66 (s, 1H); Anal. calcd for $C_{14}H_{13}ClO_6$: C, 53.77; H, 4.19. Found: C, 53.76; H, 4.05.

4.1.11. 1-*O*-Acetyl-2,3,5-tri-*O*-(4-chlorobenzoyl)-3-*C*-[2-(trimethylsilyl)ethynyl]-D-*ribo*-pentofuranose (21). To a solution of **18** (20.0 g, 29.6 mmol) in a mixture of AcOH (93 mL) and Ac_2O (12.3 mL, 130 mmol) was added conc H_2SO_4 (6.0 mL), and the mixture was stirred at room temperature for 2.5 h. After slow addition of $NaHCO_3$ (25 g), the resulting mixture was stirred at room temperature

for 15 min and then evaporated. A solution of the residue in EtOAc (150 mL) was washed with H_2O (150 mL), saturated $NaHCO_3$ solution (60 mL×2) and brine (100 mL), dried over $MgSO_4$, and evaporated. A solution of the residue in MeOH (110 mL) was poured dropwise into H_2O (230 mL). The resulting precipitates were collected by filtration and dried to give **21** (16.0 g, 77% as an amorphous): FAB-MS (KI), *m/z* 741 $(M+K)^+$; ^1H NMR (DMSO-d_6) δ 7.83–8.08 (m, 6H), 7.31–7.46 (m, 6H), 6.70 (d, 0.35H, *J*=4.4 Hz), 6.33 (d, 0.65H, *J*=1.2 Hz), 6.11 (d, 0.65H, *J*=1.2 Hz), 6.00 (d, 0.35H, *J*=4.4 Hz), 4.71–5.00 (m, 3H), 2.14 (s, 1.95H), 1.98 (s, 1.05H), 0.18 (s, 5.85H), 0.11 (s, 3.15H); α-anomer/β-anomer=35:65; Anal. calcd for $C_{33}H_{29}Cl_3O_9Si$: C, 56.30; H, 4.15. Found: C, 56.23; H, 4.08.

4.1.12. 1,2,3,5-Tetra-*O*-(4-chlorobenzoyl)-3-*C*-[2-(trimethylsilyl)ethynyl]-D-*ribo*-pentofuranose (22a). To a solution of **19** (22 g, 57.2 mmol), DMAP (210 mg, 1.72 mmol), and Et_3N (33.5 mL, 240 mmol) in CH_2Cl_2 (280 mL) was added 4-ClBzCl (29.1 mL, 229 mmol) at 0°C, and then the mixture was stirred at room temperature for 1 h. After addition of H_2O (220 mL), the resulting mixture was stirred at room temperature for 15 min and then partitioned. The organic layer was washed with H_2O, saturated $NaHCO_3$ solution, and H_2O again, dried over $MgSO_4$, and evaporated. To the residue was added isopropyl ether (IPE) (100 mL) to give precipitates, which were removed by filtration. The filtrate was evaporated, and a solution of the residue in EtOH (800 mL) was poured dropwise into H_2O (1.1 L) at room temperature under vigorous stirring. A resulting precipitated powder was collected by filtration and dried under reduced pressure to give **22a** (39.9 g, 87% as pale yellow amorphous): FAB-MS (NaI), *m/z* 823 $(M+Na)^+$; ^1H NMR (DMSO-d_6) δ 7.75–8.07 (m, 8H), 7.20–7.46 (m, 8H), 6.92 (d, 0.55H, *J*=4.4 Hz), 6.55 (s, 0.45H), 6.26 (s, 0.45H), 6.11 (d, 0.55H, *J*=4.4 Hz), 4.73–5.09 (m, 3 H), 0.17 (s, 4.05H), 0.12 (s, 4.95H); α-anomer/β-anomer=55:45; Anal. calcd for $C_{38}H_{30}Cl_4O_9Si$: C, 57.01; H, 3.78. Found: C, 56.87; H, 3.72.

4.1.13. 5-*O*-(4-Chlorobenzoyl)-1,2,3-tri-*O*-isobutyryl-3-*C*-[2-(trimethylsilyl)ethynyl]-D-*ribo*-pentofuranose (22b). To a solution of **19** (2.00 g, 5.20 mmol), Et_3N (2.10 g, 20.8 mmol), and DMAP (19 mg, 0.16 mmol) in CH_2Cl_2 (30 mL) was added isobutyryl chloride (2.17 mL, 20.8 mmol) at 0°C, and the mixture was stirred at room temperature for 5 h. After quenching the reaction with MeOH, CH_2Cl_2 (30 mL) and H_2O (50 mL) were added and partitioned. The aqueous layer was extracted with CH_2Cl_2 (30 mL), and the organic layers were combined, washed with H_2O, 10% aqueous $NaHCO_3$ solution (×2) and 25% aqueous NaCl. The organic layer was dried over $MgSO_4$ and evaporated, and the residue was purified by silica gel column chromatography (CH_2Cl_2) to give **22b** (2.68 g, 87% as colorless oil): FAB-MS (KI), *m/z* 633 $(M+K)^+$; ^1H NMR ($CDCl_3$) δ 8.01–8.05 (m, 2H), 7.41–7.45 (m, 2H), 6.52 (d, 0.5H, *J*=4.6 Hz), 6.10 (s, 0.5H), 5.75 (s, 0.5H), 5.71 (d, 0.5H, *J*=4.6 Hz), 4.57–4.79 (m, 3H), 2.51–2.65 (m, 3H), 1.12–1.22 (m, 18H), 0.15 (s, 4.5H), 0.09 (s, 4.5H); α-anomer/β-anomer=5:5; Anal. calcd for $C_{29}H_{39}ClO_9Si$: C, 58.52; H, 6.60. Found: C, 58.29; H, 6.67.

4.2. General Procedure for the synthesis of 1,2,3-tri-*O*-acyl-5-*O*-(4-chlorobenzoyl)-3-*C*-ethynyl-D-*ribo*-pentofuranose (23a–f)

To a suspension of **20** (1.0 g, 3.2 mmol), Et_3N (1.3 g, 12.8 mmol), DMAP (12 mg, 0.098 mmol) in CH_2Cl_2 (16 mL) was added an acyl chloride (12.8 mmol) at 0°C, and the mixture was stirred at room temperature for 5 h. After quenching the reaction with MeOH, to the mixture was added CH_2Cl_2 (20 mL), the resulting solution was washed with H_2O, 15% aqueous NH_4Cl solution, and 25% aqueous NaCl solution. The organic layer was dried over $MgSO_4$, evaporated, purified by silica gel column chromatography to give **23a–f**.

4.2.1. 1,2,3,5-Tetra-*O*-(4-chlorobenzoyl)-3-*C*-ethynyl-D-*ribo*-pentofuranose (23a). After purification by silica gel column chromatography (CH_2Cl_2), **23a** (1.80 g, 77% as white powder) was obtained: FAB-MS (KI), *m/z* 767 $(M+K)^+$; ^1H NMR (DMSO-d_6) δ 7.41–8.02 (m, 16H), 6.88 (d, 0.7H, *J*=4.4 Hz), 6.61 (s, 0.3H), 6.12 (m, 1H), 5.30 (dd, 0.7H, *J*=3.7, 5.6 Hz), 5.20 (dd, 0.3H, *J*=4.2, 6.8 Hz), 4.7–4.9 (m, 2H), 4.38 (s, 0.3H), 4.13 (s, 0.7H); α-anomer/β-anomer=7:3; Anal. calcd for $C_{35}H_{22}Cl_4O_9$: C, 57.72; H, 3.04. Found: C, 57.83; H, 2.93.

4.2.2. 5-*O*-(4-Chlorobenzoyl)-3-*C*-ethynyl-1,2,3-tri-*O*-isobutyryl-D-*ribo*-pentofuranose (23b). After purification by silica gel column chromatography (20% EtOAc in hexane), **23b** (1.37 g, 82% as a pale yellow oil) was obtained: FAB-MS (KI), *m/z* 561 $(M+K)^+$; ^1H NMR ($CDCl_3$) δ 8.06–7.99 (m, 2H), 7.41–7.45 (m, 2H), 6.54 (d, 0.7H, *J*=4.6 Hz), 6.12 (d, 0.3H, *J*=1.2 Hz), 5.78 (d, 0.3H, *J*=1.2 Hz), 5.71 (d, 0.7H, *J*=4.6 Hz), 4.60–4.80 (m, 3H), 2.77 (s, 0.3H), 2.69 (s, 0.7H), 2.53–2.64 (m, 3H), 1.17–1.23, (m, 18H); α-anomer/β-anomer=7:3; Anal. calcd for $C_{26}H_{31}ClO_9$: C, 59.71; H, 5.97. Found: C, 59.69; H, 5.88.

4.2.3. 1,2,3-Tri-*O*-acetyl-5-*O*-(4-chlorobenzoyl)-3-*C*-ethynyl-D-*ribo*-pentofuranose (23c). After purification by silica gel column chromatography (40% EtOAc in hexane), **23c** (1.23 g, 88% as a colorless oil) was obtained: FAB-MS (KI), *m/z* 477 $(M+K)^+$; ^1H NMR ($CDCl_3$) δ 7.99–8.05 (m, 2H), 7.42–7.99 (m, 2H), 6.52 (d, 0.6H, *J*=4.6 Hz), 6.14 (0.4H, s), 5.81 (0.4H, s), 5.74 (d, 0.6H, *J*=4.4 Hz), 4.66–4.78 (m, 3H), 2.78 (s, 0.4H), 2.72 (s, 0.6H), 2.04–2.14 (m, 9H); α-anomer/β-anomer=6:4; Anal. calcd for $C_{20}H_{19}ClO_9$: C, 54.74; H, 4.36. Found: C, 54.66; H, 4.34.

4.2.4. 5-*O*-(4-Chlorobenzoyl)-3-*C*-ethynyl-1,2,3-tri-*O*-pivaloyl-D-*ribo*-pentofuranose (23d). After purification by silica gel column chromatography (10% EtOAc in hexane), **23d** (1.73 g, 97% as a colorless foam) was obtained: FAB-MS (KI), *m/z* 603 $(M+K)^+$; ^1H NMR ($CDCl_3$) δ 8.06–7.99 (m, 2H), 7.41–7.45 (m, 2H), 6.51 (d, 0.6H, *J*=4.6 Hz), 6.07 (d, 0.4H, *J*=1.3 Hz), 5.78 (d, 0.4H, *J*=1.3 Hz), 5.62 (d, 0.6H, *J*=4.6 Hz), 4.60–4.82 (m, 3H), 2.75 (s, 0.4H), 2.64 (s, 0.6H), 1.19–1.28 (m, 27H); α-anomer/β-anomer=6:4; Anal. calcd for $C_{29}H_{37}ClO_9$: C, 61.64; H, 6.60. Found: C, 61.55; H, 6.58.

4.2.5. 1,2,3-Tri-*O*-benzoyl-5-*O*-(4-chlorobenzoyl)-3-*C*-ethynyl-D-*ribo*-pentofuranose (23e). After purification by

silica gel column chromatography (20% EtOAc in hexane), **23e** (1.63 g, 82% as a white powder) was obtained: FAB-MS (KI), *m/z* 663 $(M+K)^+$; ^1H NMR (DMSO-d_6) δ 7.27–8.08 (m, 19H), 6.91 (d, 0.7H, *J*=4.6 Hz), 6.61 (s, 0.3H), 6.13 (s, 0.3H), 6.12 (d, 0.7H, *J*=4.6 Hz), 5.16–5.27 (m, 1H), 4.70–4.95 (m, 2H), 4.40 (s, 0.3H), 4.11 (s, 0.7H); α-anomer/β-anomer=7:3; Anal. calcd for $C_{35}H_{25}ClO_9$: C, 67.26; H, 4.03. Found: C, 67.28; H, 3.86.

4.2.6. 1,2,3-Tri-*O*-(4-anisoyl)-5-*O*-(4-chlorobenzoyl)-3-*C*-ethynyl-D-*ribo*-pentofuranose (23f). After purification by silica gel column chromatography (25% EtOAc in hexane), **23f** (1.97 g, 86% as a colorless oil) was obtained: FAB-MS (KI), *m/z* 753 $(M+K)^+$; ^1H NMR (DMSO-d_6) δ 7.54–8.07 (m, 10H), 6.82–7.15 (m, 6.8H), 6.50 (s, 0.2H), 6.03–6.05 (m, 1H), 5.08–5.22 (m, 1H), 4.65–4.95 (m, 2H), 4.33 (s, 0.2H), 4.06 (s, 0.8H), 3.87 (s, 0.6H), 3.86 (s, 2.4H), 3.83 (s, 0.6H), 3.82 (s, 2.4H), 3.80 (s, 0.6H), 3.79 (s, 2.4H); α-anomer/β-anomer=8:2; Anal. calcd for $C_{38}H_{31}ClO_{12}$: C, 63.82; H, 4.37. Found: C, 63.88; H, 4.28.

4.3. Glycosylation reactions with the donor 21 (Table 2, entries 1 and 2)

A suspension of uracil (224 mg, 2.0 mmol) and $(NH_4)_2SO_4$ (8 mg), in HMDS (2.0 mL, 9.5 mmol) was heated under reflux under nitrogen atmosphere for 1 h to give a solution. The solvent was evaporated, and the residue was co-evaporated twice with toluene to give $(TMS)_2$Ura (**11**). To a solution of **11** and **21** (352 mg, 0.50 mmol) in MeCN (3.5 mL) was added a Lewis acid (2.5 mmol) at 0°C, and the mixture was then stirred at room temperature for 15 or 17 h. The resulting mixture was poured into saturated aqueous $NaHCO_3$ (20 mL), and then EtOAc was added and partitioned. The extract was diluted with EtOAc to 100 mL, which was sampled and analyzed by HPLC to determine the yield of **25a** and recovery of **21**: column, Puresil 5μ 120 Å 4.6times;150 mm^2; detection, 260 nm; eluent, 90% MeOH–H_2O, 1.0 mL/min; retention time, **21a** at 9.8 and 10.2 min, **25a** at 6.8 min.

4.4. General procedure for the glycosylation reactions (Table 2, entries 4–18)

A suspension of cytosine (222 mg, 2.0 mmol) or uracil (224 mg, 2.0 mmol) and $(NH_4)_2SO_4$ (8 mg, 0.66 μmol) in HMDS (2.0 mL, 9.5 mmol) was heated under reflux for 1 h under nitrogen atmosphere to give a solution. The solvent was evaporated, and the residue was co-evaporated twice with toluene to give $(TMS)_2$Cyt (**10**) or $(TMS)_2$Ura (**11**). To a solution of the silylated base and a glycosyl donor (1.0 mmol) in MeCN (4.0 mL) was added $SnCl_4$ (354 μL, 3.0 mmol) at 0°C, and the mixture was stirred at 30°C for 3 or 20 h. After evaporation of the solvent, the residue was partitioned between toluene (50 mL) and HCl (3 M, 50 mL×2). The organic layer was washed with saturated $NaHCO_3$ solution (50 mL×2), dried over Na_2SO_4, and evaporated. The residue was purified by silica gel column chromatography.

4.4.1. 1-{2,3,5-Tri-*O*-(4-chlorobenzoyl)-3-*C*-[2-(trimethylsilyl)ethynyl]-β-D-*ribo*-pentofuranosyl}cytosine (24a). The compound was obtained as a colorless foam after purification by silica gel column chromatography ($CHCl_3$/MeOH/EtOAc, 19/1/2): mp 131–133°C (EtOAc-IPE); FAB-MS *m/z* 754 $(M+H)^+$; ^1H NMR (DMSO-d_6) δ 8.05 (d, 2H, *J*=8.5 Hz), 7.88–7.95 (m, 4H), 7.79 (d, 1H, *J*=7.6 Hz), 7.63 (d, 2H, *J*=8.3 Hz), 7.58 (d, 2H, *J*=8.3 Hz), 7.53 (d, 2H, *J*=8.3 Hz), 7.39–7.42 (br, 2H), 6.27 (d, 1H, *J*=3.9 Hz), 5.93 (d, 1H, *J*=3.9 Hz), 5.84 (d, 1H, *J*=7.6 Hz), 5.04–5.06 (m, 1H), 4.91 (dd, 1H, *J*=4.1, 12.0 Hz), 4.77–4.81 (m, 1H), 0.11 (s, 9H); Anal. calcd for $C_{35}H_{30}Cl_3N_3O_8Si$: C, 55.67; H, 4.00; N, 5.57. Found: C, 55.32; H, 3.95; N, 5.56.

4.4.2. 1-{5-*O*-(4-Chlorobenzoyl)-2,3-di-*O*-isobutyryl-3-*C*-[2-(trimethylsilyl)ethynyl]-β-D-*ribo*-pentofuranosyl}cytosine (24b). The compound was obtained as a colorless foam after purification by silica gel column chromatography ($CHCl_3$/MeOH/EtOAc, 14/1/2): mp 97–99°C (heptane-IPE); FAB-MS *m/z* 618 $(M+H)^+$; ^1H NMR (DMSO-d_6) δ 8.03 (d, 2H, *J*=8.4 Hz), 7.68, (d, 1H, *J*=7.6 Hz), 7.63 (d, 2H, *J*=8.4 Hz), 7.35, 7.33 (each br s, each 1H), 6.08 (d, 1H, *J*=5.0 Hz), 5.78 (d, 1H, *J*=7.6 Hz), 5.61 (d, 1H, *J*=5.0 Hz), 4.64–4.76 (m, 3H), 2.52–2.67 (m, 2H), 1.08–1.12 (m, 12H), 0.07 (s, 9H); Anal. calcd for $C_{29}H_{36}ClN_3O_8Si$: C, 56.35; H, 5.87; N, 6.80. Found: C, 56.29; H, 5.83; N, 6.59.

4.4.3. 1-{2,3,5-Tri-*O*-(4-Chlorobenzoyl)-3-*C*-[2-(trimethylsilyl)ethynyl]-β-D-*ribo*-pentofuranosyl}uracil (25a). The compound was obtained as a colorless foam after purification by silica gel column chromatography (10% EtOAc in $CHCl_3$): mp 107–109°C (EtOAc-IPE); FAB-MS (KI), *m/z* 793 $(M+K)^+$; ^1H NMR (DMSO-d_6) δ 11.57 (s, 1H), 8.05 (d, 2H, *J*=8.3 Hz), 7.95 (d, 2H, *J*=8.5 Hz), 7.92 (d, 2H, *J*=8.5 Hz), 7.84 (d, 1H, *J*=8.1 Hz), 7.54–7.65 (m, 6H), 6.25 (d, 1H, *J*=4.2 Hz), 5.99 (d, 1H, *J*=4.2 Hz), 5.78 (d, 1H, *J*=8.1 Hz), 5.07–5.10 (m, 1H), 4.92 (dd, 1H, *J*=4.0, 6.0 Hz), 4.87 (dd, 1H, *J*=6.0, 12.2 Hz), 0.12 (s, 9 H); Anal. calcd for $C_{35}H_{29}Cl_3N_2O_9Si$: C, 55.60; H, 3.87; N, 3.71. Found: C, 55.46; H, 3.81; N, 3.50.

4.4.4. 1-{5-*O*-(4-Chlorobenzoyl)-2,3-di-*O*-isobutyryl-3-*C*-[2-(trimethylsilyl)ethynyl]-β-D-*ribo*-pentofuranosyl}uracil (25b). The compound was obtained as a colorless foam after purification by silica gel column chromatography (25% EtOAc in hexane): mp 160–161°C (IPE-heptane); FAB-MS *m/z* 619 $(M+H)^+$; ^1H NMR (DMSO-d_6) δ 11.51 (s, 1H), 8.02 (d, 2H, *J*=8.5 Hz), 7.75 (d, 1H, *J*=8.3 Hz), 7.63 (d, 2H, *J*=8.5 Hz), 6.01 (d, 1H, *J*=5.4 Hz), 5.72 (d, 1H, *J*=8.3 Hz), 5.63 (d, 1H, *J*=5.4 Hz), 4.64–4.76 (m, 3H), 2.60–2.68 (m, 2H), 1.08–1.13 (m, 12H), 0.06 (m, 9H); Anal. calcd for $C_{29}H_{35}ClN_2O_9Si$: C, 56.26; H, 5.70; N, 4.52. Found: C, 56.35; H, 5.60; N, 4.32.

4.4.5. 1-[2,3,5-Tri-*O*-(4-chlorobenzoyl)-3-*C*-ethynyl-β-D-*ribo*-pentofuranosyl]uracil (26a). The compound was obtained as white crystals after purification by silica gel column chromatography (50% EtOAc in hexane) followed by crystallization from IPE: mp 115–117°C; FAB-MS *m/z* 683 $(M+H)^+$; ^1H NMR (DMSO-d_6) δ 11.52 (br s, 1H), 7.83–8.04 (m, 7H), 7.56–7.66 (m, 6H), 6.26 (d, 1H, *J*=5.1 Hz), 6.00 (d, 1H, *J*=5.1 Hz), 5.79 (d, 1H, *J*=8.3 Hz), 5.07 (m, 1H), 4.93 (m, 1H), 4.82 (m, 1H), 4.21 (s, 1H); Anal. calcd for $C_{32}H_{21}Cl_3N_2O_9$: C, 56.20; H, 3.10; N, 4.10. Found: C, 56.45; H, 2.87; N, 4.00.

4.4.6. 1-[5-*O*-(4-Chlorobenzoyl)-2,3-di-*O*-isobutyryl-3-*C*-ethynyl-β-D-*ribo*-pentofuranosyl]uracil (26b). The compound was obtained as white crystals after purification by silica gel column chromatography (3% MeOH in CH_2Cl_2) followed by crystallization from EtOAc–heptane: mp 198–199°C; FAB-MS *m/z* 547 $(M+H)^+$; ^1H NMR (DMSO-d_6) δ 11.50 (s, 1H), 8.00 (d, 2H, *J*=8.8 Hz), 7.75 (d, 1H, *J*=8.1 Hz), 7.64 (d, 2H, *J*=8.8 Hz), 6.01 (d, 1H, *J*=5.6 Hz), 5.74 (d, 1H, *J*=8.1 Hz), 5.60 (d, 1H, *J*=5.6 Hz), 4.66–4.80 (m, 3H), 4.06 (s, 1H), 2.55–2.69 (m, 2H), 1.08 (m, 12H); Anal. calcd for $C_{26}H_{27}ClN_2O_9$: C, 57.09; H, 4.98; N, 5.12. Found: C, 57.09; H, 4.97; N, 5.17.

4.4.7. 1-[2,3-Di-*O*-acetyl-5-*O*-(4-chlorobenzoyl)-3-*C*-ethynyl-β-D-*ribo*-pentofuranosyl]uracil (26c). The compound was obtained as white crystals after purification by silica gel column chromatography (3% MeOH in CH_2Cl_2) followed by crystallization from IPE: mp 218–219°C; FAB-MS *m/z* 491 $(M+H)^+$; ^1H NMR (DMSO-d_6) δ 11.50 (s, 1H), 7.99 (d, 2H, *J*=6.6 Hz), 7.75 (d, 1H, *J*=8.3 Hz), 7.63 (d, 2H, *J*=6.6 Hz), 6.03 (d, 1H, *J*=5.8 Hz), 5.73 (d, 1H, *J*=8.3 Hz), 5.62 (d, 1H, *J*=5.8 Hz), 4.65–4.78 (m, 3H), 4.07 (s, 1H), 2.15 (s, 3H), 2.10 (s, 3H); Anal. calcd for $C_{22}H_{19}ClN_2O_9$: C, 53.83; H, 3.90; N, 5.71. Found: C, 54.04; H, 4.02; N, 5.55.

4.4.8. 1-[2,3-Di-*O*-benzoyl-5-*O*-(4-chlorobenzoyl)-3-*C*-ethynyl-β-D-*ribo*-pentofuranosyl]uracil (26e). The compound was obtained as white crystals after purification by silica gel column chromatography (50% EtOAc in hexane): mp 158–160°C (EtOAc–heptane); FAB-MS *m/z* 615 $(M+H)^+$; ^1H NMR (DMSO-d_6) δ 11.54 (s, 1H), 7.43–8.05 (m, 15H), 6.26 (d, 1H, *J*=4.9 Hz), 5.98 (d, 1H, *J*=4.9 Hz), 5.80 (d, 1H, *J*=8.3 Hz), 4.79–5.07 (m, 3H), 4.21 (s, 1H); Anal. calcd for $C_{32}H_{23}ClN_2O_9$: C, 62.50; H, 3.77; N, 4.56. Found: C, 62.42; H, 3.57; N, 4.52.

4.4.9. 1-[2,3-Di-*O*-(4-anisoyl)-5-*O*-(4-chlorobenzoyl)-3-*C*-ethynyl-β-D-*ribo*-pentofuranosyl]uracil (26f). The compound was obtained as a colorless foam after purification by silica gel column chromatography (60% EtOAc in hexane): FAB-MS *m/z* 675 $(M+H)^+$; ^1H NMR (DMSO-d_6) δ 11.53 (s, 1H), 7.65–7.80 (m, 9H), 6.95–7.10 (m, 4H), 6.21 (d, 1H, *J*=4.9 Hz), 5.92 (d, 1H, *J*=4.9 Hz), 5.79 (d, 1H, *J*=8.3 Hz), 4.75–5.10 (m, 3H), 4.16 (s, 1H), 3.85 (s, 3H), 3.81 (s, 3H); Anal. calcd for $C_{34}H_{27}ClN_2O_{11}$: C, 60.49; H, 4.03; N, 4.15. Found: C, 60.60; H, 4.19; N, 3.77.

4.5. Large-scale preparation of 1-{5-*O*-(4-Chlorobenzoyl)-2,3-di-*O*-isobutyryl-3-*C*-[2-(trimethylsilyl)ethynyl]-β-D-*ribo*-pentofuranosyl}cytosine (24b) from 19

To a mixture of **19** (347 g, 900 mmol) and DMAP (3.3 g, 27 mmol) in toluene (3.5 L) were added Et_3N (365 g, 3.6 mol) and isobutyryl chloride (341 g, 3.2 mol) at 0°C, and the mixture was stirred at 25–35°C for 6 h. After quenching the reaction with MeOH (88 g), H_2O (1.25 L) was added, and the resulting mixture was partitioned. The organic layer was washed with HCl (1 M, 1.25 L), aqueous $NaHCO_3$ (3%, 1.25 L), and aqueous NaCl (25%, 1.25 L), dried over $MgSO_4$, and evaporated to give crude **22b** (544 g). Besides, a mixture of cytosine (120 g, 1.08 mol), $(NH_4)_2SO_4$ (400 mg), and HMDS (870 g, 5.40 mol) was heated under reflux under nitrogen atmosphere for 1 h to give a solution. The solvent was evaporated, and the residue was co-evaporated with toluene to give $(TMS)_2$Cyt (**10**, 260 g). To a solution of **10** and the crude **22b** (544 g) in toluene (3.6 L) was added $SnCl_4$ (359 g, 1.35 mol) under nitrogen atmosphere, and the mixture was stirred at 50°C for 3 h. To the mixture was added 98% formic acid (1.8 L), H_2O (0.9 L), and HCl (6 M, 1.9 L), and then the resulting mixture was partitioned. The organic layer was washed with HCl (6 M, 1.9 L×2) and aqueous NaCl (25%, 2 L), and then evaporated. To a solution of the residue in CH_3CN (1.8 L) was added $NaHCO_3$ (757 g), and the resulting mixture was filtrated through celite pad and then PTFE membrane filter. The filtrate was evaporated to give crystals, which were recrystallized from IPE–heptane to give crystalline **24b** in a pure form (434 g, 78%).

4.6. Preparation of 25b from 19 without column chromatography

To a solution of **19** (4.23 g, 11.0 mmol), Et_3N (4.45 g, 44.0 mmol), and DMAP (40 mg, 0.33 mmol) in CH_2Cl_2 (63 mL) was added isobutyryl chloride (4.69 g, 44.0 mmol) at 0°C, and the mixture was stirred at room temperature for 4 h. After quenching the reaction with MeOH (1.35 mL), CH_2Cl_2 (50 mL) and H_2O (50 mL) were added, and the mixture was partitioned. The organic layer was washed with H_2O (50 mL), aqueous $NaHCO_3$ (10%, 60 mL), and aqueous NaCl solution (25%, 60 mL), dried over $MgSO_4$, and evaporated to give crude **22b** (6.65 g). Besides, a mixture of uracil (840 mg, 7.5 mmol), $(NH_4)_2SO_4$ (27 mg), and HMDS (5.8 g, 36 mmol) was heated under reflux under nitrogen atmosphere for 1 h to give a solution. The solvent was evaporated, and the residue was co-evaporated twice with toluene to give $(TMS)_2$Ura (**11**). To a solution of **11** and the crude **22b** (3.02 g) in CH_3CN (20 mL) was added $SnCl_4$ (885 μL, 7.50 mmol) at 0°C, and the mixture was stirred for at 50°C for 3 h. The mixture was added dropwise to aqueous $NaHCO_3$ (3%, 40 mL), and then the solvent was evaporated. To the residue were added $CHCl_3$ (100 mL) and $MgSO_4$ (10 g), and the mixture was filtrated. The filtrate was washed with saturated aqueous NaCl (100 mL), dried over $MgSO_4$, and evaporated. The residue was crystallized from heptane-IPE to give crystalline **25b** in a pure form (2.27 g, 74% from **19**).

4.6.1. 1-(3-*C*-Ethynyl-β-D-*ribo*-pentofuranosyl)cytosine (ECyd, 1). A solution of **24b** (430 g, 0.70 mol) and DBU (41.8 g, 270 mmol) in MeOH (2.8 L) was stirred at 40°C for 3 h. AcOH (19.4 g, 320 mmol) and toluene (2.8 L) were added, and the resulting mixture was stirred at 10–20°C for 1 h. The resulting crystals were collected by filtration, which were recrystallized from H_2O–MeOH to afford pure ECyd (**1**, 128 g, 68%): mp 233–235°C; FAB-MS (negative) *m/z*266 $(M-H)^-$; ^1H NMR (DMSO-d_6) δ 7.81 (d, 1H, *J*=7.6 Hz), 7.21 (br s, 1H), 7.17 (br s, 1H), 5.83 (d, 1H, *J*=6.6 Hz), 5.73–5.79 (m, 3H), 5.02 (t, 1H, *J*=4.9 Hz), 4.11 (t, 1H, *J*=6.6 Hz), 3.85–3.87 (m, 1H), 3.65–3.69 (m, 2H) 3.51 (s, 1H); Anal. calcd for $C_{11}H_{13}N_3O_5$: C, 49.44; H, 4.90; N, 15.72. Found: C, 49.58; H, 4.91; N, 15.71.

4.6.2. 1-(3-*C*-Ethynyl-β-D-*ribo*-pentofuranosyl)uracil (EUrd, 2). A solution of **25b** (2.0 g, 3.2 mmol) and DBU (2.0 g, 13 mmol) in MeOH (16 mL) was stirred at room

temperature for 1.5 h. After addition of AcOH (2.0 g), the resulting mixture was evaporated, and a solution of the residue in MeOH (4.4 mL) and $CHCl_3$ (44 mL) was stirred at room temperature for 1 h. The resulting crystals was collected by filtration to afford pure EUrd (802 mg, 92%): FAB-MS (negative) *m/z* 267 $(M-H)^-$; ^1H NMR (DMSO-d_6) δ 11.24 (br s, 1H), 7.87 (d, 1H, J=8.3 Hz), 5.91 (s, 1H), 5.82–5.86 (m, 2H), 5.68 (d, 1H, J=8.3 Hz), 5.10 (t, 1H, J=4.4 Hz), 4.17 (t, 1H, J=6.1 Hz), 3.88–3.90 (m, 1H), 3.63–3.73 (m, 2H), 3.54 (s, 1H); Anal. calcd for $C_{11}H_{12}N_2O_6$: C, 49.26; H, 4.51; N, 10.44. Found: C, 19.16; H, 4.60; N, 10.31.

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