A practical and scalable synthesis of KRN7000 by using glycosyl iodide as the glycosyl donor

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1. General Information

Unless otherwise noted, all materials and dry solvents were used as received from Adamas-beta® without further purification. $^1$H and $^{13}$C (data from HSQC) NMR spectra were recorded on Varian Mercury 300 MHz, 400 MHz or Bruker 600 MHz spectrometers. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS) ($\delta = 0$). NMR data are presented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, dd = doublet of doublet, m = multiplet and/or multiple resonances), coupling constant in hertz (Hz), integration. All NMR signals were assigned on the basis of $^1$H NMR, $^{13}$C NMR, COSY, HSQC and HMBC experiments. Mass spectra were recorded on a Q-Tof Ultima Global mass spectrometer or a Shimadzu LCMS-IT-TOF mass spectrometer. TLC-analysis was performed on silica gel 60 F$_{254}$ (Huang Hai Inc.) with detection by UV-absorption (254 nm) when applicable, and by spraying with a solution of (NH$_4$)$_6$Mo$_7$O$_{24}$·H$_2$O (25 g·L$^{-1}$) in 5% sulfuric acid in ethanol followed by charring. All reactions were carried out under an argon atmosphere.
2. Synthesis of compound Trimethylsilyl

\[ \text{2,3,4,6-tetrakis-O-trimethylsilyl-\(\alpha\)-D-galactopyranoside} \] (11)

Hexamethyldisilazane (HMDS) (50 mL, 240 mmol) and TMSCl (25 mL, 195 mmol) were added sequentially to a solution of D-galactose (5.0 g, 55.5 mmol) in pyridine (250 mL). The solution was stirred at 75 °C for 1 h under an N\(_2\) atmosphere and then cooled to rt. The mixture was poured into ice-water (250 mL) and extracted with hexane (3 × 150 mL). The combined organic extracts were washed with H\(_2\)O (5 × 150 mL), dried (Na\(_2\)SO\(_4\)) and concentrated under reduced pressure to afford per-silylated galactose 11 as viscous, colorless oil (95% crude), which was used directly in the next step without any further purification.

3. Synthesis of compound (2S, 3S, 4R)-1,3,4-tri-\(\text{tert}\)-butyldimethylsilyloxyoctadecan-1-ol (10)

A solution of phytosphingosine (18.0 g, 43.2 mmol) in CH\(_2\)Cl\(_2\) (600 mL) at 0 °C was treated sequentially with TBSOTf (49.5 mL, 217.2 mmol) and 2,6-lutidine (75 mL). There action mixture was stirred at 0 °C at first, and then warmed to 25 °C and stirred at this temperature for 4 h, after which time, CH\(_3\)OH (150 mL) was added and stirring continued for 10 min. The solvent was then removed under reduced pressure and the residue taken up in Et\(_2\)O (450 mL) and washed sequentially with H\(_2\)O (450 mL), NaHCO\(_3\) solution (450 mL) and brine (450 mL). The organic phase was dried over Na\(_2\)SO\(_4\), filtered and evaporated to give crude compound 10 as colorless oil (98% crude), which was used directly in the next step without any further purification. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 3.80 (d, \(J = 8.4\) Hz, 2H), 3.49 (d, \(J = 6.7\) Hz, 1H), 3.42 (t, \(J = 8.4\) Hz, 1H), 2.86 (s, 1H), 1.23 (s, 29H), 0.87 (s, 36H), 0.06 (s, 9H). ESI-MS: m/z calcd. for C\(_{36}\)H\(_{80}\)Si\(_3\)NO\(_3\)\(^-\) [M-H\(^-\)] 658.6, found 658.6.
4. Synthesis of compound (2S, 3S, 4R)-2-((N-tert-Butoxycarbonyl)amino)-3,4-di-tert-butyldimethylsilyloxyoctadecan-1-ol (4)

TEA (7.20 mL, 51.9 mmol) and Boc₂O (9.9 g, 45.0 mmol) were added sequentially to a crude compound 10 (28.5 g, 43.2 mmol) dissolved in THF (390 mL). After 2 h, the reaction mixture was concentrated under reduced pressure, solved in EtOAc (450 mL) and washed with H₂O (3 × 450 mL) and brine (450 mL). The organic phase was dried over Na₂SO₄, filtered and evaporated to give crude compound 7, which was used directly in the next step without any further purification (98% crude). ESI-MS: m/z calcd. for C₄₈H₈₉Si₅NNaO₅⁺ [M+Na]⁺ 782.6, found 782.6.

A solution of 7 (33.0 g, 51.9 mmol) in THF (600 mL) under a N₂ atmosphere at 0 °C was treated with HF-pyridine (7.80 mL of a 70% solution, 298.5 mmol) in THF–pyridine (41.7 mL, 65:35). The reaction mixture was stirred for 30 min at 0 °C and then allowed to warm to rt. After 1 h, the mixture was quenched by the addition of NaHCO₃ solution (28.5 mL) and stirred for 10 min. The reaction mixture was extracted with EtOAc (2 × 450 mL) and the organic phases were washed with brine (450 mL) and then filtered. Removal of the volatiles under reduced pressure and purification of the residue by flash column chromatography afforded primary alcohol 4 as a light yellowish oil (21.6g, 81% over 3 steps). ¹H NMR (400 MHz, CDCl₃): δ = 5.22 (d, J = 8.3 Hz, 1H), 4.09 (d, J = 7.4 Hz, 1H), 3.85 (s, 1H), 3.73 (s, 2H), 3.60 (s, 1H), 3.01 (s, 1H), 2.02 (s, 1H), 1.67 (s, 1H), 1.41 (s, 10H), 1.23 (s, 26H), 0.88 (d, J = 7.3 Hz, 21H), 0.08 (s, 9H).[¹] ¹³C NMR: (151 MHz, CDCl₃): δ = 79.19, 77.45, 76.06, 63.19, 60.15, 52.26, 34.07, 31.91, 29.66, 28.42, 25.97, 22.67, 20.92, 14.09, -3.78. ESI-MS: m/z calcd. for C₃₅H₇₅Si₂NNaO₅⁺ [M+Na]⁺ 668.5, found 668.5.

5. Synthesis of compound (2S, 3S, 4R)-2-((N-tert-Butoxycarbonyl)amino)-3,4-di-tert-butyldimethylsilyloxy-1-O-(2,3,4,6-tetrakis-O-trimethylsilyl-α-D-galactopyranosyl)octadecane (2)
TMSI (3.25 mL, 23.89 mmol) was added to a solution of per-silylated galactose 11 (13.0 g, 23.89 mmol) in dry CH₂Cl₂ (95 mL) at 0 °C. The reaction mixture was stirred under an N₂ atmosphere for 30 min. The solvent was removed under reduced pressure and the resulting glycosyl iodide intermediate 3 was dissolved in dry CH₂Cl₂ (50 mL) and kept under an N₂ atmosphere. In a separate flask, a mixture of activated 4 Å molecular sieves (16.0 g), n-Bu₄NI (17.55 g, 47.78 mmol), i-Pr₂NEt (15.6 mL, 35.95 mmol) and alcohol 4 (5.14 g, 8.45 mmol) in dry CH₂Cl₂ (95 mL) was prepared and stirred under an N₂ atmosphere at rt for 15 min. The solution of glycosyl iodide 3 in CH₂Cl₂ was then added dropwise over 5 min to this mixture and the resulting mixture was stirred overnight. After removal of the solvent under reduced pressure, Et₂O (130 mL) and H₂O (130 mL) were added and the phases were separated. The organic phase was dried (Na₂SO₄) and then concentrated under reduced pressure. The resulting yellow solid was purified by flash column chromatography (3% EtOAc in hexane with 0.3% TEA) to afford glycoside 2 as a colorless oil (2.40 g, 26%) and recovered 4 as a light yellowish oil (3.93 g, 72%). ¹H NMR (400 MHz, CDCl₃) δ = 5.21 (d, J = 3.9 Hz, 1H), 4.66 (d, J = 2.9 Hz, 1H), 4.12 (q, J = 7.2 Hz, 1H), 3.81 – 3.59 (m, 7H), 3.57 – 3.50 (m, 1H), 3.39 – 3.31 (m, 1H), 1.45 – 1.40 (s, 11H), 1.26 – 1.24 (m, 27H), 0.90 – 0.86 (m, 30H), 0.18 – 0.10 (m, 36H). ESI-MS: m/z calcd. for C₅₃H₁₁₈Si₆NO₁₀⁺ [M+H]⁺ 1096.7, found 1096.7.

6. Synthesis of compound (2S, 3S, 4R)-2-Amino-1-O-(α-D-galactopyranosyl)octadecane-3,4-diol (1)

HCl/MeOH (4M, 10 mL) was added to glycoside 2 (2.8 g 2.55 mmol) at rt. After 40 min, the reaction mixture was concentrated under reduced pressure to afford compound 1 as colorless oil (1.2 g crude), which was used directly in the next step without further purification. ESI-MS: m/z calcd. for C₂₄H₅₀NO₈⁺ [M+H]⁺ 480.4, found 480.4.
7. Synthesis of KRN7000

A solution of hexacosanoic acid (2.4 g, 6.0 mmol) in (COCl)\textsubscript{2} (40 mL) was stirred at 70 °C for 2 h, after which time, the solution was cooled to rt, and the (COCl)\textsubscript{2} was removed under reduced pressure. The resulting crude acyl chloride was dissolved in THF (60 mL) and added, with vigorous stirring, to a solution of amine 1 (1.50 g, 2.50 mmol) in THF/NaOAc (aq.) (8 M) (1:1, 120 mL). Vigorous stirring was maintained for 2 h, after which time, the mixture was left to stand and the phases were separated. The aqueous phase was extracted with THF (2 × 120 mL), and the combined organic phases were evaporated under reduced pressure. Purification of the residue by flash column chromatography (gradient: CHCl\textsubscript{3} to 15% MeOH in CHCl\textsubscript{3}) afforded final product KRN7000 as a white solid (2.26 g, 53% over 2 steps). The physical data matched those previously reported.\textsuperscript{[2]}
SUPPRORTING INFORMATION

Reference:

[1] Jervis, P. J.; Cox, L. R.; Besra, G. S. Synthesis of a Versatile Building Block for the Preparation of 6-N-Derivatized \( \gamma \)-Galactosyl Ceramides: Rapid Access to Biologically Active Glycolipids. *J. Org. Chem.* **2011**, *76*, 320-323.

[2] Ndonye, Rachel M.; Izmirian, Douglas P.; Dunn, Matthew F.; Yu, Karl O. A.; Porcelli, Steven A.; Khurana, A.; Kronenberg, M.; Richardson, S. K.; Howell, A. R. Synthesis and Evaluation of Sphinganine Analogues of KRN7000 and OCH. *J. Org. Chem.* **2005**, *70*, 10260-10270.
8. NMR Spectra

\[ ^1\text{H NMR of 10 (400 M, CDCl}_3) \]
$^1$H NMR of 4 (400 M, CDCl$_3$)
$^{13}$C NMR of 4 (151 M, CDCl$_3$)
$^1$H–$^1$H COSY of 4
ESI of KRN7000

858: [M+H]+
CHEMILY LLC
HPLC REPORT

HPLC of KRN7000