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Synthesis of oligomeric phosphono analogues of *Leishmania* lipophosphoglycan fragments

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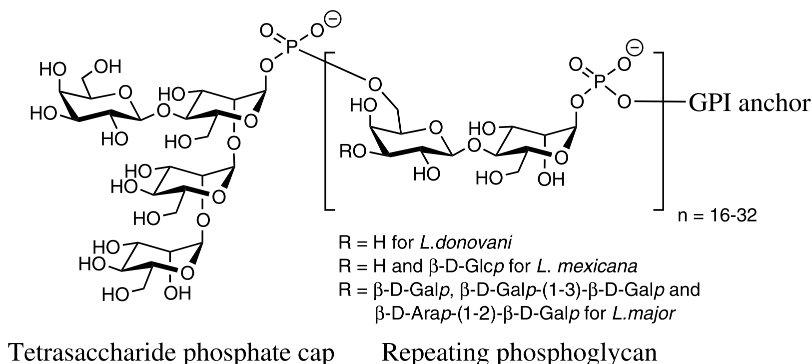
Abstract—A set of isosteric phosphono (*C*-glycoside) analogues of *Leishmania* lipophosphoglycan fragments have been synthesised by making use of a sequence of uniformly stereoselective glycosylation reactions of phosphono-oligosaccharide glycosyl acceptors with phosphono-oligosaccharide thioglycosides. © 2002 Elsevier Science Ltd. All rights reserved.

The *Leishmania* family of protozoan parasites causes severe debilitating and often fatal diseases throughout the tropics and subtropics. There is strong evidence that infectivity and survival in the mammalian host are both counted on the lipophosphoglycan (LPG) molecules which are ubiquitous on the surface of the parasite (Scheme 1).[1] The known antigenic properties of this unique glycoconjugate prompted vaccine design studies based on the different parts of the molecule.[2,3] Along this line, we reported the preparation of fully synthetic fragments of the LPG from several species of the *Leishmania*[4] and a method for conjugation of these phosphosaccharides to protein and lipid carriers.[2] Now we would like to disclose the synthesis of oligomeric phosphono (*C*-glycoside) analogues of the LPG fragments that would be more robust chemically than parent phosphosaccharides and resist the action of phosphatases as well.

Recently, we reported[5] the synthesis of dec-9-enyl β-D-galactopyranosyl-(1→4)-α-D-mannopyranosyl methanephosphonate, a phosphono analogue of the disaccharide repeating unit of the LPG. Based on these developments, an obvious approach to the targeted phosphono-oligosaccharides was devised assuming they could be obtained by stepwise 'oligomerisation' of the disaccharide phosphonic acid **6** selectively protected at 6′-OH (Scheme 2). Exploring this possibility, we have



Scheme 1. Generalised structure of the *Leishmania* lipophosphoglycan.

Keywords: carbohydrates; phosphonic acids and derivatives.

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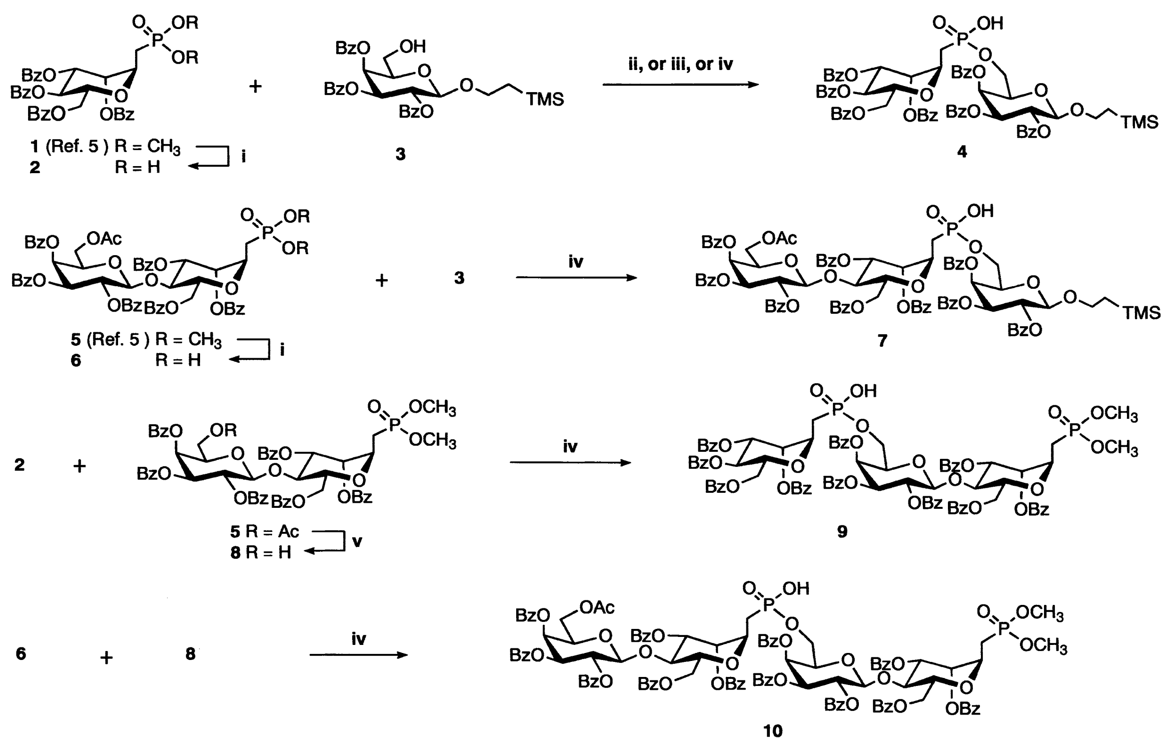
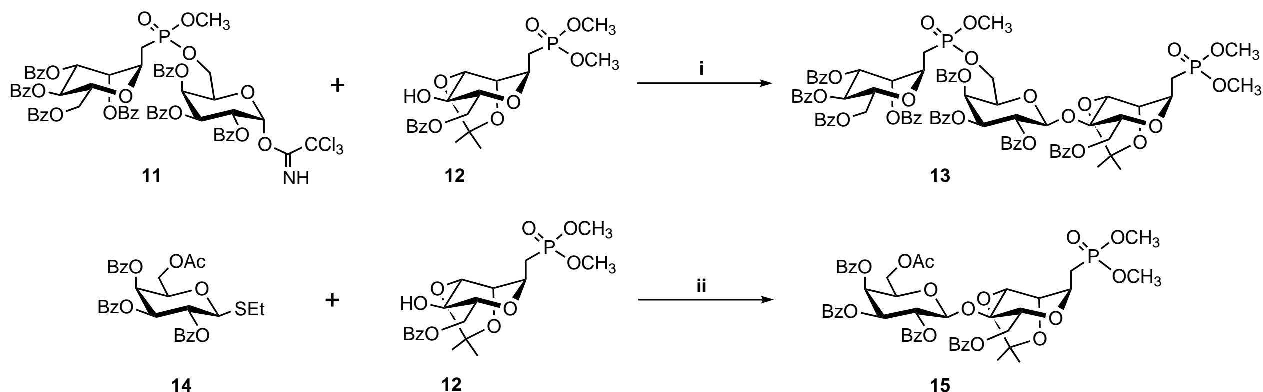


Scheme 2. *Reagents and conditions*: (i) TMSBr, DCM, 1 h, rt, quant.; (ii) DCC, THF, 65°C, 2 h, 57%; (iii) DCC, pyridine, 72 h, rt, 62%; (iv) DCC, DMAP, pyridine, 72 h, rt, 68% for **2**+**3**, 66% for **6**+**3**, 30% for **2**+**8**, 10% for **6**+**8**; (v) 2% HCl–MeOH, DCM, 12 h, rt, 88%.

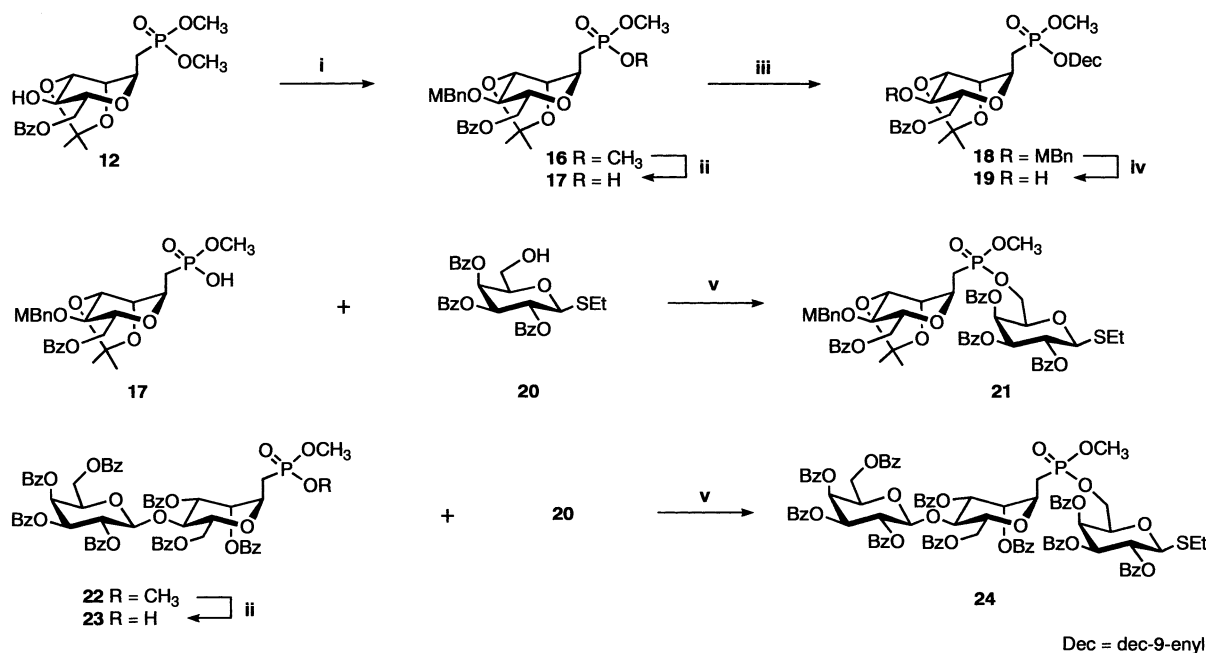
found that DCC-mediated esterification of model phosphonic acid **2** with the galactoside alcohol **3** in the presence of DMAP produced the phosphonodisaccharide **4** in 68% yield.[6] This combination of reagents was proved to be equally effective for the preparation of the phosphonotrisaccharide **7** from the phosphonic acid **6** and the monosaccharide alcohol **3**. However, attempted esterification of **2** with the monohydroxylic phosphonodisaccharide derivative **8** afforded only 30% yield of the phosphonotrisaccharide **9**. Things turned out to be even worse when coupling of the disaccharide building blocks **8** and **6** provided no more than 10% of the phosphonotetrasaccharide **10**. These findings showed that the 6′-OH group in the disaccharide **8** is disappointingly less reactive than the 6-OH group in the monosaccharide **3**, though the reason for the above inconsistency remains unclear.

Trying to circumvent this obstacle, we discovered that the phosphonotrisaccharide **13**[8] (an analogue of **9**) could be effectively obtained instead by the stereoselective *glycosylation* of the mannosyl methanephosphonate derivative **12**[5] with the phosphonodisaccharide trichloroacetimidate **11**;[7] whereas, the thiogalactoside **14**[7] was found to be the glycosyl donor of choice for the preparation of the phosphonodisaccharide **15**[8] in a separate experiment (Scheme 3). The exclusive β-selectivity of these reactions[8] clearly manifested the stereocontrolling role of the 2,3-*O*-isopropylidene group in the glycosyl acceptor **12** in accordance with our previous observations.[5]

These data brought an insight as to how the original synthetic strategy could be redesigned to achieve the ultimate objectives. Now we considered the dec-9-enyl



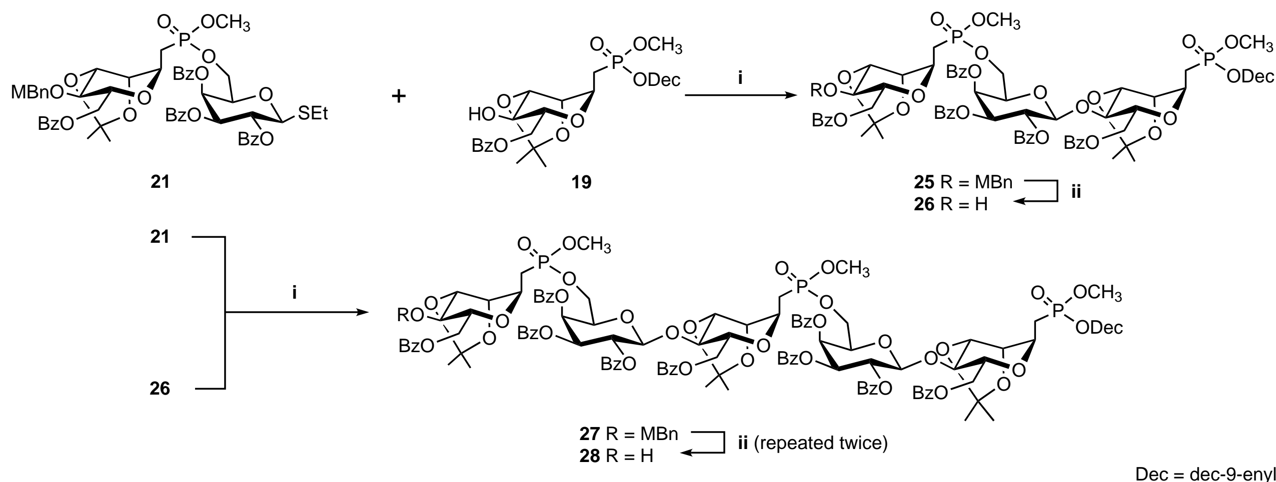
Scheme 3. *Reagents and conditions*: (i) TMSOTf, DCM, 1 h, −20°C, 72%; (ii) MeOTf, DCM, 4 Å MS, 3 h, rt, 88%.



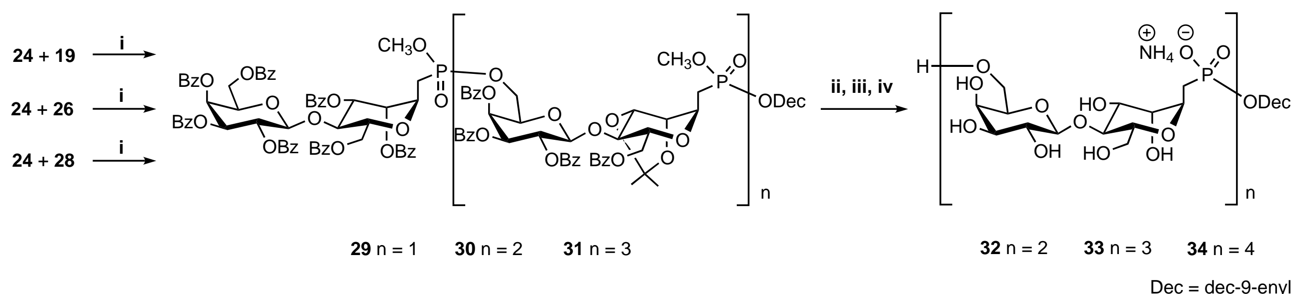
Scheme 4. *Reagents and conditions*: (i) $MBnOC(NH)CCl_3$, 0.3% TfOH, DCM, 20 min, rt, 90%; (ii) PhSH, Et_3N, DMF, 12 h, rt, quant.; (iii) dec-9-enol, Ph_3P, DIAD, THF, 2 h, rt, 88%; (iv) DDQ, DCM–H_2O, 3 h, rt, 85%; (v) Ph_3P, DIAD, THF, 16 h, 60°C, 68% for **17**+**20**, 58% for **23**+**20**.

(mannosyl methanephosphonate) **19** (Scheme 4) as a 'primer' acceptor, while the phosphonodisaccharide thioglycoside **21** orthogonally protected at 4′-OH as a 'repeating unit' for the phosphono-oligosaccharide chain assembly via repetitive glycosylation reactions. We anticipated the high level of stereoselection would be maintained all the way through using the combination of thioglycoside donors and matched glycosyl acceptors of the optimal structure. The phosphonotrisaccharide thioglycoside **24** was introduced as a 'cap' for the final glycosylation step to keep the overall convergence of the synthetic scheme.

The preparation of the mannosyl methanephosphonate **19** was accomplished in a straightforward manner starting from compound **12** (Scheme 4). Acid-catalysed installation of the *p*-methoxybenzyl (MBn) protective group[9] followed by selective monodemethylation of the phosphonic diester with PhSH–Et_3N[10] cleanly afforded the monoester **17**. Esterification of this material with dec-9-enol using the Mitsunobu protocol[11] followed by removal of the MBn protection with dichlorodicyanobenzoquinone (DDQ) produced the requisite hydroxyl derivative **19** (as a mixture of two diastereomers at phosphorus). A similar combination of PhSH–Et_3N selective demethylation/Mitsunobu esterification was successfully applied to the preparation of the phosphonodisaccharide **21** and the phosphonotrisaccharide **24**. The required phosphonic diesters were obtained (as diastereomeric mixtures at phosphorus)



Scheme 5. *Reagents and conditions*: (i) MeOTf, DCM, 4 Å MS, 7 h, rt, 87% for **21**+**19**, 75% for **21**+**26**; (ii) DDQ, DCM–H_2O, 2 h, rt, 80% for **25** to **26**, 75% for **27** to **28**.



Scheme 6. *Reagents and conditions*: (i) MeOTf, DCM, 4 Å MS, 16 h, rt, 79% for **24**+**19**, 68% for **24**+**26**, 67% for **24**+**28**; (ii) TFA, DCM–H_2O, 10 min, rt; (iii) PhSH, Et_3N, DMF, 12 h, rt; (iv) MeONa cat., MeOH, 24 h, rt.

from the reactions of the galactoside alcohol **20**[12] with phosphonic monoesters **17** (68%) and **23** (58%), respectively.

With all the principal building blocks in hand, we pursued the preparation of phosphono analogues of di-, tri- and tetrameric repeats of the *Leishmania* LPG, compounds **32**, **33** and **34**, respectively. The MeOTf-promoted glycosylation of the mannosyl methanephosphonate **19** with the phosphonodisaccharide thioglycoside **21** exclusively provided the β-linked phosphonotrisaccharide **25** (for D-Gal*p*: δ_{H1} 5.05, $J_{1,2}$ 8.2 Hz; δ_{C1} 101.2; a mixture of four possible isomers at phosphorus) in 87% yield (Scheme 5). Treatment of **25** with DDQ ensued removal of the MBn protection in 80% yield. Reiterative glycosylation of thus prepared **26** with the same thioglycoside **21** afforded the phosphonopentasaccharide **27** (as a mixture of eight isomers at phosphorus) in 75% yield, again, as the β-isomer only at the newly formed galactoside bond(s) (δ_{H1} 5.05, $J_{1,2}$ 8.0 Hz; δ_{C1} 101.3). Deprotection of **27** required DDQ oxidation to be repeated twice to achieve the conversion into monohydroxylic derivative **28** in 75% yield.

In due course, a series of uniform MeOTf-promoted reactions of the mono-, tri- and penta-saccharide derivatives **19**, **26** and **28**, respectively, with the phosphonotrisaccharide thioglycoside **24** validated the versatility of the reiterative glycosylation approach to the oligomeric phosphono analogues of the LPG: the fully protected phosphono-tetrasaccharide **29** (four isomers at phosphorus), -hexasaccharide **30** (eight isomers at phosphorus) and -octasaccharides **31** (16 isomers at phosphorus) were obtained exclusively with β-D-galactopyranoside bonds (δ_{C1} 101.1–101.2) in 79, 68, and 67% yields, respectively (Scheme 6).

Finally, stepwise deprotection of the above phosphono-oligosaccharides produced the targeted compounds **32**, **33** and **34** in 83, 75 and 65% overall yields, respectively, after isolation by anion-exchange chromatography. The structures of the oligomers were supported by NMR and ES-MS data.[13] Steady changes of integration of characteristic NMR signals (e.g. H-1, C-1, C-5 and C-6 in Gal residues and C-4 in Man residues) in terminal and internal units were consistent with chain length growing from the dimer **32** to the tetramer **34**.

The phosphono analogues **32**–**34** were used for the preparation (using a dec-9-enyl moiety as a linker[2]) of neoglycoconjugates as potential synthetic vaccines. They have also revealed a biological activity (similar to parent phosphosaccharides) as effective acceptor substrates for the elongating α-D-mannosyl phosphate transferase (MPT) in the *Leishmania*.[14] The results of the studies will be published elsewhere in due course.

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6. All new compounds showed satisfactory spectral and analytical data.
7. Compound **11** was prepared from **4** in three steps: (i) CH_2N_2, EtOAc–MeOH, 0°C, 90%; (ii) TFA, DCM, 1 h, rt; (iii) Cl_3CCN, DBU, DCM, 1 h, 0°C, 95% for ii, iii. Compound **14** was prepared from ethyl tetra-*O*-acetyl-1-thio-β-D-galactopyranoside in four steps: (i) MeONa, MeOH, 2 h, rt; (ii) DMTCl, DMAP, pyridine, 12 h, rt, then BzCl, 12 h, rt; (iii) aq. TFA, DCM, 5 min, rt; (iv) Ac_2O, pyridine, 2 h, rt, 80% for i–iv.
8. Compound **13**: ^1H NMR (300 MHz, $CDCl_3$, selected data): δ 5.05 (d, 1H, $J_{1,2}$ 8.0, H-1, Gal); ^{13}C NMR (75

MHz, $CDCl_3$, selected data): δ 101.2 (C-1, Gal). Compound **15**: ^{1}H NMR (300 MHz, $CDCl_3$, selected data): δ 5.07 (d, 1H, $J_{1,2}$ 8.0, H-1, Gal); ^{13}C NMR (75 MHz, $CDCl_3$, selected data): δ 101.3 (C-1, Gal).

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12. Prepared as described for **14** (Ref. 7) except the final acetylation was omitted.
13. Compound **32**: ^{1}H NMR (300 MHz, D_2O, selected data): δ 4.10 (m, 2H, H-1, Man, Man′), 4.25 (d, 1H, $J_{1,2}$ 8.0, H-1, Gal′), 4.30 (d, 1H, $J_{1,2}$ 8.0, H-1, Gal); ^{13}C NMR (75 MHz, D_2O): δ 25.3, 28.5, 28.6, 28.7, 28.9, 30.5 (d, $J_{C,P}$ 6.2), 33.5, 65.1 (d, $J_{C,P}$ 5.4) [8×CH_2, dec-9-enol], 27.0 (d, 2C, $J_{C,P}$ 132.6, C-1*, Man, Man′), 60.8 (2C, C-6, Man, Man′), 61.4 (C-6, Gal′), 62.8 (d, $J_{C,P}$ 6.0, C-6, Gal), 68.4 (C-4, Gal), 68.9 (C-4, Gal′), 69.4 (C-3, Man′), 69.5 (C-3, Man), 71.2 (br, 4C, C-2, Man, Man′, Gal, Gal′), 72.7 (C-3, Gal), 72.8 (C-3, Gal′), 73.1 (br, 2C, C-5, Man, Man′), 73.9 (C-1, Man), 74.2 (C-1, Man′), 74.3 (d, $J_{C,P}$ 8.1, C-5, Gal), 75.7 (C-5, Gal′), 77.3 (C-4, Man′), 78.1 (C-4, Man), 103.4 (C-1, Gal′), 103.6 (C-1, Gal), 114.2 (=CH_2), 140.8 (–CH=); ^{31}P NMR (121 MHz, D_2O): δ 22.5 (P), 23.0 (P′); ES-MS (–) data: m/z 479.28 (100%, $[M-2NH_3-2H]^{2-}$) (expected m/z 479.17); $[\alpha]_D$ +25.5 (*c* 0.5, MeOH–H_2O, 1:1).
Compound **33**: ^{1}H NMR (300 MHz, D_2O, selected data): δ 4.10 (m, 3H, H-1, Man–Man″), 4.25 (d, 1H, $J_{1,2}$ 8.0, H-1, Gal″), 4.30 (d, 2H, $J_{1,2}$ 8.0, H-1, Gal, Gal′); ^{13}C NMR (75 MHz, D_2O): δ 25.2, 28.4, 28.5, 28.7, 28.8, 30.4 (d, $J_{C,P}$ 6.1), 33.4, 65.0 (d, $J_{C,P}$ 6.0) [8×CH_2, dec-9-enol], 26.9 (br d, 3C, $J_{C,P}$ 133.2, C-1*, Man–Man″), 60.7 (3C, C-6, Man–Man″), 61.4 (C-6, Gal″), 62.7 (br, 2C, C-6, Gal, Gal′), 68.3 (br, 2C, C-4, Gal, Gal′), 68.9 (C-4, Gal″), 69.4 (br, 3C, C-3, Man–Man″), 71.1 (br, 6C, C-2, Man–Man″, Gal–Gal″), 72.6 (2C, C-3, Gal, Gal′), 72.8 (C-3, Gal″), 73.0 (2C, C-5, Man, Man″), 73.2 (C-5, Man′), 73.6 (C-1, Man′), 73.8 (C-1, Man), 74.2 (br, 3C, C-1, Man″ and C-5, Gal, Gal′), 75.6 (C-5, Gal″), 77.2 (C-4, Man″), 78.0 (2C, C-4, Man, Man′), 103.3 (C-1, Gal″), 103.5 (2C, C-1, Gal, Gal′), 114.2 (=CH_2), 140.8 (–CH=); ^{31}P NMR (121 MHz, D_2O): δ 22.5 (P), 23.0 (P″), 23.2 (P′); ES-MS (–) data: m/z 453.31 (100%, $[M-3NH_3-3H]^{3-}$) (expected m/z 453.14), 680.23 (90%, $[M-3NH_3-2H]^{2-}$) (expected m/z 680.21); $[\alpha]_D$ +23 (*c* 1.0, MeOH–H_2O, 1:1).
Compound **34**: ^{1}H NMR (300 MHz, D_2O, selected data): 4.10 (m, 4H, H-1, Man–Man‴), 4.25 (d, 1H, $J_{1,2}$ 8.0, H-1, Gal‴), 4.30 (d, 3H, $J_{1,2}$ 8.0, H-1, Gal–Gal″); ^{13}C NMR (75 MHz, D_2O): δ 25.3, 28.5, 28.6, 28.7, 28.8, 30.4 (d, $J_{C,P}$ 6.3), 33.5, 65.2 (d, $J_{C,P}$ 6.0) [8×CH_2, dec-9-enol], 26.9 (br d, 4C, $J_{C,P}$ 133.2, C-1*, Man–Man‴), 60.7 (4C, C-6, Man–Man‴), 61.4 (C-6, Gal‴), 62.7 (br, 3C, C-6, Gal–Gal″), 68.3 (br, 3C, C-4, Gal–Gal″), 68.9 (C-4, Gal‴), 69.4 (br, 4C, C-3, Man–Man‴), 71.2 (br, 8C, C-2, Man–Man‴, Gal–Gal‴), 72.7 (3C, C-3, Gal–Gal″), 72.8 (C-3, Gal‴), 73.1 (2C, C-5, Man, Man‴), 73.2 (br, 2C, C-5, Man′, Man″), 73.7 (br, 2C, C-1, Man′, Man″), 73.9 (C-1, Man), 74.1 (d, 3C, $J_{C,P}$ 8.1, C-5, Gal–Gal″), 74.2 (C-1, Man‴), 75.7 (C-5, Gal‴), 77.2 (C-4, Man‴), 78.0 (3C, C-4, Man–Man″), 103.4 (C-1, Gal‴), 103.6 (3C, C-1, Gal–Gal″), 114.2 (=CH_2), 140.8 (–CH=); ^{31}P NMR (121 MHz, D_2O): δ 22.5 (P), 23.0 (P‴), 23.2 (P′+P″); ES-MS (–) data: m/z 587.40 (100%, $[M-3NH_3-3H]^{3-}$) (expected m/z 587.16); $[\alpha]_D$ +25.7 (*c* 0.54, H_2O).
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