Supporting Information

Lithocholic acid analogues, new and potent α-2,3-sialyltransferase inhibitors

Kai-Hsuan Chang,^a Lenselot Lee,^b Jessica Chen^a and Wen-Shan Li^{*a}

^a Institute of Chemistry, Academia Sinica, Taipei 115, Taiwan; ^bDepartment of Chemistry, National Taiwan Normal University, Taipei 116, Taiwan.

Materials:

All chemicals and buffers were purchased from Sigma, Aldrich, or Acros Organics. All amino acids were purchased from Advanced Chemtech. CMP-Neu5Ac was synthesized starting with sialic acid as described by Kajihara and co-workers¹. Synthesis of lactose acceptor was achieved by procedures similar to those of Halcomb². Rat liver α -2,3-ST was obtained from CalBiochem at a concentration of 3.7 mU/ μ L, stored at – 80 °C and consumed within one week. Melting points (m.p.) were recorded with a capillary melting point apparatus (Electrothermal MEL-TEMP). FT-IR spectra were recorded with Perkin Elmer FT-IR Spectrometer (Paragon 1000) in KBr pellets. ¹H and ¹³C NMR spectra were recorded with Bruker AMX400 or 500 MHz instruments. Proton chemical shifts (δ) are reported in parts per million (ppm) relative to the methine singlet at 7.24 ppm for the residual CHCl₃ in the deuteriochloroform, or the methyl pentet at 3.30 ppm for the residual CHD₂OD in the methanol-d₄. Carbon chemical shifts are reported in parts per million relative to the internal ¹³C signals in CDCl₃ (77.0 ppm) and CD₃OD-d₄ (49.0 ppm). Mass spectra were obtained with a FAB JMS-700 double focusing mass spectrometer (JEOL, Tokyo, Japan), MALDI Voyager DE-PRO (Applied Biosystem Houston, USA) and ESI Finnigan LCQ mass spectrometer (Thermo Finnigan, San Jose, CA, United States) in negative mode. The peptide compounds were purified by reversed phase HPLC (Waters 2695 System with a 996 PDA detector), using Vydac 214TP510 C4 (1 cm × 25 cm) column to a purity greater than 95% as judged by analytical HPLC. The separation procedure was performed using H₂O/0.1% trifluoroacetic acid (TFA) (A) and CH₃CN/0.05% TFA (B) as eluents.

Determination of inhibitor IC₅₀:

Each reaction solution containing 200 mM MES buffer, 100 mM sodium chloride, 0.5

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mM disodium-EDTA, 0.01% Triton X-100, 20 μ M modified disaccharide, 1.4 mU α -2,3-ST and inhibitor (in dimethyl sulfoxide) was adjusted to a total volume of 55 μ L. The solution was incubated at 37 ^oC for 10 min, then 1mM CMP-Neu5Ac (final concentration, 3 μ L) was added to initiate the reaction. The solution was incubated at 37 ^oC until a detectable amount of product was produced. To terminate the enzyme reaction, the solution mixture was quenched by heat at 100 ^oC for 5 min. The activity was followed by measuring the quantity of sialylated disaccharide product at 348 nm by RP-HPLC (Supelco Discovery® HS C18, 5 μ m, 4.6 mm × 25 cm). Retention time of the product was 20.38 min. Assays were performed in triplicate.

Determination of compound 22 K_i : The solution concentration was the same as IC₅₀ determination, after incubating for 10 min, 0.3, 0.15 and 0.037 mM CMP-Neu5Ac was added. The inhibitor concentration was 5, 4.5, 3.5, 2.5 and 0 μ M. Assay was duplicated for each substrate and inhibitor concentration. Velocity was obtained as described under IC₅₀ assay conditions. For each inhibitor concentration, linear relations are generated between the inverse of the velocity and the inverse substrate concentration. The inhibition constant (K_i) value was determined from the secondary plot of Lineweaver-Burk plot, the slope for vertical axis, and inhibitor concentration for horizontal axis. The intercept on the horizontal axis is the absolute value of the K_i .

General procedure for the phosphorylation of steroid:

Tris(*p*-nitrophenyl)phosphate (1.30 mmol) and steroid (1.43 mmol) were dissolved in dichloromethane (20 cm³). 7,11-diazabicyclo[5.4.0]undec-11-ene (1.82 mmol) was added and the reaction was stirred at room temperature for 12 h. The reaction mixture was washed by saturated aqueous sodium bicarbonate several times. The organic extracts were dried by Na₂SO₄, then filtered and concentrated. The resulting pale yellow solid was dissolved in dichloromethane (16 cm³). Methanol (13.00 mmol, 0.54 cm³) and 7,11-diazabicyclo[5.4.0]undec-11-ene (6.5 mmol) were added and the reaction was stirred at room temperature for 15 h. The mixture was washed by saturated aqueous sodium bicarbonate several times. The organic extracts were dried by Na₂SO₄, filtered and concentrated. The residues were purified by silica gel using ethyl acetate and hexane as eluents to afford the product.

General procedure for deprotection of the methoxy of steroidal phosphate:

Phophorylated steroidal compound (0.25 mmol) was dissolved in dichloromethane (1.50 cm³). Bromotrimethylsilane (0.13 cm³, 1.00 mmol) was added and the reaction was stirred at room temperature for 30 min. The reaction was quenched by adding saturated aqueous sodium bicarbonate and the organic solvent was removed by rotary

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evaporation. The resulting mixture was precipitated by dichloromethane and hexane, filtered, concentrated, and dried under vacuum system to afford the product.

General procedure for coupling of succinic anhydride and steroid:

Steroid (0.69 mmol) was dissolved in pyridine (10 cm³). Succinic anhydride (2.07 mmol) was added, followed by 4-(dimethylamino)pyridine (0.69 mmol), and the solution was refluxed for 15h. After removal of pyridine by vacuum pump, the mixture was dissolved in dichloromethane and washed with saturated sodium bicarbonate and 6% HCl. The extracts were dried and evaporated to yield the crude product as sticky oil, which was purified by column chromatography on silica gel using ethyl acetate and hexane to give the product.

General procedure for esterification of the 3-hydroxy lithocholic acid and amino acid:

To a solution of the protected lithocholic acid (0.35 mmol), amino acid (0.45 mmol) and 4-(dimethylamino)pyridine (0.10 mmol) in dichloromethane (8 cm³) was added a solution of dicyclohexylcarbodiimide in dichloromethane (2 cm³). The reaction was stirred at room temperature for 30 min and the solvent was removed by rotary evaporation. The resulting residue was purified by column chromatography on silica gel using ethyl acetate and hexane to afford the product.

General procedure for peptide bond formation of lithocholic acid and amino acid:

To a solution of lithocholic acid (1.33 mmol) and amino acid (1.39 mmol) in dimethylformamide (DMF) (5 cm³) was added diisopropylethylamine (3.99 mmol) and 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) (1.46 mmol). The reaction was stirred at room temperature for 1 hour and the solvent was removed by vacuum system. The mixture was dissolved in dichloromethane (50 cm³) and washed with water (50 cm³) twice. The extracts were dried and evaporated to afford the crude product as sticky oil, which was purified by column chromatography on silica gel using ethyl acetate and hexane to afford the product.

General procedure for deprotection of Fmoc group:

To a solution of the Fmoc-protected steroidal compound (0.15 mmol) in dichloromethane (5 cm^3) was added neat 7,11-diazabicyclo[5.4.0]undec-11-ene (0.15 mmol) dropwise. The reaction was stirred at room temperature for 30 min. The reaction solvent was removed by rotary evaporation to yield the crude product as oil,

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which was purified by flash column using ethyl acetate and hexane to give the product.

General procedure for deprotection of Boc and *t*Bu groups:

A solution of protected compound (0.15 mmol) in TFA (2 cm^3) was treated at room temperature with 2 % water (0.04 cm^3). After 30 min, the reaction solvent was removed by rotary evaporation. The resulting mixture was neutralized with saturated sodium bicarbonate and purified by reverse phase HPLC to afford the pure product.

General procedure for reduction of methyl ester group:

The steroidal methyl ester (0.56 mmol) was dissolved in tetrahydrofuran (1.6 cm³). A solution of lithium aluminium hydride (1.13 mmol) in tetrahydrofuran (0.6 cm³) was added cautiously dropwise over 10 min at 0 $^{\circ}$ C. The reaction mixture was stirred at room temperature for 10 min and quenched by 6% HCl. The mixture was filtered and solvent was removed by rotary evaporation. The resulting solid was purified by column chromatography on silica gel using ethyl acetate and hexane to give the product.

General procedure for synthesis of diphenylphosphate steroid:

To a solution of primary steroidal alcohol (2.02 mmol) in tetrahydrofuran (12 cm³) containing diphenylphosphoryl azide (3.03 mmol) was added neat 7,11-diazabicyclo[5.4.0]undec-11-ene (5.05 mmol) dropwise. The reaction was stirred at room temperature for 10 hours and quenched by 6% HCl. The solvent was removed by rotary evaporation and the mixture was extracted by water and dichloromethane. The extracts were dried and evaporated under reduced pressure to give the crude product, which was purified by flash column using ethyl acetate and hexane to afford the pure product.

General procedure for synthesis of steroidal azide:

Diphenylphosphate steroid (0.841 mmol) was dissolved in 1,4-dioxane (15 cm³) and sodium azide (4.2 mmol), tetrabutyl ammonium iodide (0.084 mmol) and 15-crown-5 (0.1 cm³) were added. The mixture was heated under reflux in an atmosphere of nitrogen for overnight. After the mixture was cooled, the solvent was removed by rotary evaporation and the residue was extracted by water and dichloromethane. The extracts were dried and evaporated under reduced pressure to give the crude product as a powder, which was purified by column chromatography on silica gel using ethyl acetate and hexane to obtain the product.

General procedure for synthesis of steroidal 1,4-disubstituted 1,2,3-triazole:

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Steroidal azide (0.26 mmol) and alkyne (0.283 mmol) were dissolved in tetrahydrofuran and water (4 cm³, 1:1). Copper sulfate (catalytic amount) and sodium ascorbate (catalytic amount) were added and the reaction was stirred at room temperature for 3 hours. The solvent was removed by rotary evaporation and the mixture was extracted by water and dichloromethane. The extracts were dried and evaporated under reduced pressure to give the crude powder, which was purified by column chromatography on silica gel using ethyl acetate and methanol to afford the product.

Compound 5. m.p. = 140-141 °C; $\delta_{\rm H}(400 \text{ MHz}, \text{CDCl}_3) 4.15 (1 \text{ H, br}), 2.43 (1 \text{ H, dd}, J 8.9 and 19.3), 2.04-1.83 (3 H, m), 1.82-1.63 (4 H, m), 1.62-1.38 (5 H, m), 1.35-1.06 (7 H, m), 1.08-0.92 (2 H, m), 0.83 (3 H, s), 0.80 (3 H, s), 0.64(1 H, m). <math>\delta_{\rm C}(100 \text{ MHz}, \text{CDCl}_3) 220.5, 54.5, 51.5, 47.7, 44.7, 36.9, 35.7, 35.7, 35.4, 35.0, 31.6, 30.9, 29.1, 28.3, 21.7, 20.5, 13.8, 13.8, 12.2; IR (KBr, v in cm⁻¹): 2937, 2848, 1745, 1380, 1207, 1026; HRMS-FAB: calcd for C₁₉H₃₂O₅P (M+H)⁺, 371.1987; found, 371.1982.$

Compound 6. m.p. = 182-183 °C; $\delta_{H}(400 \text{ MHz}, \text{CDCl}_{3})$ 5.01 (1 H, m), 2.64 (2 H, m), 2.59 (2 H, m), 2.41 (1 H, q, *J* 8.6), 2.04 (1 H, m), 1.90 (1 H, m), 1.80-1.40 (11 H, m), 1.27-1.12 (7 H, m), 0.97 (1 H, m), 0.82 (3 H, s), 0.77 (3 H, s); $\delta_{C}(100 \text{ MHz}, \text{CDCl}_{3})$ 221.5, 177.8, 171.4, 70.5, 54.2, 51.4, 47.8, 40.0, 35.8, 35.8, 34.9, 32.8, 32.7, 31.5, 30.7, 29.3, 29.0, 28.0, 25.9, 21.8, 20.0, 13.7, 11.3; IR (KBr, v in cm⁻¹): 3130, 2926, 2852, 1730, 1447, 1405, 1345, 1249,1160; HRMS-FAB: calcd for C₂₃H₃₄O₅Na (M+Na)⁺, 413.2304; found, 413.2303.

Compound 7.³ $\delta_{\rm H}(400 \text{ MHz, CDCl}_3) 3.51 (3 \text{ H, m}), 2.02 (2 \text{ H, m}), 1.97-1.72 (4 \text{ H, m}), 1.70-1.53 (3 \text{ H, m}), 1.50-1.23 (13 \text{ H, m}), 1.21-1.04 (6 \text{ H, m}), 0.95 (6 \text{ H, m}), 0.69 (3 \text{ H, s}); <math>\delta_{\rm C}(100 \text{ MHz, CDCl}_3) \delta77.2, 71.9, 63.6, 56.5, 56.2, 42.7, 42.1, 40.4, 40.2, 35.9, 35.6, 35.3, 34.6, 31.8, 30.6, 29.4, 28.3, 27.2, 26.4, 24.2, 23.4, 20.8, 18.6, 12.0.$

Compound 8. $\delta_{\rm H}(400 \text{ MHz}, {\rm CDCl}_3)$ 7.33-7.29 (4 H, m), 7.21-7.13 (6 H, m), 4.70-4.68 (1 H, m), 4.22-4.16 (2 H, m), 1.99 (3 H, s), 1.90-1.74 (7 H, m), 1.70-1.33 (10 H, m), 1.03-0.99 (10 H, m), 0.86-0.84 (6 H, m), 0.59 (3 H, s); $\delta_{\rm C}(100 \text{ MHz}, {\rm CDCl}_3)$ 150.7, 129.8, 125.3, 120.1 (d, *J* 4.7), 71.9, 70.0, 56.6, 56.1, 42.8, 42.2, 40.5, 40.2, 36.5, 35.9, 35.4, 35.3, 34.6, 31.5, 30.6, 28.3, 27.3, 26.9, 26.5, 24.2, 23.4, 20.9, 18.6, 12.1; HRMS-FAB: calcd for C₃₆H₅₂O₅P (M+H)⁺, 595.3539; found, 595.3552.

Compound 9. m.p. = 235-236 °C; $\delta_{\rm H}(400 \text{ MHz}, \text{CD}_3\text{OD}) 3.75(2 \text{ H}, \text{ s}) 3.54 (1 \text{ H}, \text{ m}),$

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2.30 (1 H, m), 2.15 (1 H, m), 2.01 (1 H, m), 1.96-1.70 (5 H, m), 1.68-1.55 (2 H, m), 1.50-1.20 (13 H, m), 1.20-1.00 (5 H, m), 0.96 (3 H, d, *J* 6.5), 0.94 (3 H, s), 0.68 (3 H, s); $\delta_{\rm C}(100 \text{ MHz}, {\rm CD}_3{\rm OD})$ 174.6, 174.6, 70.5, 56.0, 55.6, 42.4, 42.0, 41.7, 40.0, 39.7, 35.4, 35.3, 35.0, 34.6, 33.8, 32.2, 31.2, 29.3, 27.4, 26.5, 25.8, 23.4, 22.1, 20.1, 17.1, 10.7; IR (KBr, v in cm⁻¹): 3423, 2939, 2863, 1623, 1589, 1422; HRMS-FAB: calcd for C₂₆H₄₃O₄NNa (M+Na)⁺, 456.3090; found, 456.3095.

Compound 10. m.p. = 188-189 °C; $\delta_{\rm H}(500 \text{ MHz, CD}_3\text{OD})$ 3.35 (1 H, m), 2.78 (2 H, ddd, *J* 5.5, 16.3 and 18.0), 2.29 (1 H, m), 2.15 (1 H, m), 2.01 (1 H, m), 1.90 (2 H, m), 1.82-1.72 (3 H, m), 1.60 (2 H, m), 1.50-1.06 (15 H, m), 0.96 (3 H, d, *J* 6.5), 0.94 (3 H, s), 0.69 (3 H, s); $\delta_{\rm C}(125 \text{ MHz, CD}_3\text{OD})$ 176.5, 176.4, 174.8, 72.6, 58.0, 57.6, 50.8, 44.0, 43.7, 42.0, 41.7, 38.2, 37.4, 37.3, 36.9, 36.6, 35.8, 34.0, 33.2, 31.3, 29.3, 28.5, 27.8, 25.4, 24.0, 22.1, 19.0, 12.6; IR (KBr, v in cm⁻¹): 3461, 3321, 2937, 2870, 1727, 1657, 1528, 1454, 1369, 1155; HRMS-FAB: calcd for C₂₈H₄₆O₆N (M+H)⁺, 492.3325; found, 492.3324.

Compound 11.⁴ $\delta_{\rm H}(400 \text{ MHz}, \text{CDCl}_3)$ 2.66(1 H, t, J 13.7), 2.38-2.08 (4 H, m), 2.05-1.96 (3 H, m), 1.90-1.71(4 H, m), 1.57 (1 H, m), 1.50-1.12 (16 H, m), 0.98 (3 H, s), 0.90 (3 H, d, J 6.4), 0.65 (3 H, s); $\delta_{\rm C}(100 \text{ MHz}, \text{CDCl}_3)$ 213.6, 180.0, 56.3, 55.9, 44.2, 42.7, 42.2, 40.7, 40.0, 37.1, 36.9, 35.4, 35.2, 34.8, 30.9, 30.7, 28.0, 26.5, 25.7, 24.1, 22.5, 21.5, 18.2, 12.0.

Compound 12. m.p. = 229-230 °C; $\delta_{\rm H}(500 \text{ MHz}, \text{CD}_3\text{OD/CDCl}_3)$ 4.69 (1 H, m), 2.57 (4 H, m), 2.32 (1 H, m), 2.21 (1 H, m), 2.00 (1 H, m), 1.94-1.38 (15 H, m), 1.37-1.02 (11 H, m), 0.93 (6 H, m), 0.67 (3 H, s). $\delta_{\rm C}(125 \text{ MHz}, \text{CD}_3\text{OD/CDCl}_3)$ 178.1, 175.8, 173.7, 76.0, 57.6, 57.1, 43.7, 43.1, 41.6, 41.2, 36.9, 36.4, 35.9, 35.5, 33.1, 32.1, 31.9, 30.4, 29.8, 29.1, 28.0, 27.5, 27.3, 25.1, 23.9, 21.8, 18.8, 12.6; IR (KBr, v in cm⁻¹): 2937, 2863, 1727, 1705, 1443, 1421, 1384, 1329, 1288, 1192, 1185; HRMS-FAB: calcd for C₂₈H₄₅O₆ (M+H)⁺, 477.3220; found, 477.3216.

Compound 13. m.p. = 208-209 °C; $\delta_{\rm H}(400 \text{ MHz, CD}_3\text{OD})$ 4.26 (1 H, dd, *J* 1.5 and 6.0), 2.97 (2 H, ddd, *J* 6.0, 13.2 and 17.9), 2.31 (1 H, m), 2.21 (1 H, m), 2.06-1.68 (7 H, m), 1.63-1.41 (9 H, m), 1.39-1.02 (10 H, m), 0.98 (3 H, s), 0.95 (3 H, d, *J* 6.5), 0.70 (3 H, s); $\delta_{\rm C}(100 \text{ MHz, CDCl}_3)$ 178.2, 173.1, 169.1, 78.7, 58.0, 57.6, 50.9, 44.0, 43.4, 41.9, 41.6, 37.3, 36.8, 36.0, 35.8, 35.1, 33.2, 32.4, 32.1, 29.3, 28.2, 27.6, 27.5, 25.3, 23.8, 22.0, 28.8, 12.6; IR (KBr, v in cm⁻¹): 3439, 3070, 2937, 2863, 1741, 1671, 1524, 1443, 1244, 1203, 1185; HRMS-FAB: calcd for C₂₈H₄₆O₆N (M+H)⁺, 492.3325; found, 492.3332.

Compound 14. m.p. = 163-164 °C; $\delta_{\rm H}(400 \text{ MHz}, \text{CDCl}_3) 4.71 (1 \text{ H, m}), 2.64 (2 \text{ H, m}), 2.57 (2 \text{ H, m}), 2.27 (1 \text{ H, m}), 2.10 (1 \text{ H, m}), 1.95 (1 \text{ H, m}), 1.85-1.50 (8 \text{ H, m}), 1.41 (9 \text{ H, s}), 1.40-0.90 (16 \text{ H, m}), 0.87 (6 \text{ H, m}), 0.61 (3 \text{ H, s}); <math>\delta_{\rm C}(100 \text{ MHz}, \text{CDCl}_3) 177.5, 173.7, 171.6, 79.8, 74.9, 56.4, 56.0, 42.7, 41.9, 40.4, 40.1, 35.7, 35.2, 34.9, 34.5, 32.5, 32.1, 31.0, 29.2, 28.9, 28.1, 28.1, 27.0, 26.5, 26.3, 24.1, 23.3, 20.8, 18.2, 12.0; IR (KBr, v in cm⁻¹): 2936, 2863, 1735, 1719, 1421, 1367, 1334, 1266, 1183, 1152; HRMS-FAB: calcd for <math>C_{32}H_{53}O_6 (M+H)^+$, 533.3842; found, 533.3853.

Compound 15. m.p. = 214-215 °C; $\delta_{\rm H}(400 \text{ MHz}, \text{CD}_3\text{OD}) 4.70 (1 \text{ H, m}), 3.88 (2 \text{ H, s}), 2.56 (4 \text{ H, m}), 2.28 (1 \text{ H, m}), 2.15 (1 \text{ H, m}), 2.03 (1 \text{ H, m}), 1.98-1.72 (5 \text{ H, m}), 1.76-1.32 (9 \text{ H, m}), 1.32-0.98 (12 \text{ H, m}), 0.97 (6 \text{ H, m}), 0.69 (3 \text{ H, s}); <math>\delta_{\rm C}(100 \text{ MHz}, \text{CD}_3\text{OD}) 177.3, 176.1, 173.9, 173.2, 76.2, 71.7, 58.0, 57.6, 44.0, 43.5, 41.9, 41.6, 37.3, 36.9, 36.2, 35.8, 33.9, 33.5, 33.2, 30.6, 30.0, 29.3, 28.3, 27.7, 27.7, 25.4, 23.9, 22.1, 19.0, 12.6; IR (KBr, v in cm⁻¹): 3365, 2929, 2863, 1745, 1734, 1708, 1631, 1557, 1450, 1413, 1332, 1211, 1188, 1166; HRMS-FAB: calcd for C₃₀H₄₈O₇N (M+H)⁺, 534.3431; found, 534.3426.$

Compound 16. m.p. = 173-174 °C; $\delta_{\rm H}(400 \text{ MHz}, \text{CD}_3\text{OD})$ 4.72 (1 H, m), 2.81 (2 H, m), 2.56 (4 H, m), 2.29 (1 H, m), 2.18 (1 H, m), 2.03 (1 H, m), 1.98-1.76 (5 H, m), 1.70-1.38 (11 H, m), 1.37-1.02 (13 H, m), 0.95 (6 H, m), 0.69 (3 H, s); $\delta_{\rm C}(125 \text{ MHz}, \text{CD}_3\text{OD})$ 176.7, 176.1, 174.2, 174.0, 174.0, 76.2, 58.0, 57.6, 50.3, 44.0, 43.5, 41.9, 41.6, 37.3, 37.0, 36.9, 36.2, 35.8, 34.0, 33.4, 33.2, 30.6, 29.9, 29.3, 28.3, 27.7, 27.7, 25.4, 23.9, 22.1, 19.00, 12.6; IR (KBr, v in cm⁻¹): 3386, 2926, 2863, 1735, 1711, 1628, 1385, 1186; HRMS-FAB: calcd for C₃₂H₅₀O₉N (M+H)⁺, 592.3486; found, 592.3498.

Compound 17. m.p. = 159-160 °C; $\delta_{\rm H}(400 \text{ MHz, CD}_3\text{OD})$ 4.21 (1 H, dd, *J* 1.7 and 6.0), 2.89 (2 H, ddd, *J* 6.0, 12.9 and 17.9), 2.30 (1 H, m), 2.20 (1 H, m), 2.02 (1 H, m), 1.98-1.71 (7 H, m), 1.64-1.52 (3 H, m), 1.50-1.38 (8 H, m), 1.37-1.03 (12 H, m), 0.97 (3 H, s), 0.95 (3 H, d, *J* 6.5), 0.69 (3 H, s); $\delta_{\rm C}(125 \text{ MHz, CD}_3\text{OD})$ 178.3, 173.7, 169.3, 78.6, 57.9, 57.6, 51.1, 44.0, 43.4, 41.9, 41.6, 37.3, 36.8, 36.0, 35.8, 35.5, 33.1, 32.4, 32.1, 29.3, 28.2, 27.6, 27.5, 25.3, 23.8, 22.0, 18.8, 12.5; IR (KBr, v in cm⁻¹): 3434, 3069, 2939, 2864, 1742, 1670, 1522, 1441, 1240, 1188; HRMS-FAB: calcd for C₂₈H₄₆O₆N (M+H)⁺, 492.3325; found, 492.3324.

Compound 18. m.p. = 235-236 °C; $\delta_{\text{H}}(400 \text{ MHz}, \text{CDCl}_3)$ 7.40 (1 H, s), 4.28-4.23 (2 H, m), 3.91 (1 H, s), 3.60-3.57 (1 H, m), 2.94-2.91 (2 H, m), 2.32 (2 H, s), 1.92-1.70 (8 H, m), 1.65-1.34 (9 H, m), 1.30-0.87 (11 H, m), 0.87-0.86 (6 H, m), 0.59(3 H, s); $\delta_{\text{C}}(100 \text{ MHz})$

MHz, CDCl₃) 147.1, 121.5, 71.9, 61.6, 56.5, 55.9, 51.0, 42.8, 42.1, 40.5, 40.2, 36.5, 35.9, 35.4, 35.3, 34.6, 32.7, 30.6, 28.7, 28.3, 27.2, 27.0, 26.5, 24.2, 23.4, 20.9, 18.6, 12.1; IR (KBr, v in cm⁻¹): 3392, 2931, 2857, 1733, 1453, 1376, 1048; HRMS-FAB: calcd for $C_{28}H_{48}O_2N_3$ (M+H)⁺, 458.3737; found, 458.3747.

Compound 19. m.p. = 151-152 °C; $\delta_{\rm H}(500 \text{ MHz}, \text{CD}_3\text{OD})$ 7.86 (1 H, s), 4.35 (2 H, m), 4.32 (1 H, dd, *J* 5.2 and 7.1), 3.54 (1 H, m), 3.39 (1 H, dd, *J* 5.1 and 15.7), 3.29 (1 H, dd, *J* = 5.1 and 15.7), 2.05-1.56 (9 H, m), 1.52-1.02 (19 H, m), 0.94 (6 H, m), 0.68 (3 H, s); $\delta_{\rm C}(125 \text{ MHz}, \text{CD}_3\text{OD})$ 170.9, 142.5, 124.8, 72.5, 58.0, 57.5, 54.9, 53.8, 51.9, 44.0, 43.6, 42.0, 41.6, 37.3, 36.7, 36.6, 35.8, 33.8, 31.3, 29.4, 28.4, 28.0, 27.8, 27.3, 25.3, 24.0, 22.0, 19.1, 12.5; HRMS-FAB: calcd for C₂₉H₄₉O₃N₄ (M+H)⁺, 501.3805; found, 501.3810.

Compound 20. decomposed at 285 °C; $\delta_{\rm H}(400 \text{ MHz, CDCl}_3)$ 7.91 (1 H, s), 4.5-4.42 (2 H, m), 4.33-4.24 (4 H, m), 3.73-3.67 (1 H, m), 3.49-3.40 (1 H, m), 3.35-3.19 (2 H, m), 2.12-1.99 (4 H, m), 1.92-1.63 (7 H, m), 1.63-1.49 (6 H, m), 1.47-1.42 (6 H, m), 1.38-1.11 (7 H, m), 1.06-1.05 (2 H, m), 0.78 (3 H, s). $\delta_{\rm C}(100 \text{ MHz, CDCl}_3)$ 181.1, 174.0, 146.9 (d, *J* 18), 124.2, 72.4, 64.2 (dd, *J* 6.7 and 19.4), 57.7, 57.2, 51.8, 49.9, 43.8, 43.3, 41.7, 41.4, 37.1, 36.9, 36.5 (d, *J* 2.2), 36.4, 35.6, 33.6, 31.0, 29.2, 28.3, 27.8, 27.6, 25.2, 25.1 (d, *J* 2.9), 24.4, 24.1, 21.9, 19.2, 16.8, 16.7 (d, *J* 6); IR (KBr, v in cm⁻¹): 3431, 2929, 2863, 1605, 1447, 1376, 1222, 1163, 1048, 1026, 975; HRMS-FAB: calcd for C₃₃H₅₆O₆N₃PNa (M+Na)⁺, 644.3798; found, 644.3804.

Compound 21. m.p. = 164-165 °C; $\delta_{H}(400 \text{ MHz, CDCl}_{3}/\text{ CD}_{3}\text{OD})$ 7.36 (1 H, s), 4.17-4.12 (2 H, m), 3.54-3.51 (1 H, m), 2.85-2.83 (2 H, m), 2.44 (2 H, m), 1.88-1.30 (17 H, m), 1.19-0.89 (11 H, m), 0.84- 0.82(6 H, m), 0.56 (3 H, s); $\delta_{C}(100 \text{ MHz, D}_{2}\text{O}/\text{CD}_{3}\text{OD})$ 180.1, 147.2, 121.1, 71.0, 56.1, 55.6, 50.4, 42.3, 41.7, 40.1, 39.8, 36.0, 35.6, 35.5, 34.9, 34.9, 34.2, 32.2, 29.7, 27.8, 26.8, 26.6, 26.0, 23.8, 22.9, 21.2, 20.4, 18.0, 11.5; IR (KBr, v in cm⁻¹): 3386, 2934, 2857, 1560, 1447, 1368, 1218, 1161, 1065, 1039; HRMS-FAB: calcd for C₂₉H₄₈O₃N₃ (M+H)⁺, 486.3686; found, 486.3696.

Compound 22. m.p. = 150-151 °C; $\delta_{\text{H}}(400 \text{ MHz}, \text{CDCl}_3)$ 7.34 (1 H, s), 4.23-4.18 (2 H, m), 3.59-3.55 (3 H, m), 2.67 (2 H, t, *J* 7.2), 2.28-2.26 (2 H, m), 1.91-1.34 (10 H, m), 1.34-1.22 (9 H, m), 1.22-0.99 (9 H, m), 0.87-0.86 (6 H, m), 0.59 (3 H, s); $\delta_{\text{C}}(100 \text{ MHz}, \text{CDCl}_3)$ 180.2, 148.1, 122.0, 78.2, 72.7, 57.4, 56.7, 51.7, 43.6, 43.0, 41.3, 41.1, 37.3, 36.7, 36.3, 36.2, 35.5, 35.4, 33.6, 31.4, 29.2, 28.1, 27.9, 27.3, 25.9, 25.7, 25.1, 24.3, 21.7, 19.5, 13.0; IR (KBr, v in cm⁻¹): 3387, 2936, 2858, 1565, 1450, 1362, 1212,; HRMS-FAB: calcd for C₃₀H₅₀O₃N₃ (M+H)⁺, 500.3846; found, 500.3852.

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Supplementary Material (ESI) for Chemical Communications # This journal is (c) The Royal Society of Chemistry 2006

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 10 NWR plot parameters

 CX
 22.00 cm

 CY
 14.36 cm

 F1P
 100.000 pm

 F1
 4001.30 Hz

 F2P
 -0.500 pm

 F2P
 -0.500 pm

 F2P
 -20.06 Hz

 F2PMCM
 0.47727 ppm/cm

 HZCM
 190.97112 Hz/cm
6410.256 Hz 0.195625 Hz 2.5559540 sec 64 78.000 usec 111.43 usec 300.0 K 3 dB 1.0000000 sec 400.131956 MHz 1H F2 - Processing parameters SI 2768 SF 400.1300179 MHz WDM 600.13001 0 SSB 0.00 Hz CB 0.00 Hz CB 1.00 on Parameters 20050202 6.58 spect MHZ Current Data Parameters NAME KS-Lith-Su-tBu EXPNO 1 PROCNO 1 29 32768 CDC13 24 mm Multinu F2 - Acquisition Date_____20 ហ P1 SF01 NUCLEUS Time INSTRUM PROBHD PULPROG TD SOLVENT NS SOLVENT SWH SWH FIDRES 요동문무뉴필문 88519.0-16578.0 £9968.0-Fo 85166.0 S11E0.1-1.05102 3.155 8E970.1-Þ/G.3 92291.1 60861.1 16.734 1.22896 15.444 4.37229 674.8 -1.41532 62684.1 660.1 പ 666'0 1.52174 670.0 22997.1-E1167.1-5.140 66918.1-2.044 84948.1 -m 1.95249 5.56873 -2,58442 -S.63420 2.64963 4 01469.4-1.000 4.72101 -<u></u>... -0 4 -> 7.24010 œ 0 -თ C - mdd wdd Integral















cm cm ppm Hz Hz Hz ppm/cn Hz/cm F2 - Processing parameters SI 32768 SF 400.1300176 MHz MDW 00 SSB 0.00 Hz CLB 0.00 Hz GB 0.00 Hz GB 7.00 Hz nsec K dB sec WHz F2 - Acquisition Parameters Date_____20050514 Time_____20050514 Time______26 INSTRUM 5 mm Multinu PULPHOG 5 mm Multinu PULPHOG 32768 SULVENT 00513 NS 16 DS 16 SSWH 6410.25 Hz SWH 0.195625 Hz MHZ Hz Hz Sec
 10 NMR plot parameters
 22.00 cn

 CX
 22.00 cn

 CY
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 F1P
 10.101.30 Hz

 F2P
 -0.500 pr

 F2P
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 F2P
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 F2
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6410.256 F 0.195625 F 2.5559540 € 78.000 u 111.43 u 111.43 u 300.0 3 d Current Data Parameters NAME len-lith-3buty EXPNO 1 PROCNO 1 6.00 L 1.00000000 ÷ AQ BG DW DW DE TE d1 f1 SF01 SF01 NUCLEUS 91969.0-89698.0-*14778.0-*Fo 18288.0-68569.0-01800.1-3.032 1.02054 810.3 01060.1-10.948 1.04306 77630.1-**315.01** 87970.1--1.34103 070.8 52098.1--N 06985.1-01807.1-3.444 1.72894 ₽99£7.1-87487.1-2.011 -m EE697.1-7 -1.92392 -2.32813 1.000 -5.91254 -5.92647 713.1 -5.94035 96878.6-2.007 -3.59062 -3.60248 08619.6-71755.4-~ഗ 4.25499 4.26819 4.28447 -0 8 11045.7-0.992 17907.7--00 -თ Ê - mdd wdd ísnegrai















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