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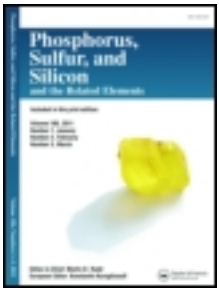

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SELECTIVE PHOSPHORYLATION ON PRIMARY ALCOHOLS OF UNPROTECTED POLYOLS

Sylvain Ladame [a], Samantha Claustre [a] & Michèle Willson [a]

[a] Groupe de Chimie Organique Biologique, LSPCMIB, UMR CNRS 5068 Université Paul Sobatier, 31062, Toulouse cédex 04, France

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SELECTIVE PHOSPHORYLATION ON PRIMARY ALCOHOLS OF UNPROTECTED POLYOLS

SYLVAIN LADAME, SAMANTHA CLAUSTRE and MICHÈLE WILLSON*

Groupe de Chimie Organique Biologique, LSPCMIB, UMR CNRS 5068 Université Paul Sabatier 31062 Toulouse cédex 04, France

(Received December 28, 2000)

The triad tribenzylphosphite-iodine-pyridine offers a general selective method for phosphorylation reactions of primary alcohols of unprotected α-diols and polyols. A mechanistic study by ^{31}P NMR allowed to evidence the formation, from iododibenzyl phosphate and pyridine, of a very reactive pyridinium salt intermediate. This analysis shows that pyridine behaves as a covalent catalyst like DMAP in acylation reactions from acylchloride. Due to its steric hindrance and high reactivity, this species appears to be the efficient selective phosphorylation reagent but leads to dibenzylphosphoric esters. Under mild conditions, the cleavage of benzyl groups gives monoester phosphoric acid.

Keywords: Selective phosphorylation; unprotected 1,2-diols and polyols; monoester phosphoric acid; dibenzyliodophosphate; dibenzylpyridiniumphosphate

INTRODUCTION

In our programme of research into glycolytic enzyme inhibitors, we have synthesized various substrate analogues of phospho-hexoses and phospho-trioses[1-3] such as fructose-6-phosphate (phosphofructo kinase), glucose-6-phosphate (hexokinase) and 1,3-*bis*phospho-D-glyceric acid (glyceraldehyde-3-phosphate dehydrogenase). For these molecules bearing several chemically reactive functions, the key reaction is the phosphorylation step that is always carried out in the last place on the synthetic pathway. Although a number of methods have already been reported[4], an

* Corresponding Author: E-mail: willson@iris.ups-tlse.fr

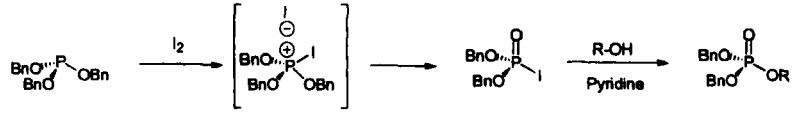
effective way to selectively phosphorylate the primary alcohol of a polyol is still a subject of great interest.

The chemical synthesis of polyhydroxy-monophosphorylated compounds has to take into account their ability to rearrange via migration of the phosphoryl group as seen in the extensive study of lysophospholipids[5]. This intra-molecular migration is pH dependent; whereas this isomerisation occurs slowly at alkaline pH, it becomes a very fast process in acidic conditions in competition with hydrolysis of the phosphate group.

To obtain the diacid phosphate monoesters in good yields, under neutral and mild conditions, we focused our interest on dibenzyl-phosphoric esters as intermediate compounds[6–7] since deprotection of diethoxy-phosphoric esters was not selective towards the third ester function around the phosphorus atom. The dibenzylphosphoramidite and dibenzylchlorophosphate routes proved to be often unsatisfactory since occurring in basic conditions and consequently leading to many by-products such as polyphosphates and pyrophosphates. In this report, we present the results obtained for the selective phosphorylation of the primary alcohol of polyols with dibenzyl-iodophosphate without protection of other sensitive functions (hydroxyl, amine, ketone).

RESULTS AND DISCUSSION

We explore here the specific reactivity of the tribenzylphosphite-iodine couple in the presence of a base[8].



SCHEME 1

The phosphorylated compounds **10–12** described in table I are analogues of two representative glycolysis metabolites: 1,3-*bis*phospho-D-glyceric acid and fructose-6-phosphate. The reported yields are given for the pure isolated phosphoric triesters and phosphoric monoesters and the structure assignments were based on ^{31}P and ^{13}C NMR, mass spectroscopy and elemental analyses.

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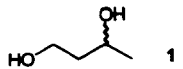
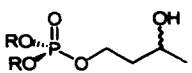
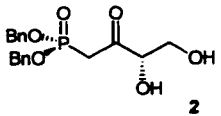
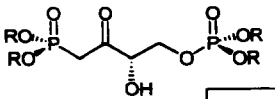
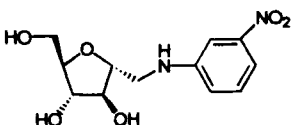
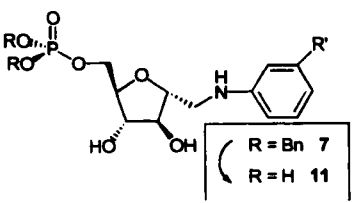
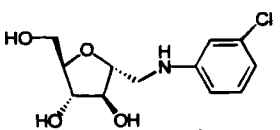
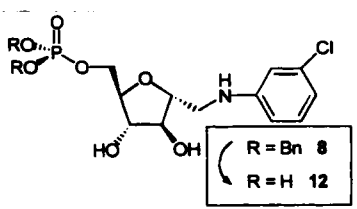
TABLE I Monophosphorylation of polyols. Yields are given for the pure isolated products

		Phosphorylation yield (%)	*Hydrogenolysis yield (%)*
 1	 R = Bn **5** R = H **9**	65	98
 2	 R = Bn **6** R = H **10**	50	98
 3	 R = Bn **7** R = H **11**	40	R′ = NO_2 30 R′ = NH_2 65
 4	 R = Bn **8** R = H **12**	60	80

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For all dibenzylphosphates **6–8** and diacid phosphates **10–12**, mass spectroscopy and elemental analysis confirmed the mono-phosphorylation of their corresponding polyhydroxylated precursors **1–4** the synthesis of which was previously described[9–10]. Phosphorylation of the primary alcohol was evidenced by comparison of ^{13}C NMR spectra of polyols with those of phosphorylated derivatives.

For the 1,3-*bis*phospho-D-glyceric acid analogue **6**, after phosphorylation, the chemical shift of the carbon of the primary alcohol function increases from 63.5 to 68.4 ppm. Moreover, only the carbon of the carbonyl group is coupled with the phosphonate's phosphorus atom (d, $^2J_{P\text{-}C} = 6.3$ Hz). No $^3J_{P\text{-}C}$ coupling with the phosphate group is observed.

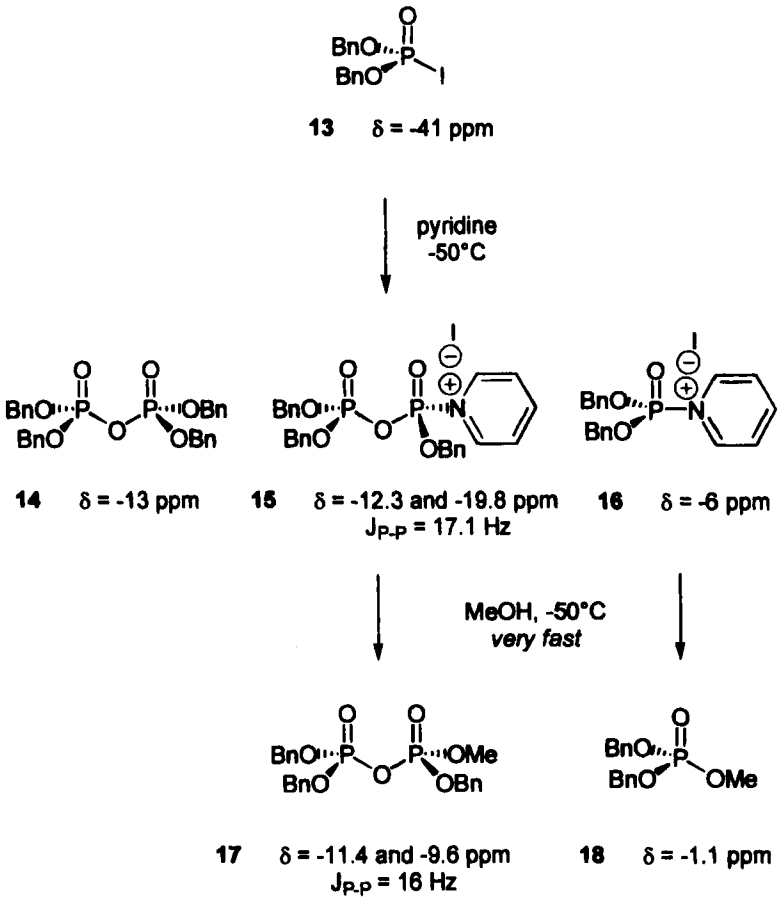
For fructose-6-phosphate analogues, dibenzylesters **7** and **8**, ^{13}C NMR spectra show that only C-6 and C-5 atoms are coupled with the phosphorus atom ($^2J_{P\text{-}C} = 6.2$ Hz and $^3J_{P\text{-}C} = 6.7$ Hz for compound **8**). No P-C coupling was observed for C-3 and C-4 atoms of the two secondary alcohols. After neutral deprotection, the monoester phosphoric acids were kept as very stable compounds under their acidic form, or as disodium or dicyclohexylammonium salts. Neither phosphate migration nor phosphate hydrolysis was observed by ^{13}C and ^{31}P NMR in water at neutral pH.

To achieve this selective phosphorylation on primary alcohols of unprotected polyhydroxylated compounds in optimal conditions, a preliminary study, carried out on the commercially available 1,3-butanediol, has been necessary. Experiments with one to four equivalents of different tertiary amines showed that the concentration of base was an essential parameter for the phosphorylation yield.

(i) With four equivalents of pyridine or triethylamine, the yields in the expected phosphotriester were 60% and 40% respectively. The formed by-products were essentially pyrophosphates identified by ^{31}P NMR (from −10 to −13 ppm).

(ii) With one equimolar amount of pyridine, the expected dibenzyl-3-hydroxybutane phosphate was the main product (70%). By-products such as dibenzylphosphite (+7 ppm; $J_{P\text{-}H} = 707$ Hz), dibenzylphosphate (+0.3 ppm) and tetrabenzylpyrophosphate (-13 ppm), arose from hydrolysis of reagents and intermediates.

After separation and purification of dibenzyl phosphates, the benzyl groups were quickly and selectively removed by hydrogenolysis in alcohol, at room temperature in less than one hour. The pure monoester phosphoric acids **9–12** were isolated in high yields (from 40 to 70%), excepted



SCHEME 2 Characterization of the main intermediates and by-products by ^{31}P NMR

for compound **7**, the hydrogenolysis of which led to 30% of the nitro compound and 65% of the amino analogue resulting from reduction of the nitro group.

The triad tribenzylphosphite-iodine-pyridine in equimolar quantities was found to give the best conditions to selectively and quickly phosphorylate the primary alcohol function of a polyol. In opposite, no selective phosphorylation was observed with the triad triethylphosphite-iodine-pyridine.

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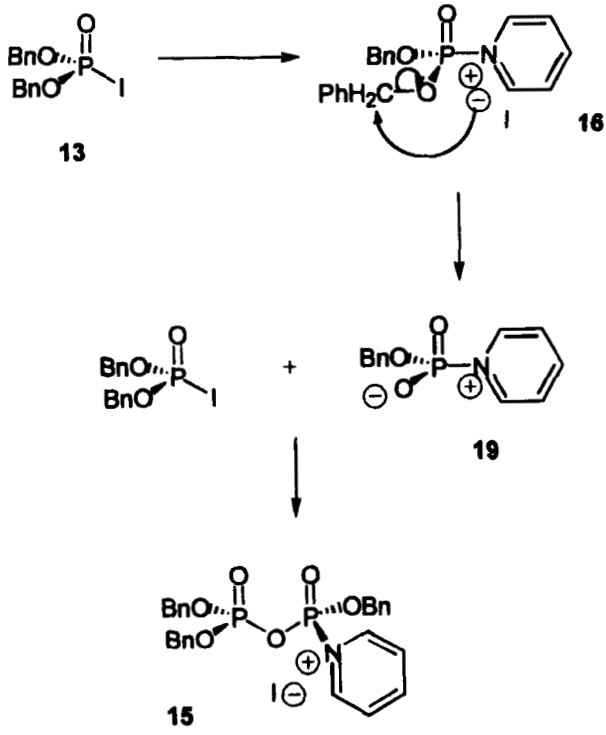
In order to understand the selectivity of this phosphorylation method, a mechanistic study was performed by using ^{31}P NMR spectroscopy. This was achieved both on triethyl and tribenzyl phosphites in deuteriated chloroform at −50°C, in order to facilitate the identification of the reaction intermediates as shown on schemes 2 and 3.



SCHEME 3

(i) Reaction of tribenzyl and triethyl phosphites with an equivalent of molecular iodine led to dibenzylphosphoroiodate **13** or the previously described diethyl phosphoroiodate,[111] both having the same chemical shift (-41 ppm).

(ii) Addition of a stoechiometric quantity of pyridine rapidly led to their pyridinium salts. Besides the pyridinium salt **16**, two pyrophosphates **14**

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(-13 ppm) and **15** (-12.3 and −19.8 ppm) were obtained from the unstable dibenzylphosphate[12]; these by-products were not observed with diethylphosphate. Pyrophosphate **14** resulted from a condensation reaction of dibenzylphosphate with iodophosphate **13**. Pyrophosphate **15** provided from mono-debenzylation of **16** by nucleophilic attack of iodine anion, leading to a zwiterionic intermediate **19**, with then formation of the pyrophosphate bridge by condensation of the latter on iodophosphate **13** (Scheme 3).

(iii) Even at −50°C, the alcoholysis of pyridinium salts **15** and **16** was quite instantaneous; the same reaction was much slower with the pyridinium salt of the diethyl analogue since it was only complete after 15 minutes. The same effect was observed in the preparative conditions at -10°C and at room temperature.

From these results, it is reasonable to assume that the formation of a very reactive pyridinium salt intermediate, with a strong steric hindrance, can account for the selective phosphorylation of the primary hydroxyl groups. The selective phosphorylation on primary alcohol of four polyols without protection of other sensitive chemical functions shows its general applicability to a number of molecules. This method appears to be an effective tool for the synthesis of many phosphorylated biomolecule analogues.

EXPERIMENTAL

NMR spectra were determined on Bruker Instruments AC-50 (^{13}C NMR) and AC-81 (^{31}P NMR) using internal TMS (^{13}C) and H_3PO_4 (^{31}P) as references. Chemical shifts, expressed in ppm, are reported for signal centers. Elementary analysis were performed on an Eager 200 instrument by the INP-ENSCT of Toulouse. High-resolution mass spectra were recorded on an AUTOSPEC 6F [Micromass, Altrichman, UK] instrument using LSIMS (resolution 5000–10,000) as ionisation mode and glycerol, trichloroacetic acid 1% in water as matrix and polyethyleneglycol (PEG) or PEG-Na as reference. Preparative column chromatography was performed using 70–230 mesh Merck silica gel. The phosphorylation reactions were performed under an argon atmosphere in dried glassware with freshly distilled CH_2Cl_2 (on P_2O_5) and pyridine (on KOH).

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General procedure of phosphorylation

To a solution of tribenzylphosphite (1 mmol) in anhydrous CH_2Cl_2 (20 mL) was added iodine (0.95 mmol) at 0°C. After stirring 10 min at 0°C and 15 min at r.t., this mixture was added via a cannula at −10°C to a solution of alcohol (1 mmol) in anhydrous CH_2Cl_2 (10 mL) and pyridine (1 mmol). After 30 min at r.t., the pyridinium salts were filtered off and the organic mixture was washed with water, dried ($MgSO_4$) and filtered. Evaporation of the solvent and purification by flash chromatography led to the expected dibenzylalkyl phosphate.

General procedure of hydrogenolysis

To a H_2-purged flask containing a solution of dibenzylalkyl phosphate (1 mmol) in methanol (25 mL) was added 10% Pd/C (100 mg). After stirring under a H_2atmosphere for 30 minutes at room temperature, Pd/C was filtered off and methanol evaporated after neutralization with cyclohexylamine (for compound **10**) or sodium hydroxide (for compound **11**) or by keeping the phosphate group under its acidic form (for compound **12**).

4, 3(R)-dihydroxy-2-oxodibenzylphosphonate (2)

^{31}P NMR ($CDCl_3$, 81 MHz): δ = 21.3 ppm.

^{13}C NMR ($CDCl_3$, 50 MHz): δ = 38.6 (C-1, $J_{P\text{-}C}$ = 130.2 Hz), 63.5 (C-4), 68.5 (CH_2benzyl, $J_{P\text{-}C}$ = 6.3 Hz), 78.7 (C-3, $J_{P\text{-}C}$ = 1.8 Hz), 128.2, 128.8, 135.6 ($J_{P\text{-}C}$ = 6.2 Hz), 204.1 (C-2, $J_{P\text{-}C}$ = 6.4 Hz).

Anal calcd for $C_{18}H_{21}O_6P$ (364): C, 59.34; H, 5.81. Found: C, 59.40; H, 5.75.

4-dibenzylphosphono, 3-oxo-2(R)-hydroxy-dibenzylphosphate (6)

^{31}P NMR ($CDCl_3$, 81 MHz): δ = −0.6 and 21.0 ppm.

^{13}C NMR ($CDCl_3$, 50 MHz): δ = 39.1 (C-4, $J_{P\text{-}C}$ = 128.4 Hz), 68.4 (CH_2benzyl, $J_{P\text{-}C}$= 6.2 Hz), 69.6 (C-1, $J_{P\text{-}C}$ = 6.0 Hz), 76.5 (C-2, $J_{P\text{-}C}$ = 6.3 Hz), 128.1, 128.2, 128.7, 128.8, 135.6 ($J_{P\text{-}C}$ = 6.2 Hz), 202.4 (C-3, $J_{P\text{-}C}$ = 6.3 Hz).

Anal calcd for $C_{32}H_{34}O_9P_2$(624): C, 61.54; H, 5.49. Found: C, 61.43; H, 5.55.

Dicyclohexylammonium(4-dicyclohexylammoniumphosphono, 3-oxo-2(R)-hydroxybutyl) phosphate (10)

^{31}P NMR (CD_3OD, 81 MHz): δ = 4.6 and 10.4 ppm.

^{13}C NMR (CD_3OD, 50 MHz): δ = 26.4, 26.9, 33.0, 47.5 (C-4, $J_{P\text{-}C}$ = 100.5 Hz), 52.8, 67.9 (C-1, $J_{P\text{-}C}$ = 4.4 Hz), 79.7 (C-2, $J_{P\text{-}C}$ = 7.3 Hz), 213.4 (C-3, $J_{P\text{-}C}$= 5.4 Hz).

Anal calcd for $C_{28}H_{62}N_4O_9P_2,4H_2O$ (732): C, 45.95; H, 9.63; N, 7.65. Found: C, 46.01; H, 9.46; N, 7.52.

2,5-anhydro-1-deoxy-1-(m-nitrophenylamino)-D-mannitol (3)

^{13}C NMR ($CDCl_3$, 50 MHz): δ = 46.7 (C-1), 63.3 (C-6), 78.9 (C-4), 80.3 (C-3), 83.1 (C-2), 85.2 (C-5), 107.3 (C-2′), 112.1 (C-4′), 119.9 (C-6′), 130.8 (C-5′), 151.0 (C-3′), 151.2 (C-1′). HRMS [M-H+] calc for $C_{12}H_{17}N_2O_6$ 285.1087; found 285.1087.

2,5-anhydro-1-deoxy-1-(m-nitrophenylamino)-D-mannitol-6-dibenzylphosphate (7)

^{31}P NMR ($CDCl_3$, 81 MHz): δ = −1.2 ppm.

^{13}C NMR ($CDCl_3$, 50 MHz): δ = 44.2 (C-1), 67.6 (C-6, $J_{P\text{-}C}$ = 6.3 Hz), 70.2 (CH_2benzyl, $J_{P\text{-}C}$ = 5.2 Hz), 72.5 (C-4), 79.0 (C-3), 80.6 (C-5, $J_{P\text{-}C}$ = 6.5 Hz), 81.5 (C-2), 107.3 (C-2′), 112.1 (C-4′), 119.9 (C-6′), 128.1, 128.8, 135.6 ($J_{P\text{-}C}$ = 6.9 Hz), 130.7 (C-5′), 150.7 (C-3′), 151.2 (C-1′). Anal calcd for $C_{26}H_{29}N_2O_9P,2H_2O$ (580): C, 53.79; H, 5.73; N, 4.83. Found: C, 53.87; H, 5.64; N, 4.75.

2,5-anhydro-1-deoxy-1-(m-nitrophenylamino)-D-mannitol-6-disodiumphosphate (11)

^{31}P NMR (D_2O, 81 MHz): δ = −3.6 ppm.

^{13}C NMR (D_2O, 50 MHz): δ = 47.1 (C-1), 68.1 (C-6, $J_{P\text{-}C}$ = 6.6 Hz), 78.8 (C-4), 80.9 (C-3), 83.2 (C-2), 84.0 (C-5, $J_{P\text{-}C}$= 6.6 Hz), 107.0 (C-2′), 113.8 (C-4′), 121.2 (C-6′), 132.2 (C-5′), 151.9 (C-3′), 152.1 (C-1′).

MS (negative FAB, glycerol): $[M\text{-}Na^+]^-$ = 385, $[M\text{-}2Na^+\text{+}H]^-$ = 363.

2,5-anhydro-1-deoxy-1-(m-chlorophenylamino)-D-mannitol (4)

^{13}C NMR ($CDCl_3$, 50 MHz): δ = 46.8 (C-1), 63.3 (C-6), 79.0 (C-4), 80.5 (C-3), 83.1 (C-2), 85.2 (C-5), 112.3 (C-6′), 113.4 (C-2′), 117.5 (C-4′),

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131.2 (C-5′), 135.9 (C-3′), 151.6 (C-1′). HRMS [M-H+] calc for $C_{12}H_{17}ClO_4$ 274.0846; found 274.0846.

2,5-anhydro-1-deoxy-1-(m-chlorophenylamino)-D-mannitol-6-dibenzylphosphate (8)

^{31}P NMR ($CDCl_3$, 81 MHz): $\delta = -1.1$ ppm.

^{13}C NMR ($CDCl_3$, 50 MHz): $\delta = 53.4$ (C-1), 67.7 (C-6, $J_{P\text{-}C} = 6.2$ Hz), 70.1 (CH_2benzyl, $J_{P\text{-}C} = 5.2$ Hz), 72.3 (C-4), 79.0 (C-3), 80.9 (C-5, $J_{P\text{-}C} = 6.7$ Hz), 81.5 (C-2), 110.7 (C-6′), 112.3 (C-2′), 116.4 (C-4′), 126.4, 128.1, 128.7, 130.1 (C-5′), 135.0 (C-3′), 135.4 ($J_{P\text{-}C} = 7.0$ Hz), 149.9 (C-1′).

Anal calcd for $C_{26}H_{29}ClNO_7P,2H_2O$ (569): C, 54.78; H, 5.84; N, 2.46. Found: C, 54.68; H, 5.92; N, 2.63.

2,5-anhydro-1-deoxy-1-(m-chlorophenylamino)-D-mannitol-6-phosphate (12)

^{31}P NMR (D_2O, 81 MHz): $\delta = 0.8$ ppm.

^{13}C NMR (D_2O, 50 MHz): $\delta = 55.3$ (C-1), 67.2 (C-6, $J_{P\text{-}C} = 5.2$ Hz), 78.7 (C-4), 79.9 (C-3), 83.7 (C-2), 84.5 (C-5, $J_{P\text{-}C} = 6.6$ Hz), 114.8 (C-6′), 116.0 (C-2′), 120.7 (C-4′), 131.7 (C-5′), 136.1 (C-3′), 148.4 (C-1′).

MS (negative FAB, glycerol): $[M\text{-}H]^- = 352$.

Acknowledgements

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