Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 2433–2435

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis of Glycosyl Phosphoramidates: Novel Isosteric Analogues of Glycosyl Phosphates

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Received 1 February 2001; accepted 23 June 2001

Abstract—Several newer isosteric analogues of glycosyl phosphates, namely of glycosyl phosphoramidates, were synthesized in good yields using Staudinger reaction of their corresponding azides with trimethyl phosphite followed by de-*O*-acetylation. The structure and conformation of the fully protected analogue synthesized, namely 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl bismethoxyphosphoramidate, was established by X-ray crystallography.

Glycosyl phosphates play a central role in carbohydrate metabolism.[1] Nature employs them as glycosyl donors in the biosynthesis of oligo- and polysaccharides and glycoconjugates. *N*-Acetyl-α-D-glucosamine 1-phosphate, for example, is the key intermediate in the biosynthesis of *N*-glycoproteins,[2] which are involved in many cell–cell and cell–pathogen recognition phenomena. α-D-Glucose 1-phosphate, on the other hand, is formed by phosphorylase catalyzed cleavage of glycogen.[3] Synthesis of isosteric analogues is a useful endeavor for the better understanding of the enzymatic pathways involving such glycosyl phosphates. More importantly, synthesis of potent analogues that could regulate the metabolism would lead to the rational development of carbohydrate-based therapeutics.

Literature survey on the synthesis of isosteric analogues of glycosyl phosphates reveals several reports[4] on the replacement of the O–P bond by a C–P bond. The multistep synthesis of the phosphonate analogues of α- and β-D-glucose 1-phosphate[4e] has been achieved in an overall yield of 20–25%. Despite the elegant methodologies developed for the phosphonate analogues, the replacement of glycosidic oxygen by methylene group did not increase but rather decreased the affinity, resulting in poor inhibition.[4c] In view of the better electronic distribution and H-bonding capacity of NH group, glycosyl phosphoramidates are expected to be potent isosteric inhibitors.[5] We describe, herein, a two-step synthesis of novel glycosyl phosphoramidates starting from the corresponding per-*O*-acetylated glycosyl azides.

The general methodology employed for the synthesis of glycosyl phosphoramidates utilizes the Staudinger reaction of per-*O*-acetylated β-D-glycosyl azides with trimethyl phosphite (Scheme 1) followed by de-*O*-acetylation. The protected glycosyl azides (**1**–**4**) were prepared following the literature procedure[6] and employing benzyltriethylammonium tetrafluoroborate as the phase transfer catalyst.

In a typical procedure, 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl azide (**1**) was reacted with trimethyl phosphite in dichloromethane at room temperature for 3 h to afford a single product. The same was isolated in 93% yield after the removal of trimethyl phosphite and dichloromethane followed by recrystallization of resultant syrup from a mixture of ethyl acetate and hexane (4:1). The NMR data of this product were entirely consistent with the structure of bismethoxyphosphoramidate **5**.[11] The β-anomeric linkage was confirmed by the appearance of a multiplet in the ^{1}H NMR spectrum at 4.54 ppm which on D_2O exchange collapsed to a triplet with a *J* value of 8.5 Hz, revealing the large H-1–H-2 vicinal coupling and the long-range H-1–P coupling. The fully decoupled ^{31}P NMR spectrum displayed a single signal at 7.2 ppm. The MALDI-FT mass

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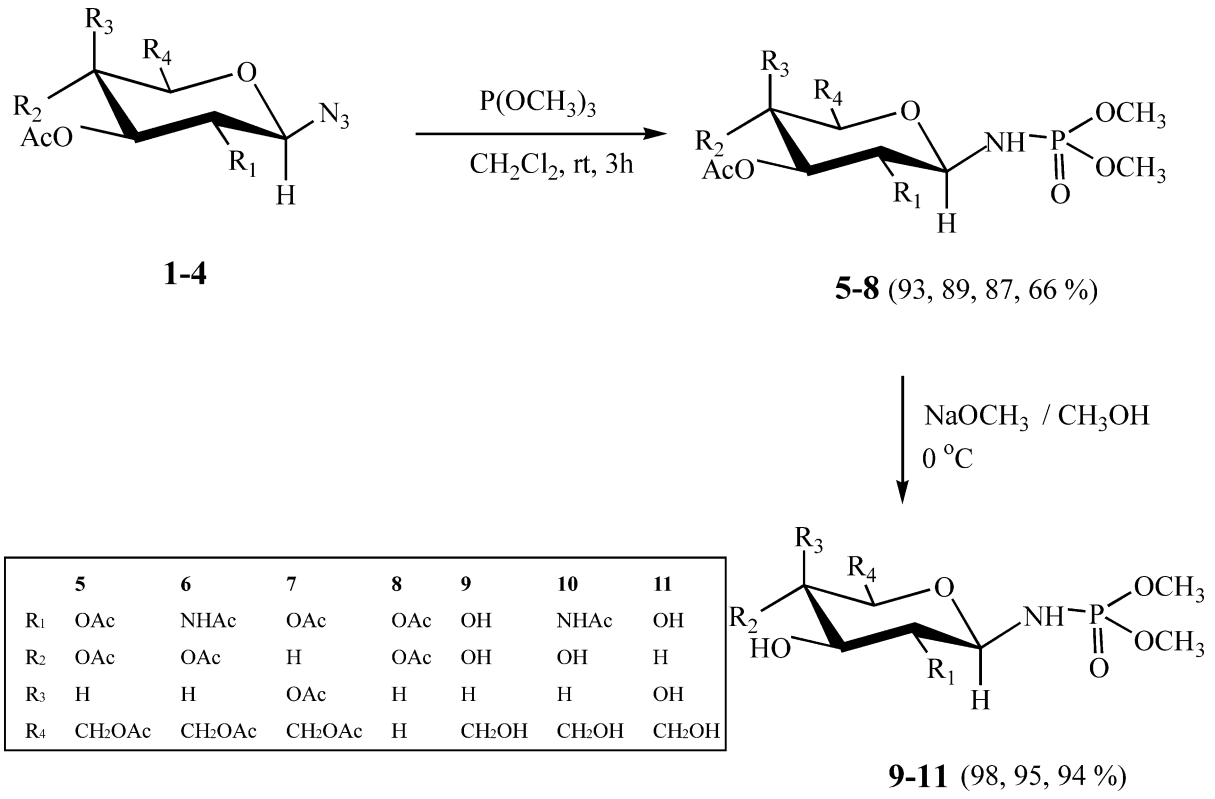
0960-894X/01/$ - see front matter
PII: S0960-894X(01)00469-3



	5	6	7	8	9	10	11
R_1	OAc	NHAc	OAc	OAc	OH	NHAc	OH
R_2	OAc	OAc	H	OAc	OH	OH	H
R_3	H	H	OAc	H	H	H	OH
R_4	CH_2OAc	CH_2OAc	CH_2OAc	H	CH_2OH	CH_2OH	CH_2OH

Scheme 1.

spectrum displayed a molecular ion $[M+Na]^+$ peak at *m/z* 478.1088, fully supporting the structure. The other per-*O*-acetylated glycosyl phosphoramidates synthesized and their yields are shown in Scheme 1.

In order to establish the structure unambiguously and also to gain knowledge of the three-dimensional structure, single crystal X-ray crystallographic analysis[7] of the novel glucosyl analogue, **5**, was carried out. The structure was solved in the space group $P2_12_12_1$. The selected geometrical parameters are given in Table 1. The ORTEP diagram of **5** with the numbering of atoms is shown in Figure 1. The dimensions of the glycosidic bond, namely distances C-1–N-1, N-1–P-1 and O-5–C-1 and angle C-1–N-1–P-1, turned out to be 1.429(5), 1.626(3) and 1.429(4) Å and 122.3(3)° as compared to the values of 1.37 (C-1–O-1), 1.59 (O-1–P-1) and 1.46 Å (O-5–C-1) and 124° (C-1–O-1–P-1) reported for dipotassium glucose-1-phosphate dihydrate.[8] The C-1–N-1 bond length in **5** agrees well with a value of 1.431(4) Å reported for β-1-*N*-acetamido-D-glucopyranose,[9] an inhibitor of glycogen phosphorylase[10] with a K_i of 0.032 mM.

The ORTEP diagram (Fig. 1) reveals the $^4C_1$ conformation of the pyranose ring in **5**. The torsion angles, particularly H-1–C-1–N-1–H(N-1) and H(N-1)–N-1–P-1–O-11 of −168.85 and −179.51°, clearly establish the *anti-Z* conformation of the phosphoramidate group with respect to the *N*-glycosidic linkage. The *anti-Z* conformation has also been observed in the case of β-1-*N*-acetamido-D-glucopyranose. The orientation of the C-6 acetoxy group is *gauche (gg)*. The molecular packing is stabilized by a strong intermolecular N–H···O11 hydrogen bond with an H···O11 distance of 2.05 Å and an angle of 168.8°. To the best of our knowledge, this is the first report on the crystal structure of any glycosyl phosphoramidate.

De-*O*-acetylation of compound **5** using NaOMe/MeOH afforded the corresponding free phosphoramidate (**9**) in quantitative yield. The ^1H NMR spectrum showed a triplet at 4.29 ppm with a coupling constant of 8.8 Hz. The fully decoupled ^{31}P NMR spectrum showed a single signal at 10.6 ppm.[11] The high resolution FAB mass

Table 1. Selected geometrical parameters for 2,3,4,6-tetra-*O*-acetyl-β-glucopyranosyl bismethoxyphosphoramidate (**5**)

Bond lengths (Å)	
C-1–N-1	1.429(5)
O-5–C-1	1.429(4)
N-1–P-1	1.626(3)
P-1–O-11	1.457(3)
P-1–O-12	1.575(4)
Bond angles (°)	
O-5–C-1–N-1	109.8(3)
C-2–C-1–N-1	110.6(3)
C-1–N-1–P-1	122.3(3)
N-1–P-1–O-11	111.96(18)
N-1–P-1–O-12	110.6(2)
Torsion angles (°)	
O-5–C-1–N-1–P-1	−81.0(4)
C-2–C-1–N-1–P-1	158.1(3)
C-1–N-1–P-1–O-11	−26.4(4)
C-1–N-1–P-1–O-12	97.5(3)
O-5–C-5–C-6–O-6	−66.9(4)
C-4–C-5–C-6–O-6	54.4(5)

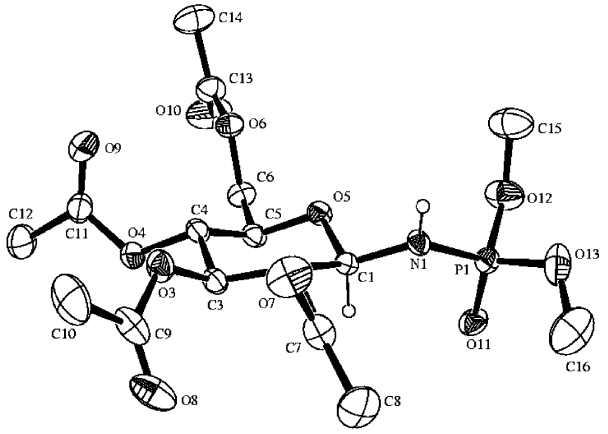


Figure 1. The ORTEP diagram of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl bismethoxyphosphoramidate (**5**); ellipsoids are set at 30% probability.

spectrum gave a molecular ion $[M+Na]^+$ peak at *m*/*z* 310.0675 which fully supported the identity of free glucosyl phosphoramidate. The de-*O*-acetylation of **6** and **7** also proceeded smoothly affording the respective free derivative as the single product, while the xylosyl analogue **8** furnished a complex mixture under NaOMe/MeOH as well as Na_2CO_3/MeOH conditions.

Considering the minimum number of steps involved and the good yields obtained, the extension of the above methodology to the synthesis of phosphoramidate analogues of other biologically important glycosyl phosphates, their crystallographic analysis and biological evaluation would prove to be very useful towards the development of carbohydrate-based drugs. Further efforts in this direction are in progress in our laboratory.

Acknowledgements

Financial support received from CSIR, New Delhi is gratefully acknowledged. We are very grateful to Professor M. N. S. Rao, Dept. of Chemistry, IIT Madras for the stimulating discussions, RSIC, IIT Madras for NMR data and Dr. Kasi V. Somayajula, University of Pittsburgh, USA for mass spectral data.

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7. X-ray diffraction data were collected at room temperature in the ω–2θ scan mode on an Enraf–Nonius CAD4 diffractometer. The crystal structure was solved by the direct methods using SHELXS-97 and refined by a full matrix least-squares method using SHELXL-97.
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11. Characteristics are given for a representative example:

5: Needles [from ethyl acetate–hexane (2:3) mixture]; mp 140–142 °C; $[\alpha]_D$ +20° (*c* 0.5, water); IR (KBr) ν_{max}: 3424(s), 1738(w), 1584(s), 1354(m), 1238(m), 1040(m), 835(w), 771(w) cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 5.26 (t, 1H, *J* = 9.5 Hz), 5.04 (t, 1H, *J* = 9.8 Hz), 4.88 (t, 1H, *J* = 9.5 Hz), 4.51–4.58 (m, 2H, H-1 and D_2O exchangeable NH), 4.24 (dd, 1H, *J* = 4.9, 12.2 Hz, H-6_a), 4.13 (dd, 1H, *J* = 2.4, 12.2 Hz, H-6_b), 3.70–3.75 (m, 7H, 2×OCH_3 and H-5), 2.02, 2.04, 2.07, 2.08 (s each, 12H, $OCOCH_3$); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.5 ($COCH_3$), 169.9, 170.4, 169.5, 82.5 (C-1), 73.1, 72.7, 71.4, 68.3, 61.9 (C-6), 53.3 (OCH_3), 53.2 (OCH_3), 20.6 ($COCH_3$), 20.6, 20.5; ^{31}P NMR ($CHCl_3$, 162 MHz) δ 7.2 ppm; MALDI-FTMS for $C_{16}H_{26}O_{12}NP$ $[M+Na]^+$: calcd 478.1085, found 478.1088.

9: Syrup, $[\alpha]_D$ +25.7° (*c* 0.5, DMSO); IR (neat) ν_{max}: 3348(b), 2919(w), 1644(m), 1464(s), 1352(w), 1224(s), 1038(s), 887(w), 841(w), 777(w) cm^{-1}; ^1H NMR (D_2O, 400 MHz) δ 4.29 (t, 1H, *J* = 8.8 Hz, H-1), 3.86 (dd,1H, *J* = 2.0, 12.2 Hz, H-6_a), 3.77 (d, 3H, *J* = 11.2 Hz, OCH_3), 3.74 (d, 3H, *J* = 11.2 Hz, OCH_3), 3.70 (dd, 1H, *J* = 4.9, 12.2 Hz, H-6_b), 3.40–3.57 (m, 3H), 3.24 (t, 1H, *J* = 8.8 Hz); ^{13}C NMR (D_2O, 100 MHz) δ 86.3 (C-1), 79.9, 79.2, 76.0, 72.2, 63.4 (C-6), 56.8 (OCH_3), 56.5 (OCH_3); ^{31}P NMR (H_2O, 162 MHz) δ 10.6 ppm; FAB-MS for $C_8H_{18}O_8NP$ $[M+Na]^+$: calcd 310.0668, found 310.0675.