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9-BBN as a convenient protecting group in functionalisation of hydroxylysine

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Abstract—9-BBN was used for regioselective protection of the α-amino and α-carboxyl groups of (5*R*)-5-hydroxy-L-lysine. The resulting 9-BBN complex was then employed in transformations such as *N*-Cbz protection, azido transfer, *O*-glycosylation, and *O*-silylation. Further manipulations led to improved methods for preparation of hydroxylysine and galactosylated hydroxylysine building blocks, suitable for direct use in peptide synthesis under standard Fmoc conditions.

1. Introduction

Lysine residues in collagen can undergo post-translational hydroxylation to give (5*R*)-5-hydroxy-L-lysine, which was first discovered in protein hydrolysates.[1,2] In collagen, hydroxylysine residues are essential for the stability of intermolecular collagen cross-links.[3] In addition, hydroxylysine in collagen may be glycosylated, either with a β-D-galactopyranosyl- or an α-D-glucopyranosyl-(1→2)-β-D-galactopyranosyl moiety.[4] Recent studies have revealed that T cell hybridomas obtained in collagen-induced arthritis (CIA), which is a common mouse model for rheumatoid arthritis (RA), specifically respond to a galactosylated hydroxylysine residue located in a peptide fragment from type II collagen.[5,6] A dominance of T cell responses to such glycopeptides from type II collagen was also recorded in a cohort of severely affected RA-patients, suggesting a crucial role for the glycosylated form of hydroxylysine in development of RA in humans.[7]

(5*R*)-N^{α}-(Fluoren-9-ylmethoxycarbonyl)-N^{ε}-benzyloxycarbonyl-5-hydroxy-L-lysine allyl ester (cf. **6**, Scheme 1) is an important intermediate for preparation of glycosylated hydroxylysine building blocks for use in solid phase glycopeptide synthesis.[5,6,8–10] Previous procedures reported from our[8,9] and other laboratories[11] for synthesis of glycosylated derivatives of hydroxylysine have involved initial formation of a cupric chelate of hydroxylysine followed by regioselective protection of the N^{ε}-amino group. The procedure developed in our laboratory allowed conversion of hydroxylysine into building block **6** in approximately 30% overall yield,[8] but it involved some practical difficulties like tedious work up and isolation of intermediates and the final product. Consequently, there is a need for developing a more convenient and high-yielding procedure for regioselective protection and glycosylation of hydroxylysine. 9-Borabicyclononane (9-BBN) has recently been reported to be convenient for simultaneous protection of the carboxyl and amino functionalities of amino acids, thereby imparting solubility of the corresponding borane complexes in various organic solvents.[12,13] We therefore turned our attention towards investigating if 9-BBN can be used as protecting group for the α-amino acid moiety of hydroxylysine and if this allows functional group transformations of the amino and hydroxyl groups in the side chain.

2. Results and discussion

Commercially available (5*R*)-5-hydroxy-L-lysine dihydrochloride was first dissolved in aqueous ammonia. Concentration and drying under high vacuum gave a solid that was treated with a slight excess of 9-BBN in refluxing methanol to give the soluble borane complex **1** in quantitative yield (Scheme 1). Interestingly, formation of a 9-BBN complex involving the δ-hydroxyl and ε-amino groups was not detected, revealing a very high selectivity for protection of the α-amino acid functionality of hydroxylysine. Previous studies have indicated that 9-BBN protected amino acids are surprisingly tolerant to a wide range of reaction conditions.[12] In line with these observations protection of the N^{ε}-amino group of **1** with benzyl chloroformate gave the

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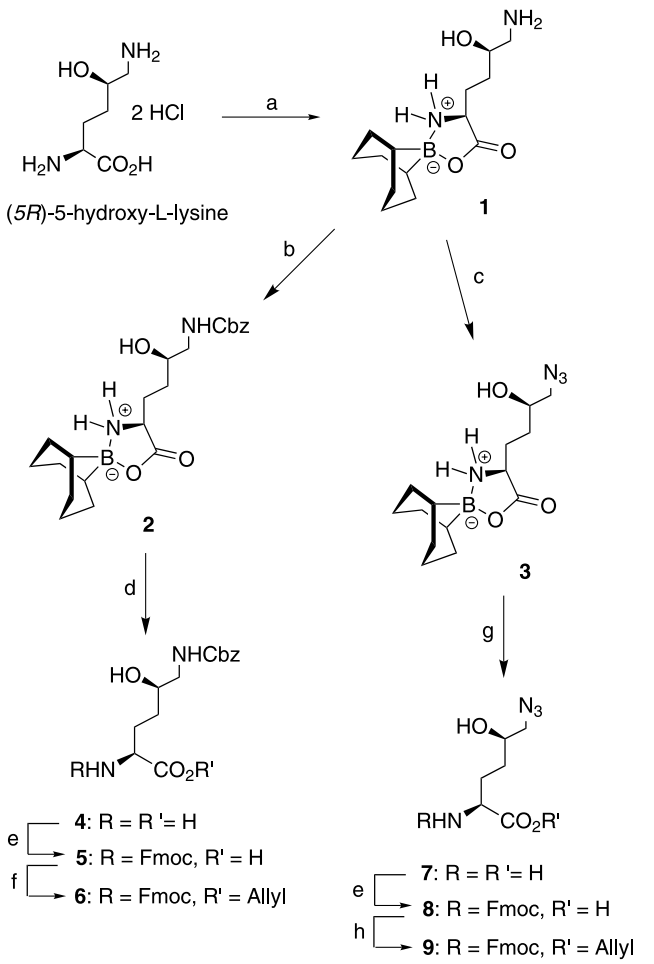
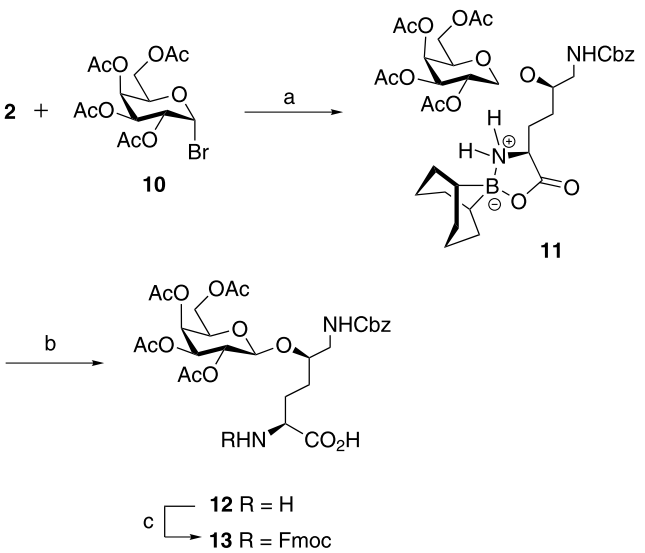


Scheme 1. (a) Aqueous NH_3 solution, 0 °C, 30 min, concentration, then 9-BBN, methanol, reflux, 4 h; (b) Cbz-Cl, $NaHCO_3$, dioxane–water, 0 °C→rt (92% yield from hydroxylysine); (c) TfN_3, K_2CO_3, $CuSO_4$, CH_2Cl_2, MeOH, 18 h, rt, (56%); (d) ethylenediamine, THF, reflux; (e) Fmoc-Cl, Na_2CO_3, dioxane–water, 1:1; (f) Cs_2CO_3, aq. EtOH, allyl bromide, DMF, rt (72% from **2**); (g) MeOH–HCl, rt, 10 min; (h) allyl alcohol, TMSCl, 0 °C→rt, 10 h (45% from **3**).

N^{ε}-Cbz protected borane complex **2** (92% yield from hydroxylysine), without any apparent reaction at the α-amino group. Complex **1** could also be treated with freshly prepared triflyl azide[14] in dichloromethane to give azido derivative **3** (56%).

Decomplexation of borane complex **2** to give **4** was obtained by heating with ethylene diamine in THF (Scheme 1). N^{α}-Fmoc protection of the α-amino group of **4** followed by conversion of the carboxyl group to an allyl ester via treatment of the corresponding cesium salt with allyl bromide in DMF yielded the differentially protected hydroxylysine derivative **6**[8] (72% from **2**). Compound **6** has previously been described as a key building block for preparation of galactosylated derivatives of (5*R*)-5-hydroxy-L-lysine, which were subsequently employed in solid-phase glycopeptide synthesis.[6,8–10] Decomplexation of azido derivative **3** was accomplished by using concentrated aqueous hydrogen chloride in methanol to give **7**. The α-amino group of **7** was then protected with an Fmoc group and the carboxyl group of **8** was converted into an allyl ester by treatment with trimethylsilyl chloride in allyl alcohol. This gave azido-hydroxylysine derivative **9** in 45% yield from **3**. Attempted glycosylation of **9** with 2,3,4,6-tetra-*O*-acetyl-galactosyl bromide as a glycosyl donor and silver silcate as a promotor failed. This was unexpected since these conditions give high and reproducible yields (appr. 80%) for the analogous Cbz-derivative **6**.[9] Efforts to perform the glycosylation using other glycosyl promoters, that is, ICl/AgOTf[15] or Br_2/AgOTf[16] proved futile and no product formation was observed. Somewhat surprisingly, it therefore had to be concluded that the azido-hydroxylysine derivative **9** is a poor glycosyl acceptor.

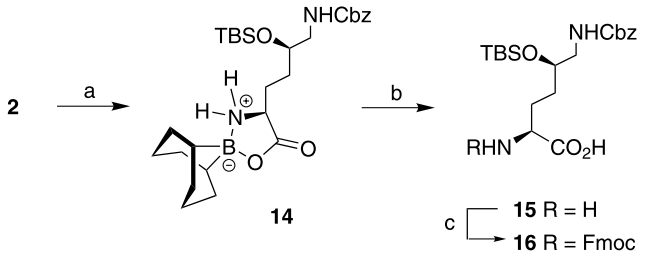
Due to our interest in developing a more efficient, reproducible and less time-consuming procedure for preparation of galactosylated hydroxylysine building blocks, glycosylation of the Cbz-protected hydroxylysine boroxazolidinone complex **2** was investigated. It was found that silver silicate promoted glycosylation of complex **2** with galactosyl bromide **10** in dichloromethane at 0 °C furnished the desired β-glycoside **11** in 68% yield (Scheme 2). Formation of detectable amounts of the corresponding α-anomer or orthoester, or decomposition of the 9-BBN complex, was not observed. The glycosylated borane complex **11** was stable to purification by column chromatography on silica gel and its identity was confirmed by NMR spectroscopy and LCMS. However, some decomposition of **11** to give the glycosylated amino acid **12** was observed upon prolonged standing of the fractions obtained after column chromatography with a mixture of chloroform and methanol as eluent. This serendipitous discovery provided a milder alternative for decomplexation[13] than those previously employed, that is, use of either ethylenediamine or concentrated HCl in methanol.[12] Such harsh conditions would have affected either the *O*-acetyl protective groups or the β-glycosidic linkage of **11**, respectively. Complete decomplexation was therefore achieved by treating **11** with a mixture of chloroform and methanol during 12 h at room temperature. Fmoc protection of **12** using 9-fluorenylmethoxycarbonyl-*N*-hydroxy-succinimide ester (Fmoc-OSu) and sodium bicarbonate as



Scheme 2. (a) Silver silicate/silver zeolite, CH_2Cl_2, 0 °C, 8 h (68%); (b) $CHCl_3$–MeOH, rt 12 h; (c) Fmoc-OSu, $NaHCO_3$, acetone–H_2O (91% from **11**).

base, followed by acidification, afforded the glycosylated hydroxylysine building block **13**[9] in 91% yield from **11**. Synthesis of **13** was thus achieved in only five steps, and 56% overall yield from hydroxylysine dihydrochloride. The present procedure is simpler to carry out than the one reported previously by us,[9] since tedious concentrations of aqueous solutions are avoided. In addition the overall yield from commercially available hydroxylysine is improved significantly.

Finally, conversion of boroxazolidinone **2** into a hydroxylysine building block for use in peptide synthesis was investigated. The hydroxyl group in the hydroxylysine part of **2** was protected to avoid potential lactonisation with the carboxyl group during peptide synthesis (Scheme 3). Protection was achieved by treatment of **2** with *tert*-butyldimethylsilyl triflouromethanesulfonate in the presence of 2,6-lutidine which furnished silyl ether **14** in 86% yield. Subsequent cleavage of the borane complex with a mixture of chloroform and methanol, as described above, followed by Fmoc protection of the α-amino group afforded the desired hydroxylysine building block **16** (89% from **14**). Building block **16** was thus prepared in five steps and 70% overall yield from hydroxylysine dihydrochloride.



Scheme 3. (a) TBSOTf, 2,6 lutidine, CH_2Cl_2, 0 °C, 2 h (86%); (b) $CHCl_3$–MeOH, rt; (c) Fmoc-OSu, $NaHCO_3$, acetone–H_2O (89% from **14**).

3. Conclusion

In conclusion, 9-BBN has been shown to be an efficient protective group for the amino acid moiety of (5*R*)-5-hydroxy-L-lysine. It was also demonstrated the 9-BBN complex of hydroxylysine is tolerant to several manipulations in the side-chain, that is, *N*-Cbz protection, azido transfer, *O*-glycosylation, and *O*-silylation. Finally, use of the 9-BBN complex of hydroxylysine allowed convenient and improved methods for preparation of hydroxylysine and galactosylated hydroxylysine building blocks to be developed. These building blocks are suitable for use in peptide and glycopeptide synthesis according to the Fmoc strategy, either in solution or on solid support.

4. Experimental

4.1. General

TLC was performed on Silica Gel 60 F_{254} (Merck) with detection by UV light or charring with anisaldehyde solution (anisaldehyde, H_2SO_4, acetic acid, ethanol). Flash column chromatography was performed on silica gel (Matrex, 60 Å, 35–70 μm, Grace Amicon) with solvents of HPLC grade, analytical grade or distilled technical grade. The ^1H and ^{13}C NMR spectra were recorded on a Bruker DRX-400 spectrometer at 400 and 100 MHz, respectively. Chemical shifts are referenced to residual $CHCl_3$ (δ_H=7.27 ppm) and $CDCl_3$ (δ_C=77.0) for solutions in $CDCl_3$. Optical rotations were recorded on a Perkin Elmer 343 polarimeter. 2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl bromide **10** was prepared from peracetylated galactose by treatment with HBr in HOAc/Ac_2O. All new compounds were determined to be >95% pure by ^1H NMR spectroscopy and LC–MS. They were also characterized by high resolution mass spectrometry.

4.1.1. (5*R*)-*N*$^{\varepsilon}$-Benzyloxycarbonyl-5-hydroxy-L-lysinato-bicyclononylboron (2). Ammonia solution (aq. 10 mL) was added to (5*R*)-5-hydroxy-L-lysine dihydrochloride monohydrate (1 g, 3.94 mmol) at 0 °C. After stirring for 30 min the solution was concentrated and the crystalline solid was dried in high vacuum before further use. The solid was added in one portion to a stirred solution of 9-BBN (1.2 g, 4.7 mmol) in hot methanol (20 mL). The reaction mixture was refluxed (ca. 3 h) under nitrogen until a clear solution was obtained. After evaporation of the solvent the residue was dissolved in hot THF and filtered. The filtrate was concentrated and the residue triturated with hot hexanes and finally with diethyl ether. The residue was dried under high vacuum to give crude **1** as an amorphous solid which was used as such in the next reaction. Sodium bicarbonate (0.50 g, 5.90 mmol), followed by Cbz-Cl (0.66 mL, 4.72 mmol), was added to a solution of **1** in a mixture of dioxane–water (1:1, 10 mL) at 0 °C. The reaction mixture was stirred for 5 h at rt and then concentrated. The compound was extracted by ethyl acetate (2×25 mL) from water (2×25 mL). The combined organic phases were washed once with brine (20 mL), water (25 mL), dried (Na_2SO_4) and concentrated. The residue was purified by flash column chromatography using hexane–ethyl acetate as eluent to afford **2** (1.51 g, 92% yield) as a white amorphous solid: $[\alpha]_D^{20}=-14.8$ (*c* 1.7, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 0.53 (brd, 2H, *J*=10.7 Hz, (B-(C*H*)$_2$), 1.30–1.89 (m, 15H, B-(CH_2)$_6$, H-β, H-γ′, H-γ), 1.92–2.07 (m, 2H, H-β, –OH), 3.01–3.13 (m, 1H, H-ε), 3.15–3.26 (m, 1H, H-ε′), 3.62–3.71 (m, 1H, H-δ), 3.74 (m, 1H, H-α), 4.95–5.07 (m, 2H, PhCH_2), 5.18 (br s, 1H, N–Hα), 5.66 (m, 2H, N–Hα′, N–Hε), 7.22–7.33 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 19.1, 21.0, 22.8, 23.8, 24.3, 26.8, 29.8, 31.1, 31.2, 31.7, 46.6, 55.1, 60.4, 67.0, 70.8, 125.2, 127.9, 128.2, 128.2, 128.5, 129.0, 136.1, 157.7, 171.3, 175.3; HR-MS (FAB): calcd for $C_{22}H_{33}BN_2O_5$ 439.2375 $[M+Na]^+$, found 439.2410.

4.1.2. (5*R*)-5-Hydroxy-6-azido-L-lysinato-bicyclononylboron (3). *Preparation of triflyl azide*.[14] Dichloromethane (10 mL) was added to an ice cold solution of sodium azide (2.5 g, 38.4 mmol) in water (6.5 mL). The biphasic mixture was stirred vigorously and treated dropwise with triflic anhydride (1.2 mL, 7.8 mmol), over a period of 5 min. The reaction mixture was stirred for 2 h at 0 °C after which the organic phase was separated and the aqueous phase was extracted twice with dichloromethane (2×5 mL). The combined organic phases were washed with aqueous sat. sodium carbonate solution and the resulting triflyl azide solution was used without concentration in the next step.

Potassium carbonate (0.80 g, 5.85 mmol), $CuSO_4$ monohydrate (6 mg, 0.024 mmol), and triflyl azide solution prepared as above were added to a solution of crude borane complex **1** (1.1 g, 3.89 mmol) in methanol (25 mL). The reaction mixture was stirred for 18 h at rt and concentrated. The residue was extracted by ethyl acetate (20 mL) from water (15 mL) and the organic phase was dried (Na_2SO_4) and concentrated. The residue was purified by flash column chromatography using $CHCl_3$–MeOH (19:1) as eluent to afford azido derivative **3** (0.67 g, 56%) as a yellow amorphous solid: $[\alpha]_D^{20}=-10.2$ (*c* 1.7, MeOH); IR (neat, cm^{-1}): 840, 960, 1214, 1261, 1357, 1450, 1697, 2098, 2842, 2919, 3114, 3222; ^1H NMR ($CDCl_3$+MeOH-d_4, 400 MHz): δ 0.56 (br s, 2H, B-$(CH)_2$), 1.35–1.88 (m, 15H, B-$(CH_2)_6$, H-β, H-γ, H-γ′), 2.03–2.17 (m, 2H, H-β′, –OH), 3.26 (dd, 1H, *J*=7.6, 12.2 Hz, H-ε), 3.38 (dd, 1H, *J*=3.6, 12.4 Hz, H-ε′), 3.77–3.84 (m, 1H, H-δ), 3.85–3.93 (m, 1H, H-α), 5.23 (dd, 1H, *J*=8.0, 12.4 Hz, –NH), 5.56 (dd, 1H, *J*=8.2, 12.4 Hz, –NH); ^{13}C NMR ($CDCl_3$+MeOH-d_4, 100 MHz): δ 23.6, 24.1, 26.4, 29.2, 30.8, 30.8, 31.0, 31.1, 54.7, 56.5, 69.7, 127.8, 128.7, 175.3; HR-MS (FAB): calcd for $C_{14}H_{27}BN_4O_3$ 309.2092 $[M+H]^+$, found 309.2103.

4.1.3. (5*R*)-*N*$^{\alpha}$-(Fluoren-9-ylmethoxycarbonyl)-*N*$^{\varepsilon}$-benzyloxycarbonyl-5-hydroxy-L-lysine allyl ester (6). Excess ethylenediamine (1 mL, 16 mmol) was added to a solution of **2** (1.51 g, 3.62 mmol) in THF (5 mL) at room temperature and heated for 1 min. The suspension was cooled and filtered. The precipitate was washed with an additional amount of THF (15 mL) and dried in vacuo to afford crude **4** (0.95 g) which was used as such for the next step. A solution of 9-fluorenylmethyl chloroformate (1.25 g, 4.84 mmol) in dioxane (5 mL) was added dropwise to a solution of **4** in dioxane–10% Na_2CO_3 (15 mL, 1:2) at 0 °C. The solution was stirred for 5 h at rt and the dioxane was evaporated. Chloroform (20 mL) was added and the mixture was acidified to pH 2 with a 1 M solution of $KHSO_4$ at 0 °C. The organic phase was separated and the aqueous phase was extracted with an additional amount of chloroform (2×10 mL). The combined organic layers were dried (Na_2SO_4) and concentrated to afford acid **5** (1.53 g). Aqueous Cs_2CO_3 was added to a solution of **5** in aqueous ethanol (80%) so that the pH was adjusted to 7. The solution was concentrated after stirring for 2 h. Allyl bromide (0.73 g, 5.43 mmol) was added to a solution of the resulting cesium salt in DMF (5 mL) and stirred for 12 h under nitrogen. The mixture was diluted with water and extracted with diethyl ether. The organic phase was dried (Na_2SO_4), concentrated and the residue was purified by column chromatography over silica gel using heptane–ethyl acetate as eluent to afford allyl ester **6** (1.45 g, 72% for three steps). Compound **6** had ^1H and ^{13}C NMR data identical to those reported previously.[8]

4.1.4. (5*R*)-*N*$^{\alpha}$-(Fluoren-9-yl-methoxycarbonyl)-6-azido-5-hydroxy-L-lysine allyl ester (9). Concentrated aqueous HCl (2 mL) was added to a solution of **3** (0.67 g, 2.14 mmol) in methanol (5 mL) at 0 °C. The solution was stirred for 10 min at rt and then concentrated. The residue was triturated with hot hexanes and finally with ether. It was dried under high vacuum to afford crude **7** (0.36 g) which was used as such in the next step. A solution of 9-fluorenylmethyl chloroformate (0.631 g, 2.44 mmol) in dioxane (2 mL) was added dropwise to a solution of crude **7** (0.36 g) in dioxane–10% Na_2CO_3 (6 mL, 1:2) at 0 °C. The solution was stirred for 4 h at room temperature and the dioxane was evaporated. Chloroform (15 mL) was added and the mixture was acidified to pH 2 with a 1 M solution of $KHSO_4$ at 0 °C. The organic phase was separated, and the aqueous phase extracted with an additional amount of chloroform (2×10 mL). The combined organic layers were dried (Na_2SO_4) and concentrated to afford crude acid **8** (0.58 g). Trimethylsilyl chloride (0.38 mL, 3.0 mmol) was added dropwise to an ice cold solution of crude **8** (0.58 g) in allyl alcohol (5 mL) under nitrogen. After stirring for 10 h at rt the solvent was evaporated and the residue was purified by flash column chromatography using hexane–ethyl acetate as eluent to afford the ester **9** (0.45 g, 45% over three steps). $[\alpha]_D^{20}=+0.58$ (*c*=2.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 1.48–1.61 (m, 2H, H-γ, γ′), 1.70–1.83 (m, 1H, H-β), 1.99–2.11 (m, 1H, H-β′), 2.16–2.56 (br s, 1H, –O*H*), 3.18–3.37 (2 m, 2H, H-ε, ε′), 3.71–3.82 (m, 1H, H-δ), 4.21 (t, 1H, *J*=7.0 Hz, Fmoc-C*H*), 4.35–4.50 (m, 3H, Fmoc-CH_2, H-α), 4.64 (d, 2H, *J*=5.3 Hz, allylic CH_2), 5.29 (ddd, 2H, *J*=17.8, 10.6, 30.2 Hz, =CH_2), 5.55 (d, 1H, *J*=7.6 Hz, N–H), 5.82–5.95 (m, 1H, –C*H*=), 7.30 (t, 2H, *J*=7.5 Hz, Fmoc), 7.39 (t, 2H, *J*=7.3 Hz, Fmoc), 7.58 (d, 2H, *J*=7.1 Hz, Fmoc), 7.75 (d, 2H, *J*=7.4 Hz, Fmoc); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 20.5, 20.5, 20.8, 21.4, 23.1, 23.9, 24.3, 26.5, 29.0, 31.0, 31.3, 31.3, 31.8, 44.1, 55.5, 61.7, 66.9, 67.0, 69.0, 70.5, 71.0, 81.4, 101.4, 125.2, 128.0, 128.2, 128.2, 128.4, 128.6, 129.0, 129.0, 136.2, 137.8, 157.1, 170.2, 170.2, 170.4, 173.5; IR (neat, cm^{-1}): 742, 1267, 1450, 1525, 1710, 2100, 2927, 3330; MS: HR-MS (FAB): calcd for $C_{24}H_{26}N_4O_5$ 450.1903 $[M+H]^+$, found 450.1898.

4.1.5. (5*R*)-*N*$^{\varepsilon}$-Benzyloxycarbonyl-5-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-5-hydroxy-L-lysinato-bicyclononylboron (11). A solution of acetobromo galactose (**10**, 1.45 g, 3.53 mmol) in dichloromethane (5 mL) was added dropwise to a stirred solution of **2** (1.02 g. 2.35 mmol) in dichloromethane (10 mL) containing silver silicate (3.2 g) and powdered molecular sieves (3 Å, 0.5 g) in the absence of light at 0 °C. The reaction mixture was stirred overnight under nitrogen at 0 °C. It was filtered and the filterate was concentrated. The residue was purified by flash column chromatography using toluene-acetonitrile as eluent to yield **11** (1.24 g, 68%) as a white amorphous solid. $[\alpha]_D^{20}=+4.9$ (*c* 1.9, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 0.55 (br s, 2H, (B-(C*H*)$_2$), 1.37–1.92 (m, 16H, B-$(CH_2)_6$, H-β, β′, H-γ, γ′), 1.95 (s, 3H, $COCH_3$), 1.97 (s, 3H, $COCH_3$), 2.09 (s, 3H, $COCH_3$), 2.12 (s, 3H, $COCH_3$), 3.30 (m, 2H, H-ε, H-ε′), 3.56–3.71 (m, 2H, H-δ, H-α), 3.82 (t, *J*=6.1 Hz, 1H, H-5), 4.03 (dd, 1H, *J*=7.5, 11.4 Hz, H-6), 4.14 (dd, 1H, *J*=5.4, 11.5 Hz, H-6′), 4.45 (d, 1H, *J*=8.0 Hz, H-1), 4.75–4.92 (m, 1H, NH-α), 4.97 (dd, 1H, *J*=3.3, 10.5 Hz, H-3), 5.07–5.14 (m, 4H, PhCH_2, H-2, NH-α′), 5.35 (d, 1H, *J*=3.0 Hz, H-4), 5.61 (t, 1H, *J*=6.1 Hz, N–Hε), 7.28–7.39 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 20.4, 20.7, 21.3, 23.0, 23.8, 24.3, 26.4, 28.9, 31.0, 31.2, 31.3, 31.7, 44.1, 55.5, 61.7, 66.9, 67.0, 68.9, 70.4, 71.0, 81.3, 101.4, 125.2, 128.0, 128.1, 128.3, 128.6, 128.9, 136.1, 137.8, 157.1, 169.9, 170.0, 170.2, 173.5; HR-MS (FAB): calcd for $C_{36}H_{51}BN_2NaO_{14}$ 769.3326 $[M+Na]^+$, found 769.3347.

4.1.6. (5*R*)-*N*$^{\alpha}$-(Fluoren-9-ylmethoxycarbonyl)-*N*$^{\varepsilon}$-benzyloxycarbonyl-5-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-5-hydroxy-L-lysine (13). Compound **11** (1.24 g, 1.66 mmol) was dissolved in methanol (2 mL) and diluted with chloroform (10 mL). The solution was stirred at room temperature for 12 h, and concentrated. The residue was triturated with hot hexanes and finally with diethyl ether (20 mL). It was dried in vacuo to afford crude **12**. A solution of 9-fluorenylmethoxycarbonyl-*N*-hydroxysuccinimide ester (Fmoc-OSu, 0.68 g, 2 mmol) in acetone (4 mL) was added to a solution of **12** and $NaHCO_3$ (0.152 g, 1.82 mmol) in water (10 mL). The mixture was stirred at room temperature for 5 h and concentrated. Chloroform was added and the mixture was acidified to pH 2 with dilute aqueous HCl at 0 °C. The compound was extracted twice with an additional amount of chloroform (2×15 mL). The combined organic layers were washed with water, dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by flash column chromatography using toluene–ethanol (30:1→10:1→4:1) as eluent to afford **13** (1.28 g, 91% over two steps). Compound **13** had ^{1}H and ^{13}C NMR data identical to those reported previously.[9]

4.1.7. (5*R*)-*N*$^{\varepsilon}$-Benzyloxycarbonyl-5-*O*-*tert*-butyldimethylsilyl-L-lysinato-bicyclononylboron (14). 2,6-Lutidine (0.16 mL, 1.38 mmol) followed by *tert*-butyldimethylsilyl triflouromethanesulfonate (0.19 mL, 0.83 mmol) was added to a stirred solution of **2** (0.30 g, 0.72 mmol) in dichloromethane (2 mL) at 0 °C under nitrogen. After 2 h, diethylether was added and the solution was washed with water (5 mL) followed by aqueous sat. NaCl (5 mL). The organic layer was dried (Na_2SO_4), concentrated and the residue purified by flash column chromatography using toluene–acetonitrile as eluent to afford **14** (0.32 g, 86%) as a yellow oil that solidified upon standing. $[\alpha]_D^{20}$=+5.4 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.06 (s, 6H, $Si(CH_3)_2$), 0.51, 0.61 (br s, each 1H, B-(C*H*)$_2$), 0.87 (s, 9H, $Si(CH_3)_3$), 1.35–1.97 (m, 15H, $B(CH_2)_6$, H-β, H-β′, H-γ), 2.10–2.24 (m, 1H, H-γ′), 3.02–3.14 (m, 1H, H-ε), 3.25–3.37 (m, 1H, H-ε′), 3.54–3.65 (m, 1H, H-α), 3.67–3.76 (m, 1H, H-δ), 4.59 (dd, *J*=8.1, 11.4 Hz, 1H, NH-α), 4.87 (dd, *J*=7.4, 11.2 Hz, 1H, NH-α′), 5.07 (d, *J*=6.0 Hz, 2H, PhC*H*$_2$), 5.14 (t, *J*=6.2 Hz, 1H, NH-ε), 7.27–7.39 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz): δ −4.6, −4.8, 18.1, 20.6, 23.9, 24.3, 25.8, 26.9, 31.0, 31.3, 32.1, 44.6, 55.5, 67.1, 70.8, 127.6, 128.3, 128.7, 136.3, 157.8, 173.1; HR-MS (FAB): calcd for $C_{28}H_{48}BN_2O_5Si$ 531.3420 $[M+H]^+$, found 531.3427.

4.1.8. (5*R*)-*N*$^{\varepsilon}$-Benzyloxycarbonyl-*N*$^{\alpha}$-(fluoren-9-ylmethoxycarbonyl-5-*O*-*tert*-butyldimethylsilyl-L-lysine (16). Compound **14** (0.32 g, 0.60 mmol) was converted to crude **15** using a mixture of $CHCl_3$–MeOH as described above in the synthesis of **13**. Treatment of crude **15** with Fmoc-OSu (0.24 g, 0.72 mmol) and $NaHCO_3$ (0.055 g, 0.663 mmol), as described for the preparation of **13**, gave **16** (0.34 g, 89%) as a white amorphous solid after purification by flash column chromatography using toluene–acetonitrile as eluent. $[\alpha]_D^{20}$=+6.1 (*c* 1.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 360 MHz): δ 0.06 (s, 6H, $Si(CH_3)_2$), 0.87 (s, 9H, $SiC(CH_3)_3$), 1.60 (m, 2H, H-γ), 1.72 (m, 1H, H-β), 1.94 (m, 1H, H-β), 3.15–3.44 (m, 2H, H-ε), 3.60–3.82 (m, 1H, H-δ), 4.20 (t, 1H, *J*=7.0 Hz, Fmoc-C*H*), 4.25–4.50 (m, 2H, Fmoc-C*H*$_2$), 5.01–5.22 (m, 3H, PhC*H*$_2$, NH), 5.59 (br s, 1H, NH), 5.76 (1H, d, *J*=7.6 Hz, H-α), 7.20–7.42 (m, 9H, Ph, Fmoc), 7.50–7.65 (m, 2H, Fmoc), 7.71–7.80 (d, 2H, *J*=7.2 Hz, Fmoc), 9.8 (br s, 1H, COOH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ −4.6, −4.5, 18.1, 25.7, 26.0, 29.9, 30.4, 63.8, 66.1, 67.3, 69.7, 115.6, 119.4, 120.0, 125.1, 126.0, 127.1, 127.7, 128.2, 128.5, 129.3, 132.0, 137.1, 141.3, 143.6, 143.7, 157.8, 168.9, 175.7; MS: HR-MS (FAB): calcd for $C_{35}H_{45}N_2O_7Si$ 633.2991 $[M+H]^+$, found 633.3000.

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References and notes

1. Van Slyke, D. D.; Hiller, A. *Proc. Natl. Acad. Sci. U.S.A.* **1921**, *7*, 185–186.
2. Van Slyke, D. D.; Hiller, A.; Dillon, A.; MacFadyen, D. A. *Proc. Soc. Exp. Biol. Med.* **1938**, *38*, 548–549.
3. Kivirikko, K. I.; Myllylä, R. Post-translational modifications. In *Collagen in Health and Disease*; Weiss, J. B., Jayson, M. I. V., Eds.; Churchill Livingstone: Edinburgh, 1982; pp 101–120.
4. Spiro, R. G. *J. Biol. Chem.* **1967**, *242*, 4813–4823.
5. Broddefalk, J.; Bäcklund, J.; Almqvist, F.; Johansson, M.; Holmdahl, R.; Kihlberg, J. *J. Am. Chem. Soc.* **1998**, *120*, 7676–7683.
6. Holm, B.; Bäcklund, J.; Recio, M. A. F.; Holmdahl, R.; Kihlberg, J. *ChemBioChem* **2002**, *3*, 1209–1222.
7. Bäcklund, J.; Carlsen, S.; Höger, T.; Holm, B.; Fugger, L.; Kihlberg, J.; Burkhardt, H.; Holmdahl, R. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 9960–9965.
8. Broddefalk, J.; Forsgren, M.; Sethson, I.; Kihlberg, J. *J. Org. Chem.* **1999**, *64*, 8948–8953.
9. Holm, B.; Broddefalk, J.; Flodell, S.; Wellner, E.; Kihlberg, J. *Tetrahedron* **2000**, *56*, 1579–1586.
10. Holm, B.; Baquer, S. M.; Holm, L.; Holmdahl, R.; Kihlberg, J. *Bioorg. Med. Chem. Lett.* **2003**, *11*, 3981–3987.
11. Malkar, N. B.; Lauer-Fields, J. L.; Fields, G. B. *Tetrahedron Lett.* **2000**, *41*, 1137–1140.
12. Dent, W. H., III; Erickson, W. R.; Fields, S. C.; Parker, M. H.; Tromiczak, E. G. *Org. Lett.* **2002**, *4*, 1249–1251.
13. Walker, W. H., IV; Rokita, S. E. *J. Org. Chem.* **2003**, *68*, 1563–1566.
14. Alper, P. B.; Hung, S.-C.; Wong, C.-H. *Tetrahedron Lett.* **1996**, *37*, 6029–6032.
15. Ercegovic, T.; Meijer, A.; Magnusson, G.; Ellervik, U. *Org. Lett.* **2001**, *3*, 913–915.
16. Kihlberg, J. O.; Leigh, D. A.; Bundle, D. R. *J. Org. Chem.* **1990**, *55*, 2860–2863.