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Synthesis of Protected Hydroxyethylamine Transition-State Analogs of *N*-Ac-Muramyl-L-alanine

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Abstract: A novel *N*-acetyl-α-D-glucosamine derivative containing an epoxide moiety **5** has been synthesized and converted into a series of protected hydroxyethylamine transition-state analogs of *N*-Ac-muramyl-L-ala peptide **6a–e** using a microwave-accelerated reaction.

Keywords: *N*-Ac-muramyl-L-ala, hydroxyethylamine, microwave-assisted synthesis, transition-state analogs

INTRODUCTION

Hydroxyethylamine (HEA) transition-state analogs are a well-established class of compounds with significant relevance in medicinal chemistry.[1–3] Our research is directed toward new HEA transition-state inhibitors of Mur ligases. The latter constitute a group of enzymes catalyzing cytoplasmic steps of bacterial peptidoglycan biosynthesis in which several amino acids are attached to the growing peptidoglycan precursor.[4] The first ligase in line is MurC ligase, which attaches L-alanine to UDP-muramic acid.[5] Thus, the tetrahedral transition state could be mimicked by an HEA analog. The most straightforward synthetic route toward this type of HEA analog involves a reaction between an epoxide and an appropriate amino acid, however, most reported procedures suffer from several drawbacks such as low yields, high excesses of reagents, and prolonged

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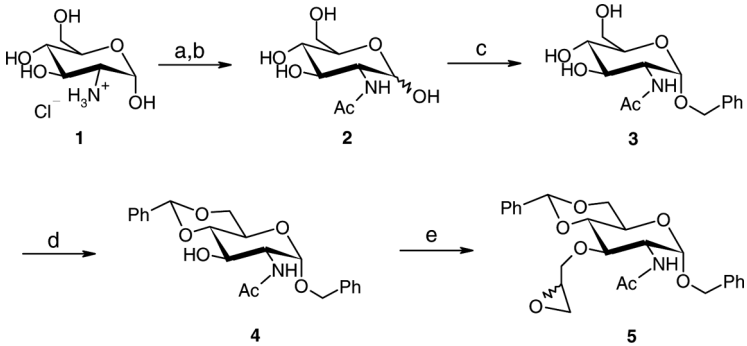
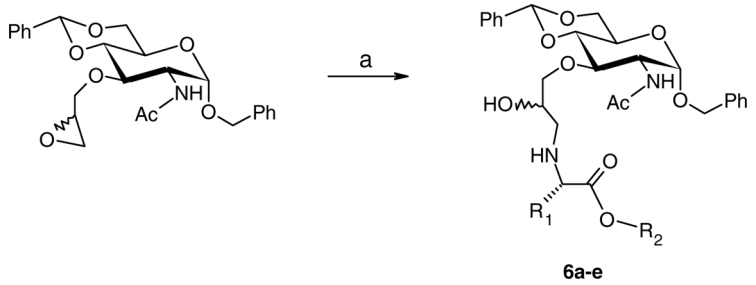
reaction times.[6–8] For that reason, we have developed a reliable and high-yield synthetic method involving a direct amino acid opening of epoxide for the synthesis of HEA derivatives.[9,10] The reaction is catalyzed by calcium trifluorosulfonate and is microwave accelerated, therefore, it is fast and appropriate for parallel synthesis. Herein we describe the application of this reaction in the synthesis of HEA transition-state analogs of *N*-Ac-muramyl-L-Ala peptide.

RESULTS AND DISCUSSION

To synthesize the desired compounds, we used the reaction pathway presented in Scheme 1. The starting material was α-D-glucosamine hydrochloride **1**, which was selectively and quantitatively *N*-acetylated with acetanhydride in methanol at ambient temperature.[11] Fischer glycosylation of **2** with benzyl alcohol gave predominantly the α-anomer of **3**. It is noteworthy that high α/β ratios were only obtainable if prolonged reaction times in refluxing benzene were used. To leave the 3′-hydroxy group of the system available for further synthetic steps, the 4′- and 6′-hydroxy groups were blocked with benzaldehyde to yield compound **4** in a good yield, according to known procedures.[12] At this point, a common way of introducing the epoxide moiety to the 3′-hydroxy group would be a two-step procedure involving Williamson ether synthesis with 3-bromopropene with subsequent oxidation of the double bond. However, a direct, one-step approach with epichlorohydrin was reasonably



Scheme 1. Reagent and conditions: (a) Na/MeOH (b) $(Ac)_2O$, rt, 1 h, quant.; (c) BnOH, PTSA, benzene, reflux, 24 h, 63%; (d) Ph-CHO, $(EtO)_3CH$, PTSA, DMF, dioxane, rt, 20 h, 76%; (e) NaH, epichlorhydrine, 15-crown-5, dioxane, rt, 30 h, 81%.



Scheme 2. Reagent and conditions: (a) $NH_2CHR_1COOR_2$, $Ca(OTf)_2$, dioxane, MW, 120 °C, 20 min, 45–60%.

successful. The reaction was carried out in dioxane with sodium hydride as the base and 15-crown-5 as catalyst.[13] The appropriate crown ether had to be used to increase the reaction rate at ambient temperature because raised reaction temperatures lead to several side products and much lower yields. Compound **5** was obtained as a mixture of two diastereoisomers (dr = 50:50) as no stereoselectivity of the reaction was observed, even at room temperature.

As Scheme 2 indicates, compound **5** was reacted with various carboxy protected L-amino acids to give compounds **6a–e** (Table 1). The reaction was carried out in a sealed reactor using microwave acceleration. Calcium trifluorosulfonate (calcium triflate) is crucial for good yields, especially considering the relatively high steric hindrance of epoxide **5**. Using this catalyst also left the protecting groups intact under the reaction conditions selected. NMR indicated that optically active carboxy protected L-amino acids reacted with both diastereoisomers without preference. No by-products, resulting from the nucleophilic attack of the more sterically hindered electrophilic carbon atom of the oxirane ring, were detected. A small excess (1.2 eq.) of carboxy protected amino acids was used to simplify purification. Regardless, some crude reaction products proved troublesome to purify by flash chromatography.

Table 1. Reaction products **6a–e** and reaction yields

Compound	R_1	R_2	Yield (%)
6a	H	Bn	57
6b	CH_3	Bn	60
6c	$CH(CH_3)_2$	Bn	58
6d	$CH_2CH(CH_3)_2$	Bn	57
6e	CH_3	*t*-Bu	45

In conclusion, we have synthesized a series of protected HEA analogs using a direct reaction between the epoxide **5** and several protected amino acids. Use of calcium trifluorosulfonate and microwave acceleration enabled the use of a small excess of amino acids and ensured short reaction times with good yields.

EXPERIMENTAL

Chemicals from Acros and SigmaAldrich were used without further purification. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel (60 F 254) plates (0.25 mm), and components were visualized with ultraviolet light and dyed with 20% sulphuric acid in ethanol, 2,4-dinitrophenalhydrazine, and ninhydrin. Microwave-accelerated reactions were carried out using a monomode CEM microwave reactor. Flash chromatography was carried out using Merck silica gel (40–63 μm). Melting points were determined on a Reichert hot-stage microscope and are uncorrected. ^{1}H, ^{13}C, DEPT-135, gradient homonuclear correlation spectroscopy (COSY), and gradient heteronuclear single quantum coherence spectroscopy (HSQC) NMR spectra were recorded on a Bruker Avance DPX_{300} spectrometer in $CDCl_3$ or dimethyl sulfoxide (DMSO)-d_6 solution with TMS as the internal standard. IR spectra were obtained on a Perkin–Elmer 1600 Fourier transform infrared spectroscopy (FTIR) spectrometer. Microanalyses were performed on a Perkin-Elmer C, H, N analyzer 240 C. Mass spectra were obtained using 1 VG-Analytical Autospec Q and Q-TOF Premier mass spectrometers.

2-Deoxy-2-acetylamido-D-glucopyranoside (2)

Compound **2** was synthesized according to Ref. 11. Amorphous solid: 30.1 g, 98%. α/β ratio = 85/15. Mp 203–205 °C. IR (KBr, cm^{-1}): 3326, 2910, 1627, 1550, 1428, 1290, 1126, 1054, 563, 726. ^{1}H NMR (DMSO-d_6, 300 MHz) (α-anomer): δ (ppm) = 1.82 (s, 3H, $COCH_3$), 3.06–3.17 (m, 1H, H-4), 3.31–4.69 (m, 5H, H-2, H-3, H-5, H-6, H-6′), 4.41 (t, 1H, OH-6, $J = 6.3$ Hz), 4.75 (d, 1H, OH-4, $J = 6.3$ Hz), 4.92 (d, 1H, OH-3, $J = 6.0$ Hz), 4.97 (t, 1H, H-1, $J = 4.1$ Hz), 6.39 (d, 1H, OH-1, $J = 5.0$ Hz), 7.68 (d, 1H, NH, $J = 7.9$ Hz). MS (FAB), $m/z = 222$ $[MH]^+$. The spectral data are in accordance with the reference material purchased from Acros.

Benzyl-2-deoxy-2-acetylamido-α-D-glucopyranoside (3)

N-Acetyl-D-glucosamine (**2**) (25 g, 113 mmol) and 1.9 g (10 mmol) of p-toluenesulfonic acid monohydrate were suspended in 300 ml of benzene.

Benzyl alcohol (180 ml) was added, and the reaction mixture was refluxed. The water produced in the reaction was removed with a Dean–Stark apparatus. After 24 h, the solvents were removed under reduced pressure. Ether–petrolether mixture (1:1) (700 ml) was added to the oily residue and stirred vigorously for 3 h. The amorphous precipitate was filtered off, and the crude product was recrystallized from ethanol to yield colorless crystals: 22.2 g, 63%. Mp 182–185 °C (ref. Mp 183–184 °C). IR (KBr, cm^{-1}): 3285, 2931, 1630, 1552, 1376, 1027, 734, 695. ^{1}H NMR (DMSO-d_6, 300 MHz): δ (ppm) = 1.84 (s, 3H, $COCH_3$), 3.09–3.20 (m, 1H, H-4), 3.41–4.75 (m, 5H, H-2, H-3, H-5, H-6, H-6'), 4.33 (t, 1H, OH-6, $J = 5.7$ Hz), 4.42 (d, 1H, CH_2Ph, $J_{gem} = 12.6$ Hz), 4.67 (d, 1H, CH_2Ph, $J_{gem} = 12.6$ Hz), 4.70–4.72 (m, 2H, H-1, OH-4), 5.0 (d, 1H, OH-3, $J = 5.6$ Hz), 7.35–7.37 (m, 5H, Ph-H), 7.79 (d, 1H, NH, $J = 8.1$ Hz). MS (FAB), $m/z = 312$ $[MH]^+$. The spectral data are in accordance with Ref. 12.

Benzyl-4,6-*O*-benzylidene-2-deoxy-2-acetylamido-α-D-glucopyranoside (4)

Colorless solid: 14.6 g, 76%. Mp 262–263 °C (ref. M. p. 262 °C). IR (KBr, cm^{-1}): 3300, 3066, 2966, 2868, 1650, 1552, 1372, 1013, 928, 727, 592. ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) = 1.99 (s, 1H, CH_3CO), 2.96 (d, 1H, OH-3, $J = 3.3$ Hz), 3.60 (app t, 1H, H-4, $J = 9.2$ Hz), 3.70–3.79 (m, 1H, H-6′), 3.86 (dd, 1H, $J = 4.6$ Hz, $J = 9.5$ Hz, H-5), 3.96 (ddd, 1H, H-3, $J = 3.0$ Hz, $J = 9.2$ Hz, $J = 9.2$ Hz), 4.20–4.28 (m, 2H, H-2, H-6), 4.50 (d, 1H, CH_2Ph, $J_{gem} = 11.8$ Hz), 4.78 (d, 1H, CH_2Ph, $J_{gem} = 11.8$ Hz), 4.94 (d, 1H, H-1, $J = 3.8$ Hz), 5.57 (s, 1H, CH-Ph), 5.79 (d, 1H, NH, $J = 8.7$ Hz), 7.26–7.52 (m, 10H, H-Ar). MS (FAB), $m/z = 400$ $[MH]^+$. The spectral data are in accordance with Ref. 12.

Benzyl-4,6-*O*-benzylidene-2-deoxy-2-acetylamido-3-*O*-(oxiran-2-yl) methyl-α-D-glucopyranoside (5)

Benzyl-4,6-*O*-benzylidene-2-deoxy-2-acetylamido-α-D-glucopyranoside (**4**) (1.0 g, 2.51 mmol) was dried with benzene co-evaporation under reduced pressure. The obtained white solid was mixed with catalytic amounts of tetrabutylammonium iodide and suspended in 0.5 g of 15-crown-5 and 30 ml of dry dioxane. The suspension was flushed with argon, and then 480 mg (20 mmol) of sodium hydride was added and left to react for 0.5 h. When the evolution of hydrogen stopped, 2.31 g (25 mmol) of epichlorohydrin was added to the reaction mixture and stirred at ambient temperature under argon. After 30 h, TLC analysis indicated the reaction was complete, and the reaction mixture was evaporated to dryness under

reduced pressure. The white solid was suspended in dichloromethane (30 ml) and cooled on an ice-water bath. Saturated sodium hydrogencarbonate solution (20 ml) was added slowly to quench the unreacted sodium hydride. The water phase was extracted with 3×30 ml of dichloromethane. The organic phase was washed with brine and dried over sodium sulfate. The crude product was recrystallized from ethanol to yield a colorless solid: 931 mg, 81%, two diastereoisomers*, mp 220–224 and 228–232* °C. IR (KBr, cm^{-1}): 3298, 2859, 1652, 1557, 1373, 726, 696. ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) = 1.98 (2.00)* (s + s, 3H, CH_3CO), 2.54 (2.72)* (dd + dd, 1H, CH_{2a}, $J = 2.7$ Hz, $J = 5.1$ Hz, ($J = 4.2$ Hz, $J = 5.2$ Hz)*), 2.76–2.80 (m, 1H, CH_{2b}), 3.05–3.12 (m, 1H, CH_2CH), 3.34 (4.21)* (dd + dd, 1H, CH_{2c}, $J = 7.3$ Hz, $J = 12.4$ Hz), 3.61–3.98 (m, 4H, H-4, H-5, H-6', H-3), 3.92 (4.06)* (dd + dd, 1H, CH_{2d}, $J = 2.5$ Hz, $J = 13.2$ Hz), 4.10–4.31 (m, 2H, H-2, H-6), 4.50 (4.72)* (dd + dd, 2H, CH_2Ph, $J_{gem} = 11.8$ Hz, $\Delta\upsilon = 74$ Hz), 4.98 (5.06)* (d + d, 1H, H-1, $J = 3.8$ Hz), 5.57 (s, 1H, CHPh), 5.89 (6.09)* (d + d, 1H, NH, $J = 8.3$ Hz), 7.36–7.48 (m, 10H, Ph-H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) = 23.6, 44.1, 44.9, 51.7, 52.1, 53.1, 53.7, 63.4, 69.3, 70.2 70.4, 70.6, 74.4, 78.6, 82.8, 83.1, 97.9, 98.1, 101.7, 126.3, 126.4, 128.5, 128.6, 128.7, 128.9, 129.0, 129.4, 171.4. MS (FAB), $m/z = 456$ $[MH]^+$. Microanalysis calcd. for $C_{25}H_{29}N_1O_7$: (%): C, 65.92; H, 6.42; N, 3.07. Found: C, 66.01; H, 6.67; N, 2.84.

General Procedure and Characterization of Products 6a–e

Entries **6a–d**: The carboxy protected amino acid salt (2.0 mmol) was suspended in 70 mL of dichloromethane and washed with 0.5 M sodium hydroxide solution (3×10 mL), water (5 mL), and brine (2×10 mL). The organic phase was dried with sodium sulphate and evaporated under reduced pressure and temperature not exceeding 30 °C. A colourless oil was obtained and dried in vacuo.

Entry **6e**: The *tert*-butyl ester of L-alanine was prepared according to known procedures.[14]

The free amino acid ester (0.66 mmol), epoxide **5** (0.55 mmol), and calcium triflate (0.14 mmol) were suspended in 6 mL of anhydrous dioxane. The reaction mixture was heated and stirred for 20 min at 120 °C and 2–3 bar in a sealed 10 ml reactor under monomode microwave irradiation of 50 W. After cooling with compressed air to ambient temperature, the solvent was evaporated under reduced pressure. The colorless crude products **6a–e** were purified with flash chromatography using dichloromethane–methanol mixtures. When necessary, the chromatographic separation was improved using gradient elution.

(**6a**) Benzyl 2-((*R,S*)-3-(1-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-acetylamido-3-α-D-glucopyranosidyloxy)-2-hydroxypropylamino)acetate

Yield: 57%, two diastereoisomers*, mp 180–186 °C. IR (KBr, cm^{-1}): 3437, 2925, 1660, 1449, 1377, 1089. ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) = 1.95 (s, 3H, CH_3CO), 2.55 (2.62)* (dd + dd, 1H, $CH_{2a}NH$, J_{gem} = 122 Hz, J = 7.3 Hz), 2.63–2.69 (m, 1H, $CH_{2b}NH$), 3.37 (3.39)* (s + s, 2H, CH_2COO), 3.48 (dd, 1H, $CH_{2c}O$, J = 7.1, 10.1 Hz), 3.60–3.91 (m, 6H, C*H*OH, $CH_{2d}O$, H-3, H-4, H-5, H-6′), 4.18–4.25 (m, 2H, H-2, H-6), 4.51 (4.71)* (d + d, 1H, OCH_2Ph, J_{gem} = 11.8 Hz), 4.97 (4.98)* (d + d, 1H, H-1, J = 3.9 Hz), 5.14 (s, 2H, $COOCH_2Ph$), 5.54 (s, 1H, CHPh), 6.05 (6.21)* (d + d, 1H, NHCO, J = 8.6 Hz), 7.26–7.48 (m, 15H, PhH). ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) = 23.70, 51.27, 53.49, 63.49, 66.98, 69.31, 69.95, 70.52, 77.64, 79.05, 82.56, 98.03, 101.83, 126.39–129.45, 135.94, 137.30, 137.59, 170.71, 172.74. LRMS (FAB), m/z = 621 $[MH]^+$. HRMS (ESI), calcd. for $C_{34}H_{41}N_2O_9$ m/z: 621.2812 $[MH]^+$, found: 621.2823. Microanalysis calcd. for $C_{34}H_{40}N_2O_9 \times 1.5H_2O$ (%): C, 63.98; H, 6.63; N, 4.39. Found: C, 63.54; H, 6.79; N, 4.77.

(**6b**) (*S*)-Benzyl-2-((*R,S*)-3-(1-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-acetylamido-3-α-D-glucopyranosidyloxy)-2-hydroxypropylamino)-propanoate

Yield: 60%, two diastereoisomers*, mp 198–205 °C. IR (KBr, cm^{-1}): 3294, 2924, 2855, 1741, 1658, 1551, 1452, 1376 1090, 734, 896. ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) = 1.26 (d, 3H, CH_3, J = 6.9 Hz), 1.95 (s, 3H, CH_3CO), 2.36 (2.65)* (dd + dd, 1H, $CH_{2a}NH$, J_{gem} = 12 Hz, J = 8.1 Hz), 2.53 (2.70)* (dd + dd, 1H, $CH_{2b}NH$, J_{gem} = 12 Hz, J = 4.2 Hz), 3.27–3.36 (m, 1H, CHCOO), 3.46 (dd, 1H, $CH_{2c}O$, J = 8.3, 11.7 Hz), 3.58–3.92 (m, 6H, C*H*OH, $CH_{2d}O$, H-3, H-4, H-5, H-6'), 4.16–4.28 (m, 2H, H-2, H-6), 4.48 (4.72)* (d + d, 1H, OCH_2Ph, J_{gem} = 11.9 Hz), 4.97 (5.01)* (d + d, 1H, H-1, J = 3.9 Hz), 5.13 (5.14)* (s + s, 2H, $COOCH_2Ph$), 5.53 (s, 1H, CHPh), 6.04 (6.31)* (d + d, 1H, NHCO, J = 8.4 Hz), 7.27–7.50 (m, 15H, PhH). ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) = 19.39, 23.66, 49.95, 53.42, 57.02, 63.53, 66.90, 69.33, 69.83, 70.52, 77.68, 78.76, 82.62, 98.04, 101.79, 126.41–129.43, 136.20, 137.44, 137.59, 170.72, 175.66. MS (FAB), m/z = 635 $[MH]^+$. HRMS (ESI), calcd. for $C_{35}H_{43}N_2O_9$ m/z: 635.2969 $[MH]^+$, found: 635.2963. Microanalysis calcd. for $C_{35}H_{42}N_2O_9 \times H_2O$(%): C, 64.44; H, 6.80; N, 4.29. Found: C, 64.73; H, 6.78; N, 3.95.

(6c) (*S*)-Benzyl-2-((*R,S*)-3-(1-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-acetylamido-3-α-D-glucopyranosidyloxy)-2-hydroxypropylamino)-3-methylbutanoate

Yield: 58%, two diastereoisomers*, mp 160–169 °C. IR (KBr, cm^{-1}): 3294, 2926, 2856, 1731, 1656, 1551, 1450, 1375, 1087, 696. ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) = 0.88–0.92 (m, 7H, $(CH_3)_2CH$), 1.96 (s, 3H, CH_3CO), 1.85–2.05 [m, 1H, $(CH_3)_2CH$], 2.36 (2.65)* (dd + dd, 1H, $CH_{2a}NH$, $J_{gem} = 12$ Hz, $J = 8.1$ Hz), 2.51 (2.70)* (dd + dd, 1H, $CH_{2b}NH$, $J_{gem} = 12$ Hz, $J = 4.2$ Hz), 3.06 (m, 1H, CHCOO), 3.49 (dd, 1H, $CH_{2c}O$, $J = 8.3$, 11.7 Hz), 3.47–3.92 (m, 6H, CHOH, $CH_{2d}O$, H-3, H-4, H-5, H-6′), 4.16–4.28 (m, 2H, H-2, H-6), 4.49 (4.72)* (d + d, 1H, OCH_2Ph, $J_{gem} = 11.9$ Hz), 4.97 (5.01)* (d + d, 1H, H-1, $J = 3.9$ Hz), 5.13 (5.14)* (s + s, 2H, $COOCH_2Ph$), 5.54 (5.55)* (s + s, 1H, CHPh), 6.05 (6.40)* (d + d, 1H, NHCO, $J = 8.4$ Hz), 7.27–7.50 (m. 15H, PhH). ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) = 18.74, 19.73, 23.64, 31.93, 50.71, 53.48, 63.51, 66.79, 67.51, 68.21, 69.33, 70.52, 77.63, 78.74, 82.70, 98.01, 101.76, 126.38–129.37, 136.09, 137.36, 137.70, 170.60, 175.09. MS (FAB), $m/z = 663$ $[MH]^+$. HRMS (ESI), calcd. for $C_{37}H_{47}N_2O_9$ m/z: 663.3282 $[MH]^+$; found: 663.3276. Microanalysis calcd. for $C_{37}H_{46}N_2O_9 \times 1/2H_2O$ (%): C, 66.19; H, 7.01; N, 4.17. Found: C, 65.94; H, 6.85; N, 3.98.

(6d) (*S*)-Benzyl-2-((*R,S*)-3-(1-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-acetylamido-3-α-D-glucopyranosidyloxy)-2-hydroxypropylamino)-4-methylpentanoate

Yield: 57%, two diastereoisomers*, mp : 169–175 °C. IR (KBr, cm^{-1}): 3289, 2925, 1737, 1654, 1551, 1454, 1375, 1089, 734, 696. ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) = 0.86 (0.90)* [d + d, 6H, $(CH_3)_2$, $J = 8.1$ Hz], 1.40–1.50 [m, 2H, $(CH_3)_2CHCH_2$], 1.60–1.75 [m, 1H, $(CH_3)_2CH$], 1.97 (s, 3H, CH_3CO), 2.28 (2.60)* (dd + dd, 1H, $CH_{2a}NH$, $J_{gem} = 12$ Hz, $J = 8.1$ Hz), 2.48 (2.72)* (dd + dd, 1H, $CH_{2b}NH$, $J_{gem} = 12$ Hz, $J = 4.2$ Hz), 3.26 (q, 1H, CHCOO, $J = 7.8$ Hz), 3.41–3.93 (m, 5H, 7H, CHOH, $CH_{2c}O$, $CH_{2d}O$, H-3, H-4, H-5, H-6′), 4.15–4.28 (m, 2H, H-2, H-6), 4.51 (4.74)* (d + d, 1H, OCH_2Ph, $J_{gem} = 11.9$ Hz), 5,00 (5.06)* (d + d, 1H, H-1, $J = 3.7$ Hz), 5.15 (s, 2H, $COOCH_2Ph$), 5.57 (s, 1H, CHPh), 6.00 (6.31)* (d + d, 1H, NHCO, $J = 7.8$ Hz), 7.25–7.53 (m. 15H, PhH). ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) = 22.49, 22.72, 23.69, 24.82, 41.19, 49.83, 53.70, 59.62, 63.52, 66.35, 66.50, 68.82, 70.53, 77.44, 78.75, 82.68, 98.02, 101.77, 126.40–129.02, 137.02, 137.22, 170.25, 175.65. MS (FAB), $m/z = 677$ $[MH]^+$. HRMS (ESI), calcd. for

$C_{38}H_{49}N_2O_9$ m/z: 677.3438 $[MH]^+$; found: 677.3448. Microanalysis calcd. for $C_{38}H_{48}N_2O_9$ (%): C, 67.44; H, 7.15; N, 4.14. Found: C, 67.13; H, 7.31; N, 4.19.

(**6e**) (*S*)-*tert*-Butyl-2-((*R,S*)-3-(1-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-2-acetylamido-3-α-D-glucopyranosidyloxy)-2-hydroxypropylamino)-propanoate

Yield: 45%, two diastereoisomers*, mp 167–173 °C. IR (KBr, cm^{-1}): 3423, 1639, 1372, 1090, 694. ^1H NMR ($CDCl_3$, 300 MHz): δ (ppm) = 1.26 (d, 3H, CH_3, $J = 6.9$ Hz), 1.47 (s, 9H, $C(CH_3)_3$), 1.99 [s, 3H, CH_3CO], 2.36 (2.65)* (dd + dd, 1H, $CH_{2a}NH$, $J_{gem} = 12$ Hz, $J = 8.1$ Hz), 2.54 (2.70)* (dd + dd, 1H, $CH_{2b}NH$, $J_{gem} = 12$ Hz, $J = 4.2$ Hz), 3.27–3.36 (m, 1H, CHCOO), 3.46 (dd, 1H, $CH_{2c}O$, $J = 7.8$, 11.5 Hz), 3.65–3.96 (m, 6H, $CHOH$, $CH_{2d}O$, H-3, H-4, H-5, H-6′), 4.16–4.27 (m, 2H, H-2, H-6), 4.51 (4.74)* (d + d, 1H, OCH_2Ph, $J_{gem} = 11.9$ Hz), 4.99 (5.03)* (d + d, 1H, H-1, $J = 3.9$ Hz), 5.59 (s, 1H, CHPh), 6.02 (6.35)* (d + d, 1H, NHCO, $J = 8.4$ Hz), 7.27–7.50 (m. 10H, PhH). ^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) = 19.50, 23.63, 28.46, 53.78, 57.92, 63.52, 67.48, 69.32, 70.51, 77.60, 78.70, 81.39, 82.68, 98.00, 101.77, 126.39–129.39, 137.35, 137.59, 170.70, 175.18. MS (FAB), $m/z = 601$ $[MH]^+$. HRMS (FAB), calcd. for $C_{32}H_{45}N_2O_9$ m/z: 601.31350 $[MH]^+$; found: 601.31251. Microanalysis calcd. for $C_{32}H_{44}N_2O_9$ (%): C, 63.98, H, 7.38; N, 4.66. Found: C, 63.44; H, 7.32; N, 4.66.

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