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Synthesis of a β-GlcN-(1→4)-MurNAc building block en route to N-deacetylated peptidoglycan fragments

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ABSTRACT

Some bacteria present a variation in their peptidoglycan structure that is the absence of the *N*-acetyl substituent in the glucosamine residue. Very recently, this structural modification was demonstrated to be critical for host innate immune evasion in *Listeria monocytogenes*. To shed light on the molecular details of the evasion mechanism, the synthesis of some N-deacetylated peptidoglycan fragments is needed. En route to this goal a high-yielding synthesis of a GlcN–MurNAc disaccharide building block has been accomplished. A careful study of the optimal protecting groups and reaction conditions was done to have a complete β-stereoselectivity in glycosylation as well as to ensure a high versatility to the disaccharide building block.

Peptidoglycan is an essential and unique structural part of the bacterial cell wall. It has a polymeric structure with alternating *N*-acetyl-glucosamine (GlcNAc) and *N*-acetyl-3-O-(*R*)-lactyl-glucosamine (muramic acid, MurNAc) linked through β-1→4-bonds. The lactyl moiety of MurNAc is covalently attached to a pentapeptide chain that is employed in polymer cross-linking.[1] Structural modifications in the glycan strand often affect bacterial recognition by hosts.[2] One of the most common modifications is N-deacetylation. It has been recently demonstrated that the absence of *N*-acetyl substituent in the glucosamine residue of peptidoglycans from *Listeria monocytogenes*—a Gram-positive human intracellular pathogen—is critical for the bacterium to evade the host innate immune system, survive in gastrointestinal environment and disseminate to various organs by surviving in human macrophages.[3] The mechanism of immune system evasion seems to be based on a protection against the bacteriolytic activity of lysozyme as well as on escaping Toll-like receptor (TLR)-2 and nucleotide-binding oligomerization domain (Nod)-1 and 2 protein detection, even if no molecular details have been reported yet. N-Deacetylation at GlcN site was also found in some other bacteria, among which *Xanthomonas campestris*,[4] a Gram-negative phytopathogen causative agent of black-rot, a disease of cruciferous plants of worldwide importance.

In the last decade there was a great deal of activity directed toward the chemical synthesis of several peptidoglycan fragments,[5] because of the lack of pure and discrete species for precise structural and biochemical studies. Nonetheless, to the best of our knowledge, there was no report on the synthesis of N-deacetylated-GlcN-containing structures, in spite of the interest in unraveling the molecular details of innate immune evasion. For this reason and in light of our interest in the chemical synthesis and phytopathological study of MAMP-related compounds,[6] we embarked in the synthesis of peptidoglycan fragments containing N-deacetylated glucosamine units. En route to this goal, the synthesis of a highly versatile β-GlcN-(1→4)-MurNAc building block is reported in this work.

The known syntheses of peptidoglycan fragments all necessitated to introduce the acetamido moiety before the coupling with the peptide chain.[5] This strategy cannot be applied to the synthesis of peptidoglycan fragments with a N-deacetylated GlcN unit. Therefore, GlcN and MurN nitrogen atoms had to be protected with orthogonal protecting groups, the GlcN one being able to liberate the amine at the final stage of the synthesis. Moreover, since the global aim of our total synthesis is the obtention of not only peptidodisaccharide fragments but also higher oligomers, the anomeric position of MurNAc unit and the position 4 of GlcN had to be protected with orthogonal protecting groups too. Finally, the eventuality of side-reactions involving the (*R*)-lactyl moiety during the manipulation of MurNAc building blocks (racemization, intramolecular lactonization at 4-hydroxy position),[7] suggested to introduce the lactyl ether at a late stage in the synthesis. All these constrains designed **A** as a proper disaccharide building block (Scheme 1). It could be obtained by a stereoselective coupling between suitably protected GlcN acceptor **B** and donor **C**.

The glycosylation of a 4-hydroxy group in glucosamine acceptors presents some well-known difficulties related to its low nucleophilicity.[8] Some methods were reported to address this problem;[9]

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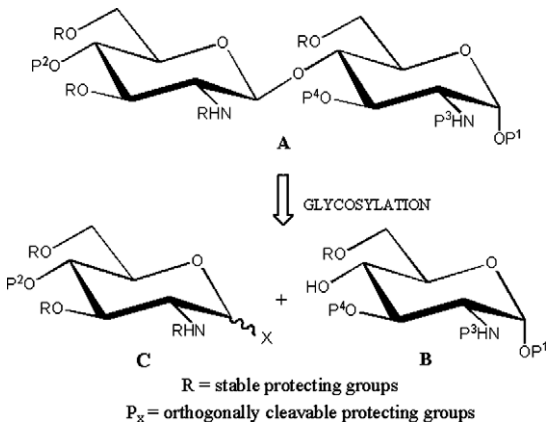
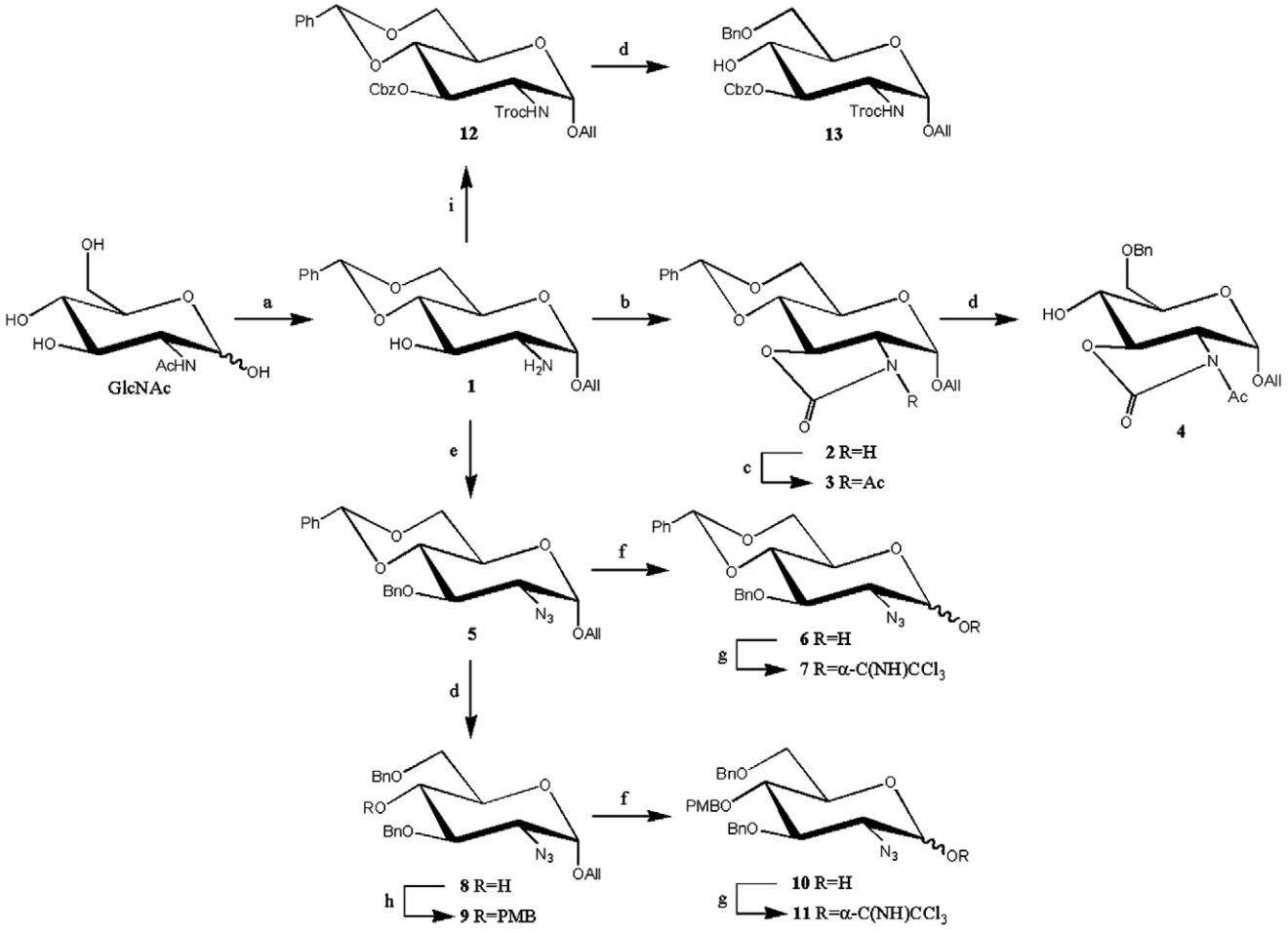


Scheme 1. Protecting group pattern on glycosyl donor, acceptor, and disaccharide.

among these protocols, we firstly focused our attention on the use of a *N*-acetyl-2,3-oxazolidinone protection[10] on the glycosyl acceptor.

Several building blocks were developed as glycosyl donors for the stereoselective synthesis of β-2-amino-2-deoxyglucosides.[11] They generally present a *N*-2 protecting group capable of efficient participation via acyloxonium ion that guarantees 1,2-trans stereoselectivity. These are very usually amido-, carbamato- or imido-protecting groups. Unfortunately they could present some problems here in surviving as stable protecting groups—that is, during the cleavage of oxazolidinone—and/or in their final deprotection to free amine in the presence of the peptide chain. To avoid any protecting group interconversion at a disaccharide level, an azide was selected as amino-masking group. Even if azide is incapable of neighboring group participation, it can be easily transformed into a free amine during final hydrogenolysis deprotecting step.

The known compound **1**[12] was prepared in three steps from *N*-acetyl-glucosamine and served as key building block for the synthesis of both acceptor **4** and donor **7** (Scheme 2). A portion of **1** was treated with triphosgene[13] to give oxazolidinone **2** in 60% yield. Subsequent N-acetylation (92%) and regioselective benzylidene ring opening under reductive conditions (Et_3SiH/TFA in CH_2Cl_2; 57%) afforded acceptor **4**. Conversely, the treatment of **1** with triflyl azide in pyridine[14] and subsequent benzylation of the 3-hydroxy group gave **5** (71% over two steps), which was de-*O*-allylated with $PdCl_2$ (58%) and then converted into the α-trichloroacetimidate donor **7**. Unfortunately the coupling between **4** and **7** in a β-directing nitrile solvent gave no disaccharide (Table 1, entries 1 and 2). Given the torsional and electronic disarming effect of a 4,6-benzylidene protection on glycosyl donors,[15] a more reactive 2-azido-2-deoxyglucosyl donor was synthesized, having a benzyl at position 6 and a selectively cleavable *p*-methoxybenzyl group at position 4. It was obtained in four steps from **5** (Scheme



Scheme 2. Reagents and conditions: (a) Ref. 13; (b) triphosgene, 2:1 v/v CH_3CN/satd aq $NaHCO_3$, rt, 60%; (c) AcCl, DIPEA, CH_2Cl_2, rt, 92%; (d) Et_3SiH, TFA, AW-300 4 Å MS, CH_2Cl_2, 5 °C, 57% for **4**, 74% for **8**, 77% for **13**; (e) (i) TfN_3, $CuSO_4$, Et_3N, py, 0 °C; (ii) BnBr, NaH, DMF, rt, 71% over two steps; (f) $PdCl_2$, 1:1 v/v CH_2Cl_2/MeOH, rt, 58% for **6**, 80% for **10**; (g) Cl_3CCN, DBU, CH_2Cl_2, rt, 78% for **7**, 70% for **11**; (h) PMBCl, NaH, DMF, 0 °C, 92%; (i) (i) TrocCl, $NaHCO_3$, 2:1 H_2O/CH_3CN, rt; (ii) CbzCl, DMAP, CH_2Cl_2, rt, 70% over two steps.

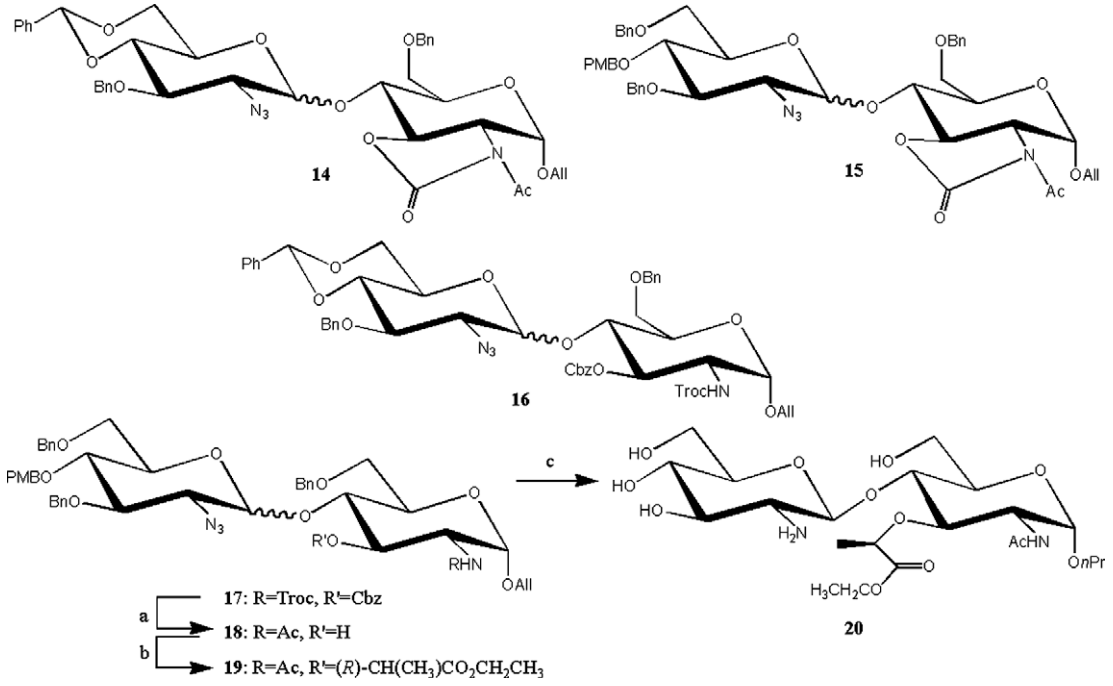
Table 1
Glycosylation reaction

Entry	Acceptor	Donor[a]	Promoter[b]	Solvent	*T*	Disaccharide product[c] (Yield)
1	**4**	**7**	TMSOTf (0.02 equiv)	CH_3CN	−20 °C	No reaction
2	**4**	**7**	TMSOTf (0.2 equiv)	CH_3CN	rt	**14** (traces)[d]
3	**4**	**11**	TMSOTf (0.02 equiv)	Pivalonitrile	rt	**15** (traces)[d]
4	**4**	**11**	$BF_3{\cdot}OEt_2$ (0.6 equiv)	3:2 v/v CH_2Cl_2/hexane	−30 °C	**15** (68%; β/α 1:1)[e]
5[f]	**4**	**11**	$BF_3{\cdot}OEt_2$ (0.15 equiv)	3:2 v/v CH_2Cl_2/hexane	−60 °C	**15** (68%; β/α 1:3:1)[e]
6	**13**	**7**	$BF_3{\cdot}OEt_2$ (0.2 equiv)	3:2 v/v CH_2Cl_2/hexane	−78 °C	**16** (16%; only β)[e]
7	**13**	**11**	$BF_3{\cdot}OEt_2$ (0.2 equiv)	3:2 v/v CH_2Cl_2/hexane	−78 °C	**17** (81%; only β)[e]
8	**13**	**11**	$BF_3{\cdot}OEt_2$ (0.2 equiv)	3:2 v/v CH_2Cl_2/hexane	−30 °C	**17** (40%; only β)[e]
9	**13**	**11**	$BF_3{\cdot}OEt_2$ (0.2 equiv)	3:2 v/v CH_2Cl_2/CH_3CN	−30 °C	**17** (43%; only β)[e]
10	**13**	**11**	TMSOTf (0.02 equiv)	3:2 v/v CH_2Cl_2/hexane	−78 °C	**17** (71%; α/β 4:1)[e]

[a] Donor/acceptor molar ratio = 1.6, unless otherwise stated.
[b] Promoter equivalents calculated with respect to the donor.
[c] Isolated yield, unless otherwise stated.
[d] Detected by TLC and MALDI analysis.
[e] Anomeric ratio measured by the isolation of two anomers.
[f] Donor/acceptor molar ratio = 2.3.

2). Regioselective reductive opening of the benzylidene ring gave **8** (74%), that was then treated with PMBCl and NaH to afford **9** in 92% yield. De-*O*-allylation (80%) and subsequent treatment with Cl_3CCN and DBU afforded the desired α-trichloroacetimidate **11** in 70% yield. Again, the reaction of **4** and **11** in nitrile solvent gave no coupling (entry 3). Some scattered examples in the literature report the β-glycosylation of 2-azido-2-deoxyglycosyl-α-trichloroacetimidates under S_N2 conditions.[16] Therefore, the coupling between **4** and **11** was attempted at low temperature in a CH_2Cl_2–hexane solvent mixture with $BF_3{\cdot}OEt_2$ as catalyst (entry 4). Disaccharide **15** was obtained in 68% yield, but without any stereoselectivity. Higher donor/acceptor molar ratio and even milder activation conditions gave the same yield with an only slight excess of β-anomer (entry 5).

The low β/α stereoselectivity is not really surprising for glycosylations involving 2,3-oxazolidinone GlcN acceptors.[9] Therefore, a new glycosyl acceptor (**13**) was designed, with the amino group protected as a trichloroethoxycarbamate (Troc), which affords greater GlcN-4-hydroxy reactivity than other carbamato- or imido-protecting groups.[17] Compound **13** was synthesized from key building block **1** by *N*-trichloroethoxycarbonylation and protection of the 3-hydroxy as Cbz to give **12** (70% over two steps), which was then subjected to Et_3SiH/TFA benzylidene ring opening (77%) (Scheme 2). The coupling between **13** and **7** was unsatisfying (entry 6), whereas the glycosylation between **13** and **11** under $BF_3{\cdot}OEt_2$ catalysis in a CH_2Cl_2 solvent system at −78 °C afforded disaccharide **17**[18] in good yield (81%) and complete β-stereoselectivity (Table 1, entry 7). Such S_N2 reaction conditions guarantee all together a high yield of **17β**. A higher temperature, a more polar solvent system or a stronger activator considerably reduced the yield of **16β** (entries 8–10). Disaccharide **17β** is a highly versatile building block. Indeed, it was readily transformed into alcohol **18** by cleavage of Troc- and Cbz-protecting groups with 2 M KOH and subsequent N-acetylation (67% over two steps) (Scheme 3). Lactylation was then performed with the triflate of ethyl (*S*)-lactate[5j] and NaH in CH_2Cl_2 to give **19**[18] (61%). GlcN–MurNAc deprotected disaccharide was obtained as ethyl ester by hydrogenolysis (65%). Moreover, an access to a disaccharide donor and acceptor



Scheme 3. Reagents and conditions: (a) (i) 5:1 v/v THF/2 M aq KOH, 50 °C; (ii) 10:10:1 v/v/v CH_2Cl_2/MeOH/Ac_2O, rt, 67% over two steps; (b) ethyl (*S*)-2-(trifluoromethanesulfonyloxy)propionate, NaH, CH_2Cl_2, rt, 61%; (c) Pd/C, 9:1 v/v MeOH/HCOOH, ultrasound bath, 40 °C, 65%.

can be opened from **17β** by cleaving selectively the allyl and *p*-methoxybenzyl, respectively. This work is currently in progress en route to the synthesis of *L. monocytogenes* and *X. campestris* peptidoglycan disaccharide and tetrasaccharide fragments.

In conclusion, the synthesis of a highly versatile GlcN–MurNAc building block was reported. Since glycosylation presented several difficulties and constrains (low reactivity at position 4 of GlcN acceptors; necessity of gain 1,2-trans stereoselectivity without the use of amido-, imido-, or carbamato-neighboring protecting group; a protecting group pattern suitable for disaccharide oligomerization) a careful study of optimal glycosyl donor and acceptor and coupling conditions was carried out. The disaccharide was finally obtained in high yield and complete β-stereoselectivity. It is suitable for further manipulations toward the first synthesis of N-deacetylated-GlcN–containing peptidoglycan fragments that are interesting molecules for the study of host innate immune system evasion mechanism in bacteria. This work is in progress and will be published elsewhere.

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18. *Compound* **17β**: $[\alpha]_D$ +7 (*c* 0.6 in CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): δ 7.37–7.25 (20H, m, H-Ar), 7.08 (2H, d, J_{ortho} = 8.4 Hz, H-Ar PMB), 6.80 (2H, d, J_{ortho} = 8.4 Hz, H-Ar PMB), 5.86 (1H, m, $OCH_2CH{=}CH_2$), 5.28 (2H, m, NH, *trans* $OCH_2CH{=}CHH$), 5.22 (1H, br d, J_{vic} = 10.4 Hz, *cis* $OCH_2CH{=}CHH$), 5.08 (1H, t, $J_{3,2} = J_{3,4}$ 10.1 Hz, H-3_A), 5.06 (1H, d, J_{gem} = 12.5 Hz, $OCHHCCl_3$), 4.97 (1H, d, J_{gem} = 12.5 Hz, $OCHHCCl_3$), 4.93 (1H, dd, $J_{1,2}$ = 3.5 Hz, H-1_A), 4.81 (2H, s, OCH_2*p*OMePh), 4.69–4.60 (4H, m, 4 OCHPh), 4.51 (1H, d, J_{gem} = 12.1 Hz, OCHPh), 4.47 (2H, s, OCH_2Ph), 4.45 (1H, d, J_{gem} = 10.5 Hz, OCHPh), 4.21 (1H, dd, J_{gem} = 12.5 Hz, J_{vic} = 5.2 Hz, $OCHHCH{=}CH_2$), 4.13 (1H, d, $J_{1,2}$ = 7.2 Hz, H-1_B), 4.07–3.91 (5H, m, H-2_A, H-4_A, H-5_A, H-$6a_A$, $OCHHCH{=}CH_2$), 3.79 (3H, s, OMe), 3.72 (1H, dd, J_{gem} = 9.8 Hz, $J_{6b,5}$ = 1.7 Hz, H-$6b_A$), 3.61 (1H, d, J_{gem} = 10.8 Hz, $J_{6a,5}$ = 1.7 Hz, H-$6a_B$), 3.47 (2H, m, H-4_B, H-$6b_B$), 3.23 (3H, m, H-2_B, H-3_B, H-5_B); ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.3 (C_{ipso} PMB), 155.3, 154.1 ($OCOOCH_2$Ph, $NCOOCH_2CCl_3$), 138.4, 138.0, 137.7, 135.2 (4 C_{ipso} Bn), 133.1 ($OCH_2CH{=}CH_2$), 130.1 (C_{ipso} PMB), 129.4–125.0 (C-Ar), 118.4 ($OCH_2CH{=}CH_2$), 113.8 (C-Ar), 100.8 (C-1_B), 96.3 (C-1_A), 83.3 (C-3_B), 77.6 (C-4_B), 75.7 (C-3_A), 75.4 (C-5_B, OCH_2*p*OMePh), 74.7 (OCH_2Ph), 74.5 (C-4_A, OCH_2Ph), 73.5 (2 OCH_2Ph), 70.3 (C-5_A), 69.5 (OCH_2CCl_3), 68.7 (C-6_B), 68.6 ($OCH_2CH{=}CH_2$), 67.7 (C-6_A), 66.4 (C-2_B), 55.3 (OMe), 54.1 (C-2_A). MALDI TOF-MS: calcd for $C_{55}H_{59}Cl_3N_4O_{14}$ (m/z), 1104.31 $[M+H]^+$; found, 1127.07 $[M+Na]^+$. Anal. Calcd for $C_{55}H_{59}Cl_3N_4O_{14}$: C, 59.70; H, 5.37; N, 5.06. Found: C, 59.55; H, 5.34; N, 4.99.
Compound **19**: $[\alpha]_D$ +25.0 (*c* 2.0 in CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$): δ 7.37–7.30 (15H, m, H-Ar), 7.07 (2H, d, J_{ortho} = 8.5 Hz, H-Ar PMB), 6.81 (2H, d, J_{ortho} = 8.5 Hz, H-Ar PMB), 5.86 (1H, m, $OCH_2CH{=}CH_2$), 5.37 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1_A), 5.25 (1H, br d, J_{vic} = 17.0 Hz, *trans* $OCH_2CH{=}CHH$), 5.15 (1H, br d, J_{vic} = 11.0 Hz, *cis* $OCH_2CH{=}CHH$), 4.83 (2H, s, OCH_2*p*OMePh), 4.73 (1H, d, J_{gem} = 12.0 Hz, OCHPh), 4.69 (1H, d, J_{gem} = 10.5 Hz, OCHPh), 4.66 (1H, q, J_{vic} = 7.5 Hz, CH_3CHO), 4.54 (1H, d, J_{gem} = 11.5 Hz, OCHPh), 4.50 (1H, d, J_{gem} = 10.5 Hz, OCHPh), 4.46 (1H, d, J_{gem} = 12.0 Hz, OCHPh), 4.43 (1H, d, J_{gem} = 11.5 Hz, OCHPh), 4.25 (1H, d, $J_{1,2}$ = 8.0 Hz, H-1_B), 4.22 (2H, m, OCH_2CH_3), 4.11 (1H, dd, J_{gem} = 13.5 Hz, J_{vic} = 5.5 Hz, $OCHHCH{=}CH_2$), 4.06 (1H, t, $J_{4,3} = J_{4,5}$ = 10.0 Hz, H-4_A), 3.99 (1H, dd, J_{gem} = 13.5 Hz, J_{vic} = 5.5 Hz, $OCHHCH{=}CH_2$), 3.95 (dd, 1H, $J_{2,3}$ = 9.0 Hz, $J_{2,1}$ = 3.5 Hz, H-2_A), 3.80–3.64 (m, 10H, H-3_A, H-4_B, H-5_A, H-$6a_A$, H-$6b_A$, H-$6a_B$, H-$6b_B$, OCH_3), 3.30 (1H, t, $J_{2,3} = J_{2,1}$ = 8.0 Hz, H-2_B), 3.25 (1H, t, $J_{3,4} = J_{3,2}$ = 9.5 Hz, H-3_B), 3.16 (1H, br d, $J_{5,4}$ = 9.6 Hz, H-5_B), 2.04 (3H, s, CH_3CO), 1.36 (3H, d, J_{vic} = 7.5 Hz, CH_3CHO), 1.30 (3H, t, J_{vic} = 7.5 Hz, OCH_2CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 176.3 (COOEt), 170.1 ($NHCOCH_3$), 159.3 (C_{ipso} PMB), 137.9–137.7 (3 C_{ipso} Bn), 134.1 ($OCH_2CH{=}CH_2$), 130.2 (C_{ipso} PMB), 129.3–127.6 (C-Ar), 116.9 ($OCH_2CH{=}CH_2$), 113.8 (C-Ar), 100.6 (C-1_B), 95.9 (C-1_A), 83.3, 77.3, 76.8, 75.4, 75.2, 75.1, 74.5, 74.4, 73.4, 73.1, 70.6, 68.7, 68.3, 67.8, 66.7 (C-2_A, C-3_A, C-3_B, C-4_A, C-4_B, C-5_A, C-5_B, C-6_A, C-6_B, $OCHCH_3$, 3 OCH_2Ph, OCH_2PhOMe, $OCH_2CH{=}CH_2$), 61.2 (OCH_2CH_3), 55.2, 54.4 (C-2_A, OCH_3), 23.1 (CH_3CO), 18.5 (CH_3CH), 14.1 (OCH_2CH_3). MALDI TOF-MS: calcd for $C_{51}H_{62}N_4O_{13}$ (*m*/*z*), 938.43 $[M+H]^+$; found, 961.21 [M+Na]. Anal. Calcd for $C_{51}H_{62}N_4O_{13}$: C, 65.23; H, 6.65; N, 5.97. Found: C, 65.35; H, 6.55; N, 6.01.