Published in final edited form as:

Nucleosides Nucleotides Nucleic Acids. 2014 December 2; 33(12): 786–799. doi: 10.1080/15257770.2014.945648.

IODINE MONOCHLORIDE FACILITATED DEGLYCOSYLATION, ANOMERIZATION, AND ISOMERIZATION OF 3-SUBSTITUTED THYMIDINE ANALOGUES

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Abstract

The reaction of thymidine, 3-mono-, and 3,3',5'-trialkylsubstituted thymidine analogues with iodine monochloride (ICl) was investigated. Treatment with ICl resulted in rapid deglycosylation, anomerization, and isomerization of thymidine and 3-substituted thymidine analogues under various reaction conditions leading to the formation of the nucleobases as the major products accompanied by minor formation of α -furanosidic-, α -pyranosidic- and β -pyranosidic nucleosides. On the other hand, 3,3',5'-trisubstituted thymidine analogues were only deglycosylated and anomerized. These results are similar to those observed for the acidic hydrolysis of the glycoside bond in nucleosides, but were presumably caused by the Lewis acid character of an iodine electrophile.

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Supplemental materials are available for this article. These include: 1) Detailed experimental procedures and compound characterization for compounds that were used as reference compounds in HPLC studies described in the main manuscript, 2) information related to HPLC studies, 3) ¹H- and ¹³C-NMR spectra of compounds **1–7**, and 4) experimental procedures, compound characterizations, and X-ray crystallographic data (CIF) for compound **13**. Go to the publisher's online edition of *Nucleosides, Nucleotides & Nucleic Acids* to view the free supplemental files

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Keywords

3-Substituted thymidine analogues; iodine monochloride; deglycosylation; anomerization; isomerization

INTRODUCTION

The Lewis acid catalyzed glycosylation of nucleobases with protected carbohydrates plays a pivotal role in nucleoside chemistry.^[1] The reaction is reversible, and, thus, has also been used in the anomerization and intra/inter-molecular transglycosylation of nucleosides.^[1, 2] Acidic- and, to a limited extent, basic conditions are also known to cause deglycosylation, anomerization, and isomerization of nucleosides.^[3–7]

Molecular iodine (I₂) and interhalogens, such as iodine monochloride (ICl), have been used extensively as reagents in organic synthesis.^[8–11] Presumably based on its Lewis acid characteristics,^[12, 13] I₂ has also found application as a catalyst in the condensation of protected carbohydrates with nucleobases.^[14, 15] In the case of preformed nucleosides, I₂ and ICl have been used widely for the introduction of iodine at the carbohydrate (e.g. C4') or the pyrimidine ring (e.g. C5) of various ribonucleosides.^[16–23] To the best of our knowledge, however, there has been no report elucidating the possible Lewis acid facilitated deglycosylation, anomerization, and isomerization of nucleosides by halogens or interhalogens.

Recently, our group reported on the (radio)iodination of the carborane cluster of a 3-carboranyl thymidine analogue (3CTA), designated as N5 (3-[5-(*o*-carboran-1-yl)pentan-1-yl]thymidine, for potential use in the radiotherapy and imaging of tumors.^[24] These thymidine bioconjugates were originally developed as boron-10 (¹⁰B) delivery agents for boron neutron capture therapy (BNCT).^[25] The iodination reaction of N5 with ICl was carried out in DCM in the presence of aluminum trichloride (AlCl₃). By adjusting the reaction conditions carefully, it was possible to control the level of iodination at the carborane cluster to some extent resulting in preferential formation of either mono- or tetraiodinated derivatives of N5. Concomitantly, however, significant decomposition (10–35%) of N5 was also observed under all applied conditions. Decomposition products appeared to include the deglycosylated nucleobases of N5. This result was expected based on the established Lewis acid characteristics of AlCl₃. Surprisingly, however, the reaction of N5 with ICl in the absence of AlCl₃ appeared to cause even higher levels of decomposition. This was somewhat unexpected, as there are apparently no reports describing the effects of ICl on the deglycosylation of deoxyribonucleosides. Thus, we decided to investigate the as yet unexplored effect of ICl on the glycosidic bond of β-thymidine, 3-mono- and 3,3',5'-trialkyl-substituted analogues of β-thymidine, as well as some other nucleosides in detail. The results of these studies and possible mechanisms of product formation are discussed in this paper.

RESULTS AND DISCUSSION

Structure elucidation

The deglycosylation phenomenon was primarily studied at the example of the model compound 3-butyl- β -thymidine (**1**, Scheme 1) because it has chemical properties similar to those of N5 and, in contrast to N5, can be synthesized readily from β -thymidine in a single step. Initially, compound **1** was subjected to reaction with ICl in DCM using various conditions. The results are summarized in Table 1 (Entries **1**, **3**, **5**, and **7**). Two products were initially separated from other components of the reaction mixture by silica gel chromatography and preparative TLC. These were eventually identified as compounds **2** and **4**. Compound **2** was readily identified as 3-butylthymine by NMR and MS. The initial ^1H -NMR spectrum of the second isolated compound (**4**) revealed a doublet of doublets (dd) at 6.04 pm for 1'-H, which had almost the same shape and chemical shift of the dd signal of α -thymidine (**10**) (6.2 ppm, data not shown). In order to rule out any ambiguity in the analysis, 3-butyl- α -thymidine (**3**) was synthesized from α -thymidine using standard reaction conditions (Supplementary materials). As shown in Figure 1, there were significant differences in the NMR spectra of **3** and **4** although basically identical HR-MS data (321.1420 Da vs. 321.1458 Da) indicated an isomeric relationship.

In order to unambiguously identify the structure of **4**, the two purported hydroxyl groups of this isomer were substituted with *tert*-butyldimethylsilyl (TBDMS) groups and the obtained product was crystallized from diethylether/pentane (Figure 2 and Supplementary materials). The X-ray crystallographic analysis identified this structure as 3,5-di-*O-tert*-butyldimethylsilyl-1-(2'-deoxy- β -D-ribosepyranosyl)-3-butylthymine (**13**), thus confirming furanose/pyranose isomerization of **1** to **4**.

At this point, HPLC analysis using method A (Table S1, Supplementary materials) revealed that **3** was also produced during the reaction and it seemed that the reaction mixtures contained overall four compounds, i.e. **1**, **2**, **3**, and **4**, with retention times of 32.08, 30.61, 31.45, and 33.77 min, respectively (Table S1, Supplementary materials). Similar deglycosylation/isomerization patterns were previously observed during the acidic hydrolysis of β -thymidine and 2'-deoxy- β -uridine.^[5, 6, 26–28] In some of these reactions, however, a 4th isomerization product, i.e. 1-(2-deoxy- α -D-ribosepyranosyl)thymine/uracil, was also found. Thus, we decided to synthesize 1-(2-deoxy- α -D-ribosepyranosyl)-3-butylthymine (**5**) for use as a standard in our HPLC studies (Supplementary materials) and, surprisingly, this compound was found to have the same retention time (31.45 min) as **3** using HPLC method A. Fortunately, the subsequently developed HPLC Method B (Table S1, Supplementary materials) allowed us to detect the presence of this isomer as well in the reaction mixture (Figure S1A, Supplementary materials).

The configuration at C-1' in compounds **1**, **3**, **4**, and **5** was also verified by Circular Dichroism (Figure S2, Supplementary materials).

Mechanistic considerations

As mentioned above, β -thymidine and 2'-deoxy- β -uridine anomerize/isomerize to their α -furanose-, α -pyranose-, and β -pyranose counterparts by acid-catalyzed hydrolysis.^[5, 6, 26–28] Decomposition of ICl or I₂ to acidic species (i.e. HCl and HI) in the presence of hydroxyl or sulfhydryl groups is well established^[10,33–39] and there has been an extensive discussion on whether the cleavage of various protective groups mediated by e.g. I₂, ICl, or IBr is caused by the intact halogen/interhalogen or by acidic species generated *in situ* via reaction with hydroxyl or sulfhydryl groups.^[29–34] Carbonates and bicarbonates were effectively used to eliminate acidic residues in deprotection reactions with I₂.^[33] Thus, in order to rule out any role of acidic species in the ICl-mediated deglycosylation, anomerization, and isomerization of **1** in DCM, we decided to carry out the reactions in the presence of three equivalents of K₂CO₃ (Table 1, entries **2**, **4**, **6**, and **8**). The obtained results indicated that acidic species may not play a significant role because there were no marked differences in the formations of compounds **1–5** in the presence or absence of K₂CO₃ (Table 1, entries **1–8**). For all experiments in DCM, deglycosylated **2** was the major product (~ 85% average). The formations of the β -isomers **1** (~ 8% average) and **4** (~ 6 % average) were significantly lower and only very small quantities of the α -isomers **3** and **5** were produced (~ 1.5 % combined average). The observed differences in product formation were probably due to normal experimental variabilities during reactions, work-up, and HPLC analysis rather than differences in reaction times or K₂CO₃ quantities. If at all, somewhat enhanced deglycosylation was observed for the 2-hour reaction times. Similar results were observed when I₂ instead of ICl was employed, although overall less transformation of **1** into nucleobase and isomeric products was observed (data not shown). When the reaction with ICl was carried out in other representative aprotic solvents, i.e. CCl₄ and acetonitrile, the ratio of reaction products was comparable with that in DCM (Table 1, Entries **9** & **10**). Furthermore, when **1** was treated with only 0.1 equivalent of ICl in DCM at room temperature less than 1% of products **2–4** were produced indicating that a stoichiometric amount of ICl facilitates the reaction (data not shown).

In order to further explore the role of acidity and substantiate the findings shown in Table 1 for the experiments with K₂CO₃, compound **1** was exposed to the same (or similar) acidic conditions previously reported for the hydrolysis of β -thymidine/2'-deoxy- β -uridine. The reaction of **1** with 2M aqueous HClO₄ for 1h at 90 °C produced a similar deglycosylation/anomerization/isomerization pattern as observed for the reaction with ICl in DCM at room temperature (Table 2, Entry **13**). The results are comparable with those previously reported by Cadet and Teoule for the reaction of β -thymidine/2'-deoxy- β -uridine with 2M HClO₄.^[6] Similar results were obtained when **1** was exposed to buffered acidic conditions at pH 1 for 12 h at 90 °C (Table 2, Entry **11**), as previously described by Shapiro et al.^[28] for reactions with β -thymidine/2'-deoxy- β -uridine. In this study, both compounds hydrolyzed rapidly at pH 1.9 but significantly slower at pH 3–7, indicating an important role of the hydrogen ion concentration in the hydrolysis, similar to our own results showing concentration-dependent efficacy of ICl. When the acidic hydrolysis reactions were carried out for 1h at room temperature, no deglycosylation/anomerization/isomerization was observed, (Table 2, Entries **12** & **14**) which also suggests that acidic species, if at all, only contribute insignificantly to the reaction of β -thymidine analogues with ICl in DCM.

It has been reported that the reaction of ICl with free hydroxyl groups produces HCl.^[35] Therefore, methylation of 3'- and 5'-OH groups of **1**, as in 3',5'-di-*O*-methyl-3-butyl- β -thymidine (**6**), should prevent the formation of HCl. The reaction of **6** with ICl in DCM at room temperature generated 3-butylthymine (**2**) and 3',5'-di-*O*-methyl-3-butyl- α -thymidine (**7**) (Table 3, Entry **17**). No pyranosidic nucleosides were detected by HPLC (Figure S1B, Supplementary materials). This finding supports the proposed mechanism shown in Scheme 2, which does not lead to and indeed prevents pyranosidic nucleoside formation of 3',5'-disubstituted β -thymidine analogues. The reaction produced markedly higher quantities of the α -furanose product **7** compared with reactions utilizing unprotected **1**. Exposure of compound **6** to aqueous acidic condition (pH 1) at 90 °C for 12 h, produced **2** and **7** in significantly lower quantities than exposure to ICl in DCM (Table 3, Entry **18** vs. Entry **17**). In contrast, compound **6** was not at all transformed at pH 1 at room temperature (Table 3, Entry **19**). The obtained results lend further strong support to the hypothesis that the reaction of **6** with ICl in DCM does not rely on the presence of acidic species.

Another important finding of our studies was that neither NaI nor KI caused any deglycosylation, isomerization or anomerization of compound **1** (Table 2, Entries **15** and **16**). This strongly suggests that an electrophilic iodine species is responsible for the observed effects.

Other pyrimidine nucleosides that were exposed to ICl in DCM or acetonitrile included 3- β -thymidine (**9**), 5-methyluridine, 2'-deoxycytidine, 2'-deoxyadenosine, and 2'-deoxyguanosine. In the case of **9**, reaction with ICl in acetonitrile (Table 3, Entry **20**) produced similar deglycosylation, anomerization, and isomerization rates as those observed for **1** (Table 1, Entry **9**). Interestingly, the reactions of 5-methyluridine, 2'-deoxycytidine, 2'-deoxyadenosine, and 2'-deoxyguanosine with ICl (1 equiv) in acetonitrile at 25 °C for 1h did not yield any products (data not shown). It is well established that 2'-deoxyribonucleosides are far more susceptible to acidic hydrolysis than their ribose counterparts and that purine nucleosides are less rapidly hydrolyzed than pyrimidine nucleosides.^[36,37] Lack of reactivity of 2'-deoxycytidine, 2'-deoxyadenosine, and 2'-deoxyguanosine also suggests the absence of acidic species in these reactions.

CONCLUSIONS

In summary, the underlying mechanism of action for the reaction of β -thymidine and 3-substituted β -thymidine analogues with ICl appears to include an electrophilic iodine species, which functions as a Lewis acid in a fashion very similar to that proposed by Cadet and Teoule^[6] for the proton in the acidic hydrolysis of β -thymidine/2'-deoxy- β -uridine. Acidic hydrolysis does not appear to play a significant role in the observed deglycosylation, anomerization, and isomerization. The hypothetical mechanism is shown in Scheme 2. In one pathway (A), electrophilic iodine attacks the C4-carbonyl oxygen followed by formation of a cyclic oxonium ion intermediate, which can be re-attacked by the released iodinated nucleobase to form both α - and β -furanosidic nucleosides. Semiempirical AM1 level calculations by Marion et al.^[38] have shown that amongst all β -thymidine atoms, the C4-carbonyl oxygen of should have the highest affinity for electrophiles. However, mechanistically it is also possible that the iodine electrophile interacts with the C2-carbonyl

oxygen or with N1. Cadet and Teoule^[6] proposed that N1 is the point of attack by a proton in the acidic hydrolysis of β -thymidine/2'-deoxy- β -uridine. In a second pathway (B), electrophilic iodine attacks O4' of the β -thymidine scaffold leading to opening of the 2'-deoxyribose ring to form a Schiff-base intermediate, which is either converted to a C-1' carbocation or hydrolyzed to nucleobase. Re-attack of either O4' or O5' at C1' could lead to the formation of α -furanosidic, β -furanosidic, α -pyranosidic, and β -pyranosidic nucleosides. Hypoiodite-type compounds, such as the one postulated in pathways (A), are unstable and have only been generated *in situ*.^[39, 40] Such species are supposedly very moisture sensitive and should hydrolyze upon aqueous work-up.^[41]

In general, 3'-keto-2'-deoxy-nucleosides are prone to β -elimination of their nucleobase^[42] and it is conceivable that ICl causes oxidation of the 3'-hydroxyl group in compounds **1** and **9**, which subsequently can lead to loss of thymine/3-alkylthymines. However, the reaction of 3',5'-di-*O*-methyl-3-butyl- β -thymidine (**6**) with ICl led to substantial formation of 3-butylthymine (**2**) and 3',5'-di-*O*-methyl-3-butyl- α -thymidine (**7**), which cannot occur via oxidation of the 3'-hydroxyl group and subsequent β -elimination. In addition, 3',5'-disubstitution, as in compound **6**, prevented the formation of pyranosidic nucleosides following ICl exposure and this finding strongly supports the mechanisms depicted in Scheme 2.

Intuitively, it can be deduced that proposed cascade may be initiated by the use of catalytic amount of the positively charged iodine species. However, our results show significant rate acceleration in these reactions by employing a stoichiometric amount of ICl. Detailed kinetic and mechanistic studies for the observed phenomena are beyond the scope of this manuscript.

As an offshoot, the developed methodology could be utilized as a novel protective group strategy for the regioselective synthesis of 3-substituted thymine or uracil analogues. Basically, the 2'-deoxyribose moiety of commercially available β -thymidine or 2'-deoxy- β -uridine would serve as the protective group for N1 in this strategy, which is removed by ICl or I₂ after introduction of a substituent at N3. This could be advantageous compared with currently utilized strategies, which rely on the initial introduction of protective groups at N1 of thymine or uracil.^[43–45] This could be particularly interesting in the context of 3CTAs, which have attracted considerable attention in biomedical research in recent years.^[25] All currently available 3CTAs contain a 2'-deoxyribose moiety at N1. Following deprotection (removal of the 2'-deoxyribose) at N1 by ICl or I₂, the resulting 3-substituted nucleobases could be condensed with different types of carbohydrates thereby potentially broadening the application range of this type of biomedical agents.

EXPERIMENTAL SECTION

General Procedures

¹H- and ¹³C NMR spectra were measured at 400 MHz for ¹H and 100 MHz for ¹³C. Chemical shifts (δ) are reported in ppm from internal deuterated chloroform or methanol. Coupling constants are reported in Hz. High Resolution Mass Spectra (HRMS) were obtained by Electrospray Ionization (ESI). Silica gel 60 (0.063–0.200 mm) was used for

gravity column chromatography and silica gel 60 (0.015–0.049 mm) was used for flash column chromatography. Analytical thin-layer chromatography was performed on silica gel 60 F₂₅₄ aluminum sheets with fluorescent indicator. Preparative TLC was performed on 20×20 silica gel 1000 μm with fluorescent indicator. Compounds were visualized by UV light ($\lambda = 254$ and 366 nm). Reagent-grade solvents were used for column chromatography. Anhydrous solvents were purchased from standard vendors. All reactions were carried out under argon atmosphere. Analytical HPLC was carried out with a 5μ C18 110A Column (250 x 4.6 mm) supplied by Phenomenex Inc. (Torrance, CA) on an HPLC system with a Windows based data acquisition and Diode array detector.

General procedure for the treatment of β -thymidine/3-substituted β -thymidine analogues with ICI and acidic conditions—To a solution of nucleoside (0.1 mmol) in 2 mL of DCM, CCl₄, or CH₃CN, ICI (0.1 or 1 molar equivalent) and K₂CO₃ (0 or 3 molar equivalent) (See Table 1) were added and the resulting mixture was stirred for 1 h at room temperature. Distilled water (2 mL) and sodium thiosulfate were added to quench the reaction. The resulting mixture was extracted with DCM (3 × 2 mL), the organic phase was evaporated, and the residue was purified by silica gel column chromatography. Further purification was performed by preparative TLC. For acidic hydrolysis, the nucleoside (0.1 mmol) was added to 2 mL of aqueous HCl buffer (pH 1, 0.2 M HCl/0.2M KCl, 2.68/1, v/v) or 2M HClO₄ and stirred for 1 h or 12 h at 25°C or 90 °C. The resulting mixture was extracted with DCM and further processed as describe above.

Chromatography data and spectroscopic analysis (information in the following is only for compounds isolated from reactions described above)

3-Butylthymine (2): The product was purified by silica gel column chromatography (DCM: MeOH, 25:1) to give a white amorphous solid, R_f: 0.32 (hexanes: ethyl acetate, 3:7). ¹H NMR (MeOH-d₄) δ : 7.24 (s, 1H, H-6), 3.72 (t, $J = 7.38$ Hz, 2H, CH₂-N), 1.70 (s, 1H, CH₃), 1.73 (m, 2H, CH₂), 1.23 (m, 2H, CH₂), 0.86 (t, $J = 7.30$ Hz, 3H, CH₃). ¹³C NMR (MeOH-d₄) δ : 166.48 (C-4), 153.56 (C-2), 137.39 (C-6), 110.03 (C-5), 41.49 (CH₂-N), 30.84 (CH₂), 21.21 (CH₂), 14.19 (CH₃), 12.98 (CH₃). MS (HR-ESI) for C₉H₁₄N₂O₂Na [(M+Na)⁺]. Calcd: m/z 205.0953 Found: m/z 205.0953.

1-(2-Deoxy- α -D-ribofuranosyl)-3-butylthymine (3): The product was purified by silica gel column chromatography (DCM: MeOH, 9:1) to give a colorless oil, R_f: 0.58 (DCM: MeOH, 9:1). ¹H NMR (MeOH-d₄) δ : 7.75 (s, 1H, H-6), 6.20 (dd, $J = 7.36$ Hz, $J = 2.50$ Hz, 1H, H-1'), 4.34–4.37 (m, 1H, H-3'), 4.29–4.32 (m, 1H, H-4'), 3.91 (t, $J = 7.51$ Hz, 2H, CH₂-N), 3.55–3.63 (m, 2H, H-5', H-5''), 2.64–2.71 (ddd, $J = 14.17$ Hz, $J = 6.44$ Hz, $J = 6.44$ Hz, 1H, H-2'), 2.05 (dm, 1H, H-2''), 1.54–1.61 (m, 2H, CH₂), 1.31–1.4 (m, 2H, CH₂), 0.95 (t, $J = 7.42$ Hz, 3H, CH₃). ¹³C NMR (MeOH-d₄) δ : 165.60 (C-4), 152.32 (C-2), 137.00 (C-6), 109.76 (C-5), 91.16 (C-1'), 89.09 (C-4'), 72.43 (C-3'), 63.47 (C-5'), 41.96 (CH₂-N), 41.83 (C-2'), 30.76 (CH₂), 21.17 (CH₂), 14.14 (CH₃), 13.31 (CH₃). MS (HR-ESI) for C₁₄H₂₂N₂O₅ [(M+Na)⁺]. Calcd: m/z 321.1426 Found: m/z 321.1420.

1-(2-Deoxy- β -D-ribofuranosyl)-3-butylthymine (4): The product was purified by silica gel column chromatography (DCM: MeOH, 19:1) to give a colorless oil, R_f: 0.41 (DCM:

MeOH, 19:1). ^1H NMR (MeOH- d_4) δ : 7.53 (s, 1H, H-6), 6.04 (dd, $J = 10.80$ Hz, $J = 2.57$ Hz, 1H, H-1'), 4.16 (m, 1H, H-4'), 3.92 (t, $J = 7.24$ Hz, 2H, $\text{CH}_2\text{-N}$), 3.75–3.84 (m, 3H, H-3', H-5', H-5''), 1.93–2.09 (m, 2H, H-2', H-2''), 1.91 (s, 3H, CH_3), 1.53–1.60 (m, 2H, CH_2), 1.29–1.34 (m, 2H, CH_2), 0.95 (t, $J = 7.28$ Hz, 3H, CH_3). ^{13}C NMR (MeOH- d_4) δ : 165.26 (C-2), 152.11 (C-4), 136.31 (C-6), 110.94 (C-5), 79.60 (C-1'), 68.24 (C-4'), 67.72 (C-3'), 67.23 (C-5'), 42.27 ($\text{CH}_2\text{-N}$), 37.14 (C-2'), 30.72 (CH_2), 21.15 (CH_2), 14.13 (CH_3), 13.06 (CH_3). MS (HR-ESI) for $\text{C}_{14}\text{H}_{22}\text{N}_2\text{O}_5$ [(M+Na) $^+$]. Calcd: m/z 321.1426 Found: m/z 321.1458.

1-(2-Deoxy-3,5-di-*O*-methyl- α -D-ribofuranosyl)-3-butylthymine (7): The product was purified by silica gel column chromatography (hexane: ethylacetate, 1:1) to give white foam, R_f : 0.54 (hexane: ethylacetate, 1:1). ^1H NMR (MeOH- d_4) δ : 7.61 (s, 1H, H-6), 6.21 (d, $J = 7.21$ Hz, 1H, H-1'), 4.54 (m, 1H, H-3'), 3.97 (m, 1H, H-4'), 3.91 (t, $J = 7.26$ Hz, 2H, $\text{CH}_2\text{-N}$), 3.46 (m, 2H, H-5', H-5''), 3.38 (s, 3H, OCH_3), 3.31 (s, 3H, OCH_3), 2.60–2.67 (m, 1H, H-2'), 2.13 (m, 1H, H-2''), 1.90 (s, 3H, CH_3), 1.54–1.59 (m, 2H, CH_2), 1.29–1.38 (m, 2H, CH_2), 0.95 (t, $J = 7.28$ Hz, 3H, CH_3). ^{13}C NMR (MeOH- d_4) δ : 165.47 (C-2), 152.29 (C-4), 136.77 (C-6), 109.90 (C-5), 88.77 (C-4'), 86.43 (C-1'), 82.91 (C-3'), 74.48 (C-5'), 59.93 (OCH_3), 56.92 (OCH_3), 41.96 ($\text{CH}_2\text{-N}$), 38.71 (C-2'), 30.74 (CH_2), 21.16 (CH_2), 14.15 (CH_3), 13.32 (CH_3). MS (HR-ESI) for $\text{C}_{16}\text{H}_{26}\text{N}_2\text{O}_5$ [(M+Na) $^+$]. Calcd: m/z 349.1739 Found: m/z 349.1724.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The present study was supported by funds from The Ohio State University College of Pharmacy and NIH Grant R01CA127935. The authors would like to thank Dr. Jed Fisher, Department of Chemistry and Biochemistry, University of Notre Dame, Dr. Christophe Mathé, Institute of Biomolecules, Max Mousseron University of Montpellier 2, Dr. Gilles Gosselin, Idenix Pharmaceuticals, and Dr. Craig McElroy, College of Pharmacy, The Ohio State University, for helpful discussions.

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Fig. 1. Partial ^1H NMR and ^{13}C NMR spectra of compounds **1**, **3**, **4**, and **5**

Fig. 2.

X-ray crystal structure of 3', 5'-diTBDMS substituted of 1-(2-deoxy- β -D-ribofuranosyl)-3-butylthymine (**13**, see also Supplementary materials).

Scheme 1.

General scheme of the deglycosylation, isomerization and anomerization reaction. Reaction conditions are listed in Tables 1–3.

Scheme 2.

The proposed mechanism of the deglycosylation, anomerization, and isomerization reaction.

Table 1

Deglycosylation, isomerization, and anomerization products of 3-butyl- β -thymidine (**1**) with 1 molar equivalent of ICl at 25 °C analyzed with HPLC using method A.

Entry	Solvent	Time (h)	K ₂ CO ₃ (eq.)	I Products %			
				2	23&5	1	4
1	DCM	0.25	0	83	1	9	7
2	DCM	0.25	3	77	2	13	8
3	DCM	0.5	0	84	2	8	6
4	DCM	0.5	3	87	1	5	7
5	DCM	1	0	77	1	21	1
3 ₆	DCM	1	3	84	2	6	9
7	DCM	2	0	97	<1	1	2
8	DCM	2	3	89	2	4	5
9	CH ₃ CN	1	0	62	12	7	19
10	CCl ₄	1	0	72	1	23	4

¹ Ratios were calculated from peak integrations of the HPLC chromatograms. In general, extinction coefficients of nucleosides and the corresponding nucleobases are in the same range.^[46, 47]

² Data for compounds **3** & **5** and were combined because of low peak intensities and/or inseparable peaks in the HPLC chromatograms.

³ Entry **6** is also shown in the chromatogram displayed in Figure S1A (Supplementary materials).

Table 2

Reaction products of 3-butyl- β -thymidine (**1**) with acids, potassium iodide, and sodium iodide (HPLC method A).

Entry	Reagent (eq.)	Solvent	Time (h) ^c	I Products %			
				2	23&5	1	4
11	³ HCl/KCl Buffer (pH 1)	H ₂ O	12/90	46	7	41	6
12	³ HCl/KCl Buffer (pH 1)	H ₂ O	1/25	No reaction			
13	HClO ₄ (2M)	H ₂ O	1/90	96	2	1	1
14	HClO ₄ (2M)	H ₂ O	1/25	No reaction			
15	KI (1.2)	DCM	12/40	No reaction			
16	NaI (1.2)	CH ₃ CN	48/85	No reaction			

¹ Ratios were calculated from peak integrations of the HPLC chromatograms. In general, extinction coefficients of nucleosides and the corresponding nucleobases are in the same range.^[46, 47]

² Data for compounds **3** & **5** were combined because of low peak intensities and/or inseparable peaks in the HPLC chromatograms.

³ HCl/KCl Buffer (pH 1) was prepared by combining 0.2 M HCl (134 mL) and 0.2 M KCl (50 mL).

Acid- and ICl-dependent deglycosylation, isomerization, and anomerization products of 3',5'-di-*O*-methyl-3-butyl-β-thymidine (**6**) and β-thymidine (**9**).

Table 3

Entry	Reactant	Reagent	Solvent	Time (h)/°C	HPLC/ Method	I Products %				
						2	7	6		
417	6	ICl (1)	DCM	1/25	C	22	44	34		
18	6	³ HCl/KCl Buffer (pH 1)	H ₂ O	12/90	C	11	3	86		
19	6	³ HCl/KCl Buffer (pH 1)	H ₂ O	1/25	C			No reaction		
						8	29&10	12	11	
20	9	ICl (1)	CH ₃ CN	1/25	A	55	32	1	12	

¹ Ratios were calculated from peak integrations of the HPLC chromatograms. In general, extinction coefficients of nucleosides and the corresponding nucleobases are in the same range.^[46, 47]

² Data for compounds **9** & **10** were combined because of low peak intensities and/or inseparable peaks in the HPLC chromatograms.

³ HCl/KCl Buffer (pH 1) was prepared by combining 0.2 M HCl (134 mL) and 0.2 M KCl (50 mL).

⁴ Entry **17** is also shown in the chromatogram displayed in Figure S1B (Supplementary materials).