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ARTICLE

A Robust Synthesis of *N*-Glycolyl Muramyl Dipeptide Via Azidonitration/Reduction

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Shuo Xing and James L. Gleason[a]

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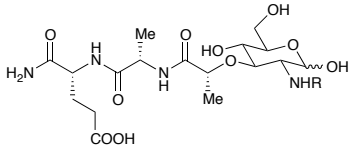
[a] Department of Chemistry, McGill University. 801 Sherbrooke St. West, Montreal, Quebec, Canada, H3A 0B8. Email: jim.gleason@mcgill.ca.

A novel synthetic route leading to *N*-glycolyl muramyl dipeptide (MDP), a bacterial glycopeptide of particular interest in studies of nucleotide-binding oligomerization domain-containing protein 2 (NOD2), is described. The synthetic strategy hinges on the alkylation of benzylidene-protected glucal with 2-bromopropionic acid and thus circumvents a challenging and non-reproducible S_N2 step at the *C*-3 position of glucosamine derivatives. The subsequent sequence includes an azidonitration and an unusual azide reduction/acylation step via an aza ylide/oxaphospholidine intermediate. This approach generates a protected *N*-glycolyl MDP that can be either subjected to a one-step global deprotection or differentially deprotected to obtain further derivatives.

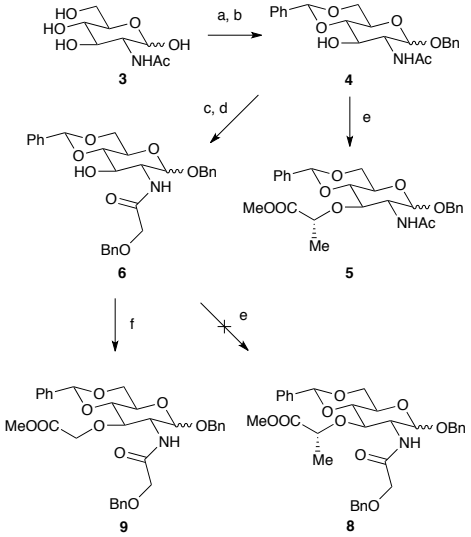
N-Acetyl muramyl dipeptide (*N*-Ac-MDP, **1**, Figure 1), a lactate linked glycopeptide, is a component of the cell wall of bacteria. *N*-Ac-MDP has been shown to trigger an immune response by activating the mammalian receptor nucleotide-binding oligomerization domain-containing protein **2** (NOD2). NOD2 is an intracellular protein that signals via the NF-κB pathway to proximally activate innate immunity through macrophage response as well as to more distally affect adaptive immunity through the production of antigen-specific T-cells.[1] Mutations in NOD2 have been associated with chronic inflammatory diseases such as Crohn's disease and Blau syndrome.[1d,2] Recent studies have shown that in certain mycobacteria and related Actinomycetes, oxidation of **1** within the peptidoglycan by *N*-acetyl muramic acid hydroxylase (NamH) results in the formation of *N*-glycolyl MDP (**2**).[3] Glycopeptide **2** has a higher potency and induces significantly higher activation of NOD2 than **1**.[4] In order to study the binding interactions and kinetics of **1**, **2** and their analogs with NOD2, we required an expeditious and flexible synthesis of these glycopeptides.



1 N-acetyl muramyl dipeptide R=Ac
2 N-glycolyl muramyl dipeptide R=$COCH_2OH$

Fig. 1 Structure of *N*-substitutued muramyl dipeptide.

The synthesis of *N*-glycolyl MDP (**2**) has been reported once previously starting from 2-acetamido-D-glucosamine (**3**) and is closely modelled after the traditional synthesis of 1.[5,6]



Scheme 1. Traditional approach to the synthesis of 8. (a) Benzyl alcohol, *p*TSA, Dean-Stark trap, C_6H_6, 80 °C, 73%. (b) benzaldehyde, $(EtO)_3CH$, *p*TSA, DMF:*p*-dioxane, 60 °C, 65%. (c) KOH, EtOH, 78 °C. (d) *O*-benzylglycolic acid, HBTU, DIPEA, DMF, 38% over two steps. (e) i. NaH, DMF, 0 °C; ii. (*S*)-2-bromopropionic acid, DMF, 0 °C to room temperature; iii. CH_2N_2, EtOAc, 0 °C. (f) i. NaH, THF; ii. methyl 2-bromoacetate, THF, 60 °C to room temperature, 71%.

Protection of **3** as its 4,6-O-benzylidine-O-benzyl glycoside affords acetamide 4 as an inseparable mixture of anomers

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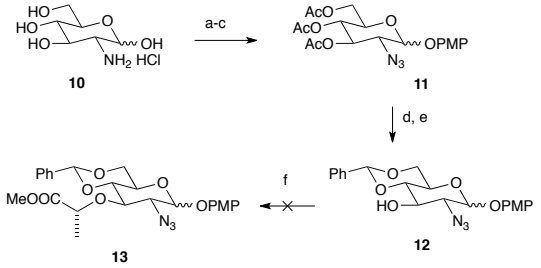
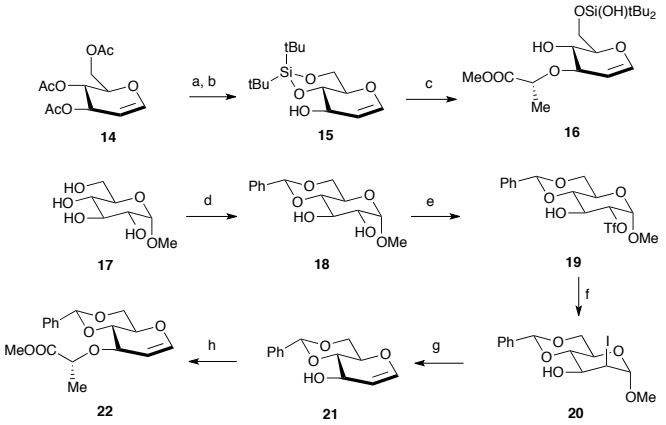
(Scheme 1). Deacetylation of **4** occurs easily under alkaline conditions, presumably due to the presence of the adjacent hydroxyl group.[7] Acylation of the resulting free amine with *O*-benzylglycolic acid affords **6** in good yield over two steps. However, while we were easily able to alkylate **4** with (*S*)-2-bromopropionic acid (**7**) in the presence of NaH to form protected *N*-Ac muramic acid **5**,[6] and in contrast to the reports of Kobayashi et al.,[5a] *N*-glycolyl functionalized **6** resisted all efforts at *O*-alkylation with **7**, including heating in the presence of excess NaH and electrophile in a variety of solvents (THF, DMF, dioxane). Attempts with 2-chloropropionic acid and methyl 2-halopropionates also afforded no alkylation products, even under forcing conditions. The difference in reactivity between **4** and **6** was surprising and although some of the difference might arise from the additional chelating oxygen, the facile alkylation of **6**, as well as **4**, with less hindered methyl bromoacetate to form **9** suggested that increased sterics from the benzyl group were the main influence hindering the S_N2 displacement.

In order to reduce the steric demands on ether formation, we prepared the protected 2-azido-2-deoxyglucose derivative **12**, as it was presumed that the smaller azide group would not interfere in the alkylation step. To this end, D-glucosamine (**10**) was azidated by treatment with triflyl azide in the presence of copper sulfate (Scheme 2). The resulting azide **11** was subjected to per-acetylation, glycosylation, deacetylation and 4,6-*O*-benzylidene formation, all under standard conditions, to afford **12**.[8] Unfortunately, **12** proved to be wholly unreactive in alkylation reactions with **7** and NaH, even in the presence of excess electrophile and prolonged heating.



Scheme 2. Attempted synthesis of 2-azido muramic acid **13**. (a) TfN_3, $CuSO_4$, $NaHCO_3$, toluene: H_2O, 0 °C. (b) acetic anhydride, pyridine, 0 °C, 97% over two steps. (c) *p*-methoxyphenol, $SnCl_4$, CH_2Cl_2, 76%. (d) NaOMe, MeOH. (e) benzaldehyde, $(EtO)_3CH$, *p*TSA, DMF:*p*-dioxane, 60 °C, 74% over two steps. (f) i. NaH, DMF, 0 °C; ii. (*S*)-2-bromopropionic acid, DMF, 0 °C to room temperature; iii. CH_2N_2, EtOAc, 0 °C.

Given the significant influence of the identity of the group at C2, we opted to remove the amine altogether and install it at a later stage in the synthesis. Preliminary studies using the 4,6-di-*tert*-butylsiloxane glucal **15**, prepared easily in two steps from 3,4,6-tri-*O*-acetyl-D-glucal, revealed that the desired alkylation with (*S*)-2-bromopropionic acid was possible, affording ether **16** where the siloxane had become partially deprotected, in moderate yield (Scheme 3). The partial deprotection was difficult to control and resulted in a significant amount of unalkylated open siloxane side product. The lability of the protecting group could be addressed through the use of a more robust 4,6-*O*-benzylidene protecting group. Direct installation of the benzylidene on D-glucal was difficult and thus we prepared **21** following the established 4-step method of Chambers starting from α-methyl D-glucoside.[9] We were pleased to find that alkylation of **21** with (*S*)-2-bromopropionic acid proceeded readily at room temperature in the presence of NaH in DMF in a much shorter reaction time compared to **4**. After esterification of the resulting acid with diazomethane, ether **22** was isolated in 96% yield. The ease and high reproducibility of this alkylation underscore the influence of the C2 substituent in slowing down alkylations of both **4** and **6**. With the lactate unit installed, we next incorporated the C2 nitrogen via an azidonitration reaction (Scheme 4).[10] Treatment of **22** with sodium azide and cerium ammonium nitrate (CAN) provided **23** as a separable mixture of three diastereomers (**23α:23β:24α/β,** 4:2.2:1) in 72% overall yield, with a 62% yield of the desired diastereomers, **23α** and **23β**. Reduction of the azide in **23α** and/or **23β** proved to be problematic under traditional conditions (Ph_3P, H2O), as the hydrolysis of the intermediate aza-ylide was quite slow.



Scheme 3. Synthesis of alkylated glucal 22. (a) NEt3, MeOH:H2O. (b) tBu2Si(OTf)2, 2,6-lutidine, 98% over two steps. (c) i. NaH, DMF, 0 °C to room temperature; ii. (S)-2-bromopropionic acid, DMF; iii. CH2N2, EtOAc, 45% (with 55% unalkylated, opened siloxylane). (d) PhCH(OMe)2, pTSA, DMF, 60 °C, 60%. (e) Tf2O, py, CH2Cl2, -30 °C, 94%. (f)TBAI, toluene, 80 °C, 80%. (g) Zn, AcOH, CH2Cl2, 46%. (h) i. NaH, DMF, 0 °C to room temperature; ii. (S)-2-bromopropionic acid, DMF; iii. CH2N2, EtOAc, 96%.

Heating the reaction or treatment with aqueous base afforded only a lactam resulting from Staudinger ligation with the lactate ester.[11] Treatment of **25** with aqueous acid was also ineffective, resulting in only slow cleavage of the benzylidene. The source of the puzzling stability of the aza ylide was eventually revealed through ^{1}H-, ^{31}P-NMR and ^{31}P/^{1}H HMBC experiments (Figure 2). As the nitrate group at C1 position is readily hydrolyzed, the free hydroxyl group stabilizes the aza ylide through the formation of an oxaphospholidine (**26**), which is in equilibrium with the open aza ylide form (**25**). Two distinct ^{31}P signals can be observed in the NMR spectrum, one of which (40.45 ppm) shows HMBC correlation to H-2 (2.74 ppm) alone, while the second ^{31}P signal (36.22 ppm) correlates to both H-2 (3.09 ppm) and an NH triplet (6.34 ppm).

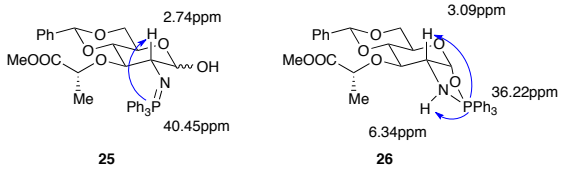


Fig. 2 ^{31}P-^{1}H HMBC correlations in **25** and **26**.

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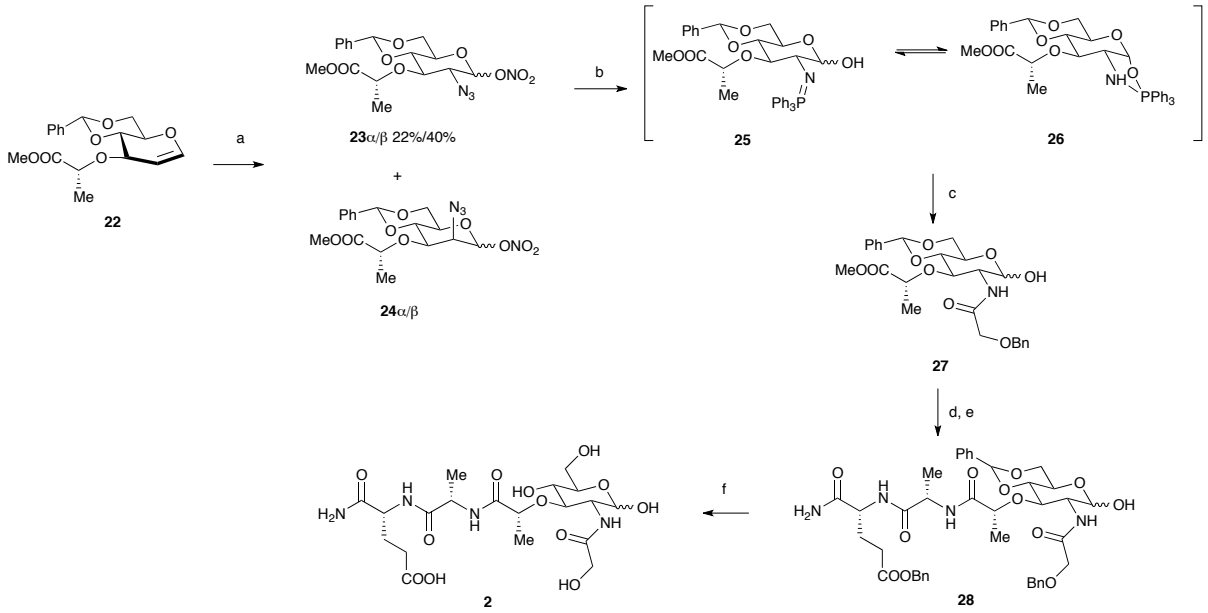

Scheme 4. Synthesis of 2. (a) NaN_3, CAN, MeCN, -15°C, 72% combined yield for all diastereomers. (b) PPh_3, THF, 60%. (c) *O*-benzylglycolic acid, HBTU, DIPEA, DMF, 60%. (d) KOH (1M aq), MeOH, THF. (e) HBTU, DIPEA, γ-benzyl ester D-isoglutamine-L-alanine TFA salt, 74% over two steps. (f) Pd/C, H_2, acetic acid, EtOAc, MeOH, 96%.

While oxaphospholidine formation slowed attack of water, we expected that N-2 would remain nucleophilic, particularly in the cyclic form, and might be acylated directly and this would in turn facilitate the desired hydrolysis.[11] To our delight, *N*-glycolylation with O-benzylglycolic acid in the presence of HBTU, although necessitating long reaction times (>48 h), proceeded to afford the desired protected glycolamide in 60% yield. Conversion of **27** to *N*-glycolyl-MDP was then achieved through a standard sequence of ester saponification with KOH, coupling the resulting acid to the g-benzyl ester of D-isoGlu-L-Ala with HBTU and then global deprotection of all benzyl and benzylidene protecting groups with H_2-Pd/C, affording **2** in 71% yield over the final three steps.

Conclusions

In conclusion we have developed a convenient 11-step synthetic route to *N*-glycolyl MDP that significantly circumvents the difficult S_N2 step in the traditional glucosamine alkylation route to MDP derivatives. This work will enable the synthesis of *N*-acetyl and *N*-glycolyl MDP derivatives for ongoing studies of NOD2 binding interactions and kinetics.

Experimental

Benzyl 2-(*O*-benzyl glycolamido)-4,6-*O*-benzylidene-D-glucopyranoside (6): Benzyl 4,6-*O*-benzylidene-D-glucosamine[12] (115.2 mg, 0.322 mmol, 1 equiv), *O*-benzyl glycolic acid (54 mg, 0.322 mmol, 1 equiv) and diisopropylethylamine (125 mg, 0.967 mmol, 3 equiv) were dissolved in DMF (0.5 mL) at room temperature. HBTU (147 mg, 0.387 mmol, 1.2 equiv) was added and the mixture stirred for 16 hours. The reaction was extracted with EtOAc (3 x 5 mL) against water. The organic layer was dried with Na_2SO_4, filtered and loaded on Celite. The crude mixture was purified using reverse phase flash chromatography (H_2O:MeCN) to give **6** as a mixture of anomers (114 mg, 67%). Data for **6**: δ_H (400MHz, $CDCl_3$) 2.99 (bs, 1H, OH), 3.48 (ddd, 1H, J_1 5.2 Hz, J_2 10.0 Hz, J_3 14.8 Hz), 3.61 (ddd, 1.5H, J_1 2.0 Hz, J_2 9.6 Hz, J_3 11.2 Hz), 3.88 (dt, 1H, J_1 10.4 Hz, J_2 24.4 Hz), 3.95 (ddd, 1H, J_1 4.8 Hz, J_2 10.0 Hz, J_3 19.6 Hz), 4.11 (m, 2H), 4.17 (s, 1H), 4.30 (m, 1H), 4.43, (m, 1H), 4.52-3.77 (m, 6H), 4.94 (m, 1H), 5,23 (s, 0.8H, anomeric H), 5.59 (s, 1H), 5.60 (s, 0.2H, anomeric H), 6.88 (d, 0.4H, J 5.2 Hz, NH), 7.05 (d, 0.6H, J 8.8 Hz, NH), 7.283-7.53 (m, 15H) δ_C (500MHz, $CDCl_3$) 53.4, 58.7, 62.7, 66.4, 66.6, 67.2, 68.6, 68.8, 69.3, 70.6, 70.9, 71.3, 73.4, 73.6, 81.5, 82.0, 96.9, 99.4, 101.98, 102.0, 126.33, 126.35, 127.9, 128.0, 128.2, 128.29, 128.31, 128.39, 128.44, 128.47, 128.5, 128.6, 129.21, 129.24, 136.5, 136.6, 136.7, 137.02, 137.05, 170.8, 171.0. HRMS (ESI) calcd for $C_{29}H_{31}NO_7$ ($M+K^+$), 544.1732, found, 544.1746.

5-*O*-ditertbutyl(hydroxy)silyl-3-*O*-*R*-(1-methyl carboxylate)ethyl)-D-glucal (16): 15[13] (75.0 mg, 0.262 mmol, 1 equiv) was dissolved in dry DMF (3 mL) at 0 °C. NaH (74 mg, 1.83 mmol, 7 equiv) was added and the mixture stirred for 45 min and allowed to warm up to room temperature. (*S*)-2-bromopropionic acid (205 mg, 1.31 mmol, 5 equiv) was added dropwise to the solution. The reaction was stirred at room temperature and followed by TLC until the disappearance of the starting material. The mixture was quenched with sat. NH_4Cl, acidified to pH 3 and quickly extracted with three portions of EtOAc (5 mL x 3). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated at reduced pressure. The crude was treated with diazomethane solution in ether to make the methyl ester for purification. The product **16** was purified by flash chromatography to give a colourless oil (46 mg, 45%). Data for **16**: δ_H (500MHz, $CDCl_3$) 1.04 (s, 18H), 1.46 (d, 3H, *J* 7 Hz), 3.06 (bs, 1H), 3.77 (s, 3H), 3.82 (dt, 2H, J_1 3 Hz, J_2 10 Hz), 3.97 (dd, 1H, J_1 8 Hz, J_2 10 Hz), 4.03 (bs, 1H), 4.13 (dd, 1H, J_1 3 Hz, J_2 12 Hz), 4.22 (dd, 1H, J_1 3 Hz, J_2 12 Hz), 4.29 (d, 1H, *J* 8 Hz), 4.41 (q, *J* 7 Hz), 4.73 (dd, 1H, J_1 2 Hz, J_2 6 Hz), 6.41 (dd, 1H, J_1 1.5 Hz, J_2 6 Hz). δ_C (500MHz, $CDCl_3$) 19.0, 20.6, 20.8, 27.4, 27.6, 52.3, 61.9, 67.0, 71.2, 76.8, 78.9, 100.9, 145.8,

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175.2. HRMS (ESI) calcd for $C_{18}H_{34}O_7Si$ (M+Na^+), 413.1983, found 413.1966.

3-*O*-*R*-(1-methylcarboxylate)ethyl)-4,6-*O*-benzylidene glucal (22): The alcohol **21**[13] (683 mg, 2.92 mmol, 1 equiv) was dissolved in dry DMF (15 mL) and cooled to 0°C using an ice bath. NaH (60% in mineral oil, 1.17 g, 29.1 mmol, 10 equiv) was added slowly to the solution with vigorous stirring. The mixture was stirred at low temperature for 15 minutes, at which point the ice bath was removed and (*S)*-2-bromopropionic acid (2.23 g, 14.6 mmol, 5 equiv) was added dropwise using a microsyringe. The reaction proceeded at room temperature until the starting material was completely consumed. The excess NaH was quenched by adding small amount of water and the reaction mixture acidified to pH 2 with 3M aqueous HCl. The resulting solution was partitioned between EtOAc and water. The aqueous layer was further extracted with EtOAc (2 x 5 mL). The combined organic layer was washed with brine, dried with Na_2SO_4, filtered and concentrated in vacuo to give a light yellow oil. The concentrate was dissolved in EtOAc and treated with a solution of diazomethane in ether to form the methyl ester. When the addition of diazomethane was complete, the solution was concentrated in vacuo without heating. The crude reaction was purified with flash chromatography (10% EtOAc in petroleum ether) to give the product as a white solid (820 mg, 88%). Data for **22**: δ_H (500MHz, $CDCl_3$) 1.43 (d, 3H, *J* 6.5 Hz, CH_3 lactate), 3.71 (s, 3H, COO*Me*), 3.81 (dd, 1H, $J_{6a,b}$ 7.5 Hz, $J_{5,6b}$ 8.5 Hz, H-6b), 3.88 (ddd, 1H, $J_{5,6a}$ 3.0 Hz, $J_{4,5}$ 8.5 Hz, $J_{5,6b}$ 8.5 Hz, H-5), 3.96 (dd, 1H, $J_{3,4}$ 8.5 Hz, $J_{4,5}$ 8.5 Hz, H-4), 4.33 (m, 2H, H-3, H-6a), 4.47 (q, 1H, $J_{CH,Me}$ 6.5 Hz, lactate CH), 4.90 (dd, 1H, $J_{2,3}$ 2.0 Hz, $J_{vinylic}$ 6.0 Hz, H-2), 5.62 (s, 1H, benzylidene CH), 6.34 (d, 1H, $J_{vinylic}$ 6.0 Hz, H-1), 7.38-7.40 (m, 3H, benzylidene), 7.41-7.49 (m, 2H, benzylidene). δ_C (500MHz, $CDCl_3$) 19.2, 51.9, 68.4, 68.5, 73.6, 74.8, 80.3, 101.1, 102.6, 125.9, 128.3, 129.0, 137.2, 144.5, 173.9. IR (cm^{-1}) 2920, 2120, 1743, 1669, 1453, 1374, 1308, 1286, 1216, 1152, 1103, 1019, 827, 751, 738, 699. MP 98-100°C. HRMS (ESI) calcd for $C_{17}H_{20}O_6$ (M+Na^+), 343.1158, found 343.1164.

α/β-nitro-2*S*-azido-4,6-*O*-benzylidene-3-(*O*-*R*-(1-methylcarboxylate)ethyl)-D-glucopyranoside (23α, β): 22 (357 mg, 1.11 mmol, 1 equiv) was dissolved in dry acetonitrile (18 mL) in a flame-dried flask and cooled to -15°C. cerium ammonium nitrate (CAN, 1.83 g, 3.34 mmol, 3 equiv) and sodium azide (109 mg, 1.67 mmol, 1.5 equiv) were added to the cooled solution simultaneously, followed by vigorous stirring. The reaction was monitored by TLC closely until starting material was completely consumed approximately 3 hours at -15°C. The reaction mixture was partitioned between water and EtOAc. Enough water was added to remove most of the residual CAN such that the organic layer became mostly colourless and the aqueous layer appeared yellow. The organic layer was then washed with saturated $NaHCO_3$ and saturated aqueous $Na_2S_2O_3$, and dried with anhydrous Na_2SO_4. The organic layer was then filtered and concentrated *in vacuo* without heating. The products were separated by flash chromatography (15% EtOAc in petroleum ether) to provide four azidonitration products: 2*R*-azido-4,6-*O*-benzylidene-α/β-methoxy-D-glucoside (**24α/β**, inseparable mixture, 49 mg ~10.5%), 2*S*-azido-4,6-*O*-benzylidene-α-methoxy-D-glucoside (**23α**, 104 mg, 22.1%), 2*S*-azido-4,6-*O*-benzylidene-β-methoxy-D-glucoside (**23β**, 189 mg, 40.0%). Data for **23α**: δ_H (500MHz, $CDCl_3$) 1.45 (d, 3H, *J* 8.5 Hz), 3.79 (s, 3H), 3.72-3.83 (m, 3H), 4.02 (dt, J_1 6 Hz, J_2 13 Hz, J_3 13 Hz), 4.10, t, 1H, *J* 12 Hz), 4.34 (dd, 1H, J_1 6 Hz, J_2 13 Hz), 4.61 (q, 1H, *J* 8.5 Hz), 5.59 (s, 1H), 6.28 (d, 1H, *J* 5.5 Hz), 7.40-7.47 (m, 5H). δ_C (500MHz, $CDCl_3$) 19.0, 52.2, 60.8, 62.0, 64.9, 68.2, 75.7, 76.2, 81.7, 97.1, 101.7, 110.0, 125.9, 128.4, 129.3, 136.5, 172.8. IR (cm-1) 2918, 2120, 1750, 1657, 1453, 1374, 1280, 1210, 1134, 1094, 1067, 996, 825, 751, 699. HRMS (ESI) calcd for $C_{17}H_{20}N_4O_9$ (M+Na+), 477.1128, found 477.1127. Data for **23β**: δ_H (500MHz, $CDCl_3$) 1.48 (d, 3H, *J* 8.5 Hz), 3.57 (dt, J_1 6 Hz, J_2 13 Hz, J_3 13 Hz), 3.61 (t, 1H, *J* 11 Hz), 4.39 (dd, 1H, J_1 6 Hz, J_2 13 Hz), 4.60 (q, 1H, *J* 8.5 Hz), 5.582 (d, 1H, *J* 11 Hz), 5.583 (s, 1H), 7.45-7.48 (m, 5H). δ_C (500MHz, $CDCl_3$) 19.0, 52.1, 60.4, 62.0, 65.6, 68.0, 75.9, 79.5, 80.6, 97.9, 101.4, 110.0, 125.8, 128.4, 129.3, 136.5, 172.8. IR (cm^{-1}) 2920, 2120, 1743, 1669, 1453, 1374, 1308, 1286, 1216, 1152, 1103, 1019, 827, 701, 738, 699, 643. HRMS (ESI) calcd for $C_{17}H_{20}N_4O_9$ (M+Na+), 477.1128, found 477.1126. Data for **24α/β**: δ_H (500MHz, $CDCl_3$) 1.50 (d, 3H, *J* 8.5 Hz), 3.77 (s, 3H), 3.84 (t, 1H, *J* 15 Hz), 3.96 (dt, 1H, J_1 6 Hz, J_2 12.5 Hz, J_3 12.5 Hz), 4.00 (dd, 1H, J_1 5 Hz, J_2 12 Hz), 4.16, (t, 1H, *J* 12 Hz), 4.31 (dd, 1H, J_1 6 Hz, J_2 13 Hz), 4.48 (m, 2H), 5.63 (s, 1H), 6.14 (s, 1H), 7.40-7.48 (m, 5H). δ_C (500MHz, $CDCl_3$) 19.0, 52.1, 60.4, 66.2, 68.1, 76.0, 76.5, 78.2, 98.6, 101.8, 125.9, 128.3, 129.2, 136.9, 173.6. HRMS (ESI) calcd for $C_{17}H_{20}N_4O_9$ (M+Na^+), 477.1128, found 477.1124.

2-azaylide-4,6-*O*-benzylidene-3-(*O*-*R*-(1-methyl carboxylate)ethyl)-D-glucoside (in equilibrium with oxazophospholidine) (25, 26): 23α/β (175 mg, 0.41 mmol, 1 equiv) was dissolved in dry THF (15 mL). PPh_3 (172.5 mg, 0.62 mmol, 1.5 equiv) was added and the mixture was stirred at room temperature for 16 hours. The crude reaction mixture was directly loaded on Celite for reverse phase purification (MeOH in H_2O, 0-95%). The product was obtained in an inseparable mixture of **25** and **26**, as an off-white fluffy powder after lyopholization (151 mg, 60%). Data for **25** and **26** as a mixture: δ_H (500MHz, $CDCl_3$) 1.28 (d, 3H, CH_3, J 7 Hz), 2.75 (ddd, 0.5H, 2H 25, J_1 9.5 Hz, J_2 9.5 Hz, J_3 19.0 Hz), 3.09 (dddd, 0.5H, 2-H 26, J_1 3.5 Hz, J_2 10.0 Hz, J_3 10.0 Hz, J_4 10.0 Hz), 3.35 (ddd, 0.5H, J_1 5 Hz, J_2 9.5 Hz, J_3 9.5 Hz), 3.42 (ddd, 1H, J_1 0.5 Hz, J_2 9.5 Hz, J_3 16.5 Hz), 3.53 (s, 1.5H, COOMe), 3.56-3.64 (m, 1H), 3.64 (s, 1.5H, COOMe), 3.68-3.74 (m, 1H), 4.07-4.27 (m, 3H), 4.62 (q, 1H, OC*H*MeCOOMe, *J* 7 Hz), 4.82 (d, 0.5H, 1-H **25**, 3.5 Hz), 5.40 (s, 0.5H, benzylidene CH), 5.42-5.47 (m, 0.5H, 1-H, **26**), 5.47 (s, 0.5H, benzylidene CH), 6.19 (dd, 1H, NH, **26**, J_1 9.5 Hz, J_2 9.5 Hz), 7.28-8.08 (m, 20H, Ar). δ_C (500MHz, $CDCl_3$) 19.0, 19.2, 51.9, 52.1, 52.2, 57.5, 60.7, 60.76, 60.78, 61.8, 65.2, 68.7, 69.1, 71.2, 74.78, 74.85, 75.2, 75.3, 77.3, 77.8, 81.8, 82.2, 82.4, 83.1, 83.2, 92.61, 92.61, 96.3, 100.98, 101.0, 101.3, 101.4, 120.6, 121.4, 121.7, 122.5, 125.98, 126.0, 126.1, 126.2, 128.1, 128.2, 128.8, 129.0, 129.4, 129.5, 130.0, 130.1, 133.9, 134.0, 134.2, 134.27, 134.33, 134.4, 135.22, 135.24, 137.2, 137.3, 173.5, 174.5. δ_P (500MHz, $CDCl_3$) 38.2 (25), 40.4 (26). HRMS (APCI) calcd for $C_{35}H_{36}NO_7P$ (M+H^+), 614.2302, found 614.2303.

2-*N*-(2-benzyloxyacetyl)-4,6-*O*-benzylidene-3-(*O*-R-(1-methyl carboxylate)ethyl)-D-glucopyranoside (27): A mixture of azaylide **25** and **26** (72 mg, 0.12 mmol, 1 equiv) was dissolved in dry DMF (1 mL). 2-Benzyloxyacetic acid (23 mg, 0.12 mmol, 1 equiv) and diisopropylethyl amine (78 µL, 0.47 mmol, 4 equiv) were added to the solution. HBTU (53 mg, 0.14 mmol, 1.2 equiv) was added at the end. The whole mixture was stirred at room temperature for 48 hours, after which the

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reaction mixture was directly loaded on Celite. The solvent was removed as much as possible on high vacuum with gentle heating. The crude reaction was purified on reverse phase chromatography (MeOH in H_2O, 0-95%). The product (1:4 mixture of α/β anomers) was obtained as a white powder after lyopholization (34 mg, 60%). Data for **27**: δ_H (500MHz, $CDCl_3$) 1.44 (d, 2.4 H, *J* 6.5 Hz), 1.54 (d, 0.6H, *J* 6.5 Hz), 3.52 (dt, *J1* 5 Hz, *J2* 9.5 Hz), 3.76 (s, 2.4H), 3.72 (s, 0.6H), 3.85 (ddd, J_1 10.5 Hz, J_2 10.5 Hz, J_3 20.5 Hz), 4.12 (q, 1H, *J* 6.5 Hz), 4.18 (d, 1H, *J* 15.5 Hz), 4.24 (d, 1H, *J* 15.5 Hz), 4.29 (m, 2H), 4.57 (q, 1H, *J* 7 Hz), 4.67 (s, 0.4H), 4.78 (2, 1.6H), 5.60 (s, 1H), 5.74 (d, 0.8H, *J* 8.5 Hz), 5.83 (d, 0.2H, *J* 8.5 Hz), 7.33-7.65 (m, 10H), 8.04 (d, 1H, *J* 8.5 Hz) δ_C (500MHz, $CDCl_3$) 18.8, 29.7, 52.0, 54.0, 66.5, 68.3, 69.7, 73.6, 75.4, 76.0, 82.0, 101.4, 106.8, 109.7, 120.0, 124.8, 125.9, 127.97, 128.0, 128.29, 128.35, 128.36. 128.50, 128.53, 128.8, 129.0, 129.2, 129.8, 136.7, 137.2, 171.5, 173.6. HRMS (ESI) calcd for $C_{26}H_{31}NO_9$ ($M+Na^+$), 524.1897, found 524.1891.

2-*N*-(2-benzyloxyacetyl)-4,6-*O*-benzylidene-3-(*O*-R-(2-propionyl-L-alanyl-D-isoglutamine benzyl ester)-D-glucopyranoside (28): To a solution of **27** (13 mg, 0.0259 mmol, 1 equiv) in dry THF (1.5 mL) 1M aqueous KOH solution (0.5 mL) and MeOH (0.5 mL) was added. The mixture was stirred at room temperature until no more starting material was observable on TLC. The solution was then acidified to pH 3 and quick extracted with EtOAc (3 mL x 3) and the combined organic layer dried with Na_2SO_4 and concentrated in vacuo. The crude carboxylic acid was then dissolved in dry DMF, to which L-alanyl-D-isoglutamine benzyl ester TFA salt was added as a solution in DMF (12 mg, 0.0285 mmol, 1.1 equiv). DIPEA (18.2 µL, 0.104 mmol, 4 equiv) and HBTU (12 mg, 0.0311 mmol, 1.2 equiv) were added in that sequence. The reaction was stirred at room temperature for 20 hrs. The mixture was extracted with EtOAc (5 mL x 3) and water. The combined organic layer was washed with brine, dried with Na_2SO_4 and concentrated in vacuo. The crude mixture was purified by reverse phase chromatography (H_2O:MeCN) to give **28** (15 mg, 74%). Data for **28**: δ_H (500MHz, $CDCl_3$) 1.34 (d, 3H, *J* 7.5 Hz), 1.41 (d, 3H, *J* 6.5 Hz), 2.02 (m, 1H), 2.21 (m, 1H), 2.49 (dt, 1H, J_1 6.5 Hz, J_2 17.5 Hz), 2.62 (m, 1H), 3.51 (td, 1H, J_1 4.5 Hz, J_2 9.5 Hz), 3.77 (d, 1H, *J* 11.5 Hz), 3.83 (d, 1H, *J* 11.5 Hz), 4.11-4.31 (m, 5H), 4.48 (dt, 1H, *J1* 4 Hz, J2 8 Hz), 4.76 (dd, 1H, *J* 12 Hz), 5.13 (d, 1H, *J* 12.5 Hz), 5.18, (d, 1H, *J* 12.5 Hz), 5.40 (bs, 1H), 5.59 (s, 1H), 5.71 (d, 1H, *J* 9 Hz), 6.70 (bs, 1H), 6.92 (d, 1H, *J* 7 Hz), 7.17 (d, 1H, *J* 8 Hz), 7.35-7.45 (m, 15H), 8.05 (d, 1H, *J* 8.5 Hz), 9.12 (s, 1H). HRMS (ESI) calcd for $C_{40}H_{48}N_4O_{12}$ ($M+Na^+$), 799.3161, found, 799.3164.

***N*-glycolyl muramyl dipeptide (2):** To a solution of **28** (10 mg) in MeOH (5 mL) and EtOAc (5 mL), was added small amount of acetic acid and catalytic amount of Pd/C. The mixture was stirred at room temperature under H_2 atmosphere for 24 hrs. The reaction mixture was filtered through Celite and purified using reverse phase HPLC (H_2O:MeOH) to give **2** as a mixture of anomers (5.8 mg, 96%). Data for **2**: δ_H (500MHz, CD_3OD) certain alcohol and carboxylic acid peaks are not detected. 1.38-1.42 (2 overlapping sets of 2 doublets, 6H) 1.40 (d, *J* 6.5 Hz), 1.41 (d, *J* 6.5 Hz), 1.42 (d, *J* 7 Hz), 1.43(d, *J* 7 Hz), 1.97 (m, 1H), 2.25 (m, 1H), 2.43 (t, 1H, *J* 7.5 Hz), 2.42 (t, 1H, *J* 7.5 Hz), 3.37 (s, 1H), 3.45-3.61 (m, 2H), 3.68-3.73 (m, 2H), 3.80-3.91 (m, 2H), 3.96-4.03 (m, 2H), 4.27-4.42 (m, 3H), 4.71 (d, 0.5H, *J* 8.5 Hz), 5.18 (d, 0.5H, *J* 3.5 Hz), 7.42 (m, 1H), 8.08 (d, 1H, *J* 8.5 Hz). δ_C (500MHz, CD_3OD) 16.2, 16.4, 18.1, 18.2, 26.5, 30.1, 49.3, 49.4, 52.4, 52.6, 53.4, 61.1, 61.27, 61.34, 61.43, 69.9, 70.2, 71.9, 76.4, 76.6, 77.0, 77.6, 77.8, 78.9, 81.8, 91.1, 95.2, 173.9, 174.0, 174.9, 175.0. HRMS (ESI) calcd for $C_{19}H_{32}N_4O_{12}$ ($M+Na^+$), 531.1914, found, 531.1909.

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