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Note

An efficient synthesis of GDP-hexanolamine, a key tool for the purification of fucosyltransferases

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Abstract

A new efficient synthesis of GDP-hexanolamine from hexanolamine is reported with an overall yield of 71%. The pyrophosphate formation, the key step of this preparation, was achieved through a sequential GMP activation procedure based on polytrifluoroacetylation of GMP followed by activation of the phosphate group by 1-methylimidazole. © 2002 Published by Elsevier Science Ltd.

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1. Introduction

Functionalization of nucleoside diphosphates (NDP) has found many important applications such as synthesis of enzyme inhibitors, mechanistic probes preparation, lipid derivatization for the 2D-crystallization of proteins as well as solid support functionalization for the purification of proteins. NDP-hexanolamine derivatives have been synthesized for the derivatization of chromatography phases such as sepharose to efficiently purify NDP binding proteins. For instance, high purity levels of glycosyltransferases can be achieved through NDP-functionalized sepharose-affinity chromatography.[1–3] Indeed the highest purity degree of purified protein is considered a crucial parameter for protein crystallization studies. This was recently exemplified by the purification and the crystallization of two galactosyltransferases, the retaining α-(1→3)-GalT and the inverting β-(1→4)-GalT1, both purified from UDP-hexanolamine functionalized columns.[4,5] To date, no three-dimensional structure of fucosyltransferase has been described in the literature. Their purification requires GDP-hexanolamine **1**, which is not commercially available, for the covalent derivatization of affinity columns. The synthesis of GDP-hexanolamine has been described in 1996 with an overall yield of 22% starting from 6-aminohexanol phosphate.[6] Here we report a synthesis of the title compound **1** starting from hexanolamine with an overall yield of 71%.

2. Results and discussion

The most common method for the preparation of functionalized NDPs at the β phosphate moiety consists in the coupling of an activated monophosphate (usually the morpholidate or the imidazolidate of NMPs)[7,8] with a lipophilized monophosphate (such as a carbohydrate-1-phosphate trialkylammonium salts), catalyzed by acidic azoles (usually 1-*H*-tetrazole).[9] The previously reported synthesis of GDP-hexanolamine suffers from a low-yielding coupling step between GMP-morpholidate and the 2,2,2-trifluoroacetamide (TFA) derivative of 6-aminohexanol phosphate **3**. The fact that coupling reactions involving GMP-morpholidate usually give lower yields than with other NMP-morpholidates may explain the difficulties encountered in GDP-hexanolamine preparation. Moreover, the synthesis of **1** addresses the general problem of preparing amine-con-

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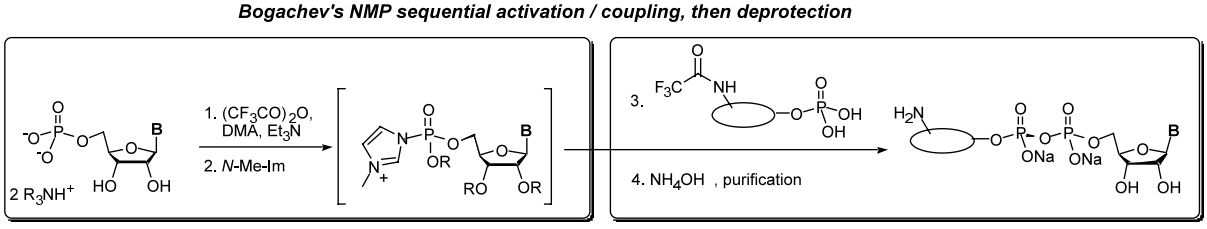
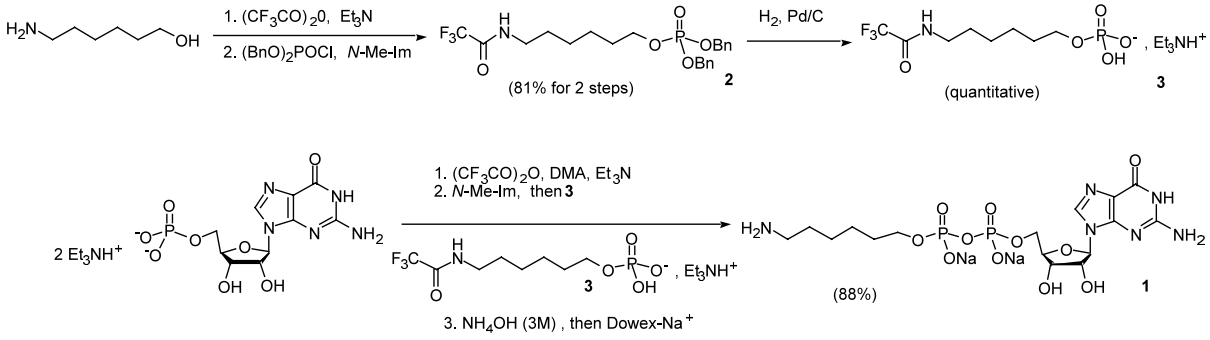


Scheme 1. General strategy for the one-pot synthesis of amine-containing nucleoside diphosphate derivatives.



Scheme 2. Synthesis of GDP-hexanolamine.

taining NDP analogues, which could find interesting extrapolations for instance for the synthesis of transition state analogues of glycosyltransferase substrates. Thus, we explored new procedures to improve the overall yield, and in particular, the coupling step between GMP and the *N*-TFA-6-aminohexanol phosphate **3**. Recently, Bogachev described an efficient new methodology for the activation of nucleoside monophosphates (NMPs) to synthesize nucleoside triphosphates (NTPs) on a large scale and with high yields.[10] We adapted this methodology to the synthesis of the title compound using the sequence in Scheme 1.

As previously described, the trifluoroacetamide *N*-protection can be maintained during the coupling step and removed at the very last step of this synthetic strategy, without degradation of the pyrophosphate unit.[6] The phosphate **3**, required for the coupling to GMP, was obtained via a straightforward sequence involving a selective *N*-acylation allowing a regiospecific phosphorylation yielding the dibenzyl phosphate **2**, followed by a quantitative benzyl hydrogenolysis (Scheme 2).

Then we applied the Bogachev's GMP activation procedure and adapted it for the coupling step. This methodology, which proved very efficient from our first attempts, is based on the quick solubilization and phosphate trifluoroacetylation of GMP in dry acetonitrile, followed by phosphate activation by an excess of 1-methylimidazole. In optimized conditions, we determined that six equivalents of trifluoroacetic anhydride were required (along with six base equivalents) instead of the five equivalents described in Bogachev's dGTP synthesis. The activated GMP intermediate was then allowed to react with phosphate **3** in anhydrous acetonitrile. The reaction was monitored by TLC and ^{31}P NMR of aliquots which showed that after 3 h the phosphate **3** was completely converted into the desired pyrophosphate. Removal of the trifluoroacetyl protective group was performed on the crude reaction mixture before the final purification by size-exclusion chromatography. The excellent 88% yield obtained for the coupling/deprotection sequence allowed a facile purification of the final pyrophosphate **1**. GDP-hexanolamine **1** has then been covalently linked to a Sepharose phase following standard procedure (data not shown).[11,12]

The procedure described in this study proved far superior to the standard morpholidate couplings and might find broad applications for the synthesis of amine-containing NDP-sugar analogues such as cationic mimics of nucleotide-sugars.[13†] This method could also be easily generalized to the synthesis of other nucleotide-hexanolamine derivatives such as UDP-, TDP-, CDP-, and ADP-hexanolamine, useful to purify proteins interacting with nucleotides.

3. Experimental

All chemicals were purchased from Fluka or Aldrich and used without further purification. Acetonitrile was

† Recently a new efficient synthesis of UDP-Galf based on Bogachev's activation/coupling procedure was described.

distilled over CaH_2 and CH_2Cl_2 over P_2O_5. NMR spectra were recorded on Bruker AC-250 or AMX-400 apparatus.

GMP-salt preparation.—GMP disodium salt was dissolved in distilled water ($c = 0.5$ mol l^{-1}) and mixed with 4 wt equiv of Dowex WX8-200, H^+ form, and shaked for 1 h. After filtration, the resin was abundantly washed with distilled water. 2 equiv of triethylamine in EtOH were added to the GMP aqueous solution and concentrated to dryness under diminished pressure. The residual crystalline white powder was dried further under high vacuum and used as such for the coupling experiments.

GDP-hexanolamine, disodium salt (**1**)[6]

GMP activation. GMP·2 Et_3NH^+ (120 mg, 2.38 mmol) was suspended under an argon atmosphere into 3 mL of freshly distilled MeCN. At 0 °C, 120 μL of *N*,*N*-dimethylaniline (9.4×10^{-4} mol, 4.0 equiv) and 50 μL of triethylamine (4.5×10^{-4} mol, 1.9 equiv) were added. A precooled solution of 200 μL of trifluoroacetic acid anhydride (1.4×10^{-3} mol, 5.9 equiv) in 1 mL anhyd MeCN were added dropwise over 5 min. The solution turned red–brown and rapidly homogenized. The reaction mixture was stirred for 15 min at 0 °C, concentrated under diminished pressure, and dried for 10 min under diminished pressure (oil pump). The polyacylated GMP was then dissolved into 3 mL anhyd MeCN. At 0 °C, 100 μL of 1-methylimidazole (1.25 mmol, 5.3 equiv), as well as 130 μL of triethylamine (1.2 mmol, 5.0 equiv) were added. A bright yellow color then appeared.

Coupling to the phosphate **3**. To the preceding solution were added 200 mg of preactivated 4 Å molecular sieves. A solution of 6-trifluoroacetamido-hexanol 1-phosphate triethylammonium salt **3** (54 mg, 1.5×10^{-4} mol, 0.63 equiv) in 1 mL anhyd MeCN was then added dropwise (over 1 min) into the reaction mixture. The mixture was stirred for 1 h at 0 °C under argon, then for 2 h at rt. The reaction could be monitored by TLC (10:11:4 $CHCl_3$–MeOH–AcO^-,NH_4^+ (1 M, pH 7)) and by ^{31}P NMR. After filtration through Celite, the reaction was quenched by 15 mL distilled water. The final mixture was extracted three times with 15 mL CH_2Cl_2. The combined organic phases were washed once by 10 mL of water. The aqueous phases were combined and concentrated under diminished pressure.

Trifluoroacetamide deprotection. The crude reaction mixture was dissolved in 20 mL NH_4OH (3 M) and stirred for 4 h at rt. After concentration by rotary evaporation, the crude mixture was dissolved in 5 mL of water and shaked with 1 g of Dowex 200WX8 (Na^+ form) for 1 h.

Purification. The crude mixture was dissolved in 2 mL of water and passed through a Sephadex G25 column (2.5 × 26 cm) using an FPLC apparatus (Äkta, Pharmacia Biotech). Chromatography conditions: the eluant was water with a flow-rate of 1 mL min^{-1}. Samples containing guanosine derivatives were detected by a UV-lamp (254 nm). Under these conditions the final GDP-hexanolamine came out first from the column, followed by GMP. Fractions containing the title compound were collected and freeze-fried yielding 141 mg (2.1×10^{-4} mol, 88% yield) of pure GDP-hexanolamine as a white viscous solid.

NMR: ^1H (400 MHz, D_2O): δ 8.29 (s, 1 H, H-8), 5.77 (d, *J* 6.0 Hz, 1 H, H-1′), 4.36 (dd, *J* 3.5, *J* 5.1 Hz, 1 H, H-3′), 4.18 (m, 1 H, H-4′), 4.04 (m, 2 H, H-5′), 3.69 (q, *J* 6.7 Hz, 2 H, CH_2–OP), 2.77 (t, *J* 7.3 Hz, 1 H, CH_2–NH_2), 1.6–0.9 (m, 8 H); ^{31}P (101 MHz, D_2O): δ −12.88 (d, *J* 21.1 Hz), −13.55 (d, *J* 21.1 Hz); MS (ES, negative mode): *m*/*z* 541.04 [M − 1].

1-O-(Dibenzyl phosphoryloxy)-6-trifluoroacetamido-1-hexanol (**2**).—6-Trifluoroacetamido-1-hexanol[14] (1.0 g, 8.6 mmol, prepared by following a known procedure[15]) was dissolved in 10 mL of anhyd CH_2Cl_2 (solution A). In a separate flask was added, dropwise at 0 °C, 2.3 mL of oxalyl chloride (26.1 mmol, 3.0 equiv) into a solution of 3.6 g of dibenzylphosphate (12.9 mmol, 1.5 equiv) and 10 μL anhyd DMF in 60 mL anhyd CH_2Cl_2. The reaction mixture was then stirred for 1 h under Ar. The crude mixture was concentrated under diminished pressure, diluted in 60 mL anhyd toluene and concentrated again. The resulting oil was dissolved in 40 mL anhyd CH_2Cl_2 and slowly added at 0 °C to solution A (6-trifluoroacetamido-1-hexanol in 10 mL anhyd CH_2Cl_2) containing 1.2 mL of 1-methylimidazole (15.0 mmol, 1.7 equiv). The solution was stirred 1 h at 0 °C, then overnight at rt. The final solution was washed once with HCl 1 N (25 mL) and once with 25 mL satd $NaHCO_3$, dried over $MgSO_4$, and concentrated to dryness under diminished pressure. The residue was chromatographed on silica gel (gradual elution 2:1 to 3:2 cyclohexane–EtOAc) to yield 3.3 g (81% over two steps) of the pure title compound (white solid).

NMR ^1H (250 MHz, $CDCl_3$): δ 7.50–7.35 (m, 10 H, Ar), 5.12 (ABX system, J_{AB} 13.4, $J_{AP} = J_{BP}$ 11.8 Hz, 4 H, $CH_2\phi$), 4.07 (q, *J* 7.3 Hz, 2 H, CH_2–OP), 3.40 (q, *J* 6.5 Hz, 2 H, CH_2–NH_2), 1.65 (hept., *J* 6.7 Hz, 4 H), 1.40 (m, 4 H); ^{13}C (50 MHz, $CDCl_3$): δ 157.4 (q, *J* 36.4 Hz, C=O), 135.80, 135.67, 129.12, 128.52, 128.35, 128.10, 127.67, 127.45, 116.05 (q, *J* 286 Hz, CF_3), 69.30 (d, *J* 5.6 Hz, $CH_2\phi$), 67.63 (d, *J* 5.6 Hz, $CH_2\phi$), 62.43, 39.58, 29.90, 28.57, 25.83, 24.79; ^{31}P (101 MHz, D_2O): δ −0.70 (s); MS (DCI-NH_3): *m*/*z* 491 [M + 18].

6-Trifluoroacetamido-hexanol 1-phosphate triethylammonium salt (**3**)[6,16].—Phosphate **2** (210 mg 4.44×10^{-4} mol) was dissolved in 4 mL MeOH and 3 mL MeOAc. Triethylamine (60 μL, 1.0 equiv) and 49 mg Pd/C (10%) were added and the resulting suspension was vigorously stirred overnight under H_2 (0.1 HPa). The final mixture was filtrated through a short pad of Celite. The filtrate

was concentrated to dryness under diminished pressure to give the pure phosphate **3** as a white solid (quantitative yield).

NMR ^{1}H (250 MHz, D_2O): δ 3.78 (q, J 6.4 Hz, 2 H, CH_2–OP), 3.15 (t, J 6.9 Hz, 2 H, CH_2–NH), 3.06 (q, J 7.3 Hz, 6 H, Et_3NH^+), 1.48 (m, 4 H), 1.20 (m, 13 H); ^{13}C (50 MHz, 2:1 CD_3OD–$CDCl_3$) δ 158.1 (q, J 36.6 Hz, C=O), 116.48 (q, J 285 Hz, CF_3), 64.28 (CH_2–OP), 48.59 (Et_3NH^+), 40.00 (CH_2–NH), 30.57, 28.86, 26.55, 25.45, 8.81 (Et_3NH^+); ^{31}P (101 MHz, D_2O): δ −1.70 (s).

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