Pergamon

Tetrahedron Letters 43 (2002) 4017–4020

TETRAHEDRON LETTERS

Synthesis of mannose-6-phosphate analogs: large-scale preparation of isosteric mannose-6-phosphonate via cyclic sulfate precursor

Nikolai A. Khanjin and Jean-Louis Montero*

Laboratoire de Chimie Biomoléculaire, UM2, ENSCM, 8 Rue de l'Ecole Normale, 34296 Montpellier Cedex 5, France

Received 14 March 2002; accepted 11 April 2002

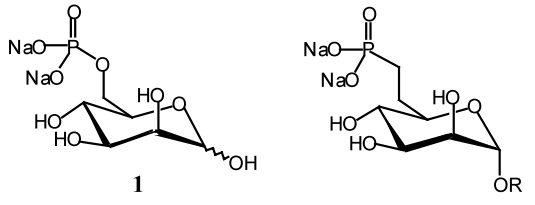
Abstract—A scale-up synthesis of isosteric phosphonate analog of mannose-6-phosphate (M6P) is presented via regioselective nucleophilic displacement of the protected 4,6-cyclic sulfate precursor by dialkyl lithiomethylphosphonates in the key step. The five-step synthesis requires only simple purification procedures after each step excluding chromatography separations. The usage of proper nucleophiles makes the method also applicable for the preparation of other isosteric M6P analogs, e.g. α,β-unsaturated phosphonate, sulfonate, etc., with the potential to expand the method for analogs of other sugar-phosphates.

Mannose-6-phosphate (M6P, **1**),[1] its isosteric phosphonate **2a** and other hydrolytically stable M6P analogs have been claimed to accelerate wound healing and prevent or mitigate scar tissue formation and other fibrotic disorders[2] (Fig. 1). The phosphonate **2a** had the binding affinity approximately three times that of M6P for the M6P receptor.[2b] From several M6P analogs tested in our laboratory, phosphonate **2b** was also better recognized by the receptor[3] (Fig. 1). In addition, breast cancer cells can be selectively targeted by bioactive molecules endowed with M6P-like moieties[4] due to the increased level of the M6P receptor in breast cancer tumors.[5] However, for any further practical application of M6P analogs, their convenient scale-up synthesis is required.

Here we report a novel and efficient five-step synthesis of **2b** (Scheme 1) starting from the commercial methyl α-D-mannopyranoside (**3**) via nucleophilic displacement of the known cyclic sulfate precursor methyl 2,3-isopropylidene-α-D-mannopyranoside-4,6-sulfate (**5**)[6] with the lithiated dialkyl methylphosphonates in the key homologation step followed by the facile deprotection of sugar hydroxyls and dealkylation of phosphonate (Scheme 1).

The existing routes to **2b** by triflate displacement at C(6)[7] or by the Horner–Emmons–Wadsworth approach[3a] were only applicable for a small scale preparation due to the unpractical purification procedures for a scale-up, the cost of reagents or the length of the synthetic route complicated in particular by the protection and deprotection of sugar hydroxyls. Other general methods of synthesis of sugar phosphonates have also been considered but have not led to a practical route to **2b**.[8]

Thus, the cyclic sulfate **5** was prepared on a 100 g scale according to the modified original procedure[6a] starting

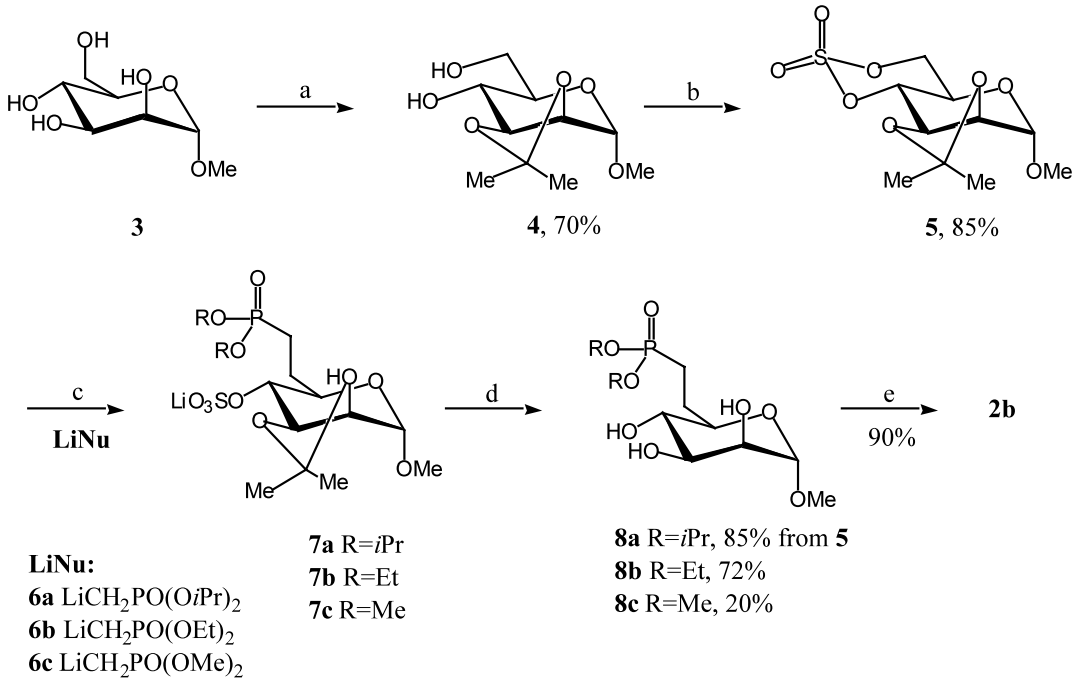


2a R=OH
2b R=OMe
2c R=$C_6H_4NHC(O)(CH_2)_2CONH$-cholesteryl

Figure 1.

* Corresponding author. Tel./fax: 33 (0)4 67 14 43 43; e-mail: montero@univ-montp2.fr

0040-4039/02/$ - see front matter
PII: S0040-4039(02)00734-7



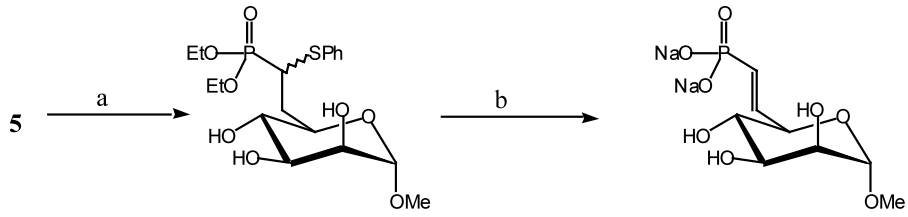
Scheme 1. *Reagents and conditions*: (a) (i) DMP, acetone, [TsOH], 3 h, (ii) H_2O; (b) (i) $SOCl_2$, Et_3N, CH_2Cl_2, 5°C, (ii) $NaIO_4$, [$RuCl_3$], $MeCN/CH_2Cl_2/H_2O$ 2:2:3; (c) HNu, BuLi (2.5 M in hexanes), DMPU or HMPA, THF, −80°C; (d) Amberlyst-15 (H^+), MeOH/THF 1:1; (e) (i) Ac_2O/Py, (ii) TMSCl/NaI/MeCN, (iii) NaOH/MeOH.

from **3** via methyl 2,3-*O*-isopropylidene-α-D-mannopyranoside (**4**).[9] In contrast to reactive protected mannose-6-triflates, solid **5** can be stored at 0°C for several months without any observable decomposition. In addition, the scale-up preparation of corresponding benzyl-protected manno-pyranoside-6-triflate used in the reported synthesis of **2b** required four steps from **3**.[7] The synthesis will be also limited by the higher cost of triflic acid derivatives.

Our initial attempts with nucleophilic substitution of **5** by lithiated dialkyl methylphosphonates **6a**–**c** showed low but observable reactivity with **5** at −80°C in THF. Longer reaction time or slow warming to ambient temperature did not improve the low yield of **7** (ca. 10%) in case of dimethyl and diethyl lithiomethylphosphonates **6b** and **6c** even with the excess of reagents (1.5–3 equiv.). On the contrary, with 1.3 equiv. of diisopropyl lithiomethylphosphonate (**6a**), the desired phosphonate product **8a** was isolated in a good yield (50% before optimization) after sugar deprotection step along with the unreacted **5**. This was in agreement with the stability of diisopropyl ester of lithiomethylphosphonate for several hours at 20°C in contrast to diethyl and dimethyl lithiomethylphosphonates (**6b**, **6c**) which undergo fast and quantitative self-condensation at >−50°C.[10] Addition of HMPA[11a] (1 equiv.) significantly accelerates the reaction which was usually completed within 5–10 minutes at −80°C similar to the fast sugar–triflate displacements with lithiomethylphosphonates catalyzed by HMPA.[8e] DMPU[11b] induced the same catalytic effect and should be used for the scale-up due to the reported toxicity of HMPA. The optimized yields at this step were varied with the three dialkyl lithiomethylphosphonates. The best results were obtained with diisopropyl ester **6a** (overall yield of **8a** was 85% from **5** after chromatography purification) compared to 72% yield (**8b**) with the diethyl ester **6b** and only 20% yield (**8c**) with dimethyl ester **6c**. The lower separated yield with **6b** and especially with **6c** was due perhaps to the competitive monodealkylation of the less hindered phosphonates **7b** and especially **7c** by the excess of nucleophile with the formation of double-charged monophosphonate–monosulfate salts.

Also, for the higher reliability and reproducibility of the yield at this step, we generated lithiated methylphosphonates **6a**–**c** from corresponding methylphosphonates with BuLi in the presence of 1,1-diphenylethylene (0.01 equiv.) for the first time as the indicator for BuLi which gave clear end points compared to other indicators investigated.[12] Thus, 100% formation of anions was secured by the in situ titration despite the uncertain exact concentration of BuLi and the possible presence of the air or the moisture in the system. In a control experiment we were able to separate the product in high yield even when THF was not anhydrous! However, a larger amount of BuLi was required in this case.

The pure intermediate monosulfate salt **7a** can be readily separated from HMPA, DMPU, unreacted nucleophile, and other uncharged impurities by partitioning between water and CH_2Cl_2 prior to the deprotection step. The formation of easily separable monosulfate salts after nucleophilic substitution is another advantage of cyclic sulfates over corresponding triflate derivatives. For the simultaneous and quantitative cleavage of monosulfate and isopropylidene groups we have used



Scheme 2. *Reagents and conditions*: (a) (i) $(OEt)_2P(O)CH_2SPh$, BuLi (2.5 M in hexanes), DMPU, THF, –80°C to rt, then Amberlyst-15, 95%; (b) (i) mcpba, 1 equiv., then C_6H_6, Δ, 80%, (iii) Ac_2O/Py, 100%, (iv) TMSCl/NaI, then NaOH/MeOH, 95%.

Amberlyst-15 (H^+) resin for the first time[13] which allows deprotection of monosulfate in 10–30 min and isopropylidene group in 3–5 h at ambient temperature in MeOH/THF. The separated **8** after extraction contained less than 5% impurities according to the NMR and it was used in the next step without further purification. Also, in anhydrous THF solution (one-pot after the reaction with lithiomethylphosphonates), intermediate monosulfates can be selectively cleaved with Amberlyst-15 in the presence of equivalent amount of water in 10–30 min at rt, keeping the isopropylidene protection intact.

The original procedure of Kim and Sharpless[14] for selective deprotection of monosulfates with catalytic conc. H_2SO_4 worked as well, but longer reaction time was needed. However, the following one-pot acidic cleavage of isopropylidene protection with an excess of water or methanol required heating at 50°C and proceeded with some side reactions. Thus, additional chromatography purification to separate pure **8** was necessary in this case compared to quantitative deprotection of both groups with Amberlyst-15.

Finally, the obtained sugar-phosphonates **8** were quantitatively converted to their peracetylated derivatives with an excess of Ac_2O in pyridine followed by one-pot phosphonate dealkylation with TMSBr[15a] or with an in situ preparation of TMSI (TMSCl/NaI)[15b] in MeCN at ambient temperature. The latter method allowed use of a smaller amount of TMSCl/NaI for the faster phosphonate dealkylation relative to the larger excess of the more expensive TMSBr. Thus, with TMSCl/NaI even hindered diisopropyl phosphonate **8a** can be cleaved quantitavely with 3 equiv. of the reagent in a few hours. For the diethyl phosphonate **8b** 2.5 equiv. of the reagent was sufficient to cleave phosphonate in 60 min at rt. After the NaOH/MeOH work up, the final disodium phosphonate **2b** was readily precipitated from the reaction mixture with ethanol. The product can be further purified by the additional precipitation from MeOH with EtOH or from diluted AcOH/MeOH for better solubility followed by precipitation with diluted ethanolic NaOH.

Additional protection of sugar hydroxyls in **8a** with acetate before the phosphonate dealkylation might not be necessary,[3a] however, at this time the procedure is not optimized for the scale-up. Also, the TMSCl/NaI/CH_3CN system was reported to readily convert alcohols into iodides at rt, which might be a problem with the unprotected sugar moiety.[16]

Cyclic sulfate **5** was also reactive towards nucleophilic displacement with several other nucleophiles including anions of diethyl difluoromethylphosphonate, phosphoroamidate, thiophosphate, *tert*-butyl acetate and isopropyl mesylate, which allows preparation of corresponding isosteric M6P analogs where P-O fragment is replaced with one or two heteroatoms or a carbon atom. Also, α,β-unsaturation can be introduced via sulfoxide elimination. In particular, α,β-unsaturated analog of **2c** was prepared by this method as outlined in Scheme 2. Since many other sugar-derived cyclic sulfates were described in the literature including pyranoside-4,6-sulfates and furanoside-5,6-sulfates, the method should be applicable for the preparation of sugar phosphate analogs in general. Currently we are working on the optimization of the method for the synthesis of other M6P analogs as well as on the biological evaluation of these compounds which will be discussed in a separate publication.

In conclusion, we described the first use of cyclic sulfate in the preparation of isosteric sugar-phosphonates via nucleophilic substitution by lithiated methylphosphonates.[17] They were quantitatively generated from methylphosphonates for the first time by the in situ titration with BuLi in the presence of 1,1-diphenylethylene as an indicator. The pure intermediate monosulfate salts after cyclic sulfate displacement were separated in the water phase. Also, Amberlyst-15 (H^+-form) was suggested for the first time for the fast and quantitative cleavage of monosulfate salts. Overall, the described short synthesis of isosteric phosphonate analog of M6P required simple purification procedures with extraction or precipitation and should be applicable for the scale-up.

Acknowledgements

We gratefully acknowledge the Laboratoire Mayoly-Spindler and the Pôle Européen Universitaire de Montpellier et du Languedoc Roussillon for the generous financial support of this work in the forms of postdoctoral fellowship (N.A.K.) and consumables.

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