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SIMULTANEOUS REMOVAL OF BENZYL AND BENZYLOXYCARBONYL PROTECTIVE GROUPS IN 5′-*O*-(2-DEOXY-α-D-GLUCOPYRANOSYL)URIDINE BY CATALYTIC TRANSFER HYDROGENOLYSIS

Ilona Wandzik, Tadeusz Bieg, and Monika Kadela

Silesian University of Technology, Faculty of Chemistry, Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology, Gliwice, Poland

□ *Synthesis of* N^3*,2′,3′-O-tris-(benzyloxycarbonyl)uridine and its use in the synthesis of 5′-O-(2-deoxy-α-D-glucopyranosyl)uridine is described. Simultaneous removal of benzyl and benzyloxycarbonyl groups was accomplished by catalytic transfer hydrogenolysis in the presence of Pearlman's catalyst without competing side reactions.*

Keywords Benzyl; benzyloxycarbonyl protective groups; hydrogenolysis; uridine; 2-deoxy-D-glucopyranose

INTRODUCTION

Chemical synthesis of biologically important complex carbohydrates usually requires using protective groups for the protection and differentiation of various O-H and N-H groups. Among them, benzyl-type protective groups are of great interest due to their stability to a variety of reagents and easy removal by catalytic hydrogenolysis.

Recently, we reported on the synthesis of 2-deoxyhexopyranosyl derivatives of uridine as donor substrate analogues of glycosyltransferases.[1] As an example compound **1** which possesses three type of protective groups: benzyl, benzoyl and acetonide is shown in Figure 1.

We originally attempted to prepare totally deprotected title compound **3** in order to ensure better interaction with the active site of glycosyltransferase, similar to that of natural substrate; therefore, three deblocking

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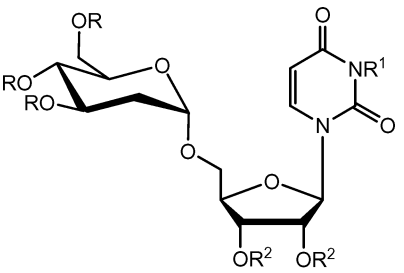
Address correspondence to Ilona Wandzik, Silesian University of Technology, Faculty of Chemistry, Department of Organic Chemistry, Bioorganic Chemistry and Biotechnology, Krzywoustego 4, 44-100 Gliwice, Poland. E-mail: ilona.wandzik@polsl.pl



1 R=Bn, R^1=Bz, R^2, R^2=$C(CH_3)_2$
2 R=H, R^1=H, R^2, R^2=$C(CH_3)_2$
3 R=R^1=R^2=H

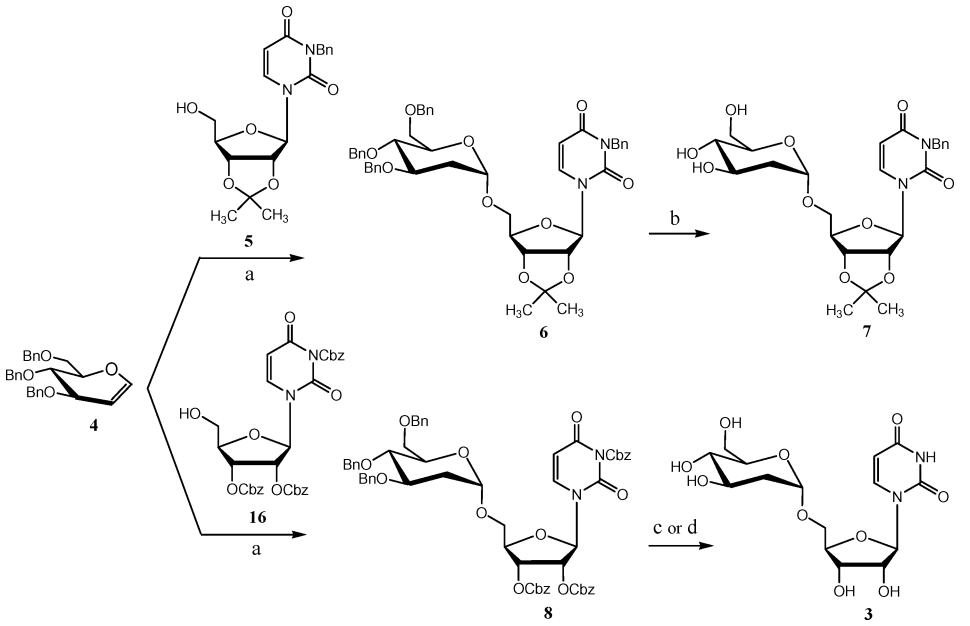
FIGURE 1 2-Deoxy-α-D-glucopyranosyl derivatires of uridine.

procedures should be undertaken. Saponification of acyl ester followed by catalytic transfer hydrogenolysis led to compound **2**. There was a concern that acetonide derivative will not fit into the active site of the enzyme owing to the steric hindrances created by the isopropylidene group. Unfortunately removal of acetonide group performed in the presence of trifluoroacetic or formic acid occurred in low yield due to the poor stability of 2-deoxyglycosidic linkage in acid medium. We considered, therefore, another way of protecting the uridine moiety. Our goal was to apply a protective group that could be removed in neutral conditions, preferably by catalytic hydrogenolysis, together with O-benzyl protection in 2-deoxy-D-glucopyranose part.

RESULTS AND DISCUSSION

Successful synthesis of compound **1** was realized in two-step regio- and stereoselective addition of uridine to D-glucal using Falck-Mioskowski protocol.[2] Our previous experiments revealed beneficial influence of using a large group as N-imide protection on α-selectivity in the addition of uridine derivatives to D-glucal.[1] Therefore, there was a need to protect not only two secondary hydroxyl groups of uridine, but also the *N*-imide function of the aglycone. First, we tried out benzyl protection of *N*-imide nitrogen hoping that in the final step it would be cleaved simultaneously to benzyl protection of the hydroxyl groups in 2-deoxy-D-glucopyranose ring. Unfortunately, we found that treatment of compound **6** with cyclohexene and Pearlman's catalyst (20% $Pd(OH)_2/C$)[5] in MeOH under reflux for 3 hours resulted in removing the *O*-benzyl groups only (Scheme 1).

Several reports discussed a similar problem in imide *N*-debenzylation by catalytic hydrogenolysis. Johnson and Widlanski[4] observed that the deprotection of *N*-imide in per-benzyluridine did not occur in the presence of hydrogen on Pearlman's catalyst.[5] Only *O*-benzyl ethers were deblocked.



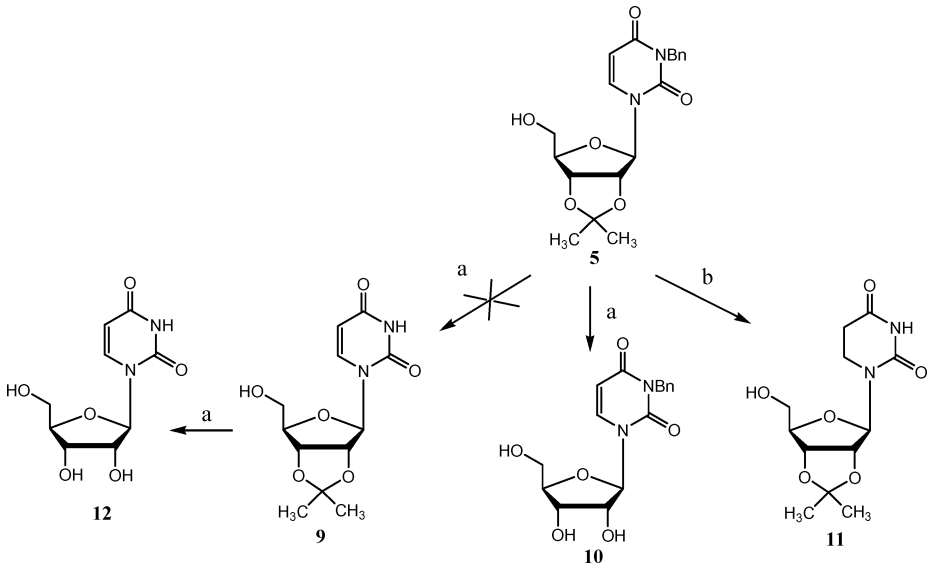
SCHEME 1 Reagents and conditions: (a) TPHB (0.1 equiv.), CH_2Cl_2, room temperature, 3 hours, (b) 20% $Pd(OH)_2$,/C, cyclohexene, MeOH, reflux, 3 hours, (c) 20% $Pd(OH)_2$/C, cyclohexene, MeOH, reflux, 15 minutes, (d) 20% $Pd(OH)_2$/C, 1,4-cyklohexadiene, MeOH, room temperature, 20 hours.

Mandal and coworkers revealed similar problem in thymidine derivatives when hydrogenolysis was carried out on 10% Pd/C in the presence of hydrogen.[6] On the other hand, Reitz and Pfleiderer[7] noted that reduction of the pyrimidine 4,5 double bond during hydrogenolytic cleavage of *O*-benzyl-substituted diuridylphosphates occurred on palladium black in the presence of hydrogen.

We revealed that hydrogenolysis of N^3-benzyl-2,3-*O*-isopropylideneuridine (**5**) by hydrogen transfer from cyclohexene or 1,4-cyclohexadiene in the presence of Pearlman's catalyst or 10% Pd/C did not lead to the desired 2,3-*O*-isopropylideneuridine (**9**). Instead of this, a preferential formation of N^3-benzyluridine (**10**) was observed (Scheme 2).

Moreover, 2,3-*O*-isopropylideneuridine (**9**) underwent deprotection to free uridine under the same conditions. This result shows that presence of N^3-benzyl group is not necessary for the cleavage of acetonide. The most striking observation was that in the presence of HCO_2NH_4 as a hydrogen donor,[8] the catalytic transfer hydrogenolysis of N^3-benzyl-2,3-*O*-isopropylideneuridine (**5**) proceeds with reduction of the 4,5 double bond of nucleobase with unaffected isopropylidene block. Compounds **10**[4] and **11**[9] were identified by comparison with literature data.

The aforementioned experiments showed that N^3-benzyl-2,3-*O*-isopropylideneuridine (**5**) is not suitable substrate for the synthesis of

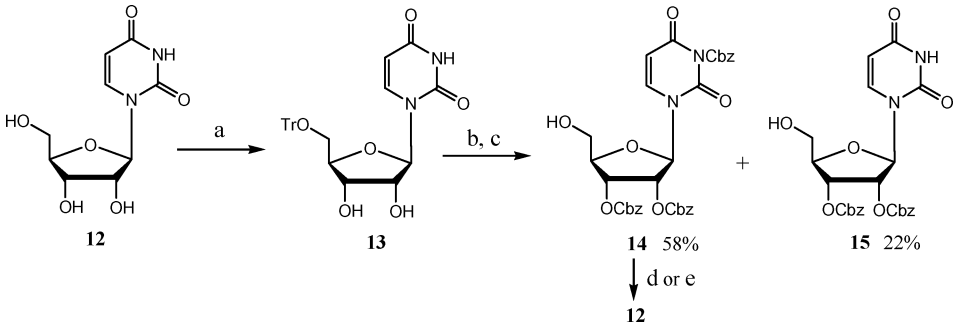


SCHEME 2 Reagents and conditions: (a) 20% $Pd(OH)_2/C$, cyclohexene, MeOH, reflux, 3 hours; (b) 10% Pd/C, HCO_2NH_4, MeOH, reflux, 2 hours then next portion of HCO_2NH_4, 2 hours.

the desired title compound **3**. We considered, therefore, other protecting groups for the imide function and the secondary hydroxyls of uridine. In this report, we turn our attention on benzyloxycarbonyl (Cbz) group. An *N*-Cbz group is one of the most common amino protective group and can be useful in protection of hydroxyl group as well. There is no report on protection of *N*-imide nitrogen in uridine by *N*-Cbz; instead, protection of two secondary hydroxyl groups for 5-O-*tert*-butyldimethylsilyluridine is reported.[4] Reaction is carried out using benzylchloroformate (CbzCl) and 4-(N,N-dimethylamino)pyridine (DMAP) in CH_2Cl_2 at room temperature for 3 days. We attempted to carry out the synthesis of totally tris-Cbz protected uridine since we believed that such derivative would be more relevant for our subsequent studies. We found that application of N,N-diisopropylethylamine (DIPEA), instead of DMAP in refluxing CH_2Cl_2, led to the desired tris-Cbz derivative **14** as a major product associated with minor di-Cbz derivative **15** (Scheme 3).[10]

To test catalytic transfer hydrogenolysis of Cbz-derivative **14**, Pearlman catalyst and cyclohexene were applied. Deprotection of **14** to free uridine **12** occurred in 20 hours at room temperature. Felix and coworkers observed beneficial influence of 1,4-cyclohexadiene over cyclohexene in deprotection of peptides.[11] We applied 1,4-cyclohexadiene as a source of hydrogen in our reaction and hydrogenolysis of **14** required 2 hours for complete deprotection (Table 1).

Uridine derivative **16** enabled addition reaction to 3,4,6-tri-O-benzyl-D-glucal (**4**) to be performed at 5-OH according to the described



SCHEME 3 Reagents and conditions: (a) TrCl, pyridine, room temperature, 4 days, (b) CbzCl, DIPEA, CH_2Cl_2, room temperature, 20 hours, (c) 40% HBF_4, MeCN, room temperature, 1 hour, (d) 20% $Pd(OH)_2/C$, cyclohexene, MeOH, reflux, 15 minutes or room temperature, 20 hours, (e) 20% $Pd(OH)_2/C$, 1,4-cyklohexadiene, MeOH, room temperature, 2 hours.

procedure (Scheme 1).[1] With the aid of ^1H NMR and ^{13}C NMR we have estimated that desirable α-product **8** was contaminated with small amount of β-anomer (<5%). Subsequently the total deprotection of **8** without the reduction of the 5,6 double bond of uridine was carried out. As anticipated, *N*-Cbz and *O*-Cbz protected nucleoside and *O*-Bn protected 2-deoxy-*D*-glucopyranose could be totally deblocked by transfer catalytic hydrogenolysis on Pearlman's catalyst affording title compound **3**. It was found that 1,4-cyclohexadiene as a hydrogen donor was more effective than cyclohexene. 1,4-cyclohexadiene was applied at room temperature[12] while cyclohexene under reflux in methanol (Table 1). It is noteworthy that the yield of deprotection was significantly better in the case of the reaction carried out at room temperature, since thermal decomposition of **8** may

TABLE 1 Reaction conditions for the hydrogenolysis on Pearlman's catalyst

Entry	Substrate	Product	Hydrogen donor	Reaction time	Temperature
1	**5**	**10**	cyclohexene	3 hours	reflux
			1,4-cyclohexadiene	3 hours	reflux
2	**5**	**11**	HCO_2NH_4[a]	4 hours[b]	reflux
3	**6**	**7**	cyclohexene	3 hours	reflux
4	**8**	**3**	cyclohexene	15 minutes	reflux
			1,4-cyclohexadiene	20 hours	room temerature
5	**9**	**12**	cyclohexene	3 hours	reflux
6	**14**	**12**	cyclohexene	20 hours	room temerature
			cyclohexene	15 minutes	reflux
			1,4-cyclohexadiene	2 hours	room temperature

[a]When 10% Pd/C instead of $Pd(OH)_2/C$ was applied better conversion was observed.
[b]Reaction not completed.

occur at elevated temperatures (90% compared with 75% in reflux). All new compounds **3**, **7**, and **14** were purified by column chromatography and their structures were elucidated with the aid of ^{1}H and ^{13}C NMR spectroscopy data and mass spectrometry analysis.[13]

CONCLUSIONS

Synthesized tris-Cbz uridine **14** can be valuable building block in the synthesis of complex carbohydrates or oligoribonucleotides when imide moiety of uridine must be protected during the course of synthesis. Its application in the synthesis of 5′-*O*-(2-deoxy-α-D-glucopyranosyl)uridine (**3**) was performed. Simultaneous removal of *N*-Cbz, *O*-Cbz, and *O*-Bn groups in compound **8** was accomplished by catalytic transfer hydrogenolysis on Pearlman's catalyst in the presence of 1,4-cyclohexadiene (room temperature) or cyclohexene (reflux) affording title compound **3** without side reactions.

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10. Procedure for the carbamoylation of uridine (Scheme 3): Trityl chloride (1.37 g, 4.91 mmol) was added to a solution of uridine (1.00 g, 4.09 mmol) in pyridine (5 mL) and reaction mixture was kept at room temperature. After 4 days, the reaction mixture was diluted with CH_2Cl_2 (300 mL), washed with water (2 × 100 mL), the organic layer was separated, dried ($MgSO_4$), concentrated in vacuo and co-evaporated with toluene (3 × 100 mL). Crude 5′-*O*-trityluridine (**13**) was dissolved in CH_2Cl_2 (15 mL), DIPEA (4.05 ml, 24.54 mmol) was added and solution was cooled to 10°C. A CbzCl (3.50 mL, 24.54 mmol) was added dropwise and the mixture was refluxed for 0.5 hour. The mixture was diluted with CH_2Cl_2 (100 mł) and washed with 1 M HCl and then water. The organic phase was dried ($MgSO_4$) and concentrated. The residue was dissolved in acetonitrile (10 mL) and 48% HBF_4 (0.65 mL, 4.09 mmol) was added. The reaction mixture was kept at room temperature for 1 hour, neutralised with $NaHCO_3$, dried ($MgSO_4$), concentrated and purified on a column of silica gel 60 (70–230 mesh) developed with the hexane/AcOEt 7:1 → 1:1 (v/v) solvent system to yield **14** (1.41 g, 58%) as a first eluting fraction (R_F toluene/AcOEt 1:1: 0.57) and **15** (0.46

g, 22%) as the second fraction (R_F: 0.17). Analytical data for **15** are in accordance with Johnson and Widlanski.[4]

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12. Procedure for final O-Cbz, N-Cbz and O-Bn deprotection (Scheme 1, procedure d): To a solution of **8** (109 mg, 0.10 mmol) in MeOH (10 mL) 1,4-cyclohexadiene (0.6 mL) and Pearlman's catalyst (75 mg) were added. Reaction mixture was stirred at room temperature overnight. After removal of the catalyst by filtration crude product were purified by column chromatography with $CHCl_3$/MeOH 10:1 → 5:1 (v/v) solvent system to yield **3** (35 mg, 90%) as a white solid. Compound **3** was prepared using protected uridine **14** and glucal **4** according to previously described procedure.[1]
13. Analytical data for new compounds: 5′-*O*-(2-deoxy-a-D-glucopyranosyl)uridine, **3**: solid; $[\alpha]_D^{20}$+36.0 (MeOH, c 1.0); ^{1}H NMR (300 MHz, CD_3OD): δ 1.70 (ddd, 1H, J 3.5, 11.8, 13.2 Hz, H-2″ax), 2.09 (ddd, 1H, J 1.1, 5.0, 13.2 Hz, H-2″eq), 3.26 (t, 1H, J 9.0 Hz, H-4″), 3.53 (ddd, 1H, J 2.3, 5.6, 9.0 Hz, H-5′), 3.67–3.74 (m, 2H, H-6″a,b), 3.79 (ddd, J 5.0, 9.0, 11.8 Hz, H-3″), 3.83 (dd, 1H, J 2.3, 11.5 Hz, H-5′a), 3.94 (dd, 1H, J 2.6, 11.5 Hz, H-5′b), 4.12–4.19 (m, 3H, H-2′, 3′, 4′), 4.99 (br d, 1H, J 3.2 Hz, H-1″), 5.80 (d, J 8.2 Hz, H-5), 5.88 (d, 1H, J 4.1 Hz, H-1′), 7.98 (d, 1H, J 8.2 Hz, H-6); ^{13}C NMR: δ 38.86 (C-2″), 62.87, 67.11, 69.99, 71.32, 73.21, 74.53, 76.12, 84.46 (C-2′, C-3′, C-4′, C-5′, C-3″, C-4″, C-5″, C-6″), 90.91 (C-1′), 98.84 (C-1″), 102.60 (C-5), 142.03 (C-6), 152.37 (C-2), 166.19 (C-4); ESI-HRMS: Calcd for $C_{15}H_{22}N_2O_{10}Na$ ([M+Na]$^+$): m/z 413.1167, found: m/z 413.1150. N^3-benzyl-2′,3′-*O*-isopropylidene-5′-(2-deoxy-α-D-glucopyranosyl)uridine, **7**: solid; $[\alpha]_D^{20}$+27.3 (MeOH, c 0.5); ^{1}H NMR ($CDCl_3$): δ 1.35; 1.54 (2s; 6H; C($\underline{CH_3}$)$_2$); 1.61 (ddd, 1H, J 3.7, 12.0, 13.4 Hz; H-2″ax), 1.98 (ddd, 1H, $J \approx 0$, 5.2, 13.4 Hz, H-2″eq), 3.22 (t, 1H, J 9.0 Hz, H-4″), 3.44–3.91 (m, 6H, H-5′a,b, H-3″, H-5″, H-6″a,b), 4.43 (m, 1H, H-4′), 4.82–4.86 (m, 2H, H-2′, H-3′), 4.91 (d, 1H, J 2.9 Hz, H-1″), 5.02–5.14 (qAB, 2H, N-$\underline{CH_2}$Ph), 5.83 (d, 1H, J 8.1 Hz, H-5), 5.86 (d, 1H, J 1.7 Hz, H-1′), 7.19–7.38 (m, 5H, Ph), 7.79 (d, 1H, J 8.1 Hz, H-6); ^{13}C NMR: d 25.55, 27.57 (C($\underline{CH_3}$)$_2$), 38.63 (C-2″), 45.14 (N-$\underline{CH_2}$Ph), 62.79 (C−5′), 68.22, 69.84, 73.13, 74.58 (C-3″, C-4″, C-5″, C-6″), 82.59, 86.78, 87.29 (C-2′, C-3′, C-4′), 95.47 (C-1′), 98.87 (C-1″), 101.77 (C-5), 114.94 ($\underline{C}$(CH$_3$)$_2$), 128.55, 129.27, 129.49 (Ph), 138.14 (Ph$_q$), 141,50 (C-6), 152.29 (C-2), 164.91 (C-4), ESI-HRMS: Calcd for $C_{25}H_{32}N_2O_{10}Na$ ([M+Na]$^+$): m/z 543.1949, found: m/z 543.1968. N^3,2′,3′-*O*-tris-(benzyloxycarbonyl)uridine, **14**: oil; $[\alpha]_D^{20}$-22.3 ($CHCl_3$, c 3.0); ^{1}H NMR: δ 2.78 (dd, 1H, J 3.6, 5.9 Hz, 5-OH), 3.78 (ddd, 1H, J 1.8, 5.9, 12.1, Hz, H-5′a), 3,89 (m, 1H, H-5′b), 4.25 (m, 1H, H-4′), 5.04–5.42 (m, 6H, 3$\underline{CH_2}$Ph), 5.39 (dd, 1H, J 3.5, 5.7 Hz, H-2′), 5.48 (dd, 1H, J 5.7, 5.7 Hz, H-3′), 5.76 (d, 1H, J 8.2 Hz, H-5), 5.95 (d, 1H, J 5.7 Hz, H-1′), 7.25–7.44 (m, 15H, Ph), 7.64 (d, J 8.2 Hz 1H, H-6), ^{13}C NMR: d 61.85 (C-5′), 70.69, 70.87 (2PhCH$_2$OC(O)O), 71.78 (PhCH$_2$OC(O)N), 74.24, 75.92 (C-2′, C-3′), 83.34 (C-4′), 89.02 (C-1′), 102.88 (C-5), 128.65–129.20 (Ph), 133.84, 134.70, 134.78 (Ph$_q$), 140.68 (C-6), 148.56 (N-C(O)O), 149.89 (C-2), 154.03, 154.36 (2O-C(O)O), 160.30 (C-4); ESI-HRMS: Calcd for $C_{33}H_{30}N_2O_{12}Na$ ([M+Na]$^+$): m/z 669.1691, found: m/z 669.1687.