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Article

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FimH Antagonists - Phosphate Prodrugs Improve Oral Bioavailability

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Abstract

The widespread occurrence of urinary tract infections has resulted in frequent antibiotic treatment, contributing to the emergence of antimicrobial resistance. Alternative approaches are therefore required. In the initial step of colonization, FimH, a lectin located at the tip of bacterial type 1 pili, interacts with mannosylated glycoproteins on the urothelial mucosa. This initial pathogen/host interaction is efficiently antagonized by biaryl α-D-mannopyranosides. However, their poor physicochemical properties, primarily resulting from low aqueous solubility, limit their suitability as oral treatment option. Herein, we report the syntheses and pharmacokinetic evaluation of phosphate prodrugs, which show an improved aqueous solubility of up to 140-fold. In a Caco-2 cell model, supersaturated solutions of the active principle were generated through hydrolysis of the phosphate esters by brush border-associated enzymes, leading to a high concentration gradient across the cell monolayer. As a result, the *in vivo* application of phosphate prodrugs led to a substantially increased C_{max} and prolonged availability of FimH antagonists in urine.

Introduction

Urinary tract infections (UTIs) are the most frequent cause of bacteriosis in humans, affecting millions of people worldwide.[1] They remain one of the most common indications for prescribing antibiotics to alleviate symptoms (dysuria, frequent and urgent urination, bacteriuria, and pyuria) and to prevent complications (pyelonephritis and urosepsis).[2] However, the frequent and repeated use of antibiotics can induce antimicrobial resistance; therefore, alternative prevention and treatment strategies are urgently needed.[3]

Uropathogenic *Escherichia coli* (UPEC) strains are the causative agent of more than 70% of all UTI episodes.[4,5] The initial step in pathogenesis involves bacterial adherence to the bladder cell surface, preventing UPEC from being cleared by the bulk flow of urine and enabling the bacteria to colonize the urothelial cells.[6] Among the different adhesins expressed on the bacterial surface, mannose-binding type 1 pili are the most prevalent. They consist of a helical rod containing 500 to 3000 copies of the structural subunit FimA, as well as one copy of the FimF, FimG, and FimH subunits.[7] The FimH subunit is expressed at the distal tip of each pilus and exhibits the carbohydrate recognition domain (CRD), which interacts with the mannosylated glycoprotein uroplakin 1a on the mucosal surface of the bladder.[8]

More than three decades ago, Sharon and co-workers described various oligomannosides and aryl α-D-mannosides as potential antagonists of FimH-mediated bacterial adhesion.[9,10] This led to subsequent reports on several monovalent mannose-based FimH antagonists containing various aglycones, such as *n*-alkyl,[11] phenyl,[12] dioxocyclobutenylaminophenyl,[13] umbelliferyl,[12] biphenyl,[14-19] indol(in)ylphenyl,[20] triazolyl[21] or thiazolylamino.[22] In addition, different multivalent presentations of mannose derivatives have been explored.[23-29] Most importantly, it was recently shown that these α-D-mannopyranosides did not elicit adverse

side effects caused by the non-selective binding of FimH antagonists to various mammalian mannose receptors.[30]

In vivo studies in a mouse disease model confirmed the therapeutic potential of biaryl mannosides for an oral treatment of UTI.[15-17,19] However, since only low oral bioavailability could be achieved, basic determinants such as aqueous solubility and membrane permeability should be further optimized.[31] One possible solution is offered by a phosphate prodrug approach, which is applied either when the active principal exhibits high membrane permeability but suffers from low aqueous solubility,[32] or when the therapeutic dose exceeds the maximum amount of drug that can be dissolved into intestinal fluids.[33] In a phosphate prodrug approach, the active principle is rapidly released by endogenous phosphatases, such as alkaline phosphatase, an enzyme particularly abundant on the brush border of enterocytes.[34]

The application of this prodrug principle has led to various marketed drugs, such as prednisolone phosphate (**1**),[35] the antiretroviral drug fosamprenavir (**2**)[34,36] or the chemotherapeutic drug fludarabine phosphate.[37]

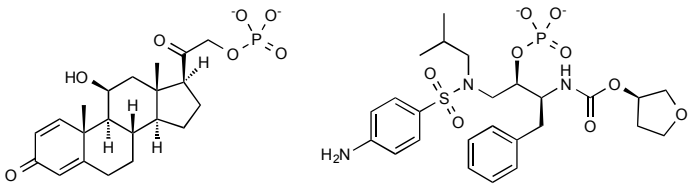


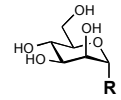
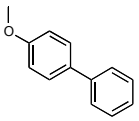
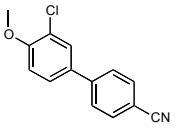
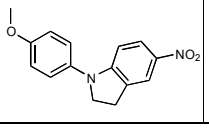
Figure 1. Phosphate prodrugs of marketed drugs.

The goal of the present study was to optimize the physicochemical profile of the biaryl mannosides **3**-**5** by phosphorylation, enhancing aqueous solubility and, consequently, the oral availability.

Results and Discussion

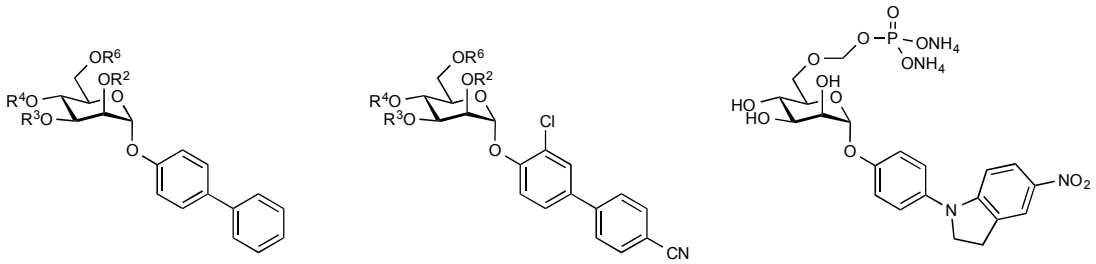
Although exhibiting nanomolar affinity and high permeability, one drawback of FimH antagonists **3**-**5**[14,18,19,20] is their low aqueous solubility, resulting in limited oral bioavailability. They are therefore perfect candidates for a phosphate prodrug approach. Table 1 summarizes their previously reported binding affinities (IC_{50})[14,18,19,20] as well as their physicochemical properties (solubility, lipophilicity, and permeability).

Table 1. Binding affinities and physicochemical properties of the biaryl α-D-mannopyranosides **3**-**5**. IC_{50} values, aqueous solubility, log *P*, PAMPA and Caco-2 cell data were adopted from references,[18-20] the IC_{50} of antagonist **4** and Caco-2 P_{app} values of antagonist **5** were obtained according to the procedure described in.[18]

Cpd		**IC_{50}**[a] [nM]	**Solubility**[b] [μg/mL]	**log *P***[c]	**PAMPA log P_e**[d] [cm/s]	**Caco-2 P_{app}**[e] [10^{-6} cm/s]	
						a→b	b→a
3[14,18]		84.9	21	2.1	-4.7	10.0 ± 0.9	19.0 ± 1.2
4[19]		10.1	192	2.1	-5.2	2.2 ± 0.4	22.1 ± 1.5
5[20]		20	24	1.9	-5.5	2.9 ± 0.6	39.3 ± 5.8

[a] IC_{50} values were determined in a cell-free competitive binding assay;[38] [b] Thermodynamic solubility for compounds **5**; kinetic solubility for compound **3** and **4**;[39] [c] Octanol-water partition coefficients (log *P*) were determined by a miniaturized shake-flask procedure;[40] [d] P_e = effective permeability: passive permeation through an artificial membrane was determined by the parallel artificial membrane permeability assay (PAMPA);[41,42] [e] P_{app} = apparent permeability: permeation through a Caco-2 cell monolayer was assessed in the absorptive (a→b) and secretory (b→a) directions in triplicate.[43,44]

For identifying the optimal position of the phosphate promoiety on the mannoside core of FimH antagonists **3**-**5**, a series of phosphate esters was synthesized (Figure 2) and their solubility determined. In the prodrugs **6a**-**d** and **7a**-**d**, the phosphate ester bond was directly linked to the various hydroxyl groups of the mannose moiety. Alternatively, an acetal linker was used in prodrugs **6e** and **8** to increase the distance between the enzymatic cleavage site and thereby enhance accessibility of the phosphate ester and subsequently the dephosphorylation rate. When prodrugs **6e** and **8** are dephosphorylated, the intermediate hemiacetals is expected to collapse spontaneously releasing the active principle **3** or **5** accompanied by formaldehyde.[45]



6a: $R^2 = PO_3(NH_4)_2$; R^3, R^4, R^6 = H
6b: $R^3 = PO_3(NH_4)_2$; R^2, R^4, R^6 = H
6c: $R^4 = PO_3(NH_4)_2$; R^2, R^3, R^6 = H
6d: $R^6 = PO_3(NH_4)_2$; R^2, R^3, R^4 = H
6e: $R^2 = CH_2OPO_3(NH_4)_2$; R^2, R^3, R^4 = H

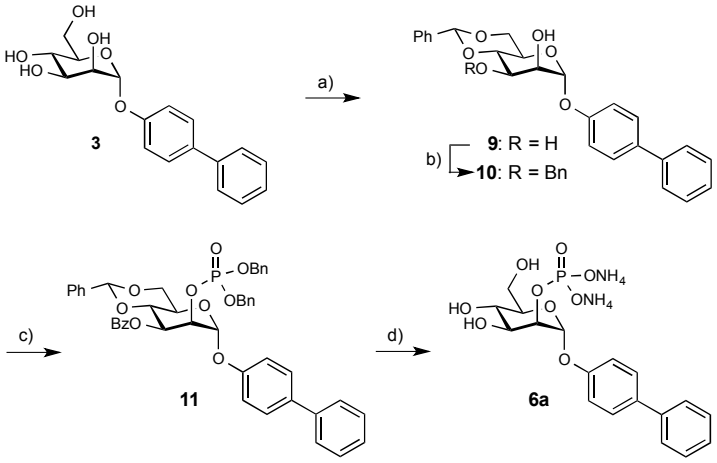
7a: $R^2 = PO_3(NH_4)_2$; R^3, R^4, R^6 = H
7b: $R^3 = PO_3(NH_4)_2$; R^2, R^4, R^6 = H
7c: $R^4 = PO_3(NH_4)_2$; R^2, R^3, R^6 = H
7d: $R^6 = PO_3(NH_4)_2$; R^2, R^3, R^4 = H

8

Figure 2. Phosphate monoesters **6a-e** of biphenyl α-D-mannopyranoside **3**, **7a-d** of substituted biphenyl α-D-mannopyranoside **4**, and **8** from indolinylphenyl α-D-mannopyranoside **5**.

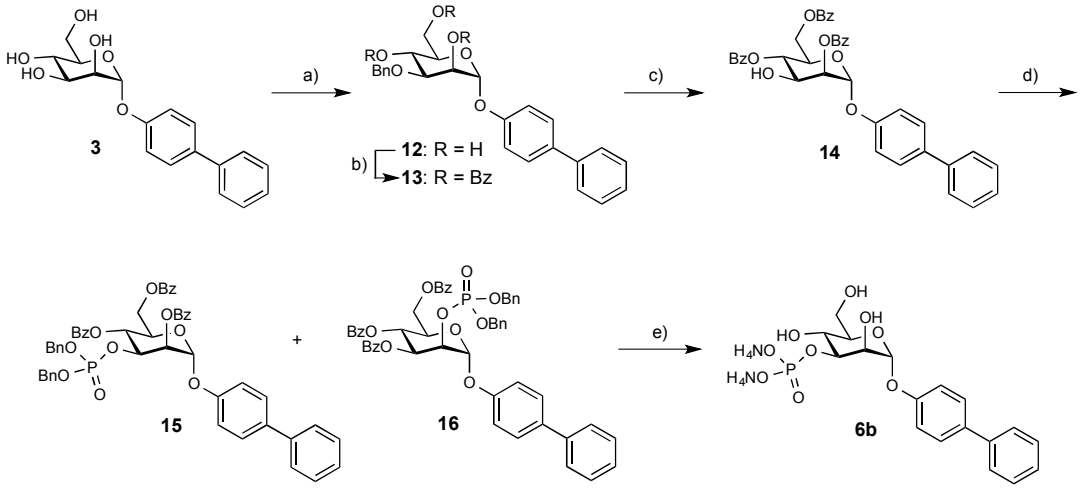
Synthesis of Phosphates 6a-d. 2-Phosphate **6a** of biphenyl α-D-mannopyranoside (**3**) was synthesized according to the procedure depicted in Scheme 1. Starting from **3**,[14,18] a benzylidene acetal (→ **9**) was formed to protect the 4- and 6-OH of the mannose moiety. The 3-position was subsequently protected by a regioselective dibutyltin oxide-mediated benzylation (→ **10**), and then phosphorylation using dibenzyl *N*,*N*-diisopropyl-phosphoramidite in the presence of 1,2,4-triazole, followed by oxidation with *tert*-

butylhydroperoxide, afforded the protected intermediate **11**. Global deprotection via catalytic hydrogenation yielded 2-phosphate **6a**.



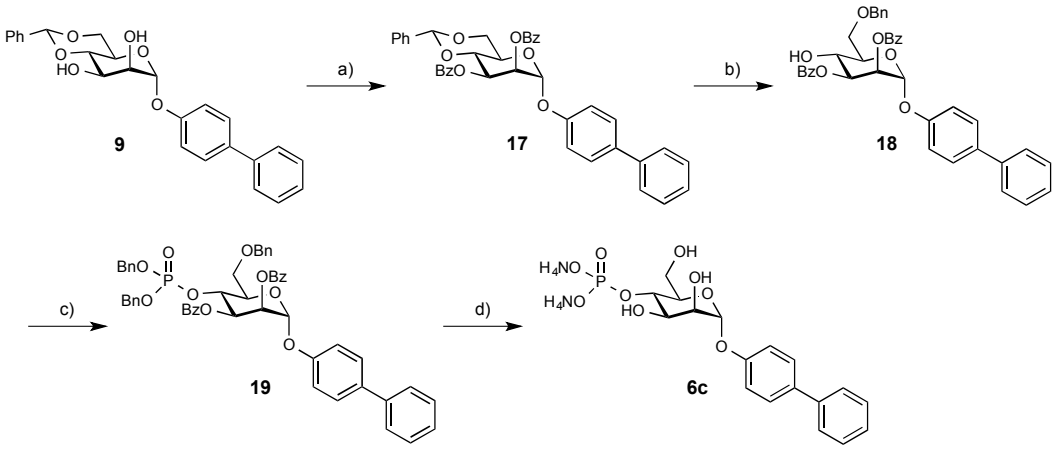
Scheme 1. a) $PhCH(OMe)_2$, *p*-TsOH, DMF, 50 °C, overnight (70%); b) i. Bu_2SnO, toluene, 135 °C, 3 h; ii. BnBr, toluene, 115 °C, overnight (80%); c) dibenzyl *N*,*N*-diisopropylphosphoramidite, 1,2,4-triazole, MeCN, 0 °C to rt, overnight; then 70% aq. *tert*-BuOOH, rt, 1 h (62%); d) i. H_2 (4 bar), $Pd(OH)_2$/C, EtOAc, cat. HOAc, overnight; ii. 25% aq. NH_3/MeOH (4:1), rt, overnight (45%).

For the synthesis of 3-phosphate **6b**, 3- position of **3** was regioselectively benzylated (→ **12**) followed by perbenzoylation to give **13** (Scheme 2). Cleavage of the benzyl group by hydrogenation (→ **14**) and subsequent phosphorylation afforded an inseparable 3:2-mixture of the protected 3- and 2-phosphates **15** & **16** due to partial migration of the 2-benzoyl moiety. Upon deprotection via catalytic hydrogenation pure 3-phosphate **6b** could be isolated.



Scheme 2. a) i. Bu_2SnO, MeOH, reflux, 5 h, ii. BnBr, toluene, 115 °C, overnight (36%); b) BzCl, cat. DMAP, pyr, rt, overnight (99%); c) H_2 (4 bar), $Pd(OH)_2/C$, dioxane/EtOAc, cat. AcOH, rt, overnight (73%); d) dibenzyl *N*,*N*-diisopropylphosphoramidite (90%), 1,2,4-triazole, MeCN, 0 °C to rt, overnight; then 70% aq. *tert*-BuOOH, rt, 1 h (80%, 3:2-mixture of 2-and 3-phosphate derivatives); e) i. H_2 (1 bar), $Pd(OH)_2/C$, EtOAc, 5 h; ii. 25% aq. NH_3/MeOH (4:1), rt, overnight (7%).

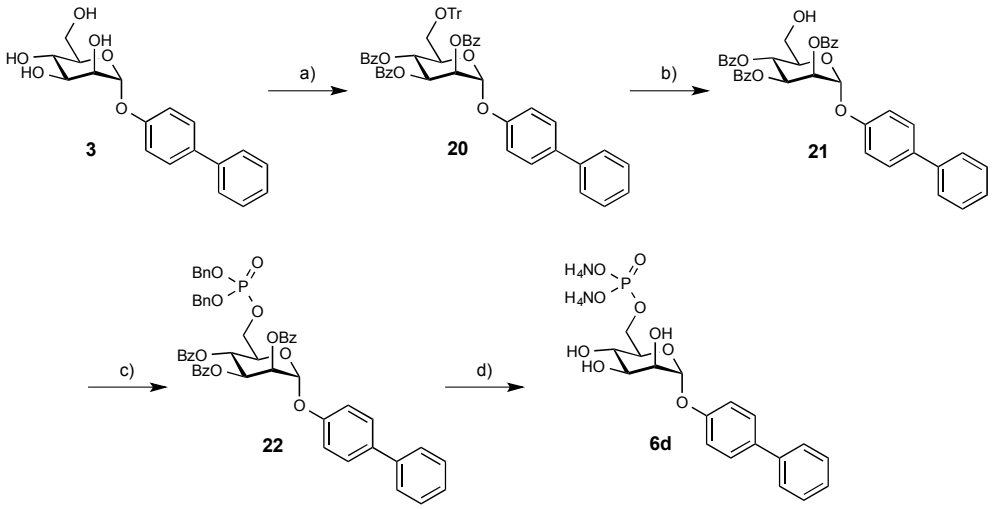
For the synthesis of 4-phosphate **6c**, **9** was benzoylated (→ **17**). Reductive opening of the benzylidene acetal with $Me_3N{\cdot}BH_3$ and $AlCl_3$ afforded precursor **18** (Scheme 3). Phosphorylation (→ **19**) and subsequent deprotection yielded 4-phosphate **6c**.



Scheme 3. a) BzCl, cat. DMAP, pyr, rt, overnight (60%); b) $Me_3N{\cdot}BH_3$, $AlCl_3$, THF/H_2O, rt, 1 h (67%); c) dibenzyl *N*,*N*-diisopropylphosphoramidite (90%), 1,2,4-triazole, MeCN, 0 °C to rt, overnight; then 70% aq. *tert*-

BuOOH, 1 h (53%); d) H_2, $Pd(OH)_2/C$, EtOH/EtOAc, 5 h, quant; e) 25% aq. NH_3/MeOH (4:1), rt, overnight (56%).

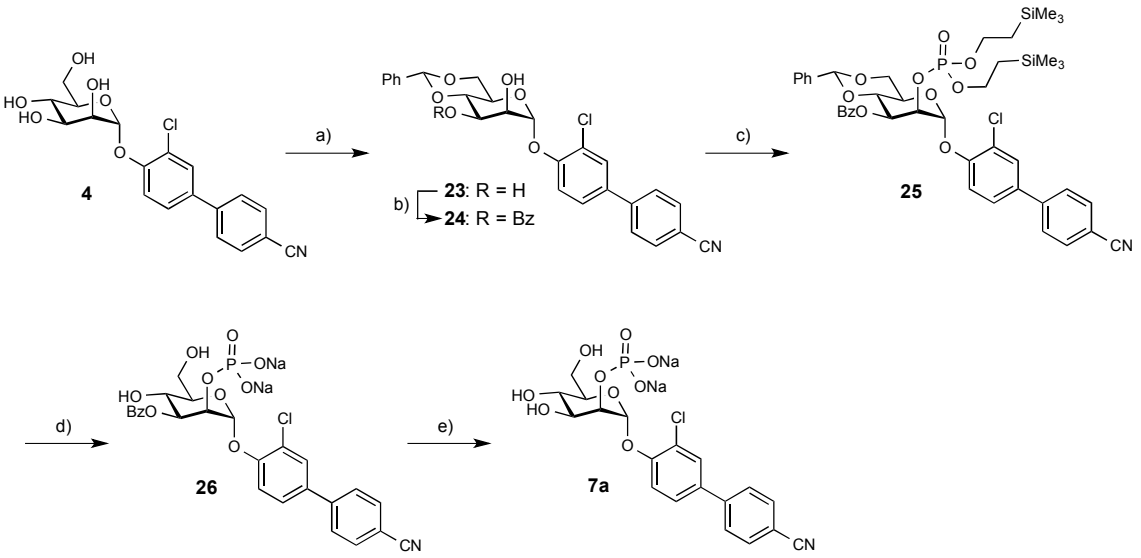
For the synthesis of 6-phosphate **6d** mannoside **3** was tritylated in the 6-position, followed by perbenzoylation (→ **20**) and removal of the trityl group (Scheme 4). Then, intermediate **21** was phosphorylated (→ **22**) and final global deprotection gave 6-phosphate **6d**.



Scheme 4. a) i. TrCl, cat. DMAP, pyr, 80 °C, overnight, ii. BzCl, 50 °C, overnight (88%); b) $FeCl_3/H_2O$, DCM, rt, 5 h (62%); c) dibenzyl *N*,*N*-diisopropylphosphoramidite (90%), 1,2,4-triazole, MeCN, 0 °C to rt, overnight; then 70% aq. *tert*-BuOOH, rt, 1 h (66%), d) i. H_2, $Pd(OH)_2/C$, EtOH/EtOAc, overnight, ii. 25% aq. NH_3/MeOH (4:1), rt, overnight (55%).

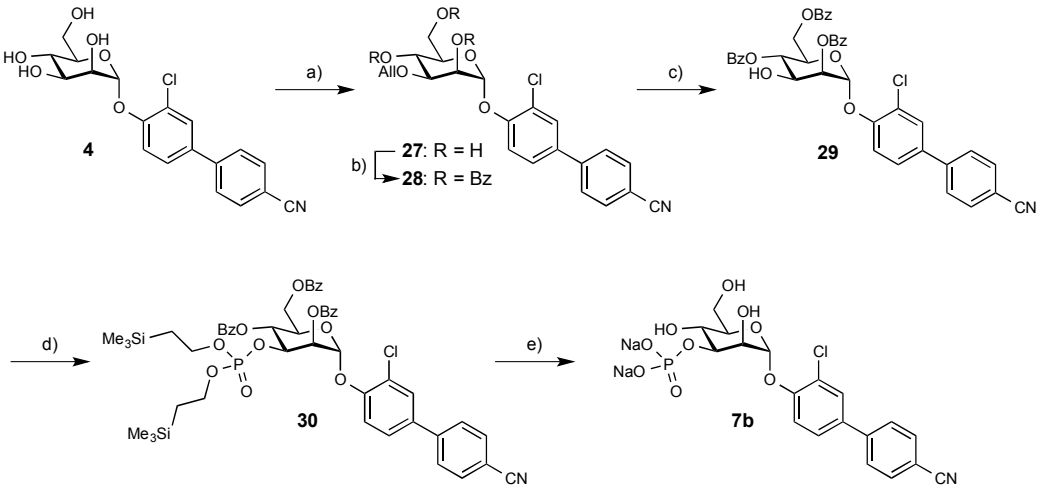
Synthesis of Phosphates 7a-d. Due to the labile chloro- and cyano-substituents present in biphenyl α-D-mannopyranoside **4**,[19] the 2-phosphate **7a** was obtained via a modified strategy (Scheme 5), omitting a potentially intractable hydrogenation step. Therefore, after protecting the 4- and 6-OH of **4** with a benzylidene acetal (→ **23**), the 3-OH of the mannose moiety was selectively benzoylated to afford **24**.[46] Phosphorylation of the 2-OH group with bis[2-(trimethylsilyl)ethyl] *N*,*N*-diisopropylphosphoramidite in the presence of 1,2,4-triazole, and subsequent oxidation with *tert*-butylhydroperoxide, yielded intermediate **25**. After cleavage of

the (trimethylsilyl)ethyl esters with TFA (→ **26**) and subsequent ester hydrolysis upon treatment with NH_3/MeOH, 2-phosphate **7a** was obtained.



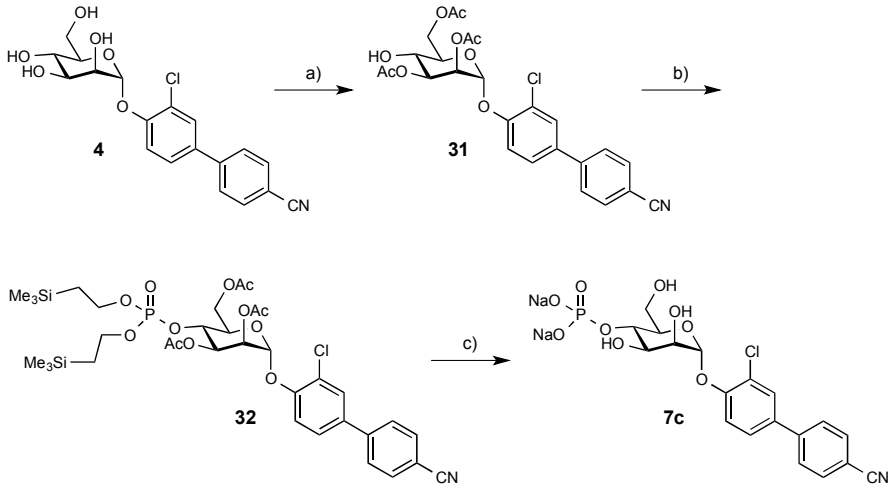
Scheme 5. a) $PhCH(OMe)_2$, *p*-TsOH, rt, 17 h (22%); b) BzCl, DCM/pyr, 0 °C to rt, 3 h (60%); c) bis[2-(trimethylsilyl)ethyl] *N*,*N*-diisopropylphosphoramidite, 1,2,4-triazole, MeCN, 0 °C to rt, 16 h; then 70% aq. *tert*-BuOOH, rt, 1 h (55%); d) TFA/DCM (1:4), rt, 2 h (61%); e) 25% aq. NH_3/MeOH (4:1), rt, 16 h (71%).

Regioselective 3-allylation of **4** (→ **27**) followed by benzoylation of the 2-, 4- and 6-OH gave **28** (Scheme 6). Subsequent cleavage of the allyl group with $PdCl_2$ (→ **29**) and phosphorylation afforded intermediate **30** and final deprotection 3-phosphate **7b**.



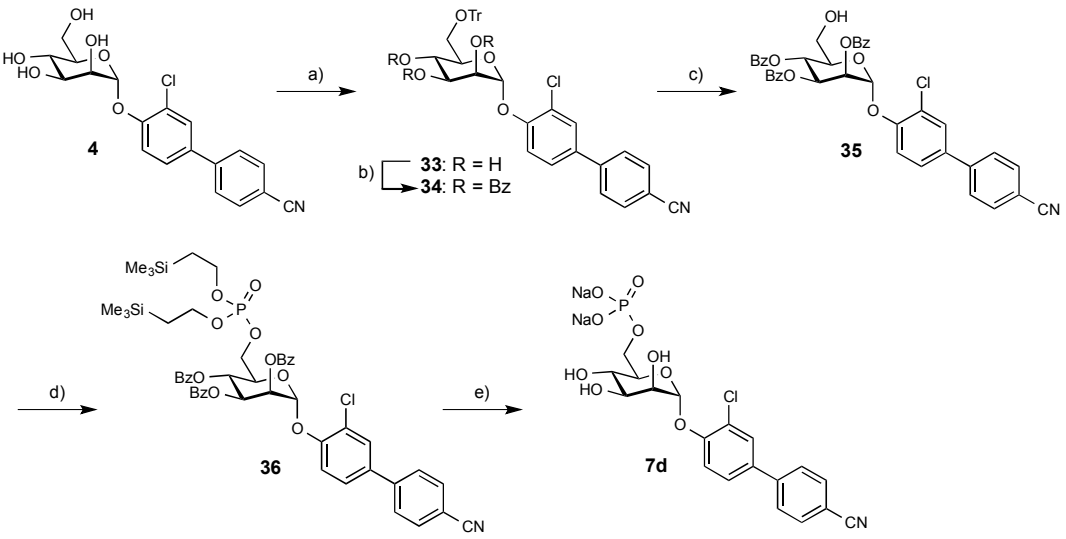
Scheme 6. a) i. Bu_2SnO, toluene, 80 °C, 6 h; ii. AllBr, Bu_4NI, toluene, 80 °C, 20 h (55%); b) BzCl, cat. DMAP, pyr, rt, overnight (87%); c) $PdCl_2$, MeOH, 40 °C, 5 h (84%); d) i. bis[2-(trimethylsilyl)ethyl] *N*,*N*-diisopropyl-phosphoramidite, 1,2,4-triazole, MeCN, 0 °C to rt, 15 h; then 70% aq. *tert*-BuOOH, rt, 2 h (32%); e) i. TFA/DCM (1:4), rt, 1.5 h; ii. 25% aq. NH_3/MeOH (4:1), rt, overnight (45%).

4-Phosphate **7c** was synthesized in three steps via regioselective dibutyltin oxide-mediated acetylation of **4** in the 2-, 3- and 6-position[47] (→ **31**), phosphorylation of the 4-OH (→ **32**) and subsequent cleavage of all protecting groups (Scheme 7).



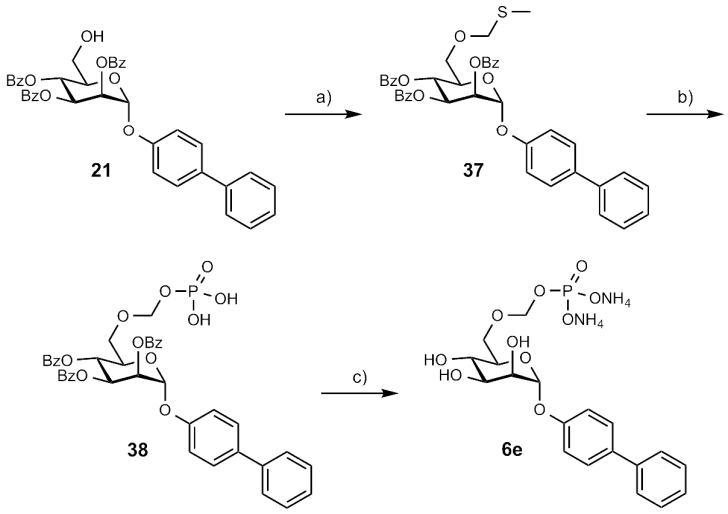
Scheme 7. a) i. Bu_2SnO, MeOH, 70 °C, 2 h; ii. Ac_2O, MeCN, rt, 16 h (21%); b) i. bis-[2-(trimethylsilyl)ethyl] *N,N*-diisopropylphosphoramidite, 1,2,4-triazole, MeCN, 0 °C to rt, 15 h; then 70% aq. *tert*-BuOOH, rt, 2 h (62%); c) i. TFA/DCM (1:4), rt, 2 h; ii. 25% aq. NH_3/MeOH (4:1), rt, 2 h (98%).

For the synthesis of 6-phosphate **7d** a similar protection strategy as for **6d** was used (Scheme 8). The parent compound **4** was tritylated in the 6-position (→ **33**), followed by perbenzoylation (→ **34**) and removal of the trityl group. Phosphorylation of intermediate **35** (→ **36**) and final global deprotection afforded 6-phosphate **7d**.



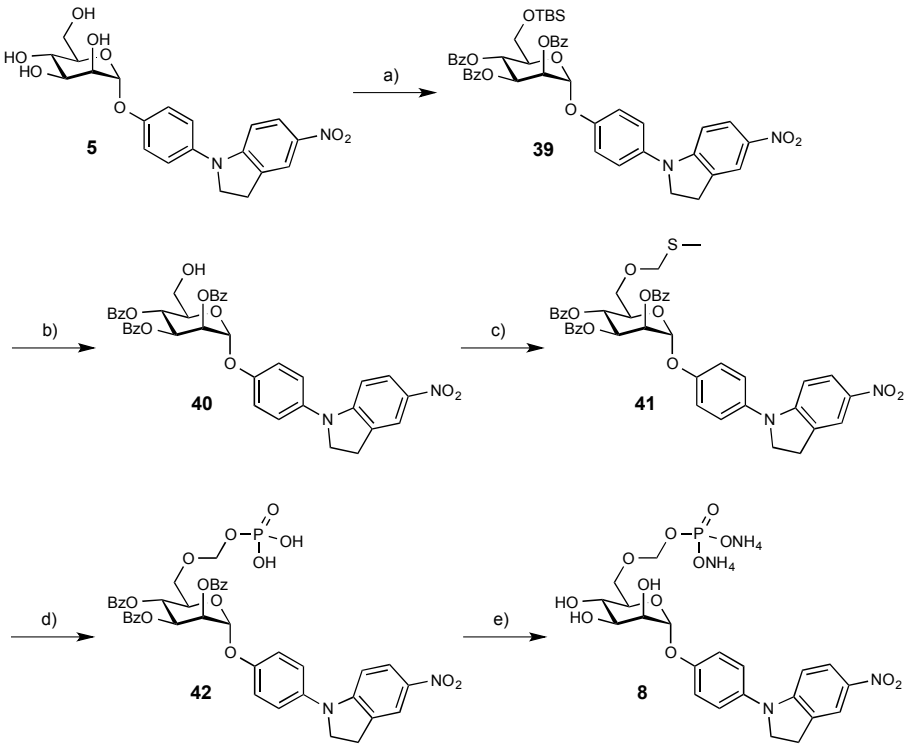
Scheme 8. a) TrCl, cat. DMAP, pyr, 80 °C, 16 h (80%); b) BzCl, cat. DMAP, pyr, rt, 6 h (79%); c) $FeCl_3$, H_2O, rt, 5 h (85%); d) i. bis-[2-(trimethylsilyl)ethyl] *N,N*-diisopropylphosphoramidite, 1,2,4-triazole, MeCN, 0 °C to rt, 15 h; then 70% aq. *tert*-BuOOH, rt, 1.5h (59%); e) i. TFA/DCM (1:4), rt, 2 h; ii. 25% aq. NH_3/MeOH (4:1), rt, overnight (68%).

Synthesis of Acetal-linked Phosphates 6e & 8. Synthesis of the acetal-linked phosphate **6e** of biphenyl α-D-mannopyranoside (**3**) was achieved by first introducing a 6-*O*-(thiomethyl)-methyl group on intermediate **21** with DMSO-acetic anhydride under acidic conditions (→ **37**, Scheme 9). Subsequent treatment with phosphoric acid and *N*-iodosuccinimide gave phosphate **38**. Finally, debenzoylation with NH_3/MeOH provided the target compound **6e**.



Scheme 9. a) DMSO, Ac_2O/AcOH, rt, overnight (35%); b) H_3PO_4, NIS, THF, 0 °C to rt, 1 h (58%); c) 25% aq. NH_3/MeOH (4:1), rt, overnight (50%).

The acetal-linked phosphate **8** was prepared analogously from indolinylphenyl α-D-mannopyranoside **5**[20] (Scheme 10). Selective TBS-protection of the 6-OH, followed by per-benzoylation (→ **39**), and cleavage of the silyl ether yielded precursor **40**. After the introduction of a 6-*O*-(thiomethyl)methyl group with DMSO-acetic anhydride (→ **41**), treatment with phosphoric acid and *N*-iodosuccinimide (→ **42**) and debenzoylation, test compound **8** was obtained.



Scheme 10. a) i. TBSCl, cat. DMAP, pyr, rt, overnight, ii. BzCl, rt, 2 h, (quant.); b) 1 M H_2SO_4/MeOH, rt, 1.5 h (73%); c) DMSO/Ac_2O/HOAc, rt, overnight (74%); d) H_3PO_4/NIS/THF, 0 °C to rt, 1 h (67%); e) 25% aq. NH_3/MeOH/DCM, rt, overnight (41%).

Solubility. The thermodynamic solubility of the phosphate prodrugs was determined in phosphate buffer (50 mM, pH 6.5). The expected improvement of aqueous solubility, which exceeds those of the active principles **3**-**5** by several orders of magnitude (Table 2), could be confirmed.

ALP-Mediated Hydrolysis. ALP-mediated hydrolysis of the various phosphate esters was studied in Caco-2 cells, which express phosphatase on the apical brush border surface of the confluent cell monolayer.[48] The experimental half-life ($t_{1/2}$) was calculated from the concentration of remaining prodrug vs. incubation time (Table 2).

Table 2. Aqueous solubility and Caco-2 phosphatase-mediated hydrolysis ($t_{1/2}$) of prodrugs **6a-e**, **7a-d**, **8**, and their active principles **3**-**5**, respectively.

Cpd	**Solubility** [μg/mL]	$t_{1/2}$ [min]
3, active principle[14,18]	21	---
6a	>3000	12
6b	>3000	13
6c	2703	> 60
6d	>3000	> 60
6e	>3000	8.7
4, active principle[19]	192	---
7a	>3000	13
7b	>3000	12
7c	>3000	43
7d	>3000	48
5, active principle[20]	24	---
8	>3000	11

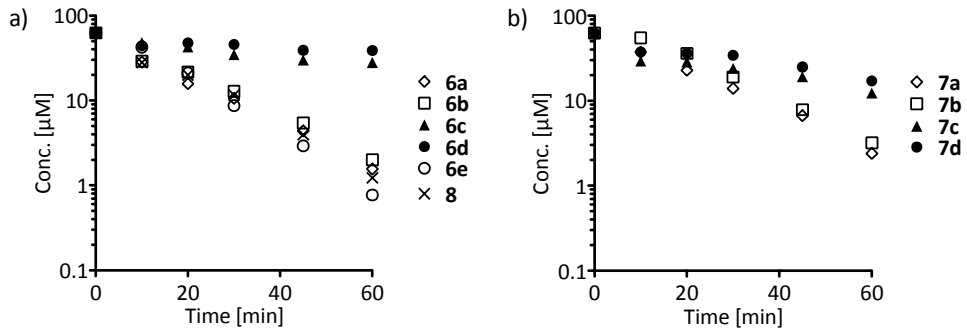


Figure 3. Decomposition of phosphomonoester prodrugs **6a-d** and **7a-d** and phosphonooxymethyl ether prodrugs **6e** and **8** in the apical compartment of the Caco-2 cell assay: (a) **6a-e**, **8**; (b) **7a-d**. Prodrugs dissolved in Dulbecco's Modified Eagle's Medium (62.5 μM) were applied to the apical chamber and the concentrations of unchanged prodrug were monitored by LC-MS.

Depending on the position of the promoiety, the prodrugs showed varying propensity to dephosphorylation (Figure 3). The 2- and 3-phosphate esters (**6a**, **6b**, **7a** and **7b**) were rapidly hydrolyzed ($t_{1/2}$ < 15 min), whereas the 4- and 6-phosphate esters (**6c**, **6d**, **7c** and **7d**) showed prolonged half-lives ($t_{1/2}$ > 40 min). Improved stability of the latter likely results from reduced

access to the ester bonds at C4 and C6 because of steric hindrance.[45,49,50] Therefore, with the introduction of a linker (→ **6e, 8**), accessibility can be improved and the susceptibility to ALP-mediated cleavage was markedly increased ($t_{1/2}$ = 8.7 min and 11 min, respectively).

Owing to their high propensity to ALP-mediated hydrolysis, the phosphate esters **6a**, **6b**, **7a, 7b** and **8** were almost entirely converted to parent drug within 60 min (Figure 3). For example, Figure 4 depicts the concentration of prodrug **7b** 60 min after application to either the apical or the basolateral side of the Caco-2 system. When applied to the apical chamber, the prodrug was almost quantitatively hydrolyzed. However, when applied to the basal chamber, the prodrug remained the prominent species detected, due to the lack of ALP on the basal Caco-2 cell membrane.[48] In addition, irrespective of dosing on the apical or basal side, the prodrug could not be detected in the receiver compartment, which corroborates the poor membrane permeability of the polar phosphate ester (Figure 4).

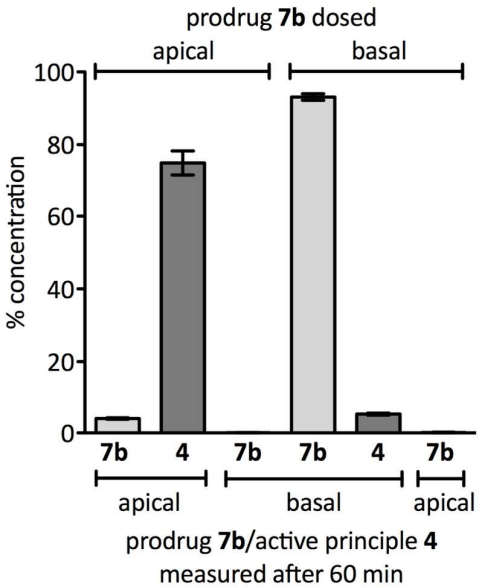


Figure 4. Conversion of prodrug **7b** to the active principle **4** in a Caco-2 cell monolayer model after 60 min of incubation. A prodrug solution (62.5 μM) was applied either into the apical or basal chamber. Columns represent the percentage concentrations of prodrug and active principle 60 min after initiation of the experiment. Concentration of prodrug **7b** determined at time point t = 0 min is defined as 100%.

Stability in biorelevant media. Since chemical stability at various pH conditions and stability for degradation by digestive enzymes (simulated gastric and intestinal fluids,[51,52] for composition see Table S1 in Supporting Information) turned out to be high (> 80% after 2 h; Figure 5), the phosphate prodrugs **7a**, **7c** and **7d** appear optimally suited to mitigate the solubility problem.

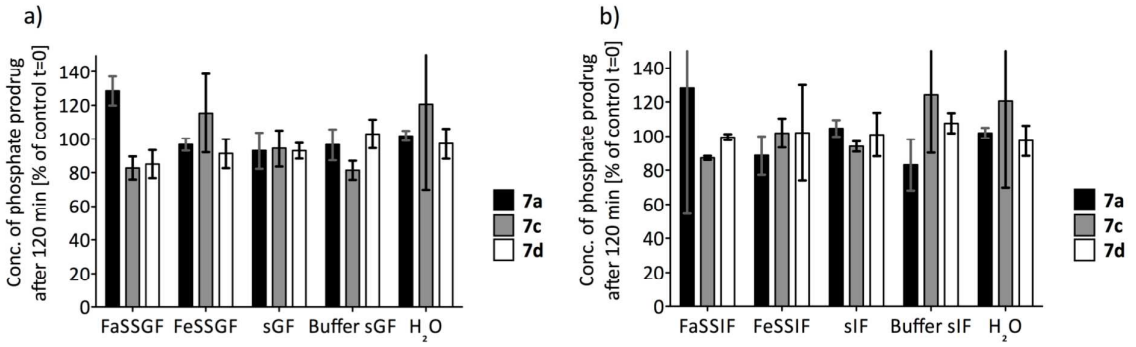


Figure 5. Stability of the phosphate prodrugs **7a** (black column), **7c** (grey column), and **7d** (white column) in biorelevant media. Percentage of the remaining compound concentration relative to the initial concentration (t = 0 min) after 120 min of incubation in a) simulated gastric fluids (FaSSGF, fasted-state simulated gastric fluid; FeSSGF, fed-state simulated gastric fluid; sGF, simulated gastric fluid; buffer sGF, prepared equally to sGF but without pepsin) and b) simulated intestinal fluids (FaSSIF, fasted-state simulated intestinal fluid; FeSSIF, fed-state simulated intestinal fluid; sIF, simulated intestinal fluid; buffer sIF, prepared equally to sIF but without pancreatin) are shown. For composition see Table S1 in Supporting Information. H_2O was used as a reference media. Data represent the mean (triplicates) with its corresponding standard deviation.

Oral Bioavailability. For oral administration of a phosphate prodrug several absorption rate-limiting factors need to be considered. In addition to unsuitably slow ALP-mediated hydrolysis of the phosphate prodrug, low solubility, poor permeability, and efflux of the active principle can also limit oral absorption.

In Vitro Pharmacokinetic Evaluation. To further examine the benefits associated with improved solubility obtained upon phosphorylation of the active principle, the most labile phosphate prodrugs **6e**, **7b**, and **8** ($t_{1/2} \leq 12$ min, Table 2) were applied to the apical chambers of the Caco-2 system, and the accumulation of active principles **3**, **4**, and **5** on the basal side of the cell monolayer was monitored (Figures 6a-c). Applying either the active principle or the respective prodrug at equal concentrations (62.5 μM) to the apical chamber resulted in similar basolateral concentrations of the active principle, i.e. the hydrolysis is not the rate limiting step. When higher apical doses of the phosphate prodrugs were applied, the basolateral concentrations were markedly increased (Figure 6a-c).

Once a prodrug has been hydrolyzed *in vivo*, physicochemical properties of the active principle (i.e. solubility and permeability) are becoming the rate-limiting steps for absorption. When after rapid hydrolysis ($t_{1/2} < 15$ min, Table 2) the active principle is precipitating due to low solubility, the available amount for absorption is reduced. Although the active principles **3** and **5** show similar solubilities (Table 1), different basolateral concentrations were observed (Figure 6a & 6c). In contrast, the basolateral concentrations of compound **3** were similar or higher compared to those of compound **4** (Figure 6a & 6b), even though compound **4** has an 8-fold higher solubility (Table 1). Based on permeability data derived from PAMPA,[39] high passive permeability is predicted for all three antagonists ($\log P_e < -5.7$ cm/s, Table 1).[42] However, for the antagonists **4** and **5**, efflux ratios (b-a/a-b) > 2 (Table 1) in bi-directional Caco-2 experiments were observed, indicating the involvement of an efflux transporter (e.g. P-gp).[44] Due to the high apical concentrations reached from fast ALP-mediated hydrolysis of prodrugs **7b** and **8,** saturation of the transporter mediated efflux occurred, leading to increased basolateral concentrations of **4** and **5** (Figure 6b & 6c). In contrast, **3** exhibited an efflux ratio < 2 (Table 1), i.e. the basolateral concentration rose proportionally to the amount of applied prodrug and did not display saturation kinetics (Figure 6a).[53]

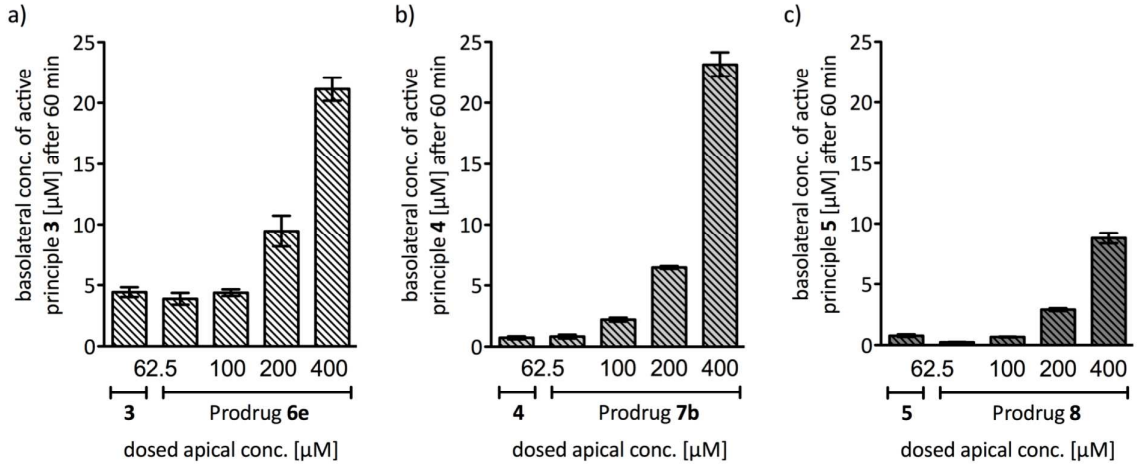


Figure 6. Accumulation of active principle (a) **3**, (b) **4**, and (c) **5** in the basal receiver chamber of a Caco-2 cell system 60 min after applying (a) active principle **3** or prodrug **6e**, (b) active principle **4** or prodrug **7b**, and (c) active principle **5** or prodrug **8** into the apical chamber. The active principles were dosed at a concentration of 62.5 µM, which corresponds to approx. the aqueous solubilities of **3** and **5**. The phosphate prodrugs were dosed at four different concentrations (62.5 µM, 100 µM, 200 µM, and 400 µM).

In vivo pharmacokinetic studies. In the mouse PK model, an increased intestinal uptake was anticipated for the prodrug **7c** due to its increased solubility and slower hydrolysis rate. The prodrugs **7b**, **7c** and **8** were administered per os at a dose of 10 mg/kg, but could not be detected in plasma or urine samples. The oral bioavailability of active principle **4** upon administering prodrug **7b** and **7c** and of active principle **5** upon administration of prodrug **8** are illustrated in Figure 7. Table 3 summarizes the pharmacokinetic parameters (C_{max}, T_{max}, and urine $AUC_{0\text{-}24}$) of the different p.o. applications.

Table 3. Pharmacokinetic parameters (C_{max}, T_{max}, and urine $AUC_{0\text{-}24}$) determined after a single p.o. application of compounds **4**, **7b**, **7c**, and **8**, in female C3H/HeN mice.[a]

Parameter	**4**		**7b** (prodrug of **4**)	**7c** (prodrug of **4**)	**8** (prodrug of **5**)
Dose (mg/kg) p.o.	1.25	7.7	10	10	10
C_{max} (µg/mL)	3.9 ± 1.9	23.6 ± 3.1	40.7 ± 11.4	57.0 ± 4.2	4.5 ± 1.6

T_{max} (h)	1.4 ± 0.6	1.4 ± 0.2	0.9 ± 0.2	0.44 ± 0.2	3.0 ± 0.0
Urine AUC_{0-24} (µg x h/mL)	21.5 ± 5.2	106.8 ± 18.0	103.9 ± 7.7	226.4* ± 42.8	24.6 ± 10.7

[a]Values were calculated using PKsolver and are shown as mean with standard deviation.[54] C_{max}, maximal concentration; T_{max}, time were C_{max} is reached; AUC, area under the curve. *Statistical differences at $p < 0.001$ (Two-Way ANOVA, Prism; GraphPad Software, Version 5.0f), compared to all other p.o. applications.

Given the almost identical propensity to ALP-mediated hydrolysis of **7b** and **8**, the different urine profiles must be related to physicochemical properties of their active principles **4** and **5**. Indeed, antagonist **5**, besides having an 8-times lower solubility and therefore a higher propensity to precipitate after release in the small intestines, also permeates biological membranes less easily (shown in the PAMPA results, Table 1). In fact, urine AUC_{0-24} and C_{max} of **5** were similar to the pharmacokinetic parameters of **4** when applied at a dose of 1.25 mg/kg (Table 3).

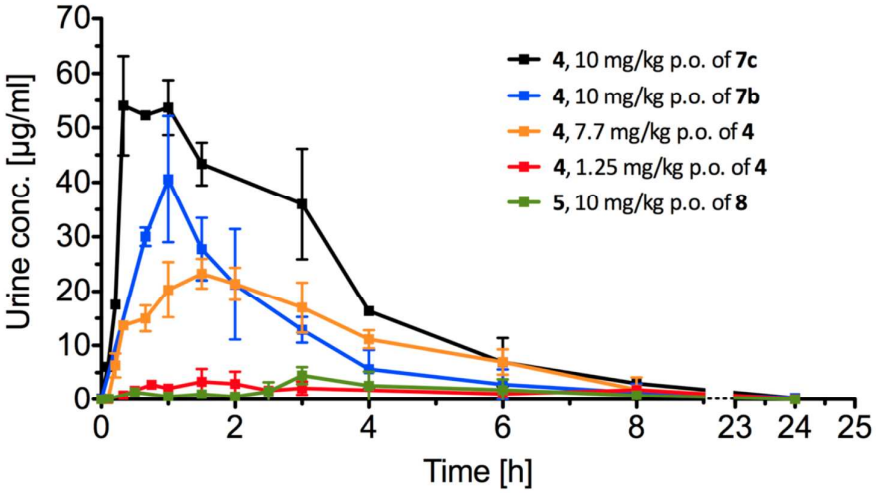


Figure 7. Urine concentrations of active principle **4** in C3H/HeN mice upon p.o. administration of **4** (1.25 mg/kg and 7.7 mg/kg p.o.) or the related phosphate prodrugs **7b**, **7c** (10 mg/kg p.o.) and the urine concentration of the active principle **5** upon p.o. administration of phosphate prodrug **8** (10 mg/kg p.o.). Shown are mean values with standard deviations for groups of three (**4**, 1.25 mg/kg, **7b**, **7c**, and **8**) or five (**4**, 7.7 mg/kg) mice.

Since prodrugs **7b** and **8** are rapidly hydrolyzed *in vitro*, we also assessed the impact of slower ALP-mediated bioactivation and availability of the related active principle in urine. For this purpose, we administered prodrug **7c** (*in vitro* $t_{1/2}$ of 43 min, Table 2) at a dose of 10 mg/kg (Figure 7). After applying **7c**, compound **4** reached C_{max} (57 µg/mL, Table 3) after only 26 min, as compared to 40.7 µg/mL at 54 min for **7b**; it then remained at elevated levels for the next 3 h. Within the observation period of 3-24 h post-administration, urine levels of the two prodrugs dropped steadily. However, at 6 h the concentration of active principle **4** originating from prodrug **7c** was approx. three times higher than the one reached with **7b**, resulting in a 2-fold increased urine $AUC_{0\text{-}24}$ of **7c**.

The lower concentration of active principle **4** upon p.o. application of **7b** as compared to **7c** can be rationalized by several pharmacokinetic effects. First, the fast dephosphorylation of **7b** leads to a local accumulation of **4** at the brush border in the small intestine. Due to insufficient solubility, the active principle **4** could suffer from precipitation. Because of the slower cleavage of the promoiety in prodrug **7c**, the solubility limit of **4** takes longer to reach, enabling an improved absorption of the active principle. Moreover, although high concentrations of **4** saturate P-gp, its efflux activity still contributes to accumulated concentrations of **4** and consequently to precipitation and intestinal elimination. A slower prodrug hydrolysis apparently optimizes the timely interplay between parent drug uptake and P-gp-mediated efflux, leading to higher net absorption and urine concentrations.

Next, we addressed the question of whether increased solubility indeed leads to higher availability of active principle in the urine. Therefore, urine concentration profiles upon p.o. application of 1.25 mg/kg (based on maximal solubility) or 7.7 mg/kg of **4** (applied as a suspension corresponding to 10 mg/kg of prodrug) were determined (Figure 7).

Oral application of the suspension of **4** resulted in a urine $AUC_{0\text{-}24}$ of 106.8 µg x h/mL (C_{max} of 23.6 µg/mL, Table 3) as compared to a urine $AUC_{0\text{-}24}$ of 226.4 µg x h/mL (C_{max} of 57 µg/mL, Table 3) achieved with the corresponding prodrug **7c**, demonstrating the beneficial effect of increased solubility on intestinal uptake. In addition, the application of **4** at two doses (1.25 mg/kg and 7.7 mg/kg; 6.16 times greater) resulted in parallel curves with a similar T_{max} and an approximate difference in urine $AUC_{0\text{-}24}$ of a factor of 5 and C_{max} of a factor of 6 (Table 3). This is a useful observation for future dose-finding in patients.

Summary and Conclusions

For the successful oral application of a phosphate prodrug of an active principle that displays moderate to high membrane permeability, but insufficient solubility, several prerequisites need to be fulfilled. Obviously, the solubility of the prodrug should allow to dissolve the required dose (e.g. in our case 10 mg/kg). Second, because phosphate prodrugs are too polar to permeate the enterocyte layer, an efficient release of the active principle is required. Finally, slow enzymatic hydrolysis is preferred to avoid precipitation of the poorly soluble active principle.

We have demonstrated the advantages of the phosphate prodrug approach as applied to FimH antagonists with an insufficient solubility but high passive permeability. An increase in solubility of up to 140-fold could be reached upon phosphorylation of the active principle. Furthermore, either fast or slow hydrolysis was observed, depending on the position of the phosphate promoiety on the mannose ring. When the phosphate ester bond was directly linked at the C2- or C3-position of mannose (→ **6a**, **6b**, **7a**, **7b**), or when an acetal linker at C6- was used (→ **6e** & **8**), enzymatic cleavage was fast ($t_{1/2}$ < 15 min). In contrast, a phosphate at the

C4- or C6-position (→ **6c**, **6d**, **7c** & **7d**) showed an enhanced enzymatic stability ($t_{1/2}$ > 40 min).

Interestingly, even when the rates of hydrolysis were similar, e.g. for prodrugs **7b** and **8**, different physicochemical properties (solubility and permeability) of their active principles influenced the *in vivo* PK properties. For antagonist **5**, which has an 8-fold lower solubility as compared to **4**, the risk of precipitation from a supersaturated solution in the small intestines needs to be taken into account.

Furthermore, a high concentration gradient across the Caco-2 cell monolayer, as was reached with the more soluble phosphate prodrugs, promotes absorptive flux of the active principles **3**, **4**, and **5**, and apparently saturates the efflux carrier activity of **4** and **5**. This observation was confirmed in an *in vivo* PK study in mice, where urine $AUC_{0\text{-}24}$ of the active principle **4** could be doubled when prodrug **7c** was applied instead of active principle **4**. Moreover, *in vivo* administration of slowly hydrolyzed prodrug **7c** ($t_{1/2}$ < 40 min) exhibited an increase in urine $AUC_{0\text{-}24}$ as compared to a phosphate prodrug with fast enzymatic cleavage (**7b**, $t_{1/2}$ < 15 min). Slower conversion to the active principle prolonged intestinal uptake and renal excretion by improving the interplay between solubility, drug uptake, and saturation of P-gp mediated efflux.

Experimental Section

Synthesis

General Methods. NMR spectra were recorded on a Bruker Avance DMX-500 (500.1 MHz) spectrometer. Assignment of ^{1}H and ^{13}C NMR spectra was achieved using 2D methods (COSY, HSQC). Chemical shifts are expressed in ppm using residual $CHCl_3$ or MeOH as

references. Optical rotations were measured with a PerkinElmer Polarimeter 341. Electrospray ionization mass spectrometry (ESI-MS) data were obtained on a Waters Micromass ZQ instrument. Microwave-assisted reactions were carried out with a CEM Discover and Explorer. Reactions were monitored by TLC using glass plates coated with silica gel 60 F254 (Merck) and visualized by UV light and/or by charring with a molybdate solution (0.02 M solution of ammonium cerium sulfate dihydrate and ammonium molybdate tetrahydrate in aqueous 10% H_2SO_4). Medium pressure chromatography (MPLC) separations were carried out on a CombiFlash Companion or R_f from Teledyne Isco equipped with RediSep normal-phase or RP-18 reversed-phase flash columns. Commercially available reagents were purchased from Fluka, Aldrich, or Alfa Aesar (Germany). Solvents were purchased from Sigma–Aldrich (Buchs, Switzerland) or Acros Organics (Geel, Belgium) and were dried prior to use where indicated. MeOH was dried by reflux with sodium methoxide and distilled and stored under argon atmosphere. Dichlormethane (DCM) and acetonitrile (MeCN) were dried by filtration over Al_2O_3 (Fluka, type 5016 A basic) and stored over molecular sieves under argon. Molecular sieves (4 Å) were activated *in vacuo* at 300 °C for 0.5 h before use.

Compound purity. Each test compound was purified by chromatography on silica (DCM/MeOH) or reversed-phase chromatography (RP-18, H_2O/MeOH) prior to HPLC, HRMS, NMR and activity testing. The purity of all test compounds was determined by NMR and HPLC [Method A: Beckman Coulter Gold, consisting of pump 126, DAD 168 (190-410 nm) and auto-sampler 508; column: Waters Atlantis T3, 3 μm, 2.1 × 100 mm; A: H_2O + 0.1% TFA; B: MeCN + 0.1% TFA; gradient: 5% B for 0.5 min, 5% B → 70% B over 19.5 min; flow rate: 0.5 mL/min. Method B: Agilent 1100/1200 with UV detection (190-410 nm); column: Waters Atlantis T3, 3 μm, 2.1 × 100 mm; A: H_2O + 0.01% TFA; B: MeCN + 0.01% TFA; gradient: 5% B for 1 min, 5% B → 95% B over 19 min; flow rate: 0.5 mL/min.

Detection: 254 nm] to be ≥ 95% (for ^{1}H NMR spectra and HPLC traces see Supporting Information).

General procedure for phosphorylation. To an ice-cooled solution (0 °C) of protected mannoside (1 eq) and 1,2,4-triazole (4 eq) in dry MeCN was added dibenzyl *N,N*-diisopropylphosphoramidite or bis[2-(trimethylsilyl)ethyl] *N,N*-diisopropylphosphoramidite (2 eq) and the mixture was stirred for 30 min at 0 °C and then overnight at rt. Then, 70% aq. *tert*-butylhydroperoxid (4 eq) was added and the solution was stirred for 1 h. The reaction was quenched with 1 M aq. $Na_2S_2O_3$ and 1 M aq. $NaHCO_3$ and the mixture was extracted twice with DCM. The combined organic layers were dried over Na_2SO_4, filtered and the solvents removed *in vacuo.* The residue was purified by MPLC on silica gel (petroleum ether/EtOAc) to yield the phosphorylated compounds.

Biphenyl 2-*O*-phosphoryl-α-D-mannopyranoside diammonium salt (6a). Hydrogenolysis of compound **11** (100 mg, 0.129 mmol) was conducted in a Parr shaker with 10% $Pd(OH)_2$/C (12 mg) and a catalytic amount of HOAc in EtOAc (6.0 mL) under hydrogen (4 bar) at rt overnight. Then, the reaction suspension was filtered through celite and the filtrate was concentrated *in vacuo*. The residue was stirred in 25% aq. NH_3 (4 mL) and MeOH (1 mL) overnight. Then, the solvents were removed under reduced pressure and the residue was purified by MPLC on silica (DCM/MeOH/H_2O, 6:4:0.6) to give **6a** (26.0 mg, 45%) as a white solid. $[\alpha]_D^{20}$ +66.7 (*c* 0.12, H_2O); ^{1}H NMR (D_2O, 500 MHz): δ = 3.77-3.82 (m, 3H, H-5, H-6), 3.87 (t, *J* = 10.0 Hz, 1H, H-4), 4.14 (ddd, *J* = 2.0, 3.0, 10.0 Hz, 1H, H-3), 4.62 (ddd, *J* = 2.0, 3.0, 8.5 Hz, 1H, H-2), 5.89 (d, *J* = 1.5 Hz, 1H, H-1), 7.31 (m, 2H, Ar-H), 7.44 (m, 1H, Ar-H), 7.55 (t, *J* = 7.5 Hz, 2H, Ar-H), 7.71 (m, 4H, Ar-H); ^{13}C NMR (D_2O, 126 MHz): δ = 60.5 (C-6), 66.6 (C-4), 70.1 (d, *J* = 5 Hz, C-3), 73.5 (C-5), 73.6 (d, *J* = 5 Hz, C-2), 97.0 (d, *J* =

3 Hz, C-1), 117.2, 117.6, 126.7, 127.4, 128.0, 128.2, 129.1, 135.3, 140.0, 155.0 (12C, Ar-C); ESI-MS: *m/z:* Calcd for $C_{18}H_{20}O_9P$ $[M-2NH_4+H]^-$: 411.08, found: 411.06.

Biphenyl 3-*O*-phosphoryl-α-D-mannopyranoside diammonium salt (6b). Hydrogenolysis of a 3:2-mixture of **15** and **16** (130 mg, 0.144 mmol) in EtOAc (6 mL) was conducted in the presence of 10% $Pd(OH)_2/C$ (15 mg) with a hydrogen balloon at rt for 5 h. Then, the reaction suspension was filtered through celite and the filtrate was concentrated *in vacuo*. The residue was stirred in 25% aq. NH_3 (4 mL) and MeOH (1 mL) at rt overnight. The solvents were removed under reduced pressure and the residue was purified by MPLC on silica gel (DCM/MeOH/H_2O, 6:4:0.6) to provide **6b** (4.5 mg, 7%, still containing some of the 2-phosphate impurity) as white solid. $[\alpha]_D^{20}$ +91.7 (*c* 0.12, D_2O); ^1H NMR (D_2O, 500 MHz): δ = 3.75-3.83 (m, 3H, H-5, H-6), 3.92 (t, *J* = 9.5 Hz, 1H, H-4), 4.39 (m, 1H, H-2), 4.55 (dt, *J* = 3.0, 9.0 Hz, 1H, H-3), 5.72 (s, 1H, H-1), 7.32 (m, 2H, Ar-H), 7.45 (m, 1H, Ar-H), 7.54 (t, *J* = 7.5 Hz, 2H, Ar-H), 7.71 (m, 4H, Ar-H); ^{13}C NMR (D_2O, 126 MHz): δ = 61.4 (C-6), 66.9 (d, *J* = 3 Hz, C-4), 70.0 (d, *J* = 3 Hz, C-2), 74.0 (C-5), 75.0 (d, *J* = 5 Hz, C-3), 98.5 (C-1), 118.1, 118.3, 127.31, 127.34, 128.0, 128.8, 129.8, 135.8, 140.6, 155.7 (12C, Ar-C); ESI-MS: *m/z*: Calcd for $C_{18}H_{20}O_9P$ $[M-2NH_4+H]^-$: 411.08, found: 411.12.

Biphenyl 4-*O*-phosphoryl-α-D-mannopyranoside diammonium salt (6c). Hydrogenolysis of **19** (80 mg, 90 μmol) was done in EtOAc (4 mL) in the presence of 10% $Pd(OH)_2/C$ (12 mg) with hydrogen balloon at rt overnight. Then the reaction suspension was filtered through celite, the filtrate was concentrated *in vacuo* to provide the debenzylated intermediate. The crude intermediate (52 mg) was stirred in MeOH (1 mL) and 25% aq. NH_3 (4 mL) overnight. The solvent was removed under reduced pressure and the residue was purified by MPLC on silica (DCM/MeOH/H_2O, 4:1:0.1) to yield **6c** (18 mg, 56%) as a white solid. $[\alpha]_D^{20}$ +104.8 (*c* 0.20, H_2O); ^1H NMR (D_2O, 500 MHz): δ = 3.75-3.87 (m, 3H, H-5, H-6), 4.24 (m, 3H, H-2,

H-3, H-4), 5.70 (s, 1H, H-1), 7.30 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.43 (t, *J* = 7.5 Hz, 1H, Ar-H), 7.54 (t, *J* = 8.0 Hz, 2H, Ar-H), 7.70 (d, *J* = 8.0 Hz, 4H, Ar-H); ^{13}C NMR (D_2O, 126 MHz): δ = 60.6 (C-6), 69.6 (C-2), 69.7 (d, *J* = 5 Hz, C-4), 70.7 (C-3), 72.6 (d, *J* = 7 Hz, C-5), 97.9 (C-1), 117.6, 126.7, 127.4, 128.2, 129.1, 135.3, 140.0, 155.1 (12C, Ar-C); ESI-MS: *m/z*: Calcd for $C_{18}H_{20}O_9P$ $[M-2NH_4+H]^-$: 411.08, found: 411.11.

Biphenyl 6-*O*-phosphoryl-α-D-mannopyranoside diammonium salt (6d). Hydrogenolysis of **22** (64.0 mg, 70.0 μmol) was done in EtOAc/EtOH (5 mL, 3:2) in the presence of 10% $Pd(OH)_2/C$ (7.5 mg) with hydrogen balloon at rt overnight. The reaction suspension was filtered through celite and the filtrate was concentrated *in vacuo*. The crude intermediate was stirred in MeOH (1 mL) and 25% aq. NH_3 (4 mL) overnight. The solvents were removed under reduced pressure and the residue was purified by MPLC on silica (DCM/MeOH/H_2O, 4:1:0.1) to yield **6d** (16.0 mg, 55%) as a white solid. $[\alpha]_D^{20}$ +74.1 (*c* 0.16, H_2O); ^{1}H NMR (D_2O, 500 MHz): δ = 3.83 (m, 1H, H-5), 3.89 (ddd, *J* = 1.9, 4.9, 12.0 Hz, 1H, H-6a), 4.01 (t, *J* = 9.9 Hz, 1H, H-4), 4.08 (m, 1H, H-6b), 4.11 (dd, *J* = 3.5, 10.0 Hz, 1H, H-3), 4.21 (dd, *J* = 1.8, 3.4 Hz, 1H, H-2), 5.69 (d, *J* = 1.4 Hz, 1H, H-1), 7.28 (m, 2H, Ar-H), 7.49 (t, *J* = 7.4 Hz, 1H, Ar-H), 7.54 (t, *J* = 7.7 Hz, 2H, Ar-H), 7.70 (d, *J* = 8.7 Hz, 4H, Ar-H); ^{13}C NMR (D_2O, 126 MHz): δ = 62.6 (*J* = 4 Hz, C-6), 65.8 (C-4), 70.0, 70.1 (C-2, C-3), 72.9 (d, *J* = 8 Hz, C-5), 98.4 (C-1), 117.5, 127.4, 126.7, 128.3, 129.1, 135.3, 140.0, 155.1 (12C, Ar-C); ESI-MS: *m/z*: Calcd for $C_{18}H_{20}O_9P$ $[M-2NH_4+H]^-$: 411.08, found: 411.09.

Biphenyl 6-*O*-(phosphonooxymethyl)-α-D-mannopyranoside diammonium salt (6e). Compound **38** (110 mg, 0.146 mmol) was stirred in MeOH (1 mL) and 25% aq. NH_3 (4 mL) overnight. The solvents were removed under reduced pressure and the residue was purified by MPLC on RP-18 (H_2O/MeOH) to yield **6e** (32.3 mg, 50%) as a white solid. $[\alpha]_D^{20}$ +75.5 (*c*

0.32, H_2O); ^1H NMR (D_2O, 500 MHz): δ = 3.85 (dd, *J* = 2.0, 11.5 Hz, 1H, H-6a), 3.88 (m, 1H, H-5), 3.93 (t, *J* = 9.5 Hz, 1H, H-4), 4.10 (dd, *J* = 3.5, 9.5 Hz, 1H, H-3), 3.97 (dd, *J* = 4.0, 11.5 Hz, 1H, H-6b), 4.22 (dd, *J* = 2.0, 3.5 Hz, 1H, H-2), 4.94 (A of ABX, *J* = 5.5, 11.0 Hz, 1H, CH_2), 5.04 (B of ABX, *J* = 5.5, 8.5 Hz, 1H, CH_2), 5.69 (d, 1.5 Hz, 1H, H-1), 7.28 (m, 2H, Ar-H), 7.44 (m, 1H, Ar-H), 7.54 (m, 2H, Ar-H), 7.69-7.72 (m, 4H, Ar-H); ^{13}C NMR (D_2O, 126 MHz): δ = 66.1 (C-4), 66.6 (C-6), 69.9 (C-2), 70.3 (C-3), 72.2 (C-5), 90.4 (d, *J* = 4 Hz, CH_2), 98.3 (C-1), 117.5, 126.7, 127.4, 128.3, 128.8, 129.1, 132.5, 135.4, 140.0, 155.0 (12C, Ar-C); ESI-MS: *m/z*: Calcd for $C_{19}H_{22}O_{10}P$ $[M-2NH_4+H]^-$: 441.10, found: 440.92.

3'-Chloro-4'-(2-*O*-phosphoryl-α-D-mannopyranosyloxy)-biphenyl-4-carbonitrile disodium salt (7a). Compound **26** (34.7 mg, 56.0 µmol) was dissolved in MeOH (0.25 mL) and 25% aq. NH_3 (1 mL). The mixture was stirred for 16 h at rt. The solvents were removed *in vacuo*, the residue was dissolved in H_2O (0.5 mL) containing a drop of 1 M aq. NaOH and purified by MPLC on RP-18 (H_2O/MeOH) to yield pure 2-phosphate **7a** (20.4 mg, 71%) as the sodium salt. $[\alpha]_D^{20}$ +17.9 (*c* 0.78, H_2O); ^1H NMR (500 MHz, D_2O): δ = 3.70-3.82 (m, 3H, H-5, H-6), 3.92 (t, *J* = 9.8 Hz, 1H, H-4), 4.13 (dd, *J* = 3.0, 9.7 Hz, 1H, H-3), 4.66 (dt, *J* = 2.4, 8.3 Hz, 1H, H-2), 5.97 (d, *J* = 1.2 Hz, 1H, H-1), 7.40 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.45-7.53 (m, 4H, Ar-H), 7.66 (d, *J* = 8.4 Hz, 2H, Ar-H); ^{13}C NMR (126 MHz, D_2O): δ = 62.0 (C-6), 68.6 (C-4), 72.3 (d, *J* = 3 Hz, C-3), 73.8 (d, *J* = 4 Hz, C-2), 99.2 (d, *J* = 5 Hz, C-1), 111.0, 118.9, 121.2, 125.5, 128.2, 128.4, 130.1, 134.4, 135.4, 144.7, 152.5 (13C, 12 Ar-C, CN); IR (KBr): ν = 3436 (vs, OH), 2230 (w, CN) cm^{-1}; ESI-MS: *m/z:* Calcd for $C_{19}H_{18}ClNO_9P$ $[M-2Na+H]^-$: 470.04, found: 469.96.

3'-Chloro-4'-(3-*O*-phosphoryl-α-D-mannopyranosyloxy)-biphenyl-4-carbonitrile disodium salt (7b). A solution of **30** (89.1 mg, 90 µmol) in dry DCM (2 mL) was treated with TFA (500 µL) for 1.5 h at rt under argon. Then, the mixture was evaporated to dryness *in*

vacuo to yield the benzoylated intermediate, which was dissolved in MeOH (1 mL) and 25% aq. NH_3 (4 mL). The mixture was stirred at rt overnight. The solvents were removed *in vacuo,* the residue was dissolved in H_2O (1 mL) containing two drops of 1 M aq. NaOH and purified by MPLC on RP-18 (H_2O/MeOH, 95:5-4:1) to yield **7b** (20.7 mg, 45%). $[\alpha]_D^{20}$ +42.5 (*c* 0.82, H_2O); ^{1}H NMR (500 MHz, D_2O): δ = 3.74 (dd, *J* = 5.8, 12.4 Hz, 1H, H-6a), 3.77-3.83 (m, 2H, H-6b, H-5), 3.91 (t, *J* = 9.6 Hz, 1H, H-4), 4.43 (dd, *J* = 1.8, 3.4 Hz, 1H, H-2), 4.55 (dt, *J* = 3.4, 8.8 Hz, 1H, H-3), 5.76 (d, *J* = 1.5 Hz, 1H, H-1), 7.42 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.58 (dd, *J* = 2.3, 8.7 Hz, 1H, Ar-H), 7.71 (d, *J* = 8.5 Hz, 2H, Ar-H), 7.75 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.80 (d, *J* = 8.5 Hz, 2H, Ar-H); ^{13}C NMR (126 MHz, D_2O): δ = 62.2 (C-6), 68.3 (d, *J* = 3 Hz, C-4), 70.9 (d, *J* = 4 Hz, C-2), 74.9 (d, *J* = 5 Hz, C-3), 75.3 (C-5), 99.8 (C-1), 111.1, 121.2, 125.7, 128.2, 128.6, 130.3, 134.4, 135.9, 144.9, 152.5 (13C, 12 Ar-C, CN); IR (KBr): ν = 3436 (vs, OH), 2230 (w, CN) cm^{-1}; ESI-MS: *m/z*: Calcd for $C_{19}H_{18}ClNO_9P$ $[M-2NH_4+H]^-$: 470.04, found: 470.12.

3'-Chloro-4'-(2,3,4-*O*-benzoyl-4-*O*-phosphoryl-α-D-mannopyranosyloxy)-biphenyl-4-carbonitrile disodium salt (7c). Prepared according to the procedure described for **7b**. Compound **32** (37.3 mg, 27 μmol) was subsequently treated with TFA (300 μL) in DCM (1.2 mL) for 2 h, and then with MeOH (0.5 mL) and 25% aq. NH_3 (2 mL) for 2 h. Purification by MPLC on RP-18 (H_2O/MeOH, 95:5-4:1) yielded **7c** (23.6 mg, 98%). $[\alpha]_D^{20}$ +70.0 (*c* 0.81, H_2O); ^{1}H NMR (500 MHz, D_2O): δ = 3.64-3.71 (m, 2H, H-6a, H-5), 3.85 (dd, *J* = 4.1, 12.5 Hz, 1H, H-6b), 4.17-4.28 (m, 3H, H-3, H-2, H-4), 5.56 (s, 1H, H-1), 7.11 (d, *J* = 1.9 Hz, 1H, Ar-H), 7.16 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.19 (m, 3H, Ar-H), 7.41 (d, *J* = 8.3 Hz, 2H, Ar-H); ^{13}C NMR (126 MHz, D_2O): δ = 60.7 (C-6), 69.2 (d, *J* = 5 Hz, C-4), 69.6, 70.9 (C-2, C-3), 73.3 (d, *J* = 7 Hz, C-5), 98.6 (C-1), 109.5, 117.5, 119.5, 124.1, 126.6, 128.2, 132.8, 134.0,

142.7, 151.4 (13C, 12 Ar-C, CN); IR (KBr): ν = 3433 (vs, OH), 2227 (w, CN) cm^{-1}; ESI-MS: *m/z*: Calcd for $C_{19}H_{18}ClNO_9P$ $[M-2Na+H]^-$: 470.04, found: 470.07.

3'-Chloro-4'-(6-*O*-phosphoryl-α-D-mannopyranosyloxy)-biphenyl-4-carbonitrile disodium salt (7d). Prepared according to the procedure described for **7b**. Compound **36** (50.8 mg, 90 μmol) was subsequently treated with TFA (400 μL) in DCM (1.6 mL) for 2 h, and then with MeOH (0.5 mL) and 25% aq. NH_3 (2 mL) overnight. Purification by MPLC on RP-18 (H_2O/MeOH, 95:5-4:1) yielded **7d** (18.1 mg, 68%). $[\alpha]_D^{20}$ +40.3 (*c* 0.58, H_2O); ^1H NMR (500 MHz, D_2O): δ = 3.75 (d, *J* = 9.6 Hz, 1H, H-5), 3.81 (m, 1H, H-6a), 4.04-4.13 (m, 2H, H-4, H-6b), 4.15 (dd, *J* = 3.4, 10.1 Hz, 1H, H-3), 4.25 (dd, *J* = 1.8, 3.3 Hz, 1H, H-2), 5.66 (d, *J* = 1.4 Hz, 1H, H-1), 7.23 (d, *J* = 8.7 Hz, 1H, Ar-H), 7.33 (d, *J* = 2.3 Hz, 1H, Ar-H), 7.35-7.41 (m, 3H, Ar-H), 7.55 (d, *J* = 8.4 Hz, 2H, Ar-H); ^{13}C NMR (126 MHz, D_2O): δ = 62.8 (d, *J* = 4 Hz, C-6), 66.2 (C-4), 70.5, 70.6 (C-2, C-3), 74.2 (d, *J* = 7 Hz, C-5), 99.3 (C-1), 110.0, 118.0, 120.3, 124.6, 127.3, 129.1, 133.4, 134.5, 143.6, 152.0 (13C, 12 Ar-C, CN); IR (KBr): ν = 3436 (vs, OH), 2225 (w, CN) cm^{-1}; ESI-MS: *m/z*: Calcd for $C_{19}H_{18}ClNO_9P$ $[M-2Na+H]^-$: 470.04, found: 470.02.

4-(5-Nitroindolin-1-yl)phenyl 6-*O*-(phosphonooxy)-methyl α-D-mannopyranoside diammonium salt (8). Compound **42** (278 mg, 0.337 mmol) was stirred in a mixture of MeOH (5 mL), DCM (4 mL) and 25% aq. NH_3 (8 mL) overnight. The solvent was removed under reduced pressure and the residue was purified by MPLC on RP-18 (H_2O/MeOH) to give **8** (78 mg, 41%). ^1H NMR (D_2O, 500 MHz): δ = 3.21 (t, *J* = 8.4 Hz, 2H, CH_2), 3.81 (dd, *J* = 1.8, 11.3 Hz, 1H, H-6a), 3.86 (m, 1H, H-5), 3.91 (t, *J* = 9.7 Hz, 2H, NCH_2), 3.96 (dd, *J* = 4.2, 11.3 Hz, 1H, H-6b), 4.04 (dd, *J* = 3.5, 9.6 Hz, 1H, H-3), 4.14 (t, *J* = 8.8 Hz, 2H, CH_2), 4.16 (dd, *J* = 1.8, 3.4 Hz, 1H, H-2), 4.89 (A of ABX, *J* = 5.6, 10.8 Hz, 1H, CH_2), 5.00 (B of

ABX, J = 5.6, 8.1 Hz, 1H, CH_2), 5.60 (s, 1H, H-1), 6.86 (d, J = 8.9 Hz, 1H, Ar-H), 7.22 (d, J = 8.9 Hz, 2H, Ar-H), 7.39 (d, J = 8.8 Hz, 2H, Ar-H), 8.01 (s, 1H, Ar-H), 8.05 (d, J = 9.3 Hz, 1H, Ar-H); ^{13}C NMR (D_2O, 126 MHz): δ = 26.3 (CH_2), 53.6 (NCH_2), 66.1, 66.3 (C-4, C-6), 69.9 (C-2), 70.2 (C-3), 72.3 (C-5), 90.1 (d, J = 4 Hz, CH_2), 98.7 (C-1), 105.6, 118.1, 121.2, 121.9, 122.0, 127.0, 132.5, 136.6, 137.5, 151.9, 154.6 (12C, 12 Ar-C), ESI-MS: *m/z*: Calcd for $C_{21}H_{24}N_2O_{12}P$ $[M-2NH_4+H]^-$: 527.11, found: 527.18.

Biphenyl 4,6-*O*-benzylidene-α-D-mannopyranoside (9). To a mixture of biphenyl α-D-mannopyranoside (**1**)[14,18] (1.16 g, 3.51 mmol) and benzaldehyde dimethyl acetal (1.58 mL, 10.5 mmol) in dry MeCN/DMF (10 mL/1 mL) was added *p*-toluenesulfonic acid (40 mg). The reaction mixture was stirred at 80 °C overnight and then neutralized with satd. aq. $NaHCO_3$. Then the mixture was diluted with DCM (20 mL) and washed with water (2 × 10 mL) and brine (10 mL). The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by MPLC on silica (DCM/MeOH, 20:1-9:1) to afford **9** (1.03 g, 70%) as a white solid. $[\alpha]_D^{20}$ +163.1 (*c* 1.09, $CHCl_3$/MeOH, 1:1); ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 3.83 (t, J = 10.0 Hz, 1H, H-6a), 3.99 (td, J = 4.5, 9.5 Hz, 1H, H-5), 4.04 (t, J = 9.5 Hz, 1H, H-4), 4.22 (dd, J = 5.0, 10.0 Hz, 1H, H-6b), 4.28 (m, 1H, H-2), 4.33 (dd, J = 3.5, 9.5 Hz, 1H, H-3), 5.60 (s, 1H, PhC*H*), 5.66 (s, 1H, H-1), 7.13 (m, 2H, Ar-H), 7.31-7.50 (m, 8H, Ar-H), 7.55 (m, 4H, Ar-H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ = 63.8 (C-5), 68.6 (C-3), 68.7 (C-6), 70.9 (C-2), 78.7 (C-4), 98.1 (C-1), 102.3 (Ph*C*H), 116.6, 126.3, 126.8, 127.0, 128.3, 128.4, 128.8, 129.0, 129.3, 129.7, 134.5, 135.7, 137.1, 140.5, 155.4 (18C, Ar-C); ESI-MS: *m/z:* Calcd for $C_{25}H_{24}NaO_6$ $[M+Na]^+$: 443.15, found: 443.12.

Biphenyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (10). A suspension of **9** (380 mg, 0.900 mmol) and dibutyl tinoxide (247 mg, 0.990 mmol) in dry toluene (6 mL) was

refluxed at 135 °C for 3 h. The mixture was concentrated to dryness and tetrabutylammonium bromide (320 mg, 0.990 mmol) and benzyl bromide (0.13 mL, 1.08 mmol) in dry toluene (6 mL) were added. The reaction mixture was stirred at 115 °C overnight, the solvent was removed under reduced pressure and the residue was purified by MPLC on silica (petroleum ether/EtOAc, 6:1-4:1) to give **10** (370 mg, 80%) as a white solid. $[\alpha]_D^{20}$ +139.6 (*c* 2.66, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 3.87 (t, *J* = 10.5 Hz, 1H, H-6a), 4.01 (td, *J* = 5.0, 10.0 Hz, 1H, H-5), 4.17 (dd, *J* = 3.0, 9.5 Hz, 1H, H-3), 4.20-4.26 (m, 2H, H-6b, H-4), 4.31 (dd, *J* = 1.5, 3.0 Hz, 1H, H-2), 4.82 (d, *J* = 12.0 Hz, 1H, OC*H*$_2$Ph), 4.97 (d, *J* = 12.0 Hz, 1H, OC*H*$_2$Ph), 5.66 (s, 1H, PhC*H*), 5.69 (d, *J* = 1.0 Hz, 1H, H-1), 7.13 (m, 2H, Ar-H), 7.37-7.46 (m, 10H, Ar-H), 7.51-7.58 (m, 7H, Ar-H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ = 64.0 (C-5), 68.7 (C-6), 70.0 (C-2), 73.3 (O*C*H$_2$Ph), 75.4 (C-3), 78.7 (C-4), 97.9 (C-1), 101.7 (Ph*C*H), 116.7, 126.1, 126.8, 127.8, 127.0, 127.8, 128.0, 128.2, 128.3, 128.5, 128.7, 128.8, 128.9, 129.0, 129.7, 134.5, 135.7, 137.4, 137.9, 140.5, 155.3 (24C, Ar-C); ESI-MS: *m/z:* Calcd for $C_{32}H_{30}NaO_6$ $[M+Na]^+$: 533.19, found: 533.17.

Biphenyl 2-*O*-dibenzylphosphoryl-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (11). According to the general procedure, compound **10** (194 mg, 0.250 mmol) was reacted with 1,2,4-triazole (69.5 mg, 1.00 mmol) and dibenzyl *N,N*-diisopropylphosphoramidite (90%, 187 µL, 0.500 mmol) in MeCN (3.0 mL), followed by treatment with 70% aq. *tert*-butylhydroperoxide (150 µL) to yield **11** (120 mg, 62%) as a white solid. $[\alpha]_D^{20}$ +55.3 (*c* 0.38, DCM); ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 3.81 (t, *J* = 10.5 Hz, 1H, H-6a), 3.99 (td, *J* = 5.0, 10.0 Hz, 1H, H-5), 4.11 (t, *J* = 10.0 Hz, 1H, H-4), 4.22 (m, 2H, H-3, H-6b), 4.85 (m, 2H, OC*H*$_2$Ph), 5.30 (m, 1H, H-2), 5.08-5.12 (m, 4H, OC*H*$_2$Ph), 5.62 (m, 2H, H-1, PhC*H*), 7.03 (m, 2H, Ar-H), 7.26-7.58 (m, 27H, Ar-H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ = 64.6 (C-5), 68.5 (C-6), 69.5 (d, *J* = 6 Hz, O*C*H$_2$Ph), 69.6 (d, *J* = 6 Hz, O*C*H$_2$Ph), 72.8 (O*C*H$_2$Ph), 73.9 (d, *J* =

5 Hz, C-3), 74.6 (d, *J* = 6 Hz, C-2), 78.2 (C-4), 97.2 (d, *J* = 3 Hz, C-1), 101.6 (Ph*C*H), 116.8, 126.0, 126.8, 126.9, 127.0, 127.6, 127.7, 127.74, 127.85, 127.92, 128.19, 128.22, 128.3, 128.45, 128.52, 128.6, 128.7, 128.9, 135.66, 135.71, 135.76, 135.8, 135.9, 137.4, 137.5, 138.0, 140.5, 155.0 (36C, Ar-C); ESI-MS: *m/z:* Calcd for $C_{46}H_{44}O_9P$ $[M+H]^+$: 771.27, found: 771.37.

Biphenyl 3-*O*-benzyl-α-D-mannopyranoside (12). Biphenyl α-D-mannopyranoside (**3**, 665 mg, 2.00 mmol) and dibutyltin oxide (548 mg, 2.20 mmol) were dissolved in dry MeOH (10 mL). The mixture was refluxed for 5 h and then concentrated to dryness under reduced pressure. To a solution of the residue in dry toluene (10 mL) were added tetrabutylammonium bromide (709 mg, 2.20 mmol) and benzyl bromide (285 μL, 2.40 mmol). The mixture was stirred at 115 °C overnight and then concentrated to dryness *in vacuo*. The residue was purified by chromatography on silica (petroleum ether/EtOAc, 4:1-1:3) to yield **12** (306 mg, 36%) as a white solid. $[\alpha]_D^{20}$ +99.8 (*c* 1.38, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 3.00 (s, 3H, 2,4,6-OH), 3.71 (m, 2H, H-5, H-6a), 3.81 (m, 1H, H-6b), 3.96 (dd, *J* = 3.0, 9.5 Hz, 1H, H-3), 4.17 (m, 2H, H-2, H-4), 4.70 (d, *J* = 11.5 Hz, 1H, OC*H*$_2$Ph), 4.79 (d, *J* = 11.5 Hz, 1H, OC*H*$_2$Ph), 5.65 (d, *J* = 1.5 Hz, 1H, H-1), 7.10 (m, 2H, Ar-H), 7.31-7.44 (m, 8H, Ar-H), 7.50-7.52 (m, 4H, Ar-H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ = 61.5 (C-6), 65.5 (C-4), 67.9 (C-2), 72.3 (O*C*H$_2$Ph), 72.8 (C-5), 79.4 (C-3), 97.7 (C-1), 116.6, 126.8, 126.9, 128.26, 128.29, 128.3, 128.7, 128.8, 135.6, 137.4, 140.5, 155.4 (18C, Ar-C); ESI-MS: *m/z*: Calcd for $C_{25}H_{26}NaO_6$ $[M+Na]^+$: 445.16, found: 445.14.

Biphenyl 2,4,6-tri-*O*-benzoyl-3-*O*-benzyl-α-D-mannopyranoside (13). To a solution of **12** (306 mg, 0.724 mmol) in pyridine (5 mL) were added benzoyl chloride (400 μL, 3.43 mmol) and DMAP (5 mg). The reaction mixture was stirred at rt overnight, then quenched with

MeOH (0.5 mL) and concentrated under reduced pressure. The residue was purified by MPLC on silica (petroleum ether/EtOAc, 4:1-3:1) to yield **13** (499 mg, 99%) as a white solid. $[\alpha]_D^{20}$ +7.1 (*c* 1.52, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 4.40-4.46 (m, 3H, H-6a, H-3, H-5), 4.62-4.65 (m, 2H, OC*H*$_2$Ph, H-6b), 4.79 (d, *J* = 12.4 Hz, 1H, OC*H*$_2$Ph), 5.80 (d, *J* = 2.0 Hz, 1H, H-1), 5.91 (dd, *J* = 2.0, 3.0 Hz, 1H, H-2), 5.97 (t, *J* = 9.7 Hz, 1H, H-4), 7.12-7.23 (m, 7H, Ar-H), 7.34 (m, 3H, Ar-H), 7.42-7.54 (m, 11H, Ar-H), 7.61 (m, 2H, Ar-H), 8.03 (m, 4H, Ar-H), 8.16 (m, 2H, Ar-H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ = 63.0 (C-6), 68.0 (C-4), 68.5 (C-5), 69.6 (C-2), 71.3 (O*C*H$_2$Ph), 74.1 (C-3), 96.3 (C-1), 116.9, 126.8, 127.0, 127.7, 127.9, 128.2, 128.26, 128.28, 128.4, 128.5, 128.7, 129.3, 129.4, 129.7, 129.8, 129.9, 130.0, 132.9, 133.3, 133.4, 135.9, 137.3, 140.3, 155.2 (36C, Ar-C), 165.4, 165.7, 166.1 (3 CO); ESI-MS: *m/z*: Calcd for $C_{46}H_{38}NaO_9$ $[M+Na]^+$: 757.24, found: 757.29.

Biphenyl 2,4,6-tri-*O*-benzoyl-α-D-mannopyranoside (14). Hydrogenolysis of **13** (499 mg, 0.679 mmol) was conducted in dioxane/EtOAc (6 mL, 5:1) in the presence of 10% $Pd(OH)_2$/C (50 mg) and a catalytic amount of AcOH in a Parr shaker under hydrogen (4 bar) at rt overnight. The reaction suspension was filtered through celite and the filtrate was concentrated *in vacuo*. The residue was purified by MPLC on silica (petroleum ether/EtOAc, 9:1-4:1) to give **14** (314 mg, 73%) as colorless syrup. $[\alpha]_D^{20}$ +56.5 (*c* 1.02, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 2.58 (d, *J* = 8.0 Hz, 1H, 3-OH), 4.48 (m, 2H, H-5, H-6a), 4.64 (m, 2H, H-3, H-6b), 5.66 (dd, *J* = 2.0, 3.5 Hz, 1H, H-2), 5.77 (t, *J* = 9.5 Hz, 1H, H-4), 5.82 (d, *J* = 2.0 Hz, 1H, H-1), 7.31-7.35 (m, 3H, Ar-H), 7.22 (m, 2H-Ar-H), 7.42-7.53 (m, 11H, Ar-H), 7.61 (m, 2H, Ar-H), 7.97 (dd, *J* = 1.0, 8.0 Hz, 2H, Ar-H), 8.10 (m, 4H, Ar-H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ = 63.0 (C-6), 69.0, 69.2 (C-5, C-3), 72.6 (C-2), 70.2 (C-4), 95.7 (C-1), 116.8, 126.8, 127.0, 128.3, 128.4, 128.6, 128.8, 129.1, 129.0, 129.7, 129.9, 130.0, 133.1,

133.69, 133.73, 136.0, 140.4, 155.2 (30C, Ar-C), 165.9, 166.1, 166.8 (3 CO); ESI-MS: *m/z*: Calcd for $C_{39}H_{32}NaO_9$ $[M+Na]^+$: 667.19, found: 667.29.

Biphenyl 2,4,6-tri-*O*-benzoyl-3-dibenzylphosphoryl-α-D-mannopyranoside (15). According to the general procedure, compound **14** (211 mg, 0.335 mmol) was reacted with 1,2,4-triazole (92.5 mg, 1.34 mmol) and dibenzyl *N,N*-diisopropylphosphoramidite (90%, 250 μL, 0.670 mmol) in MeCN (5 mL), followed by treatment with 70% aq. *tert*-butylhydroperoxide (190 μL, 1.34 mmol) to yield **15** and the 2-phosphoryl derivative **16** (245 mg, 80%) as an inseparable 3:2 mixture.

Analytical data for **15**: ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 4.62 (m, 2H, H-5, H-6a), 4.76 (dd, *J* = 7.5, 12.0 Hz, 1H, H-6b), 4.85-5.11 (m, 4H, OCH_2Ph), 5.55 (td, *J* = 3.5, 9.0 Hz, 1H, H-3), 5.81 (d, *J* = 1.5 Hz, 1H, H-1), 5.87 (dd, *J* = 1.9, 3.4 Hz, 1H, H-2), 6.04 (m, 1H, H-4), 6.92-8.11 (m, 34H, Ar-H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ = 62.8 (C-5), 67.6 (d, *J* = 6 Hz, C-4), 69.5 (C-6), 69.8 (d, *J* = 5.6 Hz, CH_2Ph), 71.0 (d, *J* = 2 Hz, C-2), 69.8 (d, *J* = 6 Hz, CH_2Ph), 73.7 (d, *J* = 5 Hz, C-3), 95.7 (C-1), 116.8, 126.8, 127.6, 127.7, 127.9, 128.0, 128.20, 128.23, 128.27, 128.32, 128.4, 128.50, 128.54, 128.57, 128.62, 129.7, 129.9, 130.0, 133.0, 133.5, 133.6, 136.1, 140.3, 155.1 (42C, Ar-C), 165.3, 165.5, 166.0 (3 CO); ESI-MS: *m/z*: Calcd for $C_{53}H_{45}NaO_{12}P$ $[M+Na]^+$: 927.25, found: 927.23.

Analytical data for **16**: ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 4.62 (m, 1H, H-6a), 4.51 (m, 1H, H-5), 4.42 (m, 1H, H-6b), 4.85-5.11 (m, 4H, OCH_2Ph), 5.20 (m, 1H, H-2), 5.66 (d, *J* = 1.5 Hz, 1H, H-1), 5.95 (td, *J* = 2.7, 10.0 Hz, 1H, H-3), 6.04 (m, 1H, H-4), 6.92-8.11 (m, 34H, Ar-H); ESI-MS: *m/z*: Calcd for $C_{53}H_{45}NaO_{12}P$ $[M+Na]^+$: 927.25, found: 927.23.

Biphenyl 2,3-di-*O*-benzoyl-4,6-*O*-benzylidene-α-D-mannopyranoside (17). To a solution of compound **9** in pyridine (5 mL) were added benzoyl chloride (0.33 mL, 2.84 mmol) and

DMAP (5 mg). The mixture was stirred at rt overnight and then concentrated under reduced pressure. The residue was purified by MPLC on silica (petroleum ether/EtOAc, 6:1-4:1) to provide **17** (270 mg, 60%) as a white solid. $[\alpha]_D^{20}$ +21.8 (*c* 1.08, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz): δ = 4.00 (t, *J* = 9.5 Hz, 1H, H-6a), 4.32-4.38 (m, 2H, H-6b, H-5), 4.48 (t, *J* = 9.5 Hz, 1H, H-4), 5.73 (s, 1H, PhC*H*), 5.82 (d, *J* = 1.5 Hz, 1H, H-1), 5.97 (dd, *J* = 1.5, 3.5 Hz, 1H, H-2), 6.10 (dd, *J* = 3.5, 10.5 Hz, 1H, H-3), 7.25 (m, 2H, Ar-H), 7.35-7.39 (m, 6H, Ar-H), 7.45-7.61 (m, 11H, Ar-H), 7.68 (t, *J* = 7.5 Hz, 1H, Ar-H), 7.99 (m, 2H, Ar-H), 8.17 (m, 2H, Ar-H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ = 64.6 (C-5), 68.7 (C-6), 68.8 (C-3), 70.8 (C-2), 76.6 (C-4), 96.7 (C-1), 102.0 (Ph*C*H), 116.8, 126.1, 126.9, 127.0, 128.2, 128.25, 128.3, 128.4, 128.6, 128.7, 129.0, 129.3, 129.6, 129.8, 129.9, 130.1, 133.1, 133.6, 136.0, 136.9, 140.5, 155.1 (30C, Ar-C), 165.36, 165.45 (2 CO); ESI-MS: *m/z*: Calcd for $C_{39}H_{32}NaO_8$ $[M+Na]^+$: 651.20, found: 651.17.

Biphenyl 2,3-di-*O*-benzoyl-6-*O*-benzyl-α-D-mannopyranoside (18). To a solution of **17** (270 mg, 0.429 mmol) in dry THF (4 mL) were added $Me_3N{\cdot}BH_3$ (125 mg, 1.72 mmol) and $AlCl_3$ (341 mg, 2.56 mmol). After 15 min, H_2O (15.5 μL) was added and the reaction mixture was stirred at rt for 45 min. The reaction was quenched with 1 M aq. HCl, diluted with DCM (20 mL) and washed with water (10 mL) and brine (10 mL). The combined organic layers were dried over Na_2SO_4. The solvent was removed under reduced pressure and the residue purified by MPLC on silica (petroleum ether/EtOAc, 6:1-3:1) to afford **18** (178 mg, 67%) as a white solid. $[\alpha]_D^{20}$ +10.8 (*c* 1.21, $CHCl_3$); ^1H NMR ($CDCl_3$, 500 MHz): δ = 3.85 (dd, *J* = 4.0, 11.0 Hz, 1H, H-6a), 3.97 (dd, *J* = 4.5, 11.0 Hz, 1H, H-6b), 4.14 (m, 1H, H-5), 4.53 (t, *J* = 10.0 Hz, 1H, H-4), 4.59 (d, *J* = 12.0 Hz, 1H, OCH_2Ph), 4.70 (d, *J* = 12.0 Hz, 1H, OCH_2Ph), 5.79 (d, *J* = 1.5 Hz, 1H, H-1), 5.81 (dd, *J* = 1.5, 3.0 Hz, 1H, H-2), 5.85 (dd, *J* = 3.5, 9.5 Hz, 1H, H-3), 7.24 (m, 2H, Ar-H), 7.33-7.39 (m, 7H, Ar-H), 7.42-7.48 (m, 5H, Ar-H), 7.54-7.57 (m, 5H,

Ar-H), 7.62 (t, J = 7.5 Hz, 1H, Ar-H), 7.98 (m, 2H, Ar-H), 8.10 (m, 2H, Ar-H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ = 67.4 (C-4), 69.8 (C-6), 70.3 (C-2), 72.0 (C-5), 72.6 (C-3), 73.7 (O*C*H_2Ph), 96.0 (C-1), 116.8, 126.9, 127.0, 127.6, 127.7, 128.3, 128.4, 128.6, 128.7, 129.88, 129.92, 133.4, 133.5, 135.9, 137.9, 140.5, 155.4 (30C, Ar-C), 165.5, 166.7 (2 CO); ESI-MS: *m/z*: Calcd for $C_{39}H_{34}NaO_8$ $[M+Na]^+$: 653.22, found: 653.25.

Biphenyl 4-*O*-dibenzylphosphoryl-2,3-di-*O*-benzoyl-6-*O*-benzyl-α-D-mannopyranoside (19). According to the general procedure, compound **18** (160 mg, 0.253 mmol) was reacted with 1,2,4-triazole (70.0 mg, 1.01 mmol) and dibenzyl *N,N*-diisopropylphosphoramidite (90%, 190 μL, 0.510 mmol) in MeCN (2 mL), followed by treatment with 70% aq. *tert*-butylhydroperoxide (200 μL) to yield **19** (120 mg, 53%) as a glassy solid. $[\alpha]_D^{20}$ +29.2 (*c* 1.18, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 3.85 (dd, J = 1.5, 11.0 Hz, 1H, H-6a), 3.94 (dd, J = 4.0, 11.5 Hz, 1H, H-6b), 4.24 (m, 1H, H-5), 4.56 (s, 2H, O*C*H_2Ph), 4.63 (dd, J = 8.5, 12.0 Hz, 1H, O*C*H_2Ph), 4.74 (dd, J = 7.0, 12.0 Hz, 1H, O*C*H_2Ph), 4.81 (dd, J = 8.5, 12.0 Hz, 1H, O*C*H_2Ph), 4.88 (dd, J = 7.5, 12.0 Hz, 1H, O*C*H_2Ph), 5.43 (q, J = 9.5 Hz, 1H, H-4), 5.79 (d, J = 2.0 Hz, 1H, H-1), 5.85 (dd, J = 2.0, 3.0 Hz, 1H, H-2), 6.04 (dd, J = 3.5, 10.0 Hz, 1H, H-3), 6.92 (m, 2H, Ar-H), 7.11-7.61 (m, 28H, Ar-H), 7.99 (dd, J = 1.0, 8.5 Hz, 2H, Ar-H), 8.04 (dd, J = 1.0, 8.0 Hz, 2H, Ar-H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ = 68.4 (C-6), 69.2 (d, J = 5 Hz, O*C*H_2Ph), 69.3 (d, J = 6 Hz, O*C*H_2Ph), 70.3 (C-2), 70.5 (d, J = 2 Hz, C-3), 71.4 (d, J = 6 Hz, C-5), 71.6 (d, J = 6 Hz, C-4), 73.4 (O*C*H_2Ph), 95.8 (C-1), 116.9, 126.9, 127.0, 127.39, 127.43, 127.45, 127.5, 127.7, 127.9, 128.2, 128.27, 128.32, 128.36, 128.40, 128.5, 128.6, 128.7, 129.4, 130.0, 133.2, 133.5, 135.3, 135.4, 135.5, 135.6, 136.0, 138.3, 140.5, 155.4 (42C, Ar-C), 165.4, 165.5 (2 CO); ESI-MS: *m/z*: Calcd for $C_{53}H_{47}NaO_{11}P$ $[M+Na]^+$: 913.28, found: 913.31.

Biphenyl 2,3,4-tri-*O*-benzoyl-6-*O*-trityl-α-D-mannopyranoside (20). To a solution of **3** (414 mg, 1.24 mmol) in pyridine were added tritylchloride (417 mg, 1.49 mmol) and DMAP (10 mg). The mixture was stirred at 80 °C overnight and then cooled to rt. Then, benzoyl chloride (50 µL, 4.92 mmol) was added and the mixture was stirred at 50 °C overnight. The mixture was diluted with DCM (50 mL) and washed with 0.1 M aq. HCl (20 mL) and satd. aq. $NaHCO_3$ (20 mL). The organic layer was dried over Na_2SO_4, filtered and the solvent removed *in vacuo*. The residue was purified by MPLC on silica (petroleum ether/EtOAc, 6:1-4:1) to give **20** (1.35 g, 88%), which contained some perbenzoylated substance as impurity, but was used in the next step without a second purification.

Biphenyl 2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside (21). To a solution of **20** (1.35 g, 1.52 mmol) in dry DCM were added anhydrous $FeCl_3$ (493 mg, 3.04 mmol) and distilled water (3.28 mL, 18.2 mmol). The mixture was stirred at rt for 5 h. Then, the mixture was diluted with DCM (50 mL) and washed with water (30 mL). The organic layer was dried over Na_2SO_4, filtered and the solvent removed *in vacuo*. The residue was purified by MPLC on silica (petroleum ether/EtOAc) to yield **21** (608 mg, 62%). $[\alpha]_D^{20}$ -1.8 (*c* 0.75, DCM); ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 2.70 (dd, *J* = 6.0, 8.4 Hz, 1H, 6-OH), 3.76 (ddd, *J* = 3.3, 5.9, 13.0 Hz, 1H, H-6a), 3.85 (ddd, *J* = 2.0, 8.5, 12.8 Hz, 1H, H-6a), 4.21 (dt, *J* = 2.6, 10.1 Hz, 1H, H-5), 5.90 (d, *J* = 1.7 Hz, 1H, H-1), 5.91 (dd, *J* = 1.9, 3.3 Hz, 1H, H-2), 5.98 (t, *J* = 10.1 Hz, 1H, H-4), 6.25 (dd, *J* = 3.4, 10.1 Hz, 1H, H-3), 7.26-7.35 (m, 5H, Ar-H), 7.39-7.48 (m, 5H, Ar-H), 7.51-7.59 (m, 7H, Ar-H), 7.65 (m, 1H, Ar-H), 7.88 (m, 2H, Ar-H), 8.01 (m, 2H, Ar-H), 8.15 (m, 2H, Ar-H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ = 61.2 (C-6), 67.1 (C-4), 69.5 (C-3), 70.5 (C-2), 71.8 (C-5), 96.1 (C-1), 116.8, 127.0, 127.1, 128.4, 128.5, 128.6, 128.8, 128.9, 129.1, 129.2, 129.8, 130.0, 130.1, 133.4, 133.8, 133.9, 136.2, 140.5, 155.4 (30C, Ar-C), 165.6, 165.7, 166.7 (3 CO); ESI-MS: *m/z*: Calcd for $C_{39}H_{32}NaO_9$ $[M+Na]^+$: 667.19, found: 667.22.

Biphenyl 6-*O*-dibenzylphosphoryl-2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside (22). According to the general procedure, compound **21** (107 mg, 0.158 mmol) was reacted with 1,2,4-triazole (44.0 mg, 0.632 mmol) and dibenzyl *N,N*-diisopropylphosphoramidite (90%, 120 µL, 0.316 mmol) in MeCN (2 mL), followed by treatment with 70% aq. *tert*-butylhydroperoxide (86 µL, 0.632 mmol) to yield **22** (94.4 mg, 66%) as a glassy solid. $[\alpha]_D^{20}$ -0.7 (*c* 0.28, DCM); ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 4.27 (m, 2H, H-6), 4.46 (m, 1H, H-5), 4.93-5.02 (m, 4H, OCH_2Ph), 5.80 (d, *J* = 1.7 Hz 1H, H-1), 5.90 (dd, *J* = 2.0, 3.1 Hz, 1H, H-2), 6.07 (t, *J* = 10.0 Hz, 1H, H-4), 6.13 (dd, *J* = 3.3, 10.0 Hz, 1H, H-3), 7.19-7.63 (m, 18H, Ar-H), 7.89 (m, 2H, Ar-H), 7.98 (m, 2H, Ar-H), 8.15 (m, 2H, Ar-H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ = 65.8 (d, *J* = 5 Hz, C-6), 66.4 (C-4), 69.4 (d, *J* = 4 Hz, O*C*H$_2$Ph), 69.5 (d, *J* = 4 Hz, O*C*H$_2$Ph), 69.9 (C-3), 70.3 (d, *J* = 8 Hz, C-5), 70.4 (C-2), 96.1 (C-1), 117.0, 127.0, 127.1, 127.8, 128.0, 128.4, 128.45, 128.46, 128.5, 128.6, 133.4, 133.7, 135.7, 135.8, 136.3, 140.4, 155.5 (42C, Ar-C), 165.4, 165.5, 165.6 (3 CO); ESI-MS: *m/z*: Calcd for $C_{53}H_{45}NaO_{12}P$ $[M+Na]^+$: 927.25, found: 927.23.

4'-(4,6-*O*-Benzylidene-α-D-mannopyranosyloxy)-3'-chloro-biphenyl-4-carbonitrile (23). To a solution of **4**[19] (500 mg, 1.28 mmol) in anhydrous DMF (20 mL) were added benzaldehyde dimethyl acetal (575 µL, 3.83 mmol) and *p*-toluenesulfonic acid (20 mg). The mixture was stirred at 50 °C overnight. Then, the reaction mixture was neutralized with satd. aq. $NaHCO_3$ (10 mL), diluted with DCM (30 mL), and washed with water (3 × 10 mL) and brine (10 mL). The organic layer was dried over Na_2SO_4, filtered and the solvents removed *in vacuo*. The residue was purified by MPLC on silica (DCM/MeOH, 1:0-5:1, +0.5% NEt_3) to yield **23** (132 mg, 22%). $[\alpha]_D^{20}$ +62.3 (*c* 0.59, $CHCl_3$/MeOH, 1:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.76 (t, *J* = 10.2 Hz, 1H, H-6a), 3.91 (td, *J* = 4.8, 9.8 Hz, 1H, H-5), 3.99 (t, *J* =

9.4 Hz, 1H, H-4), 4.14 (dd, *J* = 4.8, 10.3 Hz, 1H, H-6a), 4.28-4.33 (m, 2H, H-2, H-3), 5.53 (s, 1H, PhC*H*), 5.62 (s, 1H, H-1), 7.19 (m, 1H, Ar-H), 7.29-7.31 (m, 3H, Ar-H), 7.38 (m, 1H, Ar-H), 7.40-7.44 (m, 2H, Ar-H), 7.54-7.58 (m, 3H, Ar-H), 7.63-7.67 (m, 2H, Ar-H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 64.2 (C-5), 68.5, 70.6 (3C, C-2, C-3, C-6), 78.4 (C-4), 98.7 (C-1), 102.4 (Ph*C*H), 111.2, 116.8, 118.7, 124.6, 126.2, 126.6, 127.4, 128.4, 132.5, 143.7, 151.8 (19 C, 18 Ar-C, CN); ESI-MS: *m/z:* Calcd for $C_{33}H_{26}ClNNaO_7$ $[M+Na]^+$: 502.90, found: 502.04.

4'-(3-*O*-Benzoyl-4,6-*O*-benzylidene-α-D-mannopyranosyloxy)-3'-chloro-biphenyl-4-carbonitrile (24). To a solution of **23** (131 mg, 0.275 μmol) in DCM/pyridine (6 mL, 5:1) was added dropwise over 30 min a 0.1 M benzoyl chloride solution in dry DCM (2.8 mL, 0.280 mmol) at 0 °C under argon. The mixture was stirred another 30 min at 0 °C, then the ice-bath was removed and stirring continued for 2 h at rt. Then, the mixture was diluted with DCM (10 mL) and washed with 0.1 M aq. HCl (5 mL) and satd. aq. $NaHCO_3$ (10 mL). The organic layer was dried with Na_2SO_4, filtered and concentrated. The residue was purified by MPLC on silica (petroleum ether/EtOAc, +0.5% NEt_3) to yield **24** (96.8 mg, 60%). $[\alpha]_D^{20}$ +113.8 (*c* 1.02, DCM); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.91 (t, *J* = 10.2 Hz, 1H, H-6a), 4.17 (td, *J* = 4.9, 9.7 Hz, 1H, H-5), 4.24 (dd, *J* = 4.8, 10.2 Hz, 1H, H-6b), 4.45 (t, *J* = 9.9 Hz, 1H, H-4), 4.65 (dd, *J* = 1.6, 3.2 Hz, 1H, H-2), 5.64 (s, 1H, PhC*H*), 5.70 (d, *J* = 1.2 Hz, 1H, H-1), 7.25-7.34 (m, 4H, Ar-H), 7.37-7.47 (m, 5H, Ar-H), 7.51-7.60 (m, 4H, Ar-H), 7.51-7.60 (m, 2H, Ar-H), 8.06-8.11 (m, 2H, Ar-H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 65.1 (C-5), 68.4 (C-6), 69.1 (C-2), 71.2 (C-3), 75.6 (C-4), 99.1 (C-1), 101.8 (Ph*C*H), 110.8, 116.9, 118.6, 124.8, 126.0, 126.3, 127.2, 128.1, 128.3, 132.6, 133.2, 136.9, 143.5, 151.6 (25C, 24 Ar-C, CN), 165.7 (CO); IR (KBr): ν = 3437 (vs, OH), 2227 (m, CN), 1721 (vs, C=O) cm^{-1}; ESI-MS: *m/z:* Calcd for $C_{33}H_{26}ClNNaO_7$ $[M+Na]^+$: 606.13, found: 606.11.

4'-(3-*O*-Benzoyl-4,6-*O*-benzylidene-2-*O*-bis[2-(trimethylsilyl)ethoxy]phosphoryl-α-D-mannopyranosyloxy)-3'-chloro-biphenyl-4-carbonitrile (25). According to the general procedure, compound **24** (96.8 mg, 0.166 mmol) was reacted with 1,2,4-triazole (45.8 mg, 0.663 mmol) and bis[2-(trimethylsilyl)ethyl] *N,N*-diisopropylphosphoramidite (136 µL, 0.331 mmol) in MeCN (3.0 mL), followed by treatment with 70% aq. *tert*-butylhydroperoxide (91 µL, 0.663 mmol) to yield **25** (79.6 mg, 55%) as a 4:1-mixture of 2- and 3-phosphorylated isomers. ^{1}H NMR (500 MHz, $CDCl_3$): δ = -0.09, -0.02 (2s, 18H, 2 $Si(CH_3)_3$), 0.92-01.06 (m, 4H, 2 $SiCH_2$), 3.87 (td, *J* = 4.5, 10.1 Hz, 1H, H-6a), 4.12 (m, 6H, H-5, H-6b, 2 OCH_2), 4.36 (t, *J* = 9.9 Hz, 1H, H-4), 5.17 (ddd, *J* = 1.7, 3.1, 9.1 Hz, 1H, H-2), 5.63 (s, 1H, PhC*H*), 5.81 (m, 1H, H-3), 5.84 (d, *J* = 1.5 Hz, 1H, H-1), 7.22-7.33 (m, 4H, Ar-H), 7.37-7.45 (m, 4H, Ar-H), 7.46-7.56 (m, 2H, Ar-H), 7.57-7.64 (m, 3H, Ar-H), 7.66-7.71 (m, 2H, Ar-H), 8.08-8.14 (m, 2H, Ar-H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = -1.7, -1.6 (6C, $Si(CH_3)_3$), 19.4 (d, *J* = 5 Hz, 2C, 2 $SiCH_2$), 65.2 (C-5), 66.9 (t, *J* = 6 Hz, 2C, 2 OCH_2), 68.4 (C-6), 69.1 (d, *J* = 5 Hz, C-3), 73.2 (d, *J* = 5 Hz, C-2), 75.4 (C-4), 97.8 (d, *J* = 2 Hz, C-1), 101.9 (Ph*C*H), 111.1, 117.1, 118.6, 125.0, 126.2, 126.4, 127.3, 128.2, 128.3, 128.6, 129.0, 129.7, 129.9, 130.0, 132.6, 133.2, 134.9, 136.8, 143.5, 151.5 (25C, 24 Ar-C, CN), 165.6 (CO); ESI-MS: *m/z:* Calcd for $C_{49}H_{67}ClN_2O_{10}PSi_2$ $[M+NEt_3+H]^+$: 965.38, found: 965.53.

4'-(3-*O*-Benzoyl-2-*O*-phosphoryl-α-D-mannopyranosyloxy)-3'-chloro-biphenyl-4-carbonitrile disodium salt (26). A solution of **25** (79.6 mg, 0.275 mmol) in dry DCM (1.5 mL) was treated with TFA (150 µL) for 1 h at rt under argon. Then, a drop of water was added and stirring continued for 30 min. The solvents were removed *in vacuo,* the residue was dissolved in H_2O (1 mL) containing a drop of 1 M aq. NaOH and purified by MPLC on RP-18 (H_2O/MeOH, 95:5-4:1) to yield **26** (34.7 mg, 61%) as a 4:1-mixture of 2- and 3-phosphate. ^{1}H NMR (500 MHz, CD_3OD): δ = 3.78-3.90 (m, 3H, H-5, H-6), 4.20 (t, *J* = 9.9 Hz, 1H, H-4),

5.01 (ddd, J = 2.0, 3.1, 9.4 Hz, 1H, H-2), 5.60-5.65 (td, J = 2.6, 10.1 Hz, 1H, H-3), 5.91 (d, J = 1.6 Hz, 1H, H-1), 7.45-7.53 (m, 3H, Ar-H), 7.57-7.65 (m, 2H, Ar-H), 7.75-7.81 (m, 5H, Ar-H), 8.15-8.18 (m, 2H, Ar-H); ^{13}C NMR (126 MHz, CD_3OD): δ = 62.2 (C-6), 65.3 (C-4), 73.9 (dd, J = 5.8 Hz, 2C, C-2, C-3), 76.3 (C-5), 98.8 (d, J = 2 Hz, C-1), 112.0, 118.8, 119.7, 125.8, 127.9, 131.0, 133.9, 136.0, 145.0, 153.2 (19C, 18 Ar-C, CN), 167.8 (CO); ESI-MS: *m/z*: Calcd for $C_{26}H_{22}ClNO_{10}P$ $[M-2Na+H]^-$: 574.07, found: 574.21.

4'-(3-*O*-Allyl-α-D-mannopyranosyloxy)-3'-chloro-biphenyl-4-carbonitrile (27). A suspension of **4** (90.1 mg, 0.230 mmol) and dibutyltin oxide (62.7 mg, 0.252 mmol) in toluene (4 mL) was stirred for 6 h at 80 °C under argon. Then, tetrabutylammonium iodide (78.1 mg, 0.242 mmol) and allyl bromide (23 µL, 0.277 mmol) were added to the still turbid mixture and stirring was continued for another 20 h at 80 °C. Afterwards, the solvent was removed *in vacuo* and the residue was purified by MPLC on RP-18 (H_2O/MeOH) to yield **27** (54.7 mg, 55%) as a colorless solid. ^{1}H NMR (500 MHz, CD_3OD): δ = 3.65 (d, J = 6.3 Hz, 1H, H-5), 3.72 (dd, J = 5.3, 12.0 Hz, 1H, H-6a), 3.78 (dd, J = 2.5, 12.0 Hz, 1H, H-6b) 3.83-3.90 (m, 2H, H-3, H-4), 4.20-4.32 (m, 3H, H-2, allyl-H1a, allyl-H1b), 5.22 (dd, J = 1.7, 10.4 Hz, 1H, allyl-H3a), 5.39 (dd, J = 1.7, 17.3 Hz, 1H, allyl-H3b), 5.64 (d, J = 1.8 Hz, 1H, H-1), 6.04 (ddt, J = 5.9, 10.4, 16.3 Hz, 1H, allyl-H2), 7.48 (d, J = 8.7 Hz, 1H, Ar-H), 7.60 (dd, J = 2.3, 8.6 Hz, 1H, Ar-H), 7.75 (d, J = 2.3 Hz, 1H, Ar-H), 7.76-7.80 (m, 4H, Ar-H); ^{13}C NMR (126 MHz, CD_3OD): δ = 62.6 (C-6), 67.1 (C-4), 68.6 (C-2), 72.1 (allyl-C1), 76.1 (C-5), 79.6 (C-3), 100.5 (C-1), 111.8, 117.9 118.6, 119.8, 125.5, 127.9, 128.5, 129.8, 133.9, 135.5, 136.4, 145.1, 153.6 (15C, 12 Ar-C, CN, allyl-C2, allyl-C3); ESI-MS: *m/z*: Calcd for $C_{22}H_{22}ClNNaO_6$ $[M+Na]^+$: 454.10, found: 453.89.

4'-(3-*O*-Allyl-2,4,6-tri-*O*-benzoyl-α-D-mannopyranosyloxy)-3'-chloro-biphenyl-4-carbonitrile (28). To a solution of **27** (194 mg, 0.449 mmol) and DMAP (10 mg) in pyridine

(5 mL) was added benzoyl chloride (261 μL, 2.25 mmol) under argon. The mixture was stirred at rt overnight. MeOH (1 mL) was added and the mixture was stirred for 10 min. Then, the solvents were removed under reduced pressure, the residue was dissolved in DCM (20 mL) and washed with 1 M aq. HCl (10 mL) and satd. aq. $NaHCO_3$ (10 mL). The organic phase was dried (Na_2SO_4) and concentrated. The residue was purified by MPLC on silica (petroleum ether/EtOAc) to yield **28** (292 mg, 87%) as a foam. $[\alpha]_D^{20}$ +44.1 (*c* 1.46, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.13 (m, 1H, allyl-H1a), 4.24 (dd, *J* = 5.4, 13.0 Hz, 1H, allyl-H1b), 4.42-4.49 (m, 2H, H-5, H-6a), 4.52 (dd, *J* = 3.3, 9.8 Hz, 1H, H-3), 5.16 (dd, *J* = 1.1, 10.3 Hz, 1H, allyl-H3a), 5.29 (dd, *J* = 1.5, 17.2 Hz, 1H, allyl-H3b), 5.79 (ddt, *J* = 5.9, 10.4, 16.7 Hz, 1H, allyl-H2), 5.87 (d, *J* = 1.8 Hz, 1H, H-1), 5.91 (m, 1H, H-2), 5.98 (t, *J* = 9.8 Hz, 1H, H-4), 7.30-7.39 (m, 4H, Ar-H), 7.41-7.45 (m, 2H, Ar-H), 7.46-7.53 (m, 2H, Ar-H), 7.57-7.63 (m, 3H, Ar-H), 7.68 (m, 1H, Ar-H), 7.70-7.74 (m 2H, Ar-H), 8.00-8.05 (m, 2H, Ar-H), 8.10-8.17 (m, 4H, Ar-H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 62.8 (C-6), 67.8 (C-4), 68.8 (C-2), 70.2 (C-5), 71.1 (allyl-C1), 73.9 (C-3), 96.5 (C-1), 111.1, 116.9, 118.2, 118.6, 124.4, 127.2, 128.4, 129.9, 132.6, 132.9, 133.3, 133.8, 143.3, 151.6 (33C, 30 Ar-C, CN, allyl-C2, allyl-C3), 165.4, 165.7, 166.0 (3 CO); ESI-MS: *m/z*: Calcd for $C_{43}H_{34}ClNNaO_9$ $[M+Na]^+$: 766.18, found: 768.12.

4'-(2,4,6-Tri-*O*-benzoyl-α-D-mannopyranosyloxy)-3'-chloro-biphenyl-4-carbonitrile (29). A flask was charged with **28** (292 mg, 0.391 mmol), anhydrous $PdCl_2$ (10.8 mg, 0.118 mmol) and a magnetic stirring bar. The flask was evacuated and flushed with argon. The procedure was repeated twice. Then, dry MeOH (4 mL) was added and the mixture was stirred at 40 °C for 5 h. The mixture was filtered and the filtrate concentrated *in vacuo*. The residue was purified by MPLC on silica (petroleum ether/EtOAc) to yield **29** (231 mg, 84%). $[\alpha]_D^{20}$ +55.0 (*c* 1.01, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.35-4.41 (m, 2H, H-5, H-6a), 4.57 (m, 1H,

H-6b), 4.66 (dd, *J* = 3.4, 9.9 Hz, 1H, H-3), 5.65 (dd, *J* = 1.8, 3.4 Hz, 1H, H-2), 5.73 (t, *J* = 9.8 Hz, 1H, H-4), 5.81 (d, *J* = 1.7 Hz, 1H, H-1), 7.18-7.27 (m, 4H, Ar-H), 7.34-7.45 (m, 5H, Ar-H), 7.49-7.59 (m, 5H, Ar-H), 7.62-7.67 (m, 2H, Ar-H), 7.87-7.92 (m, 2H, Ar-H), 8.00-8.05 (m, 4H, Ar-H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 62.7 (C-6), 68.8 (C-3), 69.8 (C-5), 69.9 (C-4), 72.3 (C-2), 96.1 (C-1), 111.2, 116.9, 118.7, 124.6, 127.3, 128.6, 128.9, 129.7, 132.7, 133.1, 133.8, 134.5, 151.5 (31C, 30 Ar-C, CN), 165.8, 165.9, 166.8 (3 CO); IR (KBr): ν = 3446 (m, OH), 2228 (m, CN), 1725 (vs, C=O) cm^{-1}; ESI-MS: *m/z*: Calcd for $C_{40}H_{30}ClNNaO_9$ $[M+Na]^+$: 726.15, found: 726.49.

4'-(2,4,6-Tri-O-benzoyl-3-*O*-bis[2-(trimethylsilyl)ethoxy]phosphoryl-α-D-manno-pyranosyloxy)-3'-chloro-biphenyl-4-carbonitrile (30). According to the general procedure, compound **29** (198 mg, 0.281 mmol) was reacted with 1,2,4-triazole (77.8 mg, 1.13 mmol) and bis[2-(trimethylsilyl)ethyl] *N,N*-diisopropylphosphoramidite (232 µL, 0.563 mmol) in MeCN (3 mL), followed by treatment with 70% aq. *tert*-butylhydroperoxide (154 µL, 1.13 mmol) to yield **30** (89.1 mg, 32%). $[\alpha]_D^{20}$ +35.9 (*c* 0.99, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = -0.10, 0.00 (2s, 18H, 2 $Si(CH_3)_3$), 0.69-0.85 (m, 2H, $SiCH_2$), 1.01-1.09 (m, 2H, $SiCH_2$), 3.83-3.98 (m, 2H, OCH_2), 4.09-4.21 (m, 2H, OCH_2), 4.49-4.57 (m, 2H, H-5, H-6a), 4.67 (d, *J* = 10.1 Hz, 1H, H-6b), 5.55 (ddt, *J* = 3.4, 9.5 Hz, 1H, H-3), 5.92 (d, *J* = 1.6 Hz, 1H, H-1), 5.99 (dd, *J* = 1.9, 3.3 Hz, 1H, H-2), 6.12 (t, *J* = 9.8 Hz, 1H, H-4), 7.34-7.40 (m, 4H, Ar-H), 7.48-7.58 (m, 5H, Ar-H), 7.63-7.73 (m, 5H, Ar-H), 7.76-7.80 (m, 2H, Ar-H), 8.03-8.06 (m, 2H, Ar-H), 8.17-8.20 (m, 2H, Ar-H), 8.21-8.25 (m, 2H, Ar-H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = -1.9, -1.8 (6C, 2 $Si(CH_3)_3$), 19.0 (d, *J* = 6 Hz, $SiCH_2$), 19.3 (d, *J* = 6 Hz, $SiCH_2$), 62.5 (C-6), 66.5 (d, *J* = 1 Hz, OCH_2), 66.6 (d, *J* = 1 Hz, OCH_2), 67.3 (d, *J* = 5 Hz, C-4), 70.0 (C-5), 70.8 (d, *J* = 2 Hz, C-2), 72.6 (d, *J* = 5 Hz, C-3), 96.4 (C-1), 111.2, 117.4, 118.6, 124.9, 128.2, 129.1, 129.1, 129.6, 130.1, 132.6, 132.9, 133.5, 133.7, 135.0 (31C, 30 Ar-C, CN),

165.32, 165.34, 165.8 (3 CO); ESI-MS: *m/z*: Calcd for $C_{50}H_{55}ClNNaO_{12}PSi_2$ $[M+Na]^+$: 1006.26, found: 1006.44.

4'-(2,3,6-Tri-*O*-acetyl-α-D-mannopyranosyloxy)-3'-chloro-biphenyl-4-carbonitrile (31). According to a described procedure,[47] a solution of **4** (100 mg, 0.255 mmol) and dibutyltin oxide (340 mg, 0.562 mmol) in dry MeOH (5 mL) was stirred under reflux for 2 h at 70 °C. Afterwards, the solvent was removed *in vacuo* and the residue was dissolved in dry MeCN (5 mL) and cooled to 0 °C. Then, a solution of Ac_2O (80 µL, 0.842 mmol) in dry MeCN (1 mL) was added dropwise at 0 °C. The mixture was stirred at rt for 16 h. The reaction was quenched with MeOH (1 mL), the solvents were removed *in vacuo* and the residue was purified by MPLC on silica (petroleum ether/EtOAc) to yield **31** (27.0 mg, 21%). $[\alpha]_D^{20}$ +63.6 (*c* 0.98, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.06, 2.11, 2.16 (3s, 9H, 3 $COCH_3$), 3.94 (t, *J* = 9.8 Hz, 1H, H-4), 2.02 (ddd, *J* = 2.1, 4.6, 9.9 Hz, 1H, H-5), 4.27 (dd, *J* = 2.1, 12.3 Hz, 1H, H-6a), 4.50 (dd, *J* = 4.7, 12.3 Hz, 1H, H-6b), 5.47 (dd, *J* = 3.5, 9.7 Hz, 1H, H-3), 5.50 (dd, *J* = 1.8, 3.4 Hz, 1H, H-2), 5.58 (d, *J* = 1.6 Hz, 1H, H-1), 7.26 (m, 1H, Ar-H), 7.41 (dd, *J* = 2.3, 8.6 Hz, 1H, Ar-H), 7.59-7.62 (m, 3H, Ar-H), 7.68-7.72 (m, 2H, Ar-H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 20.7, 20.8, 20.8 (3 CO*C*H_3), 62.8 (C-6), 65.5 (C-4), 69.4 (C-2), 71.1 (C-3), 72.2 (C-5), 96.7 (C-1), 111.2, 117.2, 118.7, 125.0, 126.4, 127.4, 129.2, 132.7, 134.9, 143.5, 151.6 (13C, 12 Ar-C, CN), 169.9, 170.8, 171.3 (3 CO); IR (KBr): ν = 3436 (vs, OH), 2229 (m, CN), 1756 (vs, C=O) cm^{-1}; ESI-MS: *m/z:* Calcd for $C_{25}H_{24}ClNNaO_9$ $[M+Na]^+$: 540.10, found: 540.08.

4'-(2,3,6-Tri-*O*-acetyl-4-*O*-bis[2-(trimethylsilyl)ethoxy]phosphoryl-α-D-manno-pyranosyloxy)-3'-chloro-biphenyl-4-carbonitrile (32). According to the general procedure, compound **31** (39.3 mg, 76 µmol) was reacted with 1,2,4-triazole (21.0 mg, 0.304 mmol) and

bis[2-(trimethylsilyl)ethyl] *N,N*-diisopropylphosphoramidite (63 µL, 0.152 mmol) in MeCN (1 mL), followed by treatment with 70% aq. *tert*-butylhydroperoxide (42 µL, 0.304 mmol) to yield **32** (37.3 mg, 62%). $[\alpha]_D^{20}$ +65.0 (*c* 1.07, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 0.04, 0.05 (2s, 18H, 2 $Si(CH_3)_3$), 1.04-1.14 (m, 4H, 2 $SiCH_2$), 2.04, 2.13, 2.20 (3s, 9H, 3 $COCH_3$), 4.08-4.19 (m, 5H, H-5, 2 OCH_2), 4.33 (dd, *J* = 5.2, 12.3 Hz, 1H, H-6a), 4.42 (dd, *J* = 2.0, 12.3 Hz, 1H, H-6b), 4.79 (q, *J* = 9.6 Hz, 1H, H-4), 5.51 (dd, *J* = 1.8, 3.5 Hz, 1H, H-2), 5.59 (d, *J* = 1.7 Hz, 1H, H-1), 5.68 (dd, *J* = 3.6, 9.7 Hz, 1H, H-3) 7.27 (m, 1H, Ar-H), 7.43 (dd, *J* = 2.3, 8.6 Hz, 1H, Ar-H), 7.60-7.65 (m, 3H, Ar-H), 7.70-7.74 (m, 2H, Ar-H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = -1.6, -1.5 (6C, 2 $Si(CH_3)_3$), 19.5, 19.6 (2 $SiCH_2$), 20.7, 20.8, 20.9 (3 $COCH_3$), 62.2 (C-6), 66.7 (d, *J* = 2 Hz, OCH_2), 66.8 (d, *J* = 1 Hz, OCH_2), 68.8 (d, *J* = 2 Hz, C-3), 69.5 (C-2), 70.4 (d, *J* = 6 Hz) & 70.5 (d, *J* = 5 Hz) (C-4, C-5), 96.4 (C-1), 111.3, 117.3, 118.6, 125.0, 126.4, 127.4, 129.2, 132.7, 135.1, 143.5, 151.6 (13C, 12 Ar-C, CN), 169.7, 169.9, 170.3 (3 CO); IR (KBr): ν = 2229 (m, CN), 1753 (vs, C=O) cm^{-1}; ESI-MS: *m/z*: Calcd for $C_{35}H_{49}ClNNaO_{12}PSi_2$ $[M+Na]^+$: 820.21, found: 820.14.

3'-Chloro-4'-(6-*O*-trityl-α-D-mannopyranosyloxy)-biphenyl-4-carbonitrile (33). To a solution of **4** (150 mg, 0.383 mmol) in pyridine, were added trityl chloride (128 mg, 0.459 mmol) and DMAP (5 mg). The mixture was stirred at 80 °C for 16 h. Then, the solvent was removed *in vacuo* and the residue was purified by MPLC on silica (petroleum ether/EtOAc, +0.5% NEt_3) to yield **33** (189 mg, 80%). ^{1}H NMR (500 MHz, CD_3OD): δ = 3.30 (dd, *J* = 7.5, 16.1 Hz, 1H, H-6a), 3.46 (d, *J* = 9.3 Hz, 1H, H-6b), 3.63 (t, *J* = 9.7 Hz, 1H, H-4), 3.88 (t, *J* = 9.0 Hz, 1H, H-5), 4.02 (dd, *J* = 3.3, 9.4 Hz, 1H, H-3), 4.20 (m, 1H, H-2), 5.75 (s, 1H, H-1), 7.03-7.16 (m, 10H, Ar-H), 7.26-7.35 (m, 6H, Ar-H), 7.55 (d, *J* = 8.3 Hz, 2H, Ar-H), 7.58-7.64 (m, 3H, Ar-H), 7.70 (d, *J* = 2.0 Hz, 2H, Ar-H); ^{13}C NMR (126 MHz, CD_3OD): δ = 65.0 (C-6), 68.7 (C-4), 71.5 (C-2), 72.6 (C-3), 75.0 (C-5), 87.54 (*C*Ph_3), 99.9 (C-1), 111.7, 118.7, 119.7,

127.7, 127.8, 128.3, 129.7, 133.7, 134.9, 144.7, 145.3, 153.3 (31C, 30 Ar-C, CN); ESI-MS: *m/z*: Calcd for $C_{59}H_{44}ClNNaO_9$ $[M+Na]^+$: 656.18, found: 656.15.

4'-(2,3,4-Tri-*O*-benzoyl-6-*O*-trityl-α-D-mannopyranosyloxy)-3'-chloro-biphenyl-4-carbonitrile (34). Prepared according to the procedure described for **28** from **33** (189 mg, 0.299 mmol), benzoyl chloride (173 µL, 1.21 mmol) and DMAP (10 mg) to yield **34** (223 mg, 79%). $[\alpha]_D^{20}$ +8.1 (*c* 1.10, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.42-3.50 (m, 2H, H-6), 4.39 (m, 1H, H-5), 6.05 (d, *J* = 1.5 Hz, 1H, H-1), 6.10 (dd, *J* = 1.9, 3.2 Hz, 1H, H-2), 6.15 (dd, *J* = 3.3, 10.1 Hz, 1H, H-3), 6.23 (t, *J* = 10.1 Hz, 1H, H-4), 7.10-7.19 (m, 9H, Ar-H), 7.28-7.39 (m, 4H, Ar-H), 7.41-7.60 (m, 12H, Ar-H), 7.63-7.71 (m, 3H, Ar-H), 7.81-7.85 (m, 2H, Ar-H), 7.93-7.98 (m, 2H, Ar-H), 8.23-8.28 (m, 2H, Ar-H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = 62.0 (C-6), 66.7 (C-4), 70.0 (C-3), 70.3 (C-2), 71.6 (C-5), 86.6 (*C*Ph_3), 96.4 (C-1), 111.0, 118.6, 124.8, 126.5, 126.8, 127.2, 127.6, 128.1, 128.2, 128.4, 128.6, 128.9, 128.97, 129.02, 129.55, 129.61, 129.8, 132.5, 133.1, 133.6, 134.6, 143.4, 143.5, 151.6 (49C, 48 Ar-C, CN), 165.0, 165.3, 165.4 (3 CO); IR (KBr): ν = 2227 (m, CN), 1731 (vs, C=O) cm^{-1}; ESI-MS: *m/z*: Calcd for $C_{59}H_{44}ClNNaO_9$ $[M+Na]^+$: 968.26, found: 968.47.

4'-(2,3,4-Tri-*O*-benzoyl-α-D-mannopyranosyloxy)-3'-chloro-biphenyl-4-carbonitrile (35). Prepared according to the procedure described for **21** by reacting **34** (122 mg, 0.129 mmol) with $FeCl_3$ (41.8 mg, 0.258 mmol) and water (27.9 µL, 1.55 mmol) in DCM (10 mL) for 5 h to yield **35** (77.4 mg, 85%). $[\alpha]_D^{20}$ +6.2 (*c* 1.02, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 3.80 (qd, *J* = 2.7, 13.1 Hz, 2H, H-6) 4.25 (d, *J* = 10.0 Hz, 1H, H-5), 5.95 (s, 1H, H-1), 5.96-6.03 (m, 2H, H-2, H-4), 6.29 (dd, *J* = 3.4, 10.2 Hz, 1H, H-3), 7.25-7.32 (m, 2H, Ar-H), 7.38-7.56 (m, 8H, Ar-H), 7.61-7.73 (m, 6H, Ar-H), 7.88 (d, *J* = 7.3 Hz, 2H, Ar-H), 8.01 (d, *J* = 7.3 Hz, 2H, Ar-H), 8.14 (d, *J* = 7.2 Hz, 2H, Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 60.9 (C-6),

66.8 (C-4), 69.2 (C-3), 70.2 (C-2), 72.3 (C-5), 96.6 (C-1), 111.2, 117.0, 118.6, 124.9, 126.5, 127.3, 128.3, 128.4, 128.5, 128.7, 128.9, 129.2, 129.6, 129.90, 129.91, 132.7, 133.3, 133.7, 133.8, 134.9, 143.5, 151.6 (31C, 30 Ar-C, CN), 165.37, 165.43, 166.6 (3 CO); IR (KBr): ν = 3436 (vs, OH), 2228 (m, CN), 1731 (vs, C=O) cm^{-1}; ESI-MS: *m/z*: Calcd for $C_{40}H_{30}ClNNaO_9$ $[M+Na]^+$: 726.15, found: 726.24.

4'-(2,3,4-Tri-*O*-benzoyl-6-*O*-bis[2-(trimethylsilyl)ethoxy]phosphoryl-α-D-manno-pyranosyloxy)-3'-chloro-biphenyl-4-carbonitrile (36). According to the general procedure, compound **35** (61.3 mg, 87 μmol) was reacted with 1,2,4-triazole (24.1 mg, 0.348 mmol) and bis[2-(trimethylsilyl)ethyl] *N,N*-diisopropylphosphoramidite (72 μL, 0.174 mmol) in MeCN (2 mL), followed by treatment with 70% aq. *tert*-butylhydroperoxide (48 μL, 0.348 mmol) to yield **36** (50.8 mg, 59%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = -0.07, -0.04 (2 s, 18H, 2 $Si(CH_3)_3$) 0.94-1.03 (m, 4H, 2 $SiCH_2$), 4.03-4.15 (m, 4H, 2 OCH_2), 4.26 (dd, *J* = 3.8, 5.6 Hz, 2H, H-6), 4.52 (m, 1H, H-5), 5.86 (d, *J* = 1.7 Hz, 1H, H-1), 5.97 (dd, *J* = 2.0, 3.2 Hz, 1H, H-2), 6.07 (t, *J* = 10.1 Hz, 1H, H-4), 6.15 (dd, *J* = 3.3, 10.1 Hz, 1H, H-3), 7.27-7.33 (m, 2H, Ar-H), 7.37-7.54 (m, 8H, Ar-H), 7.61-7.76 (m, 6H, Ar-H), 7.85-7.89 (m, 2H, Ar-H), 7.95-8.00 (m, 2H, Ar-H), 8.11-8.15 (m, 2H, Ar-H); ^{13}C NMR (126 MHz, $CDCl_3$): δ = -1.65, -1.64 (6C, 2 $Si(CH_3)_3$), 19.4 (d, *J* = 6 Hz, $SiCH_2$), 19.5 (d, *J* = 6 Hz, $SiCH_2$), 65.3 (d, *J* = 5 Hz, C-6), 66.1 (C-4), 66.4 (d, *J* = 6 Hz, 2C, 2 OCH_2), 69.5 (C-3), 70.1 (C-2), 70.8 (d, *J* = 8 Hz, C-5), 96.9 (C-1), 111.3, 117.7, 118.6, 125.1, 126.7, 127.4, 128.3, 128.4, 128.7, 128.8, 128.9, 129.0, 129.2, 129.7, 129.8, 130.0, 132.7. 133.3, 133.5, 133.7, 135.2, 143.5, 151.9 (31C, 30 Ar-C, CN), 165.2, 165.35, 165.39 (3 CO); ESI-MS: *m/z*: Calcd for $C_{50}H_{55}ClNNaO_{12}PSi_2$ $[M+Na]^+$: 1006.26, found: 1006.52.

Biphenyl 2,3,4-tri-*O*-benzoyl-6-*O*-(methylthiomethyl)-α-D-mannopyranoside (37). To a solution of compound **21** (469 mg, 0.726 mmol) in Ac_2O (2.5 mL) and AcOH (0.25 mL) was added DMSO (2.5 mL). The mixture was stirred at rt for 24 h, then diluted with EtOAc (50 mL) and washed with satd. aq. $NaHCO_3$ (2 × 20 mL), H_2O (2 × 20 mL) and brine (20 mL). The organic layer was dried over Na_2SO_4 and the solvents removed under diminished pressure. The residue was purified by MPLC on silica (petroleum ether/EtOAc, 9:1-6:1) to yield **37** (171 mg, 35%), which contained some impurities but was used in the next step without further purification.

Biphenyl 2,3,4-tri-*O*-benzoyl-6-*O*-(phosphonooxy)-methyl-α-D-mannopyranoside (38). Compound **37** (170 mg, 0.250 mmol) was dissolved in a premixture of H_3PO_4 (147 mg, 1.50 mmol) in THF (1.5 mL). Then, *N*-iodosuccinimide (84.0 mg, 0.375 mmol) was added and the mixture was stirred for 15 min at 0 °C and for 1 h at rt. The reaction was then quenched with 1 M aq. $Na_2S_2O_3$, diluted with DCM/MeOH (20 mL, 4:1) and washed with satd. aq. $NaHCO_3$ (10 mL). The organic layer was dried over Na_2SO_4 and the solvents were removed *in vacuo* at < 20 °C. The residue was purified by MPLC on silica (DCM/MeOH, 1:0-3.5:1) to yield **38** (110 mg, 58%). ^{1}H NMR (CD_3OD, 500 MHz): δ = 3.90 (dd, J = 4.5, 11.6 Hz, 1H, H-6a), 4.01 (d, J = 10.2 Hz, 1H, H-6b), 4.47 (m, 1H, H-5), 5.06 (A of ABX, J = 5.5, 9.5 Hz, 1H, CH_2), 5.16 (B of ABX, J = 5.5, 9.1 Hz, 1H, CH_2), 5.89 (s, 1H, H-1), 5.92 (s, 1H, H-2), 6.05 (m, 2H, H-4, H-3), 7.26-7.68 (m, 18 H, Ar-H), 7.79 (d, J = 7.6 Hz, 2H, Ar-H), 7.96 (d, J = 7.6 Hz, 2H, Ar-H), 8.13 (d, J = 7.5 Hz, 2H, Ar-H); ^{13}C NMR (CD_3OD, 126 MHz): δ = 68.2 (C-4), 68.7 (C-6), 71.5 (C-2), 71.86 (C-5), 71.9 (C-3), 92.8 (d, J = 4 Hz, CH_2), 97.6 (C-1), 118.4, 127.7, 128.0, 129.3, 129.4, 129.6, 129.8, 129.9, 130.3, 130.4, 130.5, 130.6, 130.7, 130.9, 134.5, 134.6, 134.9, 137.4, 141.8, 156.8 (30C, Ar-C), 166.8, 167.0 (3C, 3 CO); ESI-MS: *m/z*: Calcd for $C_{40}H_{35}NaO_{13}P$ $[M+Na]^+$: 777.17, found: 777.13.

4-(5-Nitroindolin-1-yl)phenyl 2,3,4-tri-*O*-benzoyl-6-*O*-(*tert*-butylsilyldimethyl)-α-D-mannopyranoside (39). To a solution of **5**[20] (709 mg, 1.69 mmol) in pyridine were added *tert*-butyldimethylsilyl chloride (319 mg, 2.12 mmol) and DMAP (20.6 mg) and the mixture was stirred at rt overnight. Then, a solution of benzoyl chloride (0.98 mL, 8.45 mmol) in pyridine (2.0 mL) was added and the mixture was stirred at rt for 2 h. The mixture was diluted with DCM (30 mL) and subsequently washed with 0.1 M aq. HCl (10 mL) and satd. aq. $NaHCO_3$ (10 mL). The organic layer was dried over Na_2SO_4, filtered and the solvent removed *in vacuo*. The residue was purified by MPLC on silica (petroleum ether/EtOAc, 9:1-7:3) to yield crude **39** (1.43 g, quant.) as a yellow solid, which was used in the next step without further purification. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = -0.01 (s, 3H, $Si(CH_3)_2$), 0.00 (s, 3H, $Si(CH_3)_2$), 0.87 (s, 9H, $C(CH_3)_3$), 3.22 (t, J = 8.5 Hz, 2H, CH_2), 3.83 (dd, J = 2.3, 11.5 Hz, 1H, H-6a), 3.88 (dd, J = 4.7, 11.5 Hz, 1H, H-6b), 4.10 (t, J = 8.7 Hz, 2H, NCH_2), 4.43 (m, 1H, H-5), 5.77 (d, J = 1.8 Hz, 1H, H-1), 5.87 (m, 1H, H-2), 6.03-6.15 (m, 2H, H-3, H-4), 6.77 (d, J = 8.9 Hz, 1H, Ar-H), 7.22-7.31 (m, 6H, Ar-H), 7.38 (t, J = 7.8 Hz, 2H, Ar-H), 7.41-7.55 (m, 5H, Ar-H), 7.60-7.67 (m, 1H, Ar-H), 7.88 (dd, J = 1.2, 8.3 Hz, 2H, Ar-H), 7.94-8.01 (m, 3H, Ar-H), 8.03 (dd, J = 2.3, 8.9 Hz, 1H, Ar-H), 8.14 (dd, J = 1.2, 8.3 Hz, 1H, Ar-H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ = -5.51, -5.50 ($Si(CH_3)_2$), 25.8 (3C, $C(CH_3)_3$), 27.1 (CH_2), 53.7 (NCH_2), 62.1 (C-6), 66.6 (C-3), 70.3, 70.4 (C-2, C-4), 72.3 (C-5), 96.4 (C-1), 105.3, 118.0, 121.1, 122.0, 126.1, 128.3, 128.4, 128.43, 128.6, 129.1, 129.2, 129.3, 129.7, 129.8, 130.0, 131.0, 133.2, 133.3, 133.6, 136.8, 139.1, 152.8, 153.8 (30C, Ar-C), 165.3, 165.6, 165.7 (3 CO); ESI-MS: *m/z*: Calcd for $C_{47}H_{48}N_2NaO_{11}Si$ $[M+Na]^+$: 867.29, found: 867.25.

4-(5-Nitroindolin-1-yl)phenyl 2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside (40). A solution of **39** (1.43 g, 1.69 mmol) in DCM/MeOH (16 mL, 1:1) was treated with 1 M H_2SO_4 in MeOH (1.6 mL) for 1.5 h at rt. The reaction mixture was neutralized with NEt_3 and the solvents were removed *in vacuo*. The residue was purified by MPLC on silica (petroleum

ether/EtOAc, 3:1-3:2) to yield **40** (900 mg, 73%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 3.22 (t, *J* = 8.6 Hz, 2H, CH_2), 3.77 (dd, *J* = 3.3, 13.0 Hz, 1H, H-6a), 3.84 (dd, *J* = 1.8, 13.0 Hz, 1H, H-6b), 4.11 (t, *J* = 9.4 Hz, 2H, NCH_2), 4.20 (m, 1H, H-5), 5.83 (d, *J* = 1.6 Hz, 1H, H-1), 5.88 (dd, *J* = 1.9, 3.3 Hz, 1H, H-2), 5.96 (t, *J* = 10.1 Hz, 1H, H-4), 6.22 (dd, *J* = 3.4, 10.2 Hz, 1H, H-3), 6.78 (d, *J* = 8.9 Hz, 1H, Ar-H), 7.21-7.34 (m, 6H, Ar-H), 7.37-7.43 (m, 3H, Ar-H), 7.45 (t, *J* = 7.4 Hz, 1H, Ar-H), 7.50-7.57 (m, 3H, Ar-H), 7.65 (t, *J* = 7.5 Hz, 1H, Ar-H), 7.84-7.89 (m, 2H, Ar-H), 7.97-8.02 (m, 3H, Ar-H), 8.04 (dd, *J* = 2.3, 8.9 Hz, 1H, Ar-H), 8.12-8.16 (m, 2H, Ar-H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ = 27.1 (CH_2), 53.7 (NCH_2), 61.1 (C-6), 67.0 (C-4), 69.3 (C-3), 70.4 (C-2), 71.8 (C-5), 96.4 (C-1), 105.4, 117.6, 121.1, 122.0, 126.1, 127.0, 128.3, 128.4, 128.5, 128.6, 128.7, 129.0, 129.03, 129.5, 129.7, 129.9, 130.0, 131.1, 133.4, 133.77, 133.8, 137.0, 139.2, 152.5, 153.6 (30C, Ar-C), 165.5, 165.6, 166.6 (3 CO); ESI-MS: *m/z*: Calcd for $C_{41}H_{34}N_2NaO_{11}$ $[M+Na]^+$: 753.21, found: 753.33.

4-(5-Nitroindolin-1-yl)phenyl 2,3,4-tri-*O*-benzoyl-6-*O*-(methylthio)methyl α-D-manno-pyranoside (41). Degassed DMSO (2.5 mL) was added to a degassed mixture of **40** (200 mg, 0.273 mmol) in Ac_2O (1.65 mL) and HOAc (0.5 mL). The mixture was stirred at rt overnight, then diluted with EtOAc (20 mL), and subsequently washed with satd. aq. $NaHCO_3$ (2 × 10 mL), H_2O (2 × 10 mL) and brine (10 mL). The organic layer was dried over Na_2SO_4 and concentrated. The residue was purified by MPLC on silica (petroleum ether/EtOAc, 3:1-7:3) to yield **41** (160 mg, 74%). ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 2.08 (s, 3H, CH_3), 3.23 (t, *J* = 8.6 Hz, 2H, CH_2), 3.72 (dd, *J* = 2.4, 11.1 Hz, 1H, H-6a), 3.89 (dd, *J* = 4.5, 11.2 Hz, 1H, H-6b), 4.12 (m, 2H, NCH_2), 4.43 (m, 1H, H-5), 4.61, 4.72 (A, B of ABX, *J* = 11.6 Hz, 2H, CH_2), 5.79 (d, *J* = 1.6 Hz, 1H, H-1), 5.86 (m, 1H, H-2), 6.03-6.11 (m, 2H, H-3, H-4), 6.78 (d, *J* = 8.9 Hz, 1H, Ar-H), 7.22-7.33 (m, 7H, Ar-H), 7.36-7.42 (m, 2H, Ar-H), 7.46 (t, *J* = 7.4 Hz, 1H, Ar-H), 7.48-7.56 (m, 3H, Ar-H), 7.64 (t, *J* = 7.5 Hz, 1H, Ar-H), 7.85-7.91 (m, 2H, Ar-H), 7.96-8.01 (m, 3H, Ar-H), 8.04 (dd, *J* = 2.2, 8.8 Hz, 1H, Ar-H), 8.10-8.17 (m, 2H, Ar-H); ^{13}C

NMR ($CDCl_3$, 126 MHz): δ = 13.9 (CH_3), 27.1 (CH_2), 53.7 (NCH_2), 66.3 (C-6), 67.8 (C-4), 70.1 (C-3), 70.4 (C-2), 71.7 (C-5), 75.9 (CH_2), 96.5 (C-1), 105.4, 117.9, 121.1, 122.0, 126.1, 128.4, 128.5, 128.7, 129.0, 129.2, 129.7, 129.8, 131.0, 131.1, 133.3, 133.4, 133.7, 137.0, 139.2, 152.7, 153.7 (30C, Ar-C), 165.5, 165.60, 165.62 (3 CO); ESI-MS: *m/z*: Calcd for $C_{43}H_{38}N_2NaO_{11}S$ $[M+Na]^+$: 813.21, found: 813.32.

4-(5-Nitroindolin-1-yl)phenyl 2,3,4-tri-*O*-benzoyl-6-*O*-(phosphonooxy)-methyl α-D-manno-pyranoside (42). Compound **41** (400 mg, 0.500 mmol) was dissolved in a mixture of H_3PO_4 (366 mg, 3.73 mmol) in THF (5 mL). Then, *N*-iodosuccinimide (225 mg, 1.00 mmol) was added and the mixture was stirred for 15 min at 0 °C and for 1 h at rt. The reaction was quenched with 1 M aq. $Na_2S_2O_3$ and 28% aq. ammonia (2 mL), then the volatiles were removed *in vacuo* at < 30 °C. The residue was purified by MPLC on silica (DCM/[MeOH/ H_2O 10:1], 1:0-3.5:1) to yield slightly impure **42** (278 mg, 67%), which was used in the next step without further purification.

Physicochemical and pharmacokinetic characterization

Materials. Dimethyl sulfoxide (DMSO), hydrochloric acid ≥ 37% (HCl), pepsin (from porcine gastric mucosa, ≥ 250 units/mg solid), pancreatin (from porcine pancreas, 4x USP specifications), acetic acid, sodium taurocholate hydrate, lecithin, sodium acetate trihydrate, maleic acid, glyceryl monooleate, sodium oleate, Dulbecco's Modified Eagle's Medium (DMEM) - high glucose, L-glutamine solution, penicillin-streptomycin solution, Dulbecco's Phosphate Buffered Saline (DPBS), and trypsin-EDTA solution were purchased from Sigma-Aldrich(Sigma-Aldrich, St. Louis, MA, USA). MEM nonessential amino acid (MEM-NEAA) solution, fetal bovine serum (FBS), and DMEM without sodium pyruvate and phenol red were bought from Invitrogen (Carlsbad, CA, USA). Methanol (MeOH), acetonitrile (MeCN),

and dichloromethane (DCM) were obtained from Acros Organics (Geel, Belgium). Monopotassium phosphate (KH_2PO_4) and sodium hydroxide (NaOH) were bought from Merck (Merck KGaA, Darmstadt, Germany). Sodium chloride (NaCl) was purchased from Hänseler (Hänseler AG, AR, Switzerland). Long-life, heat-treated and homogenized milk (UHTmilk) containing 3.5% fat was bought from Coop (Coop Qualité & Prix, Switzerland). The Caco-2 cells were kindly provided by Prof. G. Imanidis, FHNW, Muttenz, and originated from the American Type Culture Collection (Rockville, MD, USA).

Aqueous Solubility. Microanalysis tubes (LaboTech J. Stofer LTS AG, Muttenz, Switzerland) were charged with 500 µg of solid substance and 100 µL of phosphate buffer (50 mM, pH 6.5). The tubes were briefly shaken by hand, sonicated for 15 min, and vigorously shaken (600 rpm, 25 °C, 2 h) on an Eppendorf Thermomixer Comfort (Eppendorf, Hamburg, Germany). Afterwards, they were left undisturbed for 24 h. Then, the compound solutions were filtered (MultiScreen HTS 96-well Filtration System, Millipore, Billerica, MA) by centrifugation (1500 rpm, 25 °C, 3 min). The filtrates were further diluted with buffer (1:1000 and 1:10000), and the concentrations were determined by LC-MS (see below).

Colorectal adenocarcinoma (Caco-2) cell permeation assay and hydrolysis studies. Caco-2 cells were cultivated in tissue culture flasks (BD Biosciences, Franklin Lakes, NJ, USA) with DMEM high glucose medium, containing L-glutamine (2 mM), nonessential amino acids (0.1 mM), penicillin (100 U/mL), streptomycin (100 µg/mL), and fetal bovine serum (10%). The cells were kept at 37 °C in humidified air containing 5% CO_2, and the medium was changed every second day. When approximately 90% confluence was reached, the cells were split in a 1:10 ratio and distributed to new tissue culture flasks. At passage numbers between 60 and 65, they were seeded at a density of 5.3×10^5 cells per well to Transwell six-well plates (Corning Inc., Corning, NY, USA) with 2.5 mL of culture medium in the basolateral

and 2.0 mL in the apical compartment. The medium was renewed on alternate days. Enzymatic hydrolysis and permeation experiments were performed between days 19 and 21 post seeding. Prior to the experiment, the integrity of the Caco-2 monolayers was evaluated by measuring the transepithelial electrical resistance (TEER) with an Endohm tissue resistance instrument (World Precision Instruments Inc., Sarasota, FL, USA). Only wells with TEER values higher than 250 Ω cm^2 were used. After the experiment, TEER values were assessed again for each well and results from wells with values below 250 Ω cm^2 were discarded.

Permeation experiments with the compounds **3-5** were performed in the apical-to-basolateral and basolateral-to-apical directions in triplicates. Transport medium (DMEM without sodium pyruvate and phenol red) was withdrawn from the donor compartments of three wells and replaced by the same volume of compound stock solution (10 mM in DMSO) to reach an initial sample concentration of 62.5 µM. The Transwell plate was shaken (600 rpm, 37 °C) on a Heidolph Titramax 1000 plate-shaker (Heidolph Instruments GmbH & Co. KG, Schwabach, Germany). Samples (40 µL) were withdrawn from the donor and acceptor compartments 30 min after initiation of the experiment and the compound concentrations were determined by LC-MS. Apparent permeability (P_{app}) was calculated according to Equation 1:

$$P_{app} = \frac{dQ}{dt} \times \frac{1}{A \times c_0} \quad (1)$$

where dQ/dt is the compound flux (mol s^{-1}), A the surface area of the monolayer (cm^2), and c_0 the initial concentration in the donor compartment (mol cm^{-3}).[19]

Hydrolysis studies with the compounds **6a-e, 7a-d** and **8** were performed in triplicates. Transport medium was withdrawn from the apical compartments of three wells and replaced by the same volume of compound stock solution (10 mM in H_2O) to reach an initial sample

concentration of 62.5 μM. The Transwell plate was shaken (600 rpm, 37 °C) on a Heidolph Titramax 1000 plate-shaker. Samples (40 μL) were withdrawn from the apical compartment 10, 20, 30, 45, and 60 min after the initiation of the experiment and the concentrations of prodrug were determined by LC-MS. Metabolic half-life ($t_{1/2}$) was calculated from the slope of the linear regression from the natural log remaining compound concentration versus incubation time relationship.

Studies of hydrolysis and subsequent permeation in the apical-to-basolateral and basolateral-to-apical directions were performed with compound **7b** in triplicate. Transport medium was withdrawn from the apical or basal donor compartments of three wells and replaced by the same volume of compound stock solution (10 mM in H_2O) to reach an initial sample concentration of 62.5 μM. The Transwell plate was shaken (600 rpm, 37 °C) on a Heidolph Titramax 1000 plate-shaker. Samples (40 μL) were withdrawn form the apical and basal compartments 60 min after the initiation of the experiment and the concentrations of prodrug **7b** and active principle **4** were determined by LC-MS.

Studies of hydrolysis and subsequent permeation in the apical-to-basolateral direction were performed with the compounds **6e**, **7b** and **8** at different concentrations (100, 200, or 400 μM) in duplicate. Transport medium was withdrawn from the apical compartments of two wells and replaced by the same volume of compound stock solution (16, 32, or 64 mM in H_2O) to reach initial sample concentrations of 100, 200, or 400 μM. The Transwell plate was shaken (600 rpm, 37 °C) on a Heidolph Titramax 1000 plate-shaker. Samples (40 μL) were withdrawn from the basal compartments 60 min after the initiation of the experiment and the concentrations of prodrug **6e**, **7b** and **8** as well as active principle **3**-**5**, respectively, were determined by LC-MS.

Stability Studies in Biorelevant Media. Biorelevant media were prepared according to United States Pharmacopea (USP) specifications and Dressman *et al.*[51,52] as described below and are considered to be stable at ambient storage conditions for at least 72 h.[55] Table S1 (see Supporting Information) shows the composition of these biorelevant media used to mimic gastric and intestinal conditions.[51,52,55]

Simulated Gastric Fluid (sGF) and Simulated Intestinal Fluid (sIF). sGF and sIF were prepared according to the United States Pharmacopeia (USP 28).[51] For the preparation of sGF, sodium chloride and pepsin were mixed in bidistilled water and then the pH was adjusted to 1.2 by adding 37% aq. HCl. For sIF, monopotassium phosphate and pancreatin were mixed in bidistilled water and then the p*H* was adjusted to 6.8 by adding 0.2 M NaOH. In parallel, two buffer solutions were prepared equally to sGF and sIF, without pepsin (buffer sGF) and pancreatin (buffer sIF), respectively.

Fasted State Simulated Gastric Fluid (FaSSGF). First, a NaCl solution was prepared and its p*H* was adjusted to 1.6 with 37% aq. HCl. The solution was then transferred into a round bottom flask and sodium taurocholate hydrate was dissolved by continuous stirring. Then, a freshly prepared solution of lecithin in dichloromethane (DCM) (100 mg/mL) was added. The resulting emulsion was turbid. The DCM was then evaporated at 40 °C. For the first 15 min the pressure was kept at 650 mbar. It was then decreased stepwise to a final pressure of 100 mbar and maintained for another 15 min. The product was a clear solution, having no perceptible smell of DCM. Next, pepsin was added under continuous stirring and, as a last step, the pH (1.6) and the volume of the solution were adjusted.

Fed State Simulated Gastric Fluid (FeSSGF). Sodium acetate trihydrate and NaCl were dissolved in bidistilled water. Acetic acid was added followed by a pH adjustment to 5.0 with

0.2 M NaOH. The resulting solution was mixed 1:1 with ultra-high temperature milk.

Fasted State Simulated Intestinal Fluid (FaSSIF). Blank buffer was prepared using appropriate amounts of NaCl, sodium hydroxide, and maleic acid in bidistilled water and the pH was then adjusted to 6.5 with 0.2 M NaOH. The solution was then transferred into a round bottom flask and sodium taurocholate hydrate was added under continuous stirring. Afterwards, a freshly prepared solution of lecithin in DCM (100 mg/mL) was added. The resulting emulsion was turbid. The DCM was then evaporated at 40 °C (same procedure as for the FaSSGF). As a last step, the volume and pH (6.5) of the solution were adjusted again.

Fed State Simulated Intestinal Fluid (FeSSIF). Blank buffer was prepared using appropriate amounts of NaCl, sodium hydroxide, and maleic acid in bidistilled water and the pH was then adjusted to 5.8 with 0.2 M NaOH. The solution was transferred into a round bottom flask and sodium taurocholate hydrate was added under continuous stirring. Afterwards, a freshly prepared solution of lecithin in dichloromethane (DCM) (100 mg/mL) was added. The resulting emulsion was turbid. The DCM was then evaporated at 40 °C (same procedure as for the FaSSGF). A freshly prepared solution of glyceryl monooleate in DCM (50 mg/mL) was added and a second evaporation step was performed. Next, appropriate amounts of sodium oleate and pancreatin were added slowly under continuous stirring and, as a last step, the volume and pH (5.8) of the solution were adjusted again.

Stability Assay. All fluids were preheated at 37 °C. The compounds (**7a**, **7c**, and **7d**) were then added to yield 20 μM solutions (t = 0 min). Incubations were performed on a Heidolph 1000 incubator (500 rpm, 37 °C). After an incubation time of 0, 10, 20, 30, 60, and 120 min, samples (30 μL) were withdrawn, precipitated with ice-cooled methanol (120 μL), put into the freezer (-20 °C, 10 min), and then centrifuged (13,200 rpm, 3 min). The supernatant was

transferred into a 96-well plate. The concentration of analyte in the supernatant was analyzed by LC-MS.

LC-MS Measurement. Analyses were performed using an 1100/1200 Series HPLC System coupled to a 6410 Triple Quadrupole mass detector (Agilent Technologies, Inc., Santa Clara, CA, USA) equipped with electrospray ionization. The system was controlled with the Agilent MassHunter Workstation Data Acquisition software (version B.01.04). The column used was an Atlantis® T3 C18 column (2.1 x 50 mm) with a 3 µm particle size (Waters Corp., Milford, MA, USA). The mobile phase consisted of two eluents: Eluent A (H_2O, containing 0.1% formic acid, v/v for compounds **5**, **6a-e**, **7c**, **7d** and **8**; ammonium acetate buffer, 10 mM, pH 5 for compounds **3** and **4**; formiate buffer, 10 mM, pH 3 for compounds **7a** and **7b**) and eluent B (MeCN, containing 0.1% formic acid, v/v), delivered at 0.6 mL/min. The gradient was ramped from 95% A/5% B to 5% A/95% B over 1 min, and then held at 5% A/95% B for 0.1 min. The system was then brought back to 95% A/5% B, resulting in a total duration of 4 min. MS parameters such as fragmentor voltage, collision energy, polarity were optimized individually for each analyte, and the molecular ion was followed for each compound in the multiple reaction monitoring mode. The concentrations of the analytes were quantified by the Agilent Mass Hunter Quantitative Analysis software (version B.01.04).

***In vivo* pharmacokinetics.** For the PK studies, eight-week-old female C3H/HeN mice (21-27 g) from Harlan (Venray, The Netherlands) were purchased. The mice were housed in groups of three to five per cage and kept under specific pathogen-free conditions in the Animal House of the Department of Biomedicine, University Hospital of Basel. For experimentation, all guidelines according to the Swiss veterinary law were followed. The animals were kept in a 12 h/12 h light/dark cycle and had chow and water *ad libitum*. After one week of acclimatization, the mice were used in groups of three (five for **4**, 7.7 mg/kg) for the

pharmacokinetic studies. Compounds were diluted in PBS and applied using an oral gavage (1.25 and 7.7 mg/kg for **4**, and 10 mg/kg for **8** and **7b**, **7c**). Prodrug solutions consisted of prodrug (min. 94%) and active principle (max. 6%). Blood and urine samples (10 µL) were taken before the experiment (0 min) and at 6, 13, 20, 40 min, 1, 1.5, 2, 3, 4, 6, 8, and 24 h after administration. Directly after sampling, the samples were diluted in methanol (1:5) to precipitate proteins. After centrifugation (11 min, 13000 rpm) the supernatant was transferred to a 96-well plate and analyzed by LC-MS as described before. The samples at 0 min were used to define the detection limit in plasma and urine. Sampling and administration was performed following the guidelines in reference.[56]

Supporting Information. Composition of biorelevant media, HPLC data and chromatograms for target compounds, ^{1}H NMR spectra for target compounds.

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Notes

The authors declare no competing financial interest.

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Abbreviations Used: ALP, alkaline phosphatase; C_{max}, maximum concentration; Caco-2 cells, colorectal adenocarcinoma cells; cpd, compound; CRD, carbohydrate recognition domain; EtOAc, ethyl acetate; FimH, fimbrial adhesive protein H; FaSSGF, fasted state simulated gastric fluid; FeSSGF, fed state simulated gastric fluid; LC-MS, liquid chromatography-mass spectrometry; MeCN, acetonitrile; MPLC, medium pressure liquid chromatography; NIS, *N*-iodosuccinimide; *P*, octanol-water partition coefficient; P_{app}, apparent permeability; P_e, effective permeability; PAMPA, parallel artificial membrane permeability assay; PK, pharmacokinetic; $t_{1/2}$, half-life; T_{max}, time when maximum concentration is observed; UPEC, uropathogenic *Escherichia coli*; UTI, urinary tract infection.

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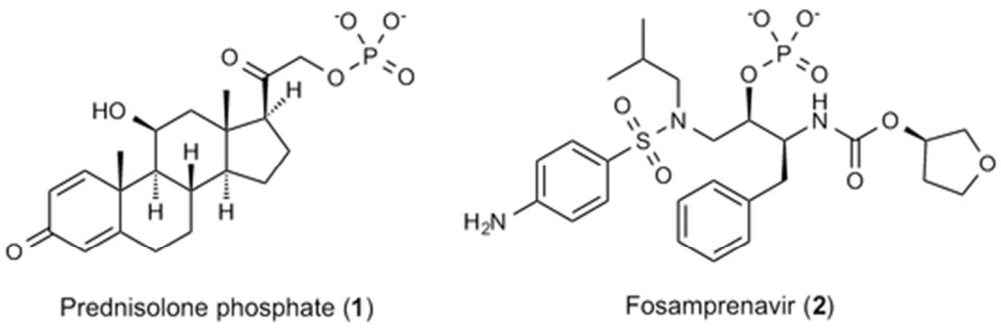
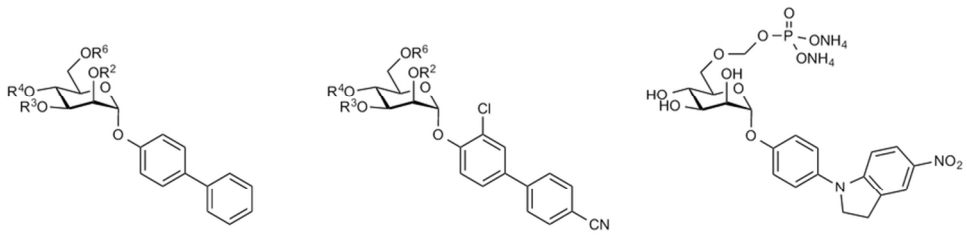


Figure 1. Phosphate prodrugs of marketed drugs.
45x14mm (300 x 300 DPI)



6a: $R^2 = PO_3(NH_4)_2$; R^3, R^4, R^6 = H
6b: $R^3 = PO_3(NH_4)_2$; R^2, R^4, R^6 = H
6c: $R^4 = PO_3(NH_4)_2$; R^2, R^3, R^6 = H
6d: $R^6 = PO_3(NH_4)_2$; R^2, R^3, R^4 = H
6e: $R^2 = CH_2OPO_3(NH_4)_2$; R^2, R^3, R^4 = H

7a: $R^2 = PO_3(NH_4)_2$; R^3, R^4, R^6 = H
7b: $R^3 = PO_3(NH_4)_2$; R^2, R^4, R^6 = H
7c: $R^4 = PO_3(NH_4)_2$; R^2, R^3, R^6 = H
7d: $R^6 = PO_3(NH_4)_2$; R^2, R^3, R^4 = H

8

Figure 2. Phosphate monoesters 6a-e of biphenyl α-D-mannopyranoside 3, 7a-d of substituted biphenyl α-D-mannopyranoside 4, and 8 from indolinylphenyl α-D-mannopyranoside 5.
81x30mm (300 x 300 DPI)

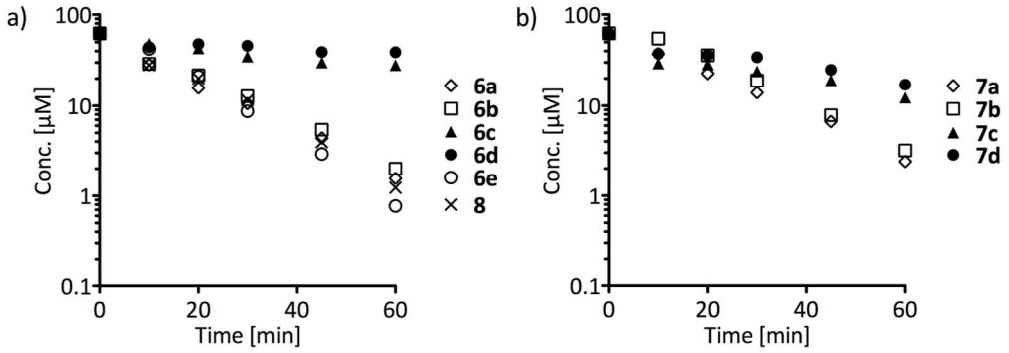


Figure 3. Decomposition of phosphomonoester prodrugs 6a-d and 7a-d and phosphonooxymethyl ether prodrugs 6e and 8 in the apical compartment of the Caco-2 cell assay: (a) 6a-e, 8; (b) 7a-d. Prodrugs dissolved in Dulbecco's Modified Eagle's Medium (62.5 µM) were applied to the apical chamber and the concentrations of unchanged prodrug were monitored by LC-MS.
248x87mm (300 x 300 DPI)

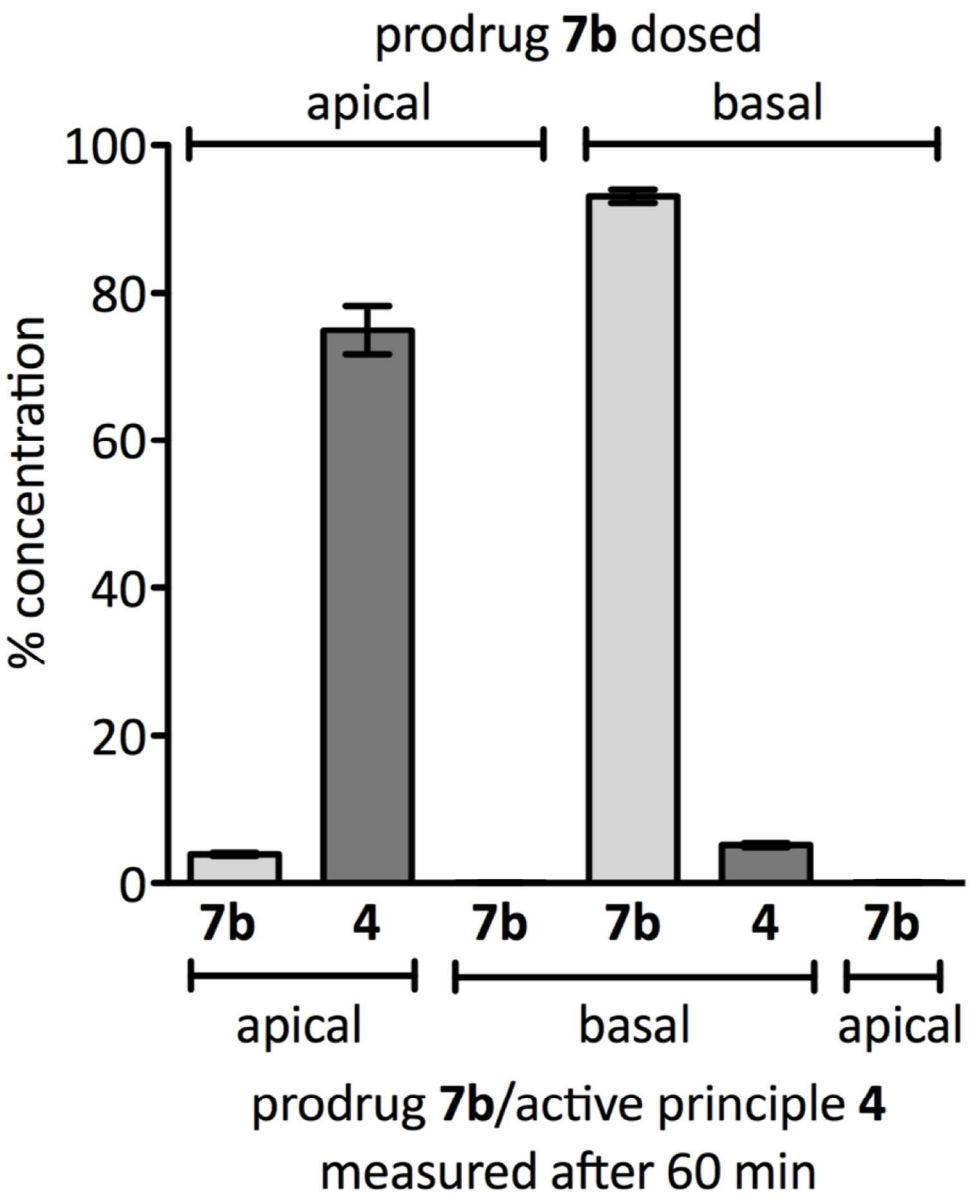


Figure 4. Conversion of prodrug 7b to the active principle 4 in a Caco-2 cell monolayer model after 60 min of incubation. A prodrug solution (62.5 µM) was applied either into the apical or basal chamber. Columns represent the percentage concentrations of prodrug and active principle 60 min after initiation of the experiment. Concentration of prodrug 7b determined at time point t = 0 min is defined as 100%.
112x137mm (300 x 300 DPI)

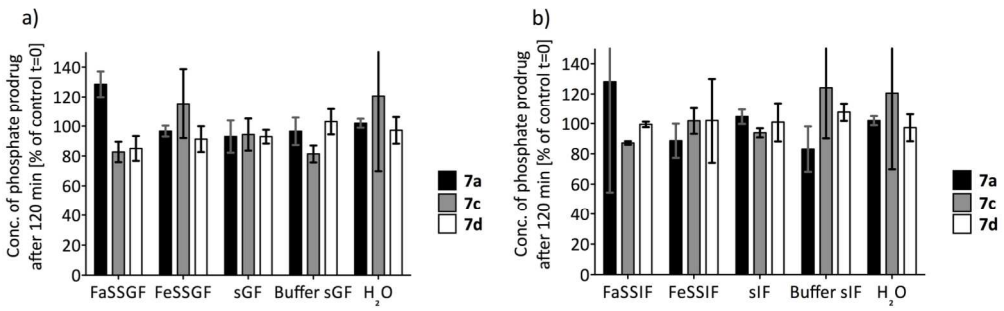


Figure 5. Stability of the phosphate prodrugs 7a (black column), 7c (grey column), and 7d (white column) in biorelevant media. Percentage of the remaining compound concentration relative to the initial concentration (t = 0 min) after 120 min of incubation in a) simulated gastric fluids (FaSSGF, fasted-state simulated gastric fluid; FeSSGF, fed-state simulated gastric fluid; sGF, simulated gastric fluid; buffer sGF, prepared equally to sGF but without pepsin) and b) simulated intestinal fluids (FaSSIF, fasted-state simulated intestinal fluid; FeSSIF, fed-state simulated intestinal fluid; sIF, simulated intestinal fluid; buffer sIF, prepared equally to sIF but without pancreatin) are shown. For composition see Table S1 in Supporting Information. H2O was used as a reference media. Data represent the mean (triplicates) with its corresponding standard deviation.
242x75mm (300 x 300 DPI)

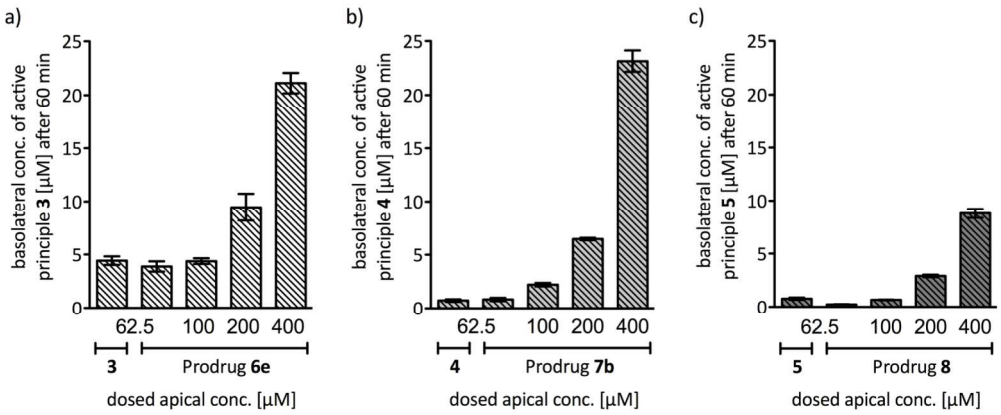


Figure 6. Accumulation of active principle (a) 3, (b) 4, and (c) 5 in the basal receiver chamber of a Caco-2 cell system 60 min after applying (a) active principle 3 or prodrug 6e, (b) active principle 4 or prodrug 7b, and (c) active principle 5 or prodrug 8 into the apical chamber. The active principles were dosed at a concentration of 62.5 µM, which corresponds to approx. the aqueous solubilities of 3 and 5. The phosphate prodrugs were dosed at four different concentrations (62.5 µM, 100 µM, 200 µM, and 400 µM).
229x94mm (300 x 300 DPI)

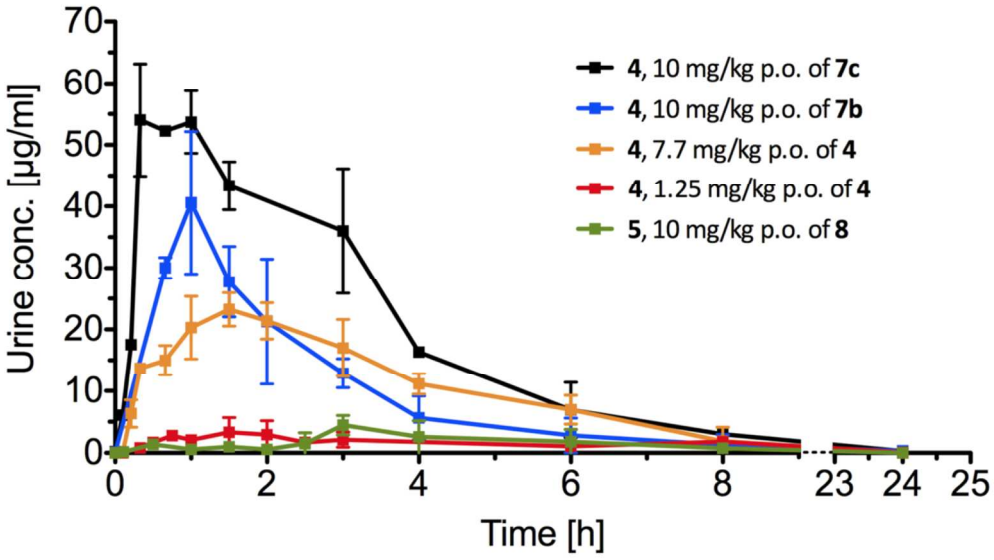
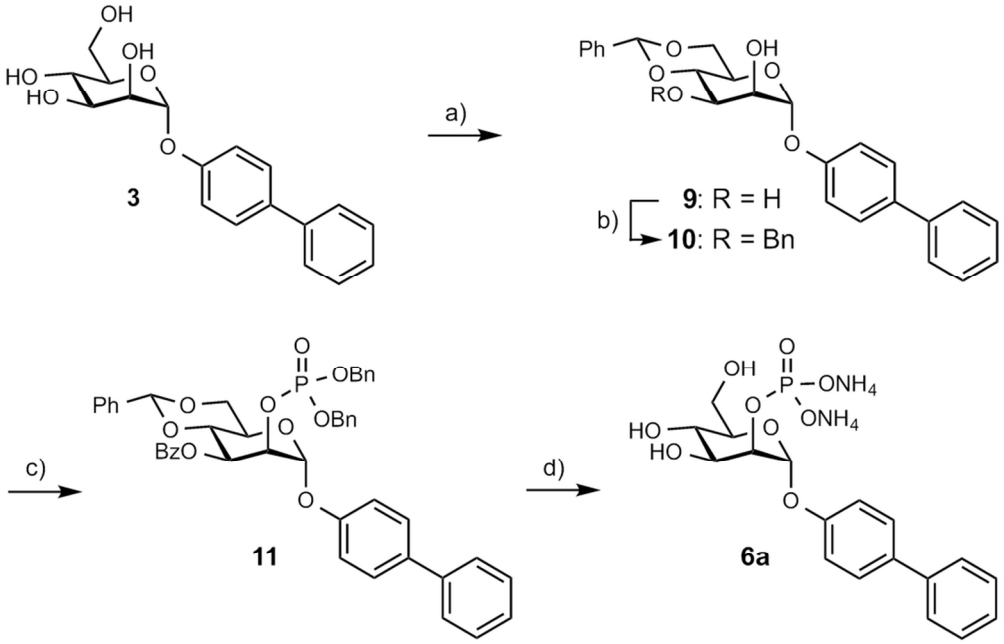
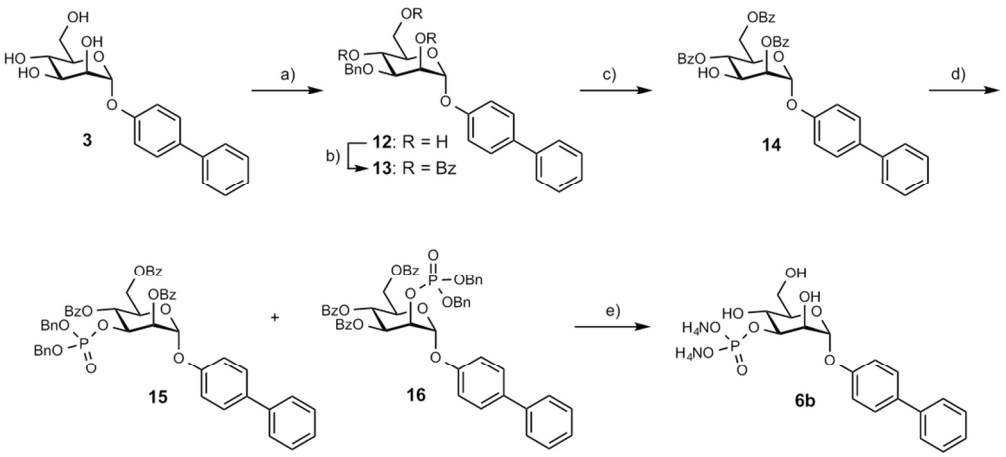


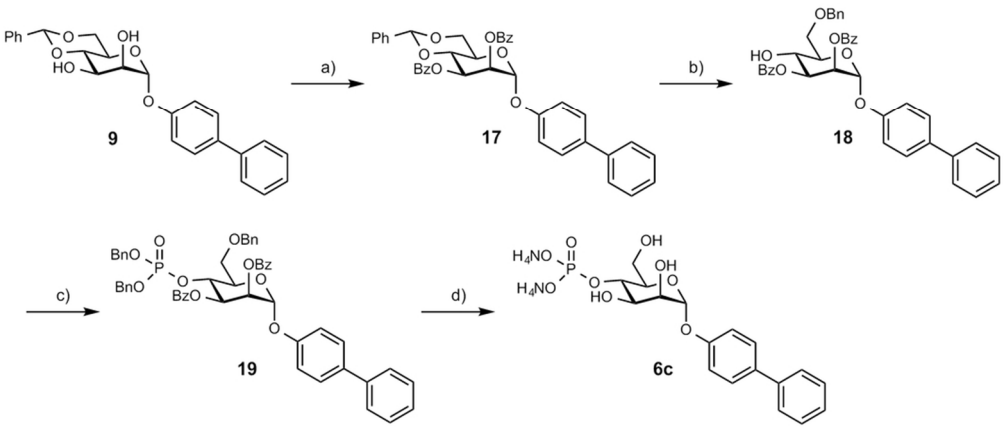
Figure 7. Urine concentrations of active principle 4 in C3H/HeN mice upon p.o. administration of 4 (1.25 mg/kg and 7.7 mg/kg p.o.) or the related phosphate prodrugs 7b, 7c (10 mg/kg p.o.) and the urine concentration of the active principle 5 upon p.o. administration of phosphate prodrug 8 (10 mg/kg p.o.). Shown are mean values with standard deviations for groups of three (4, 1.25 mg/kg, 7b, 7c, and 8) or five (4, 7.7 mg/kg) mice.
177x100mm (300 x 300 DPI)



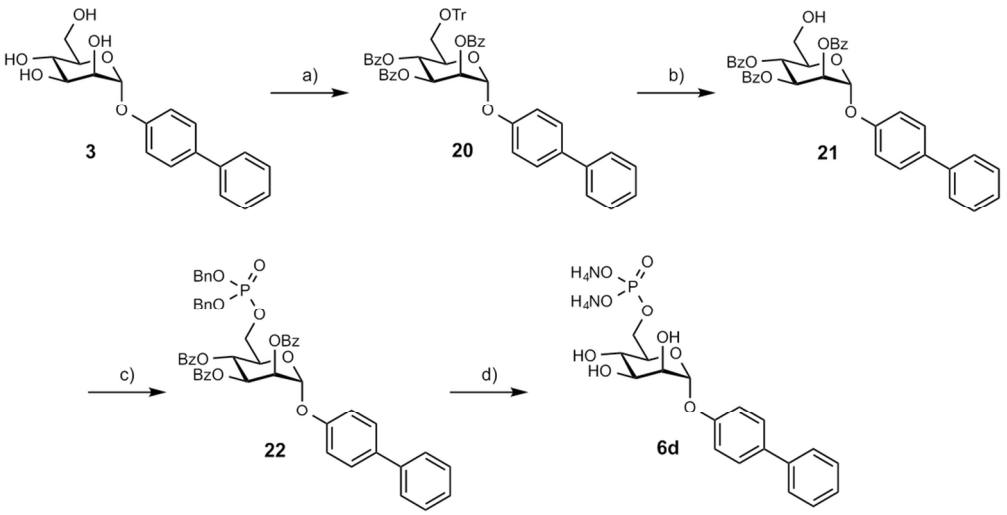
Scheme 1. a) PhCH(OMe)2, p-TsOH, DMF, 50 °C, overnight (70%); b) i. Bu2SnO, toluene, 135 °C, 3 h; ii. BnBr, toluene, 115 °C, overnight (80%); c) dibenzyl N,N-diisopropylphosphoramidite, 1,2,4-triazole, MeCN, 0 °C to rt, overnight; then 70% aq. tert-BuOOH, rt, 1 h (62%); d) i. H2 (4 bar), Pd(OH)2/C, EtOAc, cat. HOAc, overnight; ii. 25% aq. NH3/MeOH (4:1), rt, overnight (45%).
92x59mm (300 x 300 DPI)



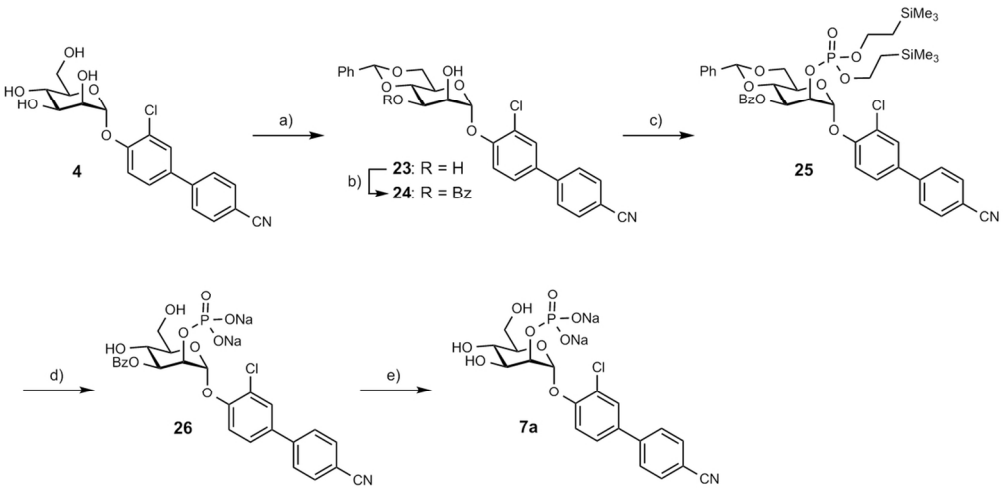
Scheme 2. a) i. Bu2SnO, MeOH, reflux, 5 h, ii. BnBr, toluene, 115 °C, overnight (36%); b) BzCl, cat. DMAP, pyr, rt, overnight (99%); c) H2 (4 bar), Pd(OH)2/C, dioxane/EtOAc, cat. AcOH, rt, overnight (73%); d) dibenzyl N,N-diisopropylphosphoramidite (90%), 1,2,4-triazole, MeCN, 0 °C to rt, overnight; then 70% aq. tert-BuOOH, rt, 1 h (80%, 3:2-mixture of 2-and 3-phosphate derivatives); e) i. H2 (1 bar), Pd(OH)2/C, EtOAc, 5 h; ii. 25% aq. NH3/MeOH (4:1), rt, overnight (7%).
97x44mm (300 x 300 DPI)



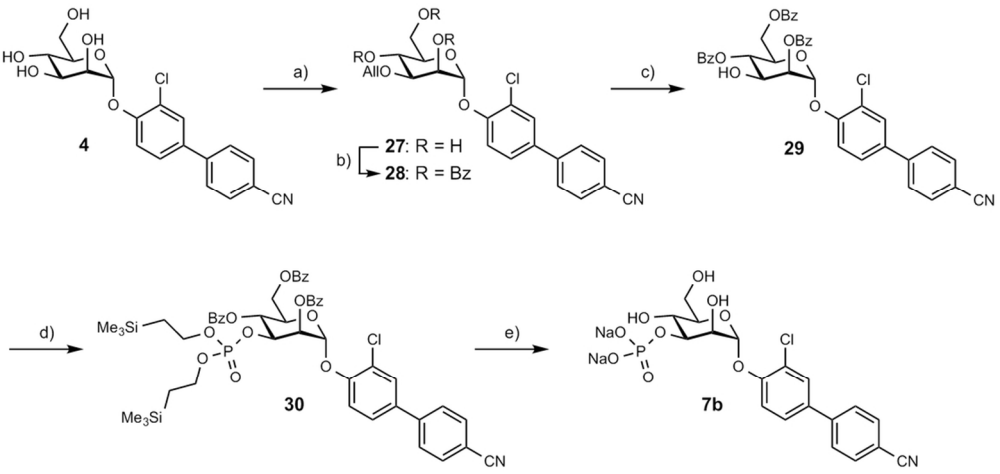
Scheme 3. a) BzCl, cat. DMAP, pyr, rt, overnight (60%); b) Me3N·BH3, AlCl3, THF/H2O, rt, 1 h (67%); c) dibenzyl N,N-diisopropylphosphoramidite (90%), 1,2,4-triazole, MeCN, 0 °C to rt, overnight; then 70% aq. tert-BuOOH, 1 h (53%); d) H2, Pd(OH)2/C, EtOH/EtOAc, 5 h, quant; e) 25% aq. NH3/MeOH (4:1), rt, overnight (56%).
89x38mm (300 x 300 DPI)



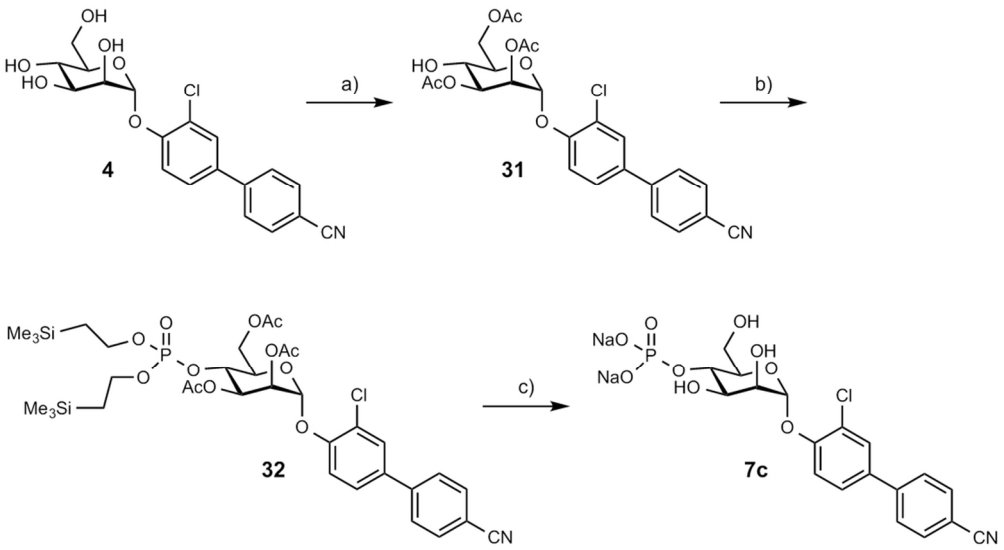
Scheme 4. a) i. TrCl, cat. DMAP, pyr, 80 °C, overnight, ii. BzCl, 50 °C, overnight (88%); b) FeCl3/H2O, DCM, rt, 5 h (62%); c) dibenzyl N,N-diisopropylphosphoramidite (90%), 1,2,4-triazole, MeCN, 0 °C to rt, overnight; then 70% aq. tert-BuOOH, rt, 1 h (66%), d) i. H2, Pd(OH)2/C, EtOH/EtOAc, overnight, ii. 25% aq. NH3/MeOH (4:1), rt, overnight (55%).
101x51mm (300 x 300 DPI)



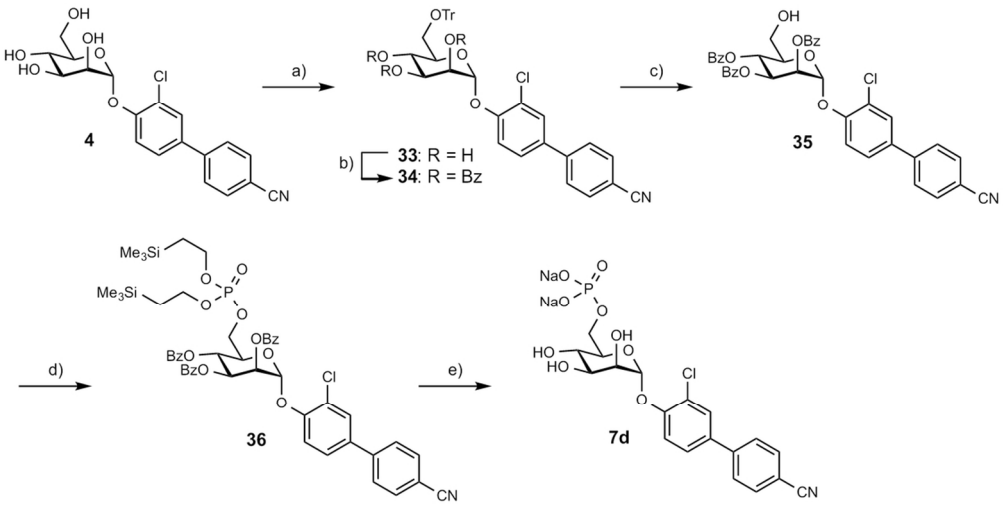
Scheme 5. a) PhCH(OMe)2, p-TsOH, rt, 17 h (22%); b) BzCl, DCM/pyr, 0 °C to rt, 3 h (60%); c) bis[2-(trimethylsilyl)ethyl] N,N-diisopropylphosphoramidite, 1,2,4-triazole, MeCN, 0 °C to rt, 16 h; then 70% aq. tert-BuOOH, rt, 1 h (55%); d) TFA/DCM (1:4), rt, 2 h (61%); e) 25% aq. NH3/MeOH (4:1), rt, 16 h (71%).
110x53mm (300 x 300 DPI)



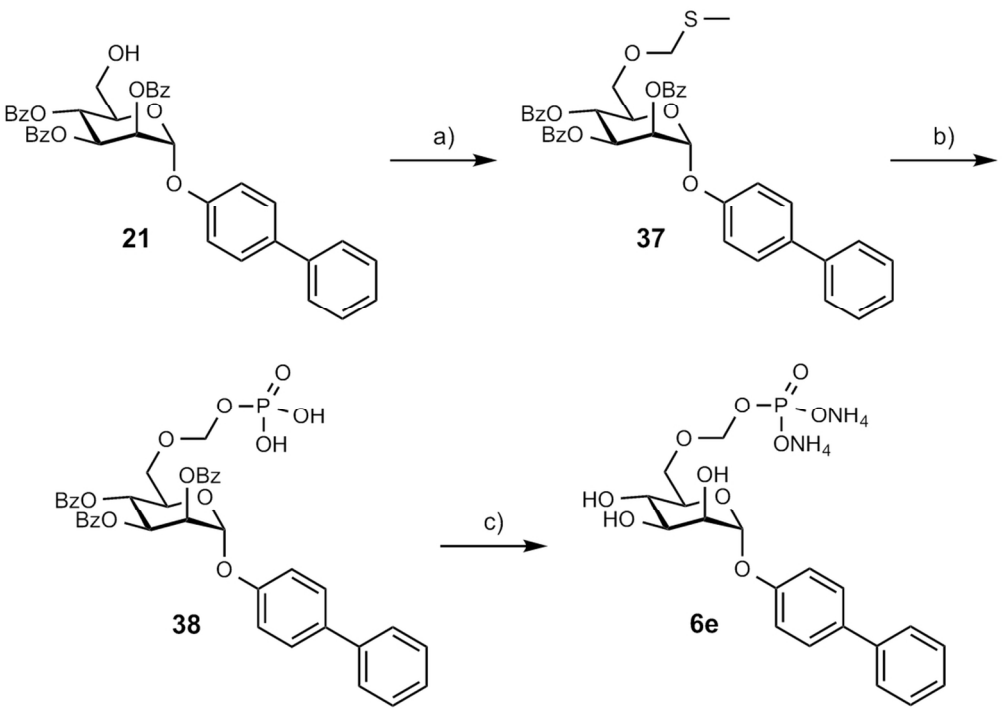
Scheme 6. a) i. Bu2SnO, toluene, 80 °C, 6 h; ii. AllBr, Bu4NI, toluene, 80 °C, 20 h (55%); b) BzCl, cat. DMAP, pyr, rt, overnight (87%); c) PdCl2, MeOH, 40 °C, 5 h (84%); d) i. bis[2-(trimethylsilyl)ethyl] N,N-diisopropyl-phosphoramidite, 1,2,4-triazole, MeCN, 0 °C to rt, 15 h; then 70% aq. tert-BuOOH, rt, 2 h (32%); e) i. TFA/DCM (1:4), rt, 1.5 h; ii. 25% aq. NH3/MeOH (4:1), rt, overnight (45%).
97x45mm (300 x 300 DPI)



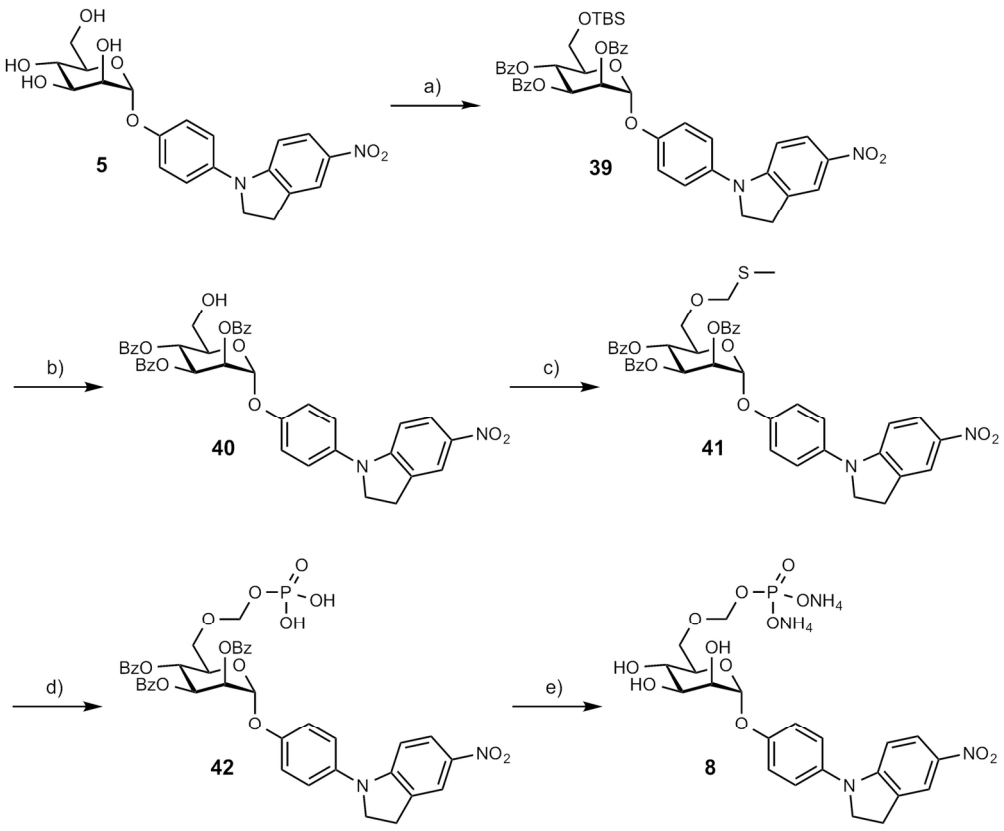
Scheme 7. a) i. Bu2SnO, MeOH, 70 °C, 2 h; ii. Ac2O, MeCN, rt, 16 h (21%); b) i. bis-[2-(trimethylsilyl)ethyl] N,N-diisopropylphosphoramidite, 1,2,4-triazole, MeCN, 0 °C to rt, 15 h; then 70% aq. tert-BuOOH, rt, 2 h (62%); c) i. TFA/DCM (1:4), rt, 2 h; ii. 25% aq. NH3/MeOH (4:1), rt, 2 h (98%).
97x53mm (300 x 300 DPI)



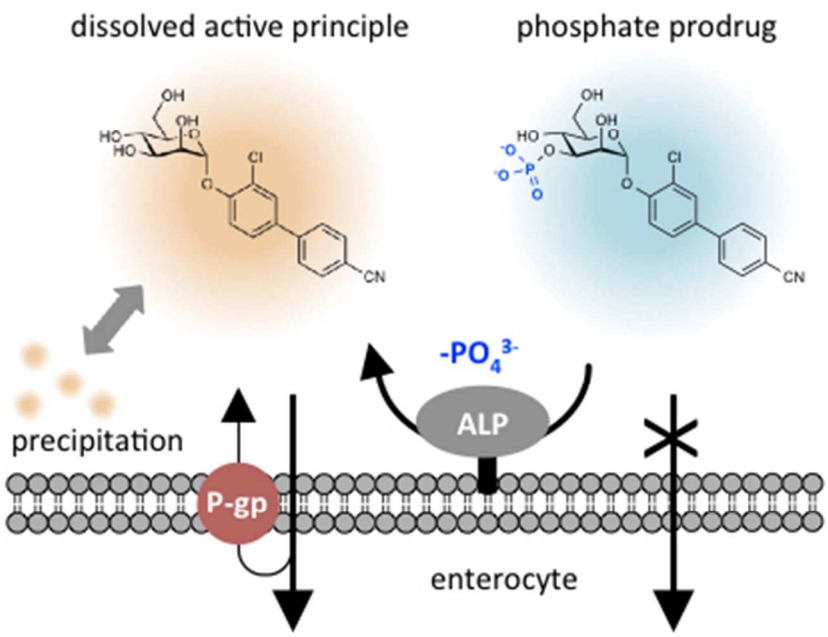
Scheme 8. a) TrCl, cat. DMAP, pyr, 80 °C, 16 h (80%); b) BzCl, cat. DMAP, pyr, rt, 6 h (79%); c) FeCl3, H2O, rt, 5 h (85%); d) i. bis-[2-(trimethylsilyl)ethyl] N,N-diisopropylphosphoramidite, 1,2,4-triazole, MeCN, 0 °C to rt, 15 h; then 70% aq. tert-BuOOH, rt, 1.5h (59%); e) i. TFA/DCM (1:4), rt, 2 h; ii. 25% aq. NH3/MeOH (4:1), rt, overnight (68%).
107x53mm (300 x 300 DPI)



Scheme 9. a) DMSO, Ac2O/AcOH, rt, overnight (35%); b) H3PO4, NIS, THF, 0 °C to rt, 1 h (58%); c) 25% aq. NH3/MeOH (4:1), rt, overnight (50%).
102x72mm (300 x 300 DPI)



Scheme 10. a) i. TBSCl, cat. DMAP, pyr, rt, overnight, ii. BzCl, rt, 2 h, (quant.); b) 1 M H2SO4/MeOH, rt, 1.5 h (73%); c) DMSO/Ac2O/HOAc, rt, overnight (74%); d) H3PO4/NIS/THF, 0 °C to rt, 1 h (67%); e) 25% aq. NH3/MeOH/DCM, rt, overnight (41%).
149x123mm (300 x 300 DPI)



70x53mm (150 x 150 DPI)