PERKIN 1

Practical syntheses of [^{13}C]- and [^{14}C]-labelled glucosphingolipids

Gordon R. Duffin,† George J. Ellames, Sascha Hartmann, John M. Herbert * and David I. Smith

Isotope Chemistry and Metabolite Synthesis Department, Sanofi-Synthélabo, Willowburn Ave., Alnwick, Northumberland, UK NE66 2JH

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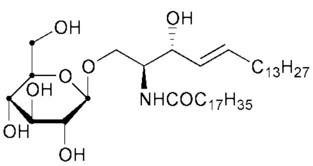
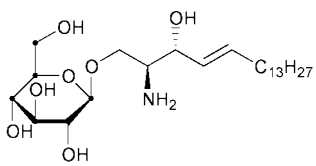
Synthetic routes to [*glucose*-U-^{14}C]-1-*O*-(β-D-glucopyranosyl)-*N*-stearoyl-D-*erythro*-sphingosine **1b** and to [*glucose*-$^{13}C_6$]-1-*O*-(β-D-glucopyranosyl)sphingosine ([*glucose*-$^{13}C_6$]glucopsychosine, **2b**) are described. Whereas the protected ceramide precursor for **1b** was prepared using conventional methodology, two new strategies were developed in the course of the synthesis of **2b**. Of these, one relies on keeping a protecting group in place at all times to avoid the handling difficulties associated with sphingosine **4**, while the other generates a protected derivative (**24**) of sphingosine indirectly by means of a Mitsunobu inversion.

Deficiency in the activity of the enzyme glucocerebrosidase, which cleaves glucosylceramide **1a**, results in an accumulation of **1a** in human spleen, characteristic of the inherited lipidosis disorder, Gaucher's disease.[1] In contrast, 1-*O*-β-D-glucosylsphingosine (glucopsychosine, **2a**) is a potent inhibitor of the same enzyme.[2] In order to facilitate pharmacological studies involving mammalian glucocerebrosidase, supplies of [^{14}C]glucosylceramide **1b** were needed, while a multiple [^{13}C]-labelled form, such as **2b**, of glucopsychosine was required for use as an internal standard in mass spectrometric assays.

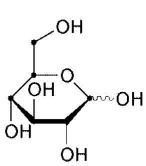


1a (no label)
1b (• denotes U-^{14}C)

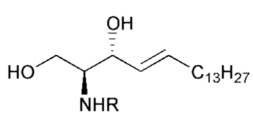


2a (no label)
2b (• denotes ^{13}C)

With U-^{14}C and $^{13}C_6$-labelled forms of glucose (**3a** and **3b** respectively) available commercially, glucosylation of protected forms of D-*erythro*-sphingosine **4** and of the corresponding stearamide (ceramide, **5**) would provide a convenient approach to **2b** and **1b** respectively, with the labelled moiety being introduced at a late stage of the synthesis in both cases. Such an approach has been used to prepare **2a**[3] and lower homologues of **1a**[4,5] but, to date, [^{14}C]glucosylceramide has been prepared only in very small quantities by glucocerebrosidase-mediated coupling of 4-methylumbelliferyl-β-D-glucose (as a glucose donor) with [^{14}C]-labelled **5**,[6] an approach which would not have provided the quantities required.



3a (U-^{14}C). **3b** ($^{13}C_6$)

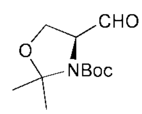


4 (R = H)
5 (R = $COC_{17}H_{35}$)

† *Current address*: Development Centre, ChiRex, Cramlington, Northumberland, UK NE23 7QG.

Results and discussion

Since only limited quantities of **4** are available commercially, its synthesis was necessary. Of the various available routes to **4**,[7] that described by Garner *et al.*[8] was chosen, and initial results were satisfactory. This route involves addition of pentadec-1-ynyllithium to the aldehyde **6** to give the acetylenic alcohol **7** in a moderate diastereomeric excess. The enantiomeric excess of this material was typically between 92 and 95%, as determined by ^{19}F NMR spectroscopy of the ester with (*R*)-MTPA.‡ Dissolving metal reduction of **7** (lithium–ethylamine) and acid hydrolysis provided **4**. In the course of resyntheses, however, several side-products were observed of which the urea **8**§ and the reductive cleavage product **9**¶ were identified. The over-reduction product **10** was produced in small quantities, but proved to be extremely difficult to separate from **4**. A modified route, which we found to be more reliable, involved partial deprotection of **7** to give the Boc-protected species **11**.[9] Red-Al



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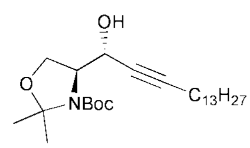

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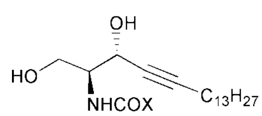
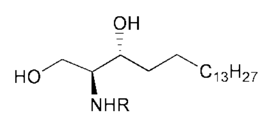


8 (X = NHEt)
11 (X = O-*t*-Bu)



9 (R = *i*-Pr)
10 (R = H)

‡ The ^{19}F NMR spectrum ($CDCl_3$) of the (*S*)-MTPA ester typically contains two signals (attributed to two rotational isomers) arising from the desired diastereomer at δ −71.48 and −71.60, the corresponding signals arising from the minor isomer being observed at δ −71.76 and −72.27.

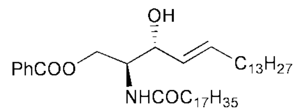
§ Data for **8**: mp 120–121 °C; δ_H($CDCl_3$) 0.90 (3H, t), 1.15 (3H, t), 1.2–1.45 (20H, m), 1.52 (2H, m), 2.22 (2H, t), 3.21 (2H, q), 3.55 (4H, br s), 3.71 (1H, dd), 3.78 (1H, dd), 3.86 (1H, dd), 4.54 (1H, d); *m/z* (FAB$^+$) 369 ([MH]$^+$, 100%), 351 ([MH − H_2O]$^+$); HRMS 369.3112 (Calc. for $C_{21}H_{41}N_2O_3$: *m/z* 369.3117).

¶ Data for **9**: mp 44–47 °C; δ_H($CDCl_3$) 0.85 (3H, t), 1.1–1.4 (34H, m), 1.47 (2H, m), 2.81 (1H, m, NC**H**Me_2), 3.26 (1H, m), 3.80 (2H, m), 3.87 (1H, m), 4.16 (3H, br s); *m/z* (CI$^+$) 344 (100%), 102, 60. HRMS 344.3526 (MH$^+$) (Calc. for $C_{21}H_{46}NO_2$: *m/z*, 344.3529). This type of process has been reported previously under other reductive conditions, *e.g.* ref. 27.

Table 1 Acylation conditions for the conversion of sphingosine **4** into 3-*O*-benzoyl-*N*-stearoyl-D-*erythro*-sphingosine **14**

Acylation conditions	Yield (%)
Stearic acid, HBTU[a]–HOBt, Pr^i_2NEt, DCM, room temp.	47
Stearic acid, TPTU[b]–HOBt, Pr^i_2NEt, DCM, room temp.	28
Stearic acid, BOP, Et_3N, THF, room temp.	70
4-Nitrophenyl stearate, DCM (ref. 11)	53
Stearic acid *N*-hydroxysuccinimide ester (ref. 12)	27
Stearoyl chloride, aq. NaOH, DCM, room temp.	0
Stearoyl chloride, pyridine[c]	26
Stearic anhydride, pyridine	46

[a] HBTU refers to *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate. [b] TPTU refers to *O*-(1,2-dihydro-2-oxo-1-pyridyl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate. [c] Using this method, even with a single molar equivalent of stearoyl chloride, diacyl products were isolated also, and starting material was recovered.



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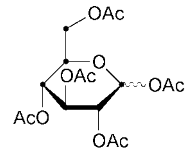
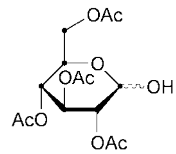
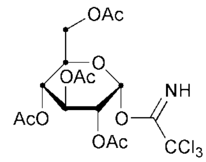


16a (• denotes U-^{14}C)
16b (• denotes ^{13}C)
16c (unlabelled)



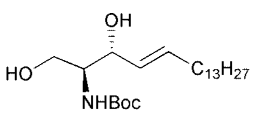
17a (• denotes U-^{14}C)
17b (• denotes ^{13}C)
17c (unlabelled)



18a (• denotes U-^{14}C)
18b (• denotes ^{13}C)
18c (unlabelled)

reduction[9] of this intermediate proceeded smoothly to give Boc-D-*erythro*-sphingosine **12** in 90% yield; exposure of **12** to hydrogen chloride in methanol resulted in acid hydrolysis to provide **4** (89%).[10]

Several methods were compared for the acylation of **4** to form ceramide **5** (Table 1), of which the best yielding proved to be a coupling using benzotriazol-1-yloxytris(dimethylamino)-phosphonium hexafluorophosphate (BOP). The major drawback of this method proved to be that HMPA formed in the reaction could not be removed completely from the product, although this did not interfere with subsequent steps. Protection of **5** was carried out in the manner described by Schmidt and Kläger[4] for the corresponding palmitamide. Thus, protection of the primary alcohol as the trityl ether, followed by benzoylation of the secondary alcohol, gave the fully protected material **13**, which was detritylated with acid to provide the known intermediate **14**.[13] A protection sequence[14] whereby the



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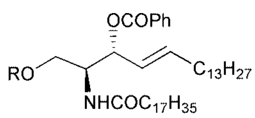
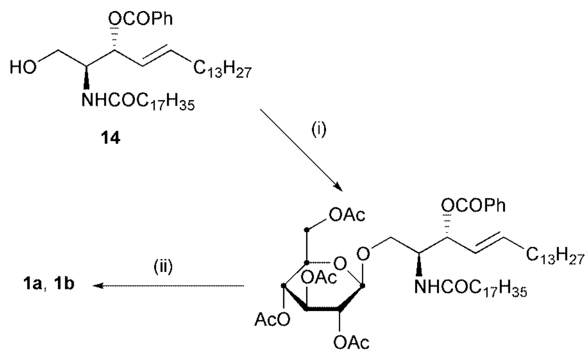


13 (R = CPh_3)
14 (R = H)

primary alcohol is protected as a *tert*-butyldiphenylsilyl ether rather than a trityl ether was examined, but was abandoned since removal of the silyl ether with tetrabutylammonium fluoride in THF resulted in a significant degree of acyl migration to give the primary benzoate **15**. A comparable process operates during the acid-promoted detritylation step, but the isomerisation is very much slower and only very small quantities of **15** were formed even after extended reaction times.‖

Protected stearoylsphingosine **14** was coupled with labelled glucose by activation of the protected sugar as a trichloroacetimidate.[15] Thus, [U-^{14}C]glucose **3a** was acetylated to give pentaacetate **16a** in 93% radiochemical yield, and this was treated with benzylamine to deprotect the anomeric position selectively.[16] The resulting tetraacetate, **17a**, was isolated in 70% radiochemical yield; unlabelled material **17c** prepared in the same manner was found from ^{1}H NMR analysis to have an anomeric ratio of 3:1 (α:β). **17a** was converted into the corresponding trichloroacetimide **18a**, which appeared to be the α-anomer exclusively (^{1}H NMR), in 78% radiochemical yield by treatment with trichloroacetonitrile in the presence of caesium carbonate.[17]

With both the protected sphingosine **14** and the glycosylating agent **18a** in hand, it was a relatively straightforward matter to assemble **1b** as outlined in Scheme 1. Lewis acid-promoted



19a (• denotes U-^{14}C)

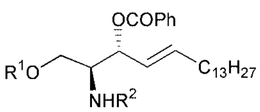
19b (unlabelled)

Scheme 1 Assembly of fragments **14** and **18**, and deprotection to give **1**: (i) **18a** or **18c**, $BF_3{\cdot}OEt_2$, DCM, 4 Å molecular sieves; (ii) MeONa, MeOH.

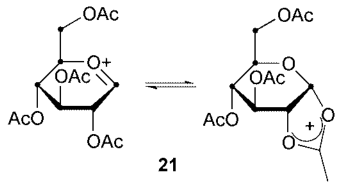
coupling of **14** with **18a**[18] afforded the fully protected species **19a** in 53% radiochemical yield. This was accompanied by a small quantity of the acetate **20**, presumably formed by nucleophilic attack of **14** upon the 2-*O*-acetate of the sugar, *via* the cyclic tautomer of intermediate **21**. The yield of this undesired material was significantly lower when boron trifluoride–diethyl ether was used to promote the coupling step than when the reaction was carried out in the presence of trimethylsilyl triflate.[19] In addition, the product distribution appeared to be dependent, at least in part, upon the temperature at which the Lewis acid was added; formation of **20** was minimised by maintaining the temperature below 0 °C during addition of the Lewis acid. Final deprotection of **19a**, using an excess of sodium methoxide in methanol, gave the desired product, **1b**, in 77% radiochemical yield. The same coupling process was carried out using unlabelled glucosylimidate **18c** to provide **1a**.

Detailed ^{1}H NMR studies were carried out on **1a**. Definitive assignments of all the resonances, other than those contained in the methylene envelope, in the ^{1}H NMR spectrum of **1a** were made with the assistance of the ^{1}H/^{1}H COSY spectrum. The stereochemistry at the anomeric centre could be assigned as β from the coupling constant (7.6 Hz) from 1-H to 2-H of the pyranose; further evidence for this assignment was provided by NÖE enhancements in the signals due to 3-H and 5-H of the

‖ Data for **15**: $\delta_H(CDCl_3)$ 0.85 (6H, t), 1.1–1.35 (48H, m), 1.5–1.65 (4H, m), 1.99 (2H, m), 2.17 (2H, t), 2.85 (1H, d), 4.25 (1H, m), 4.40 (2H, m), 4.55 (1H, m), 5.50 (1H, dd), 5.76 (1H, dt), 5.97 (1H, d), 7.43 (2H, m), 7.57 (1H, m), 8.01 (2H, d); *m*/*z* (CI$^+$) 670 (MH$^+$), 652 (670 − H_2O), 530 (652 − PhCOOH).



20 (R^1 = Ac, R^2 = $COC_{17}H_{35}$)
22 (R^1 = H, R^2 = $COCF_3$)
23 (R^1 = CPh_3, R^2 = Boc)
24 (R^1 = R^2 = H).
25 (R^1 = H, R^2 = Boc)



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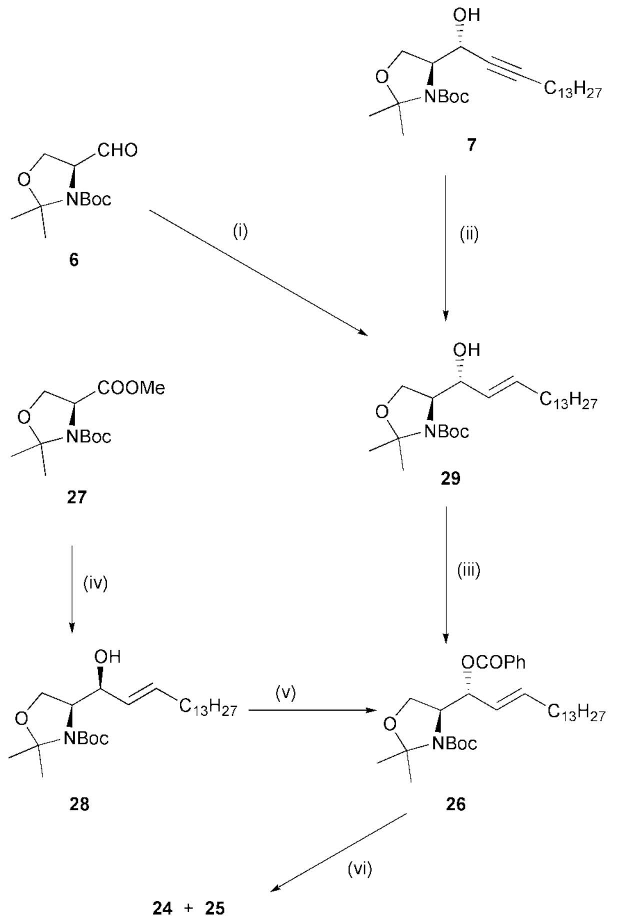
pyranose, observed upon irradiation at the resonance frequency of the anomeric proton. Since these can only occur if all three protons are on the same face of the ring, the material must be the β-anomer. The absence of any signals due to other diastereomers also constitutes evidence that the route used to prepare **4** had not resulted in isomerisation. The observed shifts in the ^{13}C NMR spectrum were also in good agreement with those reported previously for **1a**.[20]

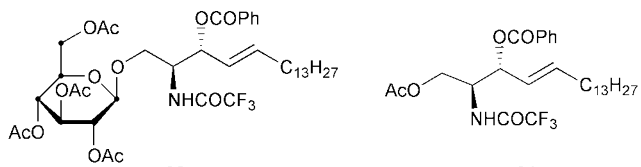
With preparations of **1a** and **1b** complete, a similar approach was chosen for that of the primary amine **2b**. Nevertheless, since the handling and solubility characteristics of **4** are poor, a route was developed to the protected sphingosine **22** starting from *N*-Boc-sphingosine **12**, with the intention of maintaining at least one protecting group in place at all times. Hence, **12** was converted into fully protected derivative **23** in an analogous sequence to that used already. Treatment with hydrogen chloride in methanol gave the known[21] 3-*O*-benzoyl-D-*erythro*-sphingosine **24**, which was acylated to give **22**, identical to material obtained using the published route.[3] Hydrolysis of the trityl group from **23** was very much faster than subsequent hydrolysis of the *tert*-butyl carbamate, and so the alcohol **25** could be isolated by use of a smaller excess of hydrogen chloride or by hydrolysis in trifluoroacetic acid. Once again, despite the use of extended reaction times, acyl migration was not observed during deprotection.

More direct routes to the monoprotected species **24** are outlined in Scheme 2. The fully protected intermediate **26** is available from three different routes: addition of (*E*)-pentadec-1-enyllithium[22] to the complex formed by addition of diisobutylaluminium hydride and triisobutylaluminium to the protected serine **27** gave the protected *threo*-sphingosine **28**.[23] This was successfully converted into the diastereomeric benzoate **26** by a Mitsunobu reaction, as previously reported[24] for the *erythro*-to-*threo* conversion. Alternatively, reduction of **7** by Red-Al in the same manner as that already described gave the allylic alcohol **29** in modest yield; the same intermediate is also available by addition of (*E*)-pentadec-1-enyllithium to the Garner aldehyde **6** but, in this case, the yield was poor at best and this does not appear to be a viable method. Thus, Mitsunobu inversion (with concomitant esterification) of **28** and subsequent hydrolysis of the acetonide provides a viable route to **26** and thereby to **24**.

Final assembly of [*glucose*-$^{13}C_6$]-1-*O*-(β-D-glucospyranoyl)-D-sphingosine **2b** followed essentially the same sequence as that described already for **1b**. The glycosylating agent **18b** was prepared from [$^{13}C_6$]glucose *via* **16b** and **17b**, although in this case the α- and β-anomeric forms were isolated. Since couplings to ^{13}C complicate the signals observed due to pyranose protons in the ^1H NMR spectra of **16b**–**18b**, making measurement of the coupling constant $J_{1,2}$ impractical, the stereochemistry at the anomeric centre of these intermediates was assigned on the basis of the coupling constant, $^1J_{CH}$, for the anomeric proton. In the case of **18b**, for example, the (major) α-anomer has a $^1J_{CH}$-value of 180 Hz, in line with that reported previously.[25] Coupling of the α-anomer of **18b** with **22** gave the protected glucosylsphingosine **30**, accompanied by the acetate **31**. Deprotection of **30** with sodium methoxide in methanol[3] gave **2b**. The NMR spectrum of this material is rather more complex than



Scheme 2 Alternative routes to **24**: (i) (*E*)-$C_{13}H_{27}CH{=}CHLi$, THF; (ii) Red-Al, toluene–Et_2O, 0–5 °C to room temp.; (iii) PhCOCl, Py–DMAP; (iv) Ref. 23; (v) $Pr^iOOCN{=}NCOOPr^i$, PPh_3, PhCOOH, THF; (vi) HCl, MeOH.



30 **31**

that of the unlabelled form **2a**, due to ^{13}C–^1H coupling in the glucose moiety. Nevertheless, the signal arising from the anomeric proton is sufficiently separated from other signals to enable confirmation that the material was the β-glycoside on the basis of the observed $J_{1,2}$-value of 7.8 Hz. The value of $^1J_{CH}$ (117.7 Hz) is surprisingly low, but is still consistent with an axial, rather than an equatorial, proton.[25] The ^{13}C NMR spectrum (acquired for the labelled portion of the molecule only) also contained peaks corresponding to a single diastereomer only.

Experimental

Mps were measured using a Buchi 510 capillary apparatus and are uncorrected. IR spectra were recorded using a Nicolet 510 FTIR Instrument. Optical rotation measurements were made using an Optical Activity digital polarimeter; $[a]_D$-values are given in units of 10^{-1} deg cm^2 g^{-1}, and concentrations (*c*) in g/100 ml. NMR spectra were recorded using a JEOL GSX-270 spectrometer. Mass spectra were recorded using a VG Autospec magnetic sector instrument at the University of York. [U-^{14}C]-D-Glucose was obtained from Amersham International, Little Chalfont, Buckinghamshire, UK, and [$^{13}C_6$]-D-glucose was obtained from Cambridge Isotope Laboratories, Andover, MA, USA.

N-Stearoyl-D-*erythro*-sphingosine 5

To a solution of octadecanoic acid (3.53 g, 12.43 mmol) in dichloromethane (DCM) (45 ml) and triethylamine (1.75 ml) was added a solution of BOP (5.57 g, 12.6 mmol) in DCM (25 ml), and the mixture was stirred at room temperature for 1 h. After this time DCM (40 ml) and a solution of sphingosine **4** (3.12 g, 10.36 mmol) in THF (80 ml) were added and the reaction mixture was stirred overnight at room temperature. Solvents were removed under reduced pressure; the residue was dissolved in chloroform, washed with aq. $NaHCO_3$, and the organic phase dried over $MgSO_4$. After evaporation, the residue was chromatographed on silica gel in methanol–chloroform (3:100) to afford **5** (4.15 g, 70%), mp 85–87 °C (lit.,[9] 88–89 °C); $[\alpha]_D^{23}$ −2.0 (*c* 1.0 in EtOH; lit.,[9] −2.4, *c* 1.1 in $CHCl_3$); $\delta_H(CDCl_3)$ 0.97 (6H, t), 1.1–1.4 (50H, m), 1.62 (2H, m), 2.04 (2H, m, CH_2CH=), 2.21 (2H, t, *J* 8.2 Hz, CH_2CONH), 2.71 (2H, m), 3.69 (1H, m), 3.8–4.0 (2H, m), 4.28 [1H, m, **CH**(OH)CH=], 5.52 [1H, ddt, *J* 15.4, 6.4, 1.0 Hz, CH(OH)C**H**=], 5.77 (1H, dtd, *J* 15.4, 6.7, 1.1 Hz, CH_2C**H**=), 6.22 (1H, d, *J* 6.8 Hz, NH).

3-*O*-Benzoyl-*N*-stearoyl-1-*O*-trityl-D-*erythro*-sphingosine 13

A suspension of **5** (6.52 g, 11.5 mmol), triethylamine (12.8 ml), DMAP (122 mg) and trityl chloride (4.80 g, 17.2 mmol) in DCM (150 ml) was heated at reflux for 60 h. Volatile materials were evaporated, the residue was re-dissolved in ethyl acetate, and the mixture was washed successively with 1 M hydrochloric acid, aq. $NaHCO_3$ and brine. The organic phase was dried ($MgSO_4$) and evaporated, and the residue was chromatographed on silica gel in ethyl acetate–hexane (3:7) to afford *N*-stearoyl-1-*O*-trityl-D-*erythro*-sphingosine (7.20 g, 77%) as an oil, $\delta_H(CDCl_3)$ 0.88 (6H, t), 1.15–1.4 (m), 1.64 (2H, m), 1.91 (2H, m), 2.20 (2H, t, *J* 8.2 Hz), 3.28 (1H, dd, *J* 9.6, 4.0 Hz), 3.35–3.4 (2H, m), 4.04 (1H, m), 4.17 (1H, m), 5.24 (1H, dd, *J* 15.4, 6.2 Hz), 5.62 (1H, dt, *J* 15.4, 6.6 Hz), 6.06 (1H, d, *J* 7.5 Hz, NH), 7.2–7.35 (9H, m), 7.35–7.45 (6H, m).

To a solution of this intermediate in pyridine (100 ml) under nitrogen were added DMAP (163 mg) and benzoyl chloride (1.74 ml, 15 mmol) and the mixture was stirred for 20 h. Solvent was removed under reduced pressure and the residue was partitioned between aq. $NaHCO_3$ and ethyl acetate. The organic phase was washed with brine, dried ($MgSO_4$), and evaporated, and the residue chromatographed on silica gel in ethyl acetate–hexane (15:85 to 1:1) to give **13** (5.61 g, 69%) as an oil, $\delta_H(CDCl_3)$ 0.87 (3H, t), 1.1–1.35 (50H, m), 1.54 (2H, m), 1.99 (2H, m), 2.08 (2H, t), 3.17 [1H, dd, *J* 7.4, 3.9 Hz, **CH**(H′)OH], 3.43 [1H, dd, *J* 9.7, 3.9 Hz, CH(**H**′)OH], 4.47 [1H, m, **CH**-(NHCOR)], 5.43 [1H, dd, *J* 15.3, 7.3 Hz, CH(OCOPh)C**H**=], 5.6–5.75 [2H, m, NH, **CH**(OCOPh)], 5.86 (1H, dt, *J* 15.3, 7.9 Hz, CH_2C**H**=), 7.1–7.25 (9H, m), 7.3–7.4 (8H, m), 7.54 (1H, t, *J* 7.5 Hz), 7.92 (2H, d, *J* 7.3 Hz); *m*/*z* (NaI-FAB) 934.6690 (MNa^+. $C_{62}H_{89}NNaO_4$ requires *m*/*z*, 934.6689), 796, 548, 264 (100%).

3-*O*-Benzoyl-*N*-stearoyl-D-*erythro*-sphingosine 14

(i) A solution of **13** (5.60 g, 6.1 mmol) and toluene-*p*-sulfonic acid monohydrate (1.3 g, 6.7 mmol) in DCM (100 ml) and methanol (100 ml) was stirred under nitrogen for 3 h. Solvent was evaporated and the residue was partitioned between aq. $NaHCO_3$ and chloroform. The organic phase was washed with brine, dried over $MgSO_4$, and evaporated to dryness. The residue was chromatographed on silica gel and eluted with ethyl acetate–hexane (1:1) to give **14** (3.70 g, 91%), mp 85.5–86 °C (lit.,[13] 86–88 °C); $[\alpha]_D^{20}$ +16.0 (*c* 0.4 in EtOH; lit.,[13] +16.7, *c* 1.5 in $CHCl_3$); $\delta_H(CDCl_3)$ 0.87 (6H, t), 1.1–1.3 (50H, m), 1.54 (2H, m), 1.96 (2H, m), 2.14 (2H, m), 2.77 (2H, br s), 3.71 (2H, m, CH_2O), 4.24 (1H, m, CHN), 5.4–5.6 [2H, m, **CH**(OC-OPh)C**H**=], 5.79 (1H, dt, *J* 15.0, 6.8 Hz, CH_2C**H**=), 6.18 (1H, d, *J* 9.6 Hz, NH), 7.38 (2H, dd, *J* 7.6, 7.2 Hz), 7.52 (1H, dd, *J* 7.6, 7.6 Hz), 7.96 (1H, d, *J* 7.2 Hz); *m*/*z* (NaI-FAB) 692.5594 (MNa^+. $C_{43}H_{75}NNaO_4$ requires *m*/*z*, 692.5594), 652 (692 − NaOH), 548 (670 − PhCOONa, 100%).

(ii) A solution of 4-nitrophenyl stearate[26] (0.223 g, 0.55 mmol) and **24** (0.202 g, 0.50 mmol) (see below) in pyridine (2 ml) was heated under nitrogen at 60 °C for 6 h, then stirred overnight at room temperature. Pyridine was evaporated and the residue was re-dissolved in ethyl acetate, washed twice with 1 M aq. sodium hydroxide and once each with water and brine. The organic phase was dried ($MgSO_4$) and evaporated to give **14** (0.197 g, 59%).

[*glucose*-U-^{14}C]-1,2,3,4,6-Penta-*O*-acetyl-α-D-glucopyranose 16a

A solution of [U-^{14}C]-glucose (20 mCi @ 302 mCi mmol^{-1}, 0.066 mmol) in pyridine (10 ml) and acetic anhydride (6.5 ml) under nitrogen was stirred at room temperature for 24 h. Volatile materials were removed under reduced pressure and the residue was partitioned between ethyl acetate and aq. $NaHCO_3$. The organic phase was washed with brine before being dried over $MgSO_4$. Solvent was removed under reduced pressure and the residue was chromatographed on silica gel and eluted with ethyl acetate–hexane (2:3) to afford **16a** (18.75 mCi, 93% radiochemical yield).

Similarly prepared, from [$^{13}C_6$]glucose (1.0 g, 5.4 mmol), was **16b** (2:1 ratio of α- to β-anomer; 2.141 g, 100%), $\delta_H(CDCl_3)$ 2.00 (9H, s), 2.00 (3H, s), 2.07 (3H, s), 3.5–6.0 (6H, m), 5.41 (0.33H, dm, $^1J_{CH}$ 147 Hz, H-1 of β-anomer), 6.32 (0.66H, dm, $^1J_{CH}$ 177 Hz, H-1 of α-anomer); $\delta_C(CDCl_3)$ 61.3 (d, ^1J 45 Hz), 67.6 (br dd, ^1J 53, 44 Hz), 69–71 (2C, m), 72.6 (dd, ^1J 45, 42 Hz), 89.0 (br d, ^1J 46 Hz, C-1 of α-anomer), 91.6 (ddd, ^1J 48 Hz, ^2J 5.5, 5.5 Hz, C-1 of β-anomer).

[*glucose*-U-^{14}C]-2,3,4,6-Tetra-*O*-acetyl-D-glucopyranose 17a

Compound **16a** (18.75 mCi, 0.62 mmol) was diluted to 50 mCi mmol^{-1} by addition of unlabelled 1,2,3,4,6-penta-*O*-acetyl-α-D-glucopyranose (**16c**; 121 mg) in THF (3 ml) under nitrogen, and this material (18.75 mCi, 155 mg, 0.371 mmol) was treated with benzylamine (55 ml, 0.49 mmol) and stirred at room temperature for 24 h. Solvent was removed under reduced pressure and the residue was chromatographed on silica gel and eluted with ethyl acetate–hexane (2:3) to afford the desired [*glucose*-U-^{14}C]-**17a** (13.2 mCi, 70% radiochemical yield).

Similarly prepared, from **16b** (2.124 g, 5.4 mmol), was **17b** (1.942 g, 100%), $\delta_H(CDCl_3)$ 2.03 (6H, s), 2.06 (6H, s), 3.4–5.6 (6H, m), 5.45 (1H, dm, $^1J_{CH}$ 174 Hz, 1-H); $\delta_C(CDCl_3)$ 61.9 (d, ^1J 41 Hz), 66–74 (m, 4C), 90.1 (d, ^1J 44 Hz, C-1 of α-anomer), 95.5 (dt, ^1J 46 Hz, ^2J 5.5, 5.5 Hz, C-1 of β-anomer); *m*/*z* (NH_3-CI^+) 372 (MNH_4^+, 100%), 337; HRMS 372.1602 (Calc. for $C_8{}^{13}C_6H_{20}O_{10}$: *M*, 372.1602).

[*glucose*-U-^{14}C]-2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate 18a

To a solution of **17a** (13.2 mCi, 0.264 mmol) in DCM (3 ml) under nitrogen were added trichloroacetonitrile (48 ml, 0.61 mmol) and caesium carbonate (23 mg, 0.07 mmol) and the mixture was stirred at room temperature for 5 h. Additional DCM was added and the solution was washed with aq. $NaHCO_3$, dried ($MgSO_4$) and evaporated. The residue was chromatographed on silica gel in ethyl acetate–hexane (2:3) to afford **18a** (10.35 mCi, 78% radiochemical yield).

Similarly prepared, from **17b** (1.388 g, 3.9 mmol), was **18b**. α-Anomer (1.108 g, 57%): $\delta_H(CDCl_3)$ 2.02 (9H, s), 2.06 (3H, s), 3.7–6.0 (6H, m), 6.52 (1H, dm, $^1J_{CH}$ 180 Hz, 1-H), 8.68 (1H, s). β-Anomer (0.286 g, 15%): $\delta_H(CDCl_3)$ 2.02 (9H, s), 2.06 (3H, s), 3.7–6.0 (6H, m), 5.82 (1H, dm, $^1J_{CH}$ 162 Hz, 1-H), 8.70 (1H, s).

[*glucose*-U-[14]C]-3-*O*-Benzoyl-1-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-*N*-stearoyl-D-*erythro*-sphingosine 19a

A suspension of **14** (169 mg, 0.252 mmol) and dried, powdered 4 Å molecular sieves (643 mg) in DCM (6 ml) was stirred under nitrogen for 90 min before the addition of **18a** (10.3 mCi, 0.207 mmol) in DCM (5 ml). The mixture was cooled to −10 °C, boron trifluoride–diethyl ether (35 ml, 0.288 mmol) was added, and the mixture was allowed to warm to room temperature and was stirred for 10 h. After this time the molecular sieves were removed by filtration and washed with further DCM. The filtrate was washed with aq. $NaHCO_3$ and dried over $MgSO_4$ before evaporation under reduced pressure. The residue was chromatographed on silica gel in acetone–toluene (4:96) to give **19a** (5.46 mCi, 53% radiochemical yield).

Similarly prepared, from **18c**,[17] was **19b** (55%), mp 74–74.5 °C, $[\alpha]_D^{22}$ +11.25 (*c* 0.4 in EtOH); δ_H($CDCl_3$) 0.86 (6H, t), 1.15–1.35 (50H, m), 1.95 (3H, s), 1.99 (6H, s), 2.00 (3H, s), 2.14 (2H, t), 3.6–3.7 (2H, m), 3.9–4.3 (4H, m), 4.45 (1H, d, *J* 7.8 Hz, H-6), 4.85–5.0 (1H, m), 5.04 (1H, dd, *J* 9.2, 4.3 Hz), 5.15 (1H, d, *J* 9.5 Hz), 5.4–5.6 (2H, m), 5.75–5.9 (2H, m), 7.41 (2H, dd, *J* 8.1, 7.6 Hz), 7.54 (1H, dd, *J* 7.6, 7.6 Hz), 7.99 (2H, dd, *J* 8.1, 1.1 Hz); *m*/*z* (NaI-FAB) 1022.6551 (MNa^+. $C_{57}H_{93}NNaO_{13}$ requires *m*/*z*, 1022.6545), 878, 548, 331, 264, 169 (100%). This was accompanied by acetylated aglycone **20** (10%), mp 88–89 °C; δ_H($CDCl_3$) 0.87 (6H, t), 1.1–1.4 (50H, m), 1.57 (2H, m), 2.00 (2H, m), 2.01 (3H, s), 2.17 (2H, t, *J* 8.5 Hz), 4.16 [1H, dd, *J* 11.6, 5.4 Hz, C**H**(H′)OH], 4.34 [1H, dd, *J* 11.6, 7.5 Hz, CH(**H**′)OH], 4.58 (1H, m, CHN), 5.4–5.6 (2H, m), 5.77 (1H, d, *J* 9.6 Hz, NH), 5.88 [1H, dt, *J* 15.0, 7.5 Hz, CH(OH)C**H**=], 7.45 (2H, dd, *J* 7.6, 7.2 Hz), 7.56 (1H, dd, *J* 7.6, 7.6 Hz), 8.01 (1H, d, *J* 7.2 Hz); *m*/*z* (NaI-FAB) 734.5699 (100%, MNa^+. $C_{45}H_{77}NNaO_5$ requires *m*/*z*, 734.5699), 612, 264, 176.

[*glucose*-U-[14]C]-1-*O*-(β-D-Glucopyranosyl)-*N*-stearoyl-D-*erythro*-sphingosine 1b

A suspension of **19a** (5.46 mCi, 0.11 mmol) and sodium methoxide (30.8 mg, 0.57 mmol) in methanol (5 ml) was stirred for 24 h. Additional methanol (25 ml) and chloroform (5 ml) were added followed by Dowex 50W-X8 cation-exchange resin (532 mg). The mixture was stirred for 10 min, the exchange resin was removed by filtration, and solvent was removed under reduced pressure. The residue was chromatographed on silica gel in methanol–chloroform (1:4) to afford **1b** (4.2 mCi, 77% radiochemical yield).

Similarly prepared, from **19b**, was **1a** (95%) as a white solid, mp 194–195 °C; δ_H($CDCl_3$–CD_3OD, 1:1) 0.89 (6H, t), 1.2–1.45 (50H, m), 1.60 (2H, m, $COCH_2CH_2$), 2.03 (2H, dt, =CHC**H**$_2$), 2.17 (2H, t, $COCH_2$), 3.20 (1H, dd, 3′-H), ≈3.28 (2H, partly obscured by solvent, **CH**$_2$OH), 3.35 (1H, dd, *J* 10.1, 2.8 Hz, 4-H), 3.64 (1H, m, 6-H), 3.68 (1H, m, NC**H**), 3.86 (1H, dd, 6-H), 3.98 (1H, ddd, 6-H′), 4.08 [1H, m, =CHC**H**(OH)], 4.11 (1H, m, 5-H), 4.26 (1H, d, *J* 7.6 Hz, 1-H), 5.46 [1H, br dd, *J* 15.4, 7.3 Hz, =C**H**CH(OH)], 5.69 (1H, dt, *J* 15.4, 6.5 Hz, =C**H**CH$_2$); *m*/*z* 750 (MH^+), 710 (750 − NaOH), 548, 264; HRMS 750.5864 (Calc. for $C_{42}H_{81}NNaO_8$: *m*/*z*, 750.5860).

3-*O*-Benzoyl-*N*-*tert*-butoxycarbonyl-1-*O*-tritylsphingosine 23

A solution of *N*-Boc-sphingosine **12** (2.002 g, 5 mmol), DMAP (107 mg), pyridine (2 ml) and trityl chloride (1.422 g, 5.1 mmol) in DCM (40 ml) was stirred for 24 h, then heated to 75 °C for 2.5 h. Solvent was removed and the residue was chromatographed on silica gel in hexane–ethyl acetate (95:5, then 9:1) to give *N*-*tert*-butoxycarbonyl-1-*O*-tritylsphingosine (2.452 g, 77%), δ_H($CDCl_3$) 0.89 (3H, t), 1.1–1.4 (22H, m), 1.48 (9H, s), 1.93 (2H, m), 3.24 (1H, m), 3.40 (1H, m), 3.70 (1H, m), 4.25 (1H, m), 5.15 (1H, dd, *J* 15.3, 6.7 Hz), 5.24 (1H, m), 5.64 (1H, dt, *J*. 15.3, 6.6 Hz), 7.15–7.35 (9H, m), 7.35–7.5 (6H, m); *m*/*z* (NaI-FAB+) 664 (MNa^+), 484, 243 (Ph_3C^+); HRMS 664.4340 (Calc. for $C_{42}H_{59}NNaO_4$: *m*/*z*, 664.4342).

This intermediate was dissolved in pyridine (10 ml) and DMAP (0.888 g) was added, followed by benzoyl chloride (0.551 ml, 4.8 mmol). The suspension was stirred for 2 h, pyridine was removed under reduced pressure, and the residue was chromatographed on silica gel in hexane–ethyl acetate (from 85:15 to 1:1) to give **23** (2.775 g, 98%), δ_H($CDCl_3$) 0.88 (3H, t), 1.1–1.5 (22H, m), 1.43 (9H, s), 1.97 (2H, m), 3.20 [1H, dd, *J* 10.9, 4.1 Hz, C**H**(H′)OTr], 3.32 [1H, dd, *J* 10.2, 4.1 Hz, CH(**H**′)OTr], 4.13 (1H, m), 4.77 (1H, br d, *J* 10.2 Hz), 5.41 (1H, dd, *J* 15.3, 7.5 Hz), 5.65 (1H, m), 5.85 (1H, dt, *J* 15.3, 7.9 Hz), 7.1–7.5 (17H, m), 7.52 (1H, dd, *J* 8.2, 4.0 Hz), 7.89 (2H, d, *J* 7.5 Hz); *m*/*z* (NaI-FAB) 768.4601 (MNa^+. $C_{49}H_{63}NNaO_5$ requires *m*/*z*, 768.4604), 664, 526, 326, 243 (100%).

***tert*-Butyl 4-[(1*R*,2*E*)-1-hydroxyhexadec-2-enyl]-2,2-dimethyloxazolidine-3-carboxylate 29**

Red-Al in toluene (3.2 M; 3.5 ml, 11 mmol) was added under nitrogen to an ice-cooled solution of **7** (0.969 g, 2.2 mmol) in diethyl ether (20 ml). The mixture was warmed to room temperature and stirred for 22 h. Methanol (1 ml) was added, followed by an excess of aq. ammonium chloride, and the mixture was filtered through Celite. The phases were separated, the aqueous phase was re-extracted with ethyl acetate, and the combined organic phases were dried ($MgSO_4$), solvent was removed under reduced pressure, and the residue was chromatographed on silica in 9:1 hexane–ethyl acetate to give **29** (0.337 g, 35%), δ_H($CDCl_3$) 0.88 (3H, t), 1.1–1.65 (37H, m), 1.99 (2H, m), 3.52 (2H, m), 3.61 (1H, m), 4.06 (1H, m), 5.34 (1H, m), 5.53 (1H, m); *m*/*z* (CI^+) 440.3736 (MH^+. $C_{26}H_{50}NO_4$ requires *m*/*z*, 440.3740), 366, 340 (100%), 100.

***tert*-Butyl 4-[(1*R*,2*E*)-1-benzoyloxyhexadec-2-enyl]-2,2-dimethyloxazolidine-3-carboxylate 26**

(i) A solution of benzoyl chloride (116 ml, 1 mmol), DMAP (0.184 g) and **29** (0.320 g, 0.73 mmol) in pyridine (5 ml) was stirred for 2 h, concentrated to dryness, and the residue chromatographed on silica gel in 1:9 ethyl acetate–hexane to give **26** (0.210 g, 53%) as a colourless oil, δ_H($CDCl_3$) 0.87 (3H, t), 1.1–1.4 (22H, m), 1.45 (9H, s), 1.46 (3H, s), 1.50 (3H, s), 2.03 (2H, m), 4.0–4.3 (3H, m), 5.4–5.65 (1H, m), 5.7–5.9 (2H, m), 7.45 (2H, m), 7.54 (1H, m), 8.04 (1H, m), 8.09 (1H, d); *m*/*z* (NaI-FAB) 566.3814 (100%, MNa^+. $C_{33}H_{53}NNaO_5$ requires *m*/*z*, 566.3821).

(ii) A solution of diisopropyl azodicarboxylate (20 ml, 0.1 mmol) and triphenylphosphine (26 mg, 0.1 mmol) in THF (2 ml) was stirred for 20 min, following which the *S*-alcohol **28**[23] (20 mg, 46 μmol) as a solution in THF (1 ml) was added followed, immediately, by benzoic acid (31 mg). The mixture was stirred for 36 h, then purified by preparative TLC on silica gel in 1:3 ethyl acetate–hexane to give **26** (18 mg, 72%).

3-*O*-Benzoyl-D-*erythro*-sphingosine 24

(i) Acetyl chloride (0.995 ml, 14 mmol) was added to methanol (25 ml) and, after 5 min, the solution was added to the fully protected sphingosine **23** (2.638 g, 3.5 mmol) to give a suspension, which was stirred for 90 min. The mixture was concentrated to dryness, the residue was partitioned between ethyl acetate and aq. $NaHCO_3$, and the organic extract was washed with brine, dried over ($MgSO_4$), and solvent removed under vacuum. The residue was purified by chromatography on silica gel in 99:1 ethyl acetate–acetic acid followed by 96:2:2 propan-2-ol–methanol–acetic acid to give **24** (809 mg, 57%) as a white solid, mp 96–97 °C; $[\alpha]_D^{22}$ +3.2 (*c* 3.1 in EtOH); δ_H($CDCl_3$) 0.92 (3H, t), 1.1–1.5 (22H, m), 2.13 (2H, dt, *J* 8.2, 7.2 Hz), 3.51 (1H, dd, *J* 13.0, 5.5 Hz, CHN), 3.76 [1H, m, C**H**(H′)OH], 3.90 [1H, m, CH(**H**′)OH], 5.59 (1H, dd, *J* 15.6, 7.5 Hz), 5.71 [1H, dd, *J* 7.2, 6.2 Hz, C**H**(OCOPh)], 6.00 (1H, dt, *J* 15.6, 7.1 Hz), 7.51 (2H, dd, *J* 8.2, 7.5 Hz), 7.66 (1H, dd, *J* 7.5, 7.5 Hz), 8.09

(2H, d, *J* 8.2 Hz); δ_H for HCl salt (CD_3OD) 0.90 (3H, t), 1.1–1.5 (22H, m), 2.11 (2H, m), 3.58 (1H, m), 3.79 [1H, dd, *J* 10.8, 3.4 Hz, C**H**(H′)OH], 3.94 [1H, dd, *J* 10.8, 2.7 Hz, CH(**H**′)OH], 5.56 [1H, dd, *J* 15.6, 7.1 Hz, CH(OCOPh)C**H**=], 5.74 [1H, dd, *J* 7.1, 3.0 Hz, C**H**(OCOPh)], 6.03 (1H, dt, *J* 15.6, 6.8 Hz, =C**H**CH_2), 7.51 (2H, dd, *J* 7.1, 7.5 Hz), 7.64 (1H, dd, *J* 7.1, 7.1 Hz), 8.07 (2H, d, *J* 7.5 Hz); *m*/*z* (FAB) 404.3166 (MH^+. $C_{25}H_{42}NO_3$ requires *m*/*z*, 404.3165), 281 (100%).

(ii) A solution of **26** (0.210 g, 0.37 mmol) in trifluoroacetic acid (4.2 ml) was stored for 3 h. Volatile materials were removed under reduced pressure and the residue was partitioned between ethyl acetate and aq. $NaHCO_3$. The organic phase was dried ($MgSO_4$) and evaporated. Preparative TLC of the residue on silica gel in 99 : 1 ethyl acetate–acetic acid gave **25** (84 mg, 43%); δ_H($CDCl_3$) 0.88 (3H, t), 1.1–1.5 (31H, m), 2.35 (2H, m), 3.55–4.10 (4H, m), 5.03 [1H, m, C**H**(OCOPh)], 5.64 (1H, dd, *J* 15.3, 7.3 Hz), 5.84 (1H, dt, *J* 15.3, 7.1 Hz), 7.44 (2H, m), 7.57 (1H, m), 8.06 (2H, m); *m*/*z* (NaI-FAB) 526.3507 (MNa^+. $C_{30}H_{49}NNaO_5$ requires *m*/*z*, 526.3508), 326 (100%), 154, and **24** (60 mg, 39%).

3-*O*-Benzoyl-*N*-trifluoroacetyl-D-*erythro*-sphingosine 22

A solution of 4-nitrophenyl trifluoroacetate (1.014 g, 4.1 mmol) and **24** (664 mg, 1.65 mmol) in pyridine (10 ml) under nitrogen was stirred for 2 h at 55 °C. Volatile material was removed under reduced pressure, and the residue was partitioned between ethyl acetate and 0.3 M aq. $KHSO_4$. The organic phase was washed twice with 2 M aq. NaOH, three times with water, and once with brine, then dried ($MgSO_4$). Evaporation under reduced pressure, and column chromatography of the residue on silica gel in ethyl acetate–hexane (1 : 9), gave **22** (444 mg, 54%), mp 80–81 °C (lit.,[2] 80.5–81.5 °C); $[\alpha]_D^{21}$ +11.2 (*c* 1.0 in EtOH) (lit.,[2] $[\alpha]_D$ +11.1, *c* 1.0, $CHCl_3$); δ_H($CDCl_3$) 0.88 (3H, t), 1.15–1.4 (22H, m), 2.05 (2H, dt, CH_2CH=), 2.69 (1H, br s, OH), 3.69 [1H, dd, *J* 12.0, 3.4 Hz, C**H**(H′)OH], 3.80 [1H, dd, *J* 12.0, 2.8 Hz, CH(**H**′)OH], 4.26 (1H, m, CHN), 5.55 [1H, m, CH-(OCOPh)], 5.60 [1H, dd, *J* 14.4, 7.2 Hz, =C**H**CH(OH)], 5.90 (1H, dt, *J* 14.4, 6.7 Hz, =C**H**CH_2), 6.95 (1H, d, *J* 8.95 Hz, NH), 7.47 (2H, dd, *J* 8.5, 7.5 Hz), 7.61 (1H, dd, *J* 7.5, 7.5 Hz), 8.04 (1H, d, *J* 8.5 Hz); *m*/*z* (NaI-FAB) 522.2809 (MNa^+. $C_{27}H_{40}F_3NNaO_4$ requires *m*/*z*, 522.2807), 173. Further elution returned **24** (100 mg, 15% recovery).

[*glucose*-U-^{13}C]-3-*O*-Benzoyl-1-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-*N*-trifluoroacetyl-D-*erythro*-sphingosine 30

As described for **19a**, coupling of **18b** (316 mg, 0.6 mmol) with **22** gave **30** (140 mg, 27%), mp 100–102 °C (lit.,[2] 103–105 °C); $[\alpha]_D^{22}$ −4.3 (*c* 0.8 in EtOH; lit.,[2] −4.5, *c* 0.75 in $CHCl_3$); δ_H($CDCl_3$) 0.88 (3H, t), 1.05–1.3 (22H, m), 1.95 (3H, s), 1.98 (6H, s), 2.00 (3H, s), 2.08 (2H, m), 3.0–5.3 (11H, m), 5.34 (1H, t, *J* 7.9 Hz), 5.45 (1H, dd, *J* 14.4, 6.8 Hz), 5.80 (1H, dt, *J* 14.4, 7.7 Hz), 7.30 (2H, m), 7.45 (1H, m), 7.88 (2H, d, *J* 7.9 Hz); *m*/*z* (NH_3-FAB^+) 853.4408 (MNH_4^+, 100%. $C_{35}{}^{13}C_6H_{62}F_3N_2O_{13}$ requires *m*/*z*, 853.4405), 733, 337. Also recovered was **31** (58 mg, 18%), δ_H($CDCl_3$) 0.87 (3H, t), 1.1–1.4 (22H, m), 2.05 (3H, s), 2.06 (2H, m), 4.26 (1H, dd), 4.37 (1H, dd), 4.57 (1H, m), 5.47 (1H, t), 5.57 (1H, dd), 5.96 (1H, dd), 6.84 (1H, d), 7.46 (2H, t), 7.60 (1H, t), 8.01 (2H, d).

[*glucose*-$^{13}C_6$]-1-*O*-(β-D-Glucopyranosyl)-D-*erythro*-sphingosine 2b

Hydrolysis of **30**[7] (0.240 g, 0.29 mmol) gave **2b** (105 mg, 78%), δ_H(CD_3OD) 0.90 (3H, t), 1.15–1.5 (2H, m), 2.11 (2H, dt, CH_2CH=), 2.9–3.2 (2.5H, m), 3.3–3.65 (4H, m, part obscured by solvent), 3.8–4.05 (2.5H, m), 4.11 [1H, br d, *J* 6.5 Hz, C**H**(OH)CH=], 4.36 (1H, dd, $^1J_{CH}$ 117.7 Hz, *J* 7.8 Hz, 1-H), 5.48 [1H, dd, *J* 15.6, 7.5 Hz, CH(OH)C**H**=], 5.80 (1H, dt, *J* 15.6, 6.5 Hz, =C**H**CH_2); δ_C(CD_3OD) 62.6 (d, *J* 170 Hz), 71.5 (dd, *J* 162, 153 Hz), 74.9 (dd, *J* 184, 158 Hz), 77.9 (2C, 2 dd), 104.25 (d, *J* 188 Hz); *m*/*z* (NaI-FAB^+) 490.3455 (MNa^+. $C_{18}{}^{13}C_6H_{47}NO_7Na$ requires *m*/*z*, 490.3452), 473, 323, 282; (CI) 468, 450, 282 (468 − $C_{13}H_{27}C{\equiv}CH$), 264.

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