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Synthesis of lipid A analogues containing glucose instead of glucosamine and their LPS-antagonistic activities

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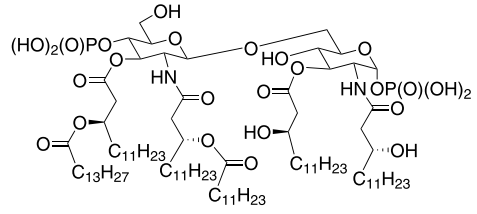
Abstract—Lipid A analogues containing glucose in substitution for glucosamine on the reducing end were synthesized, and the inhibitory activities on LPS-induced TNFα production (LPS-antagonistic activity) in vitro using human whole blood cells were measured. The IC_{50} values (nM) of these ten compounds, **8**, **14**, **21**, **31**, **40**, **51**, **57**, **62**, **67** and **72**, were 11.2, 15.4, 2.7, 0.1, 0.4, 1.3, 3.2, 3.2, 1.4 and 14.4, respectively. And also inhibitory activities (ID_{50}) on TNFα production toward galactosamine loaded C3H/HeN mice in vivo of compounds **21**, **31**, **57**, **62** and **67** were measured. The values of these compounds were 0.29, 0.50, 0.61, not dose-dependent and 0.33 mg/kg, respectively.

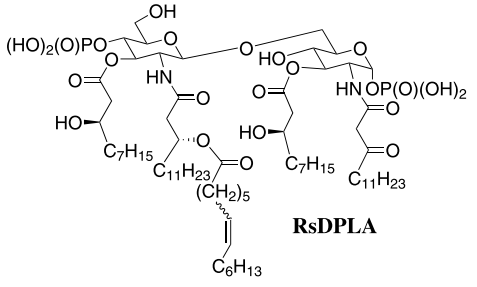
1. Introduction

The study of endotoxin has proceeded extensively[1] since Shiba and Kusumoto's[2] total synthesis of lipid A, a toxic component of endotoxin (lipopolysaccharide, LPS) existing in the outer surface membrane of Gram-negative bacteria. On the contrary, a nontoxic natural lipid A-related compound (RsDPLA)[3] was isolated from *Rhodobacter sphaeroides* by an Eisai group. This compound unlike lipid A has a unique structural feature, that is, it contains two amides composed of an unsaturated fatty acid ((*R*)-3-(7-tetradecenoyloxy)tetradecanoic acid) and a 3-oxotetradecanoic acid in its long fatty acid chains, and shows LPS-agonistic activity toward neither human nor mouse macrophages.[4] Furthermore, the Eisai group found that many RsDPLA-related compounds having an olefinic double bond in their molecules behave as LPS antagonists toward human and murine macrophages,[4] and E5564,[5] a compound related to RsDPLA, has been developed as a highly potent anti-septicemia drug (Fig. 1).

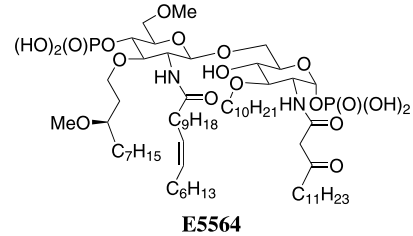
The active structures of all natural lipid A- and also RsDPLA-related compounds are constructed with an β(1-6)-linked glucosamine–glucosamine disaccharide moiety, and the configuration of the anomeric phosphate



Lipid A



RsDPLA



E5564

Figure 1. Structures of Lipid A, RsDPLA, and E5564.

Keywords: LPS-antagonist; RsDPLA.
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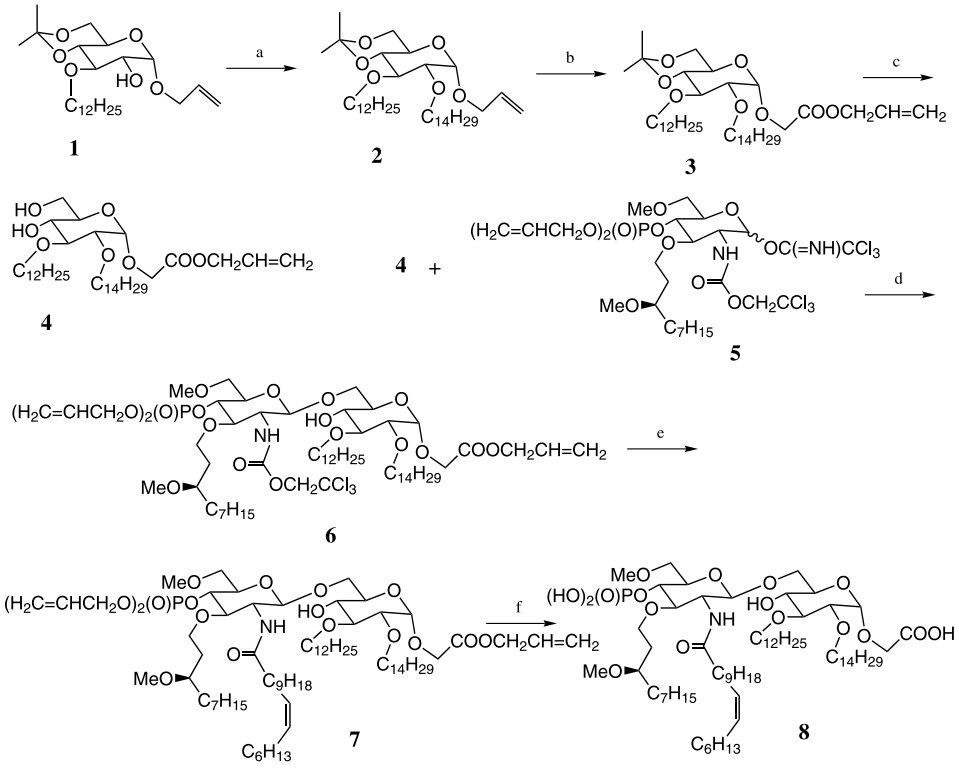
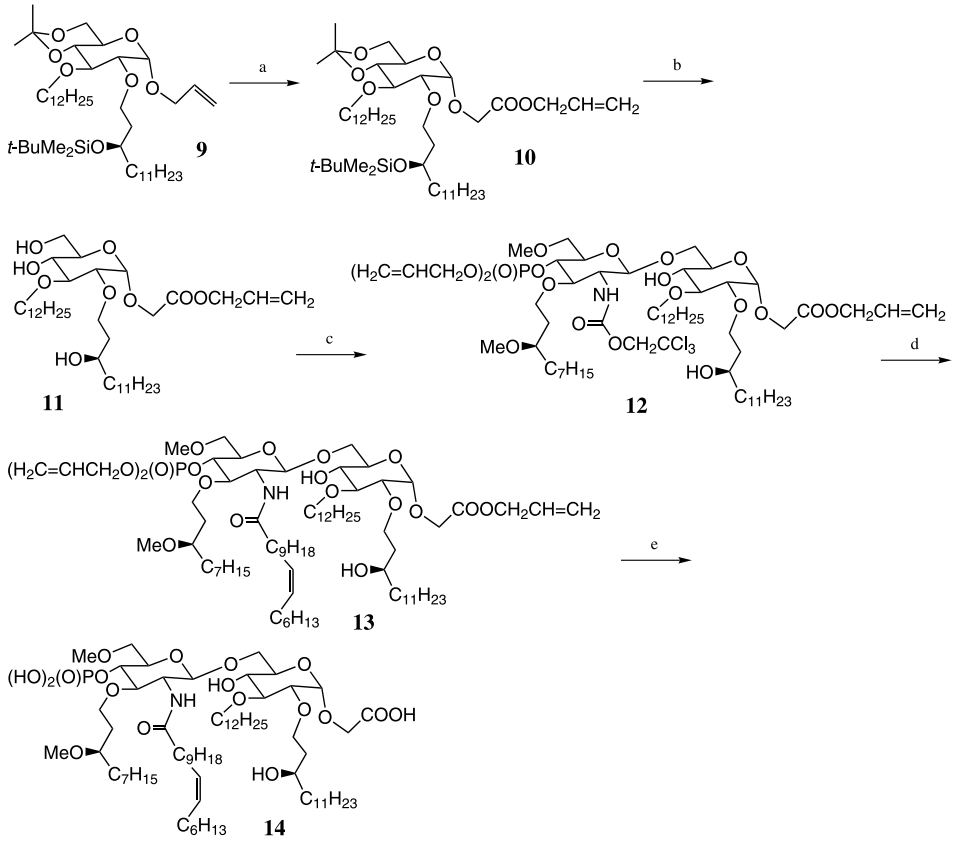
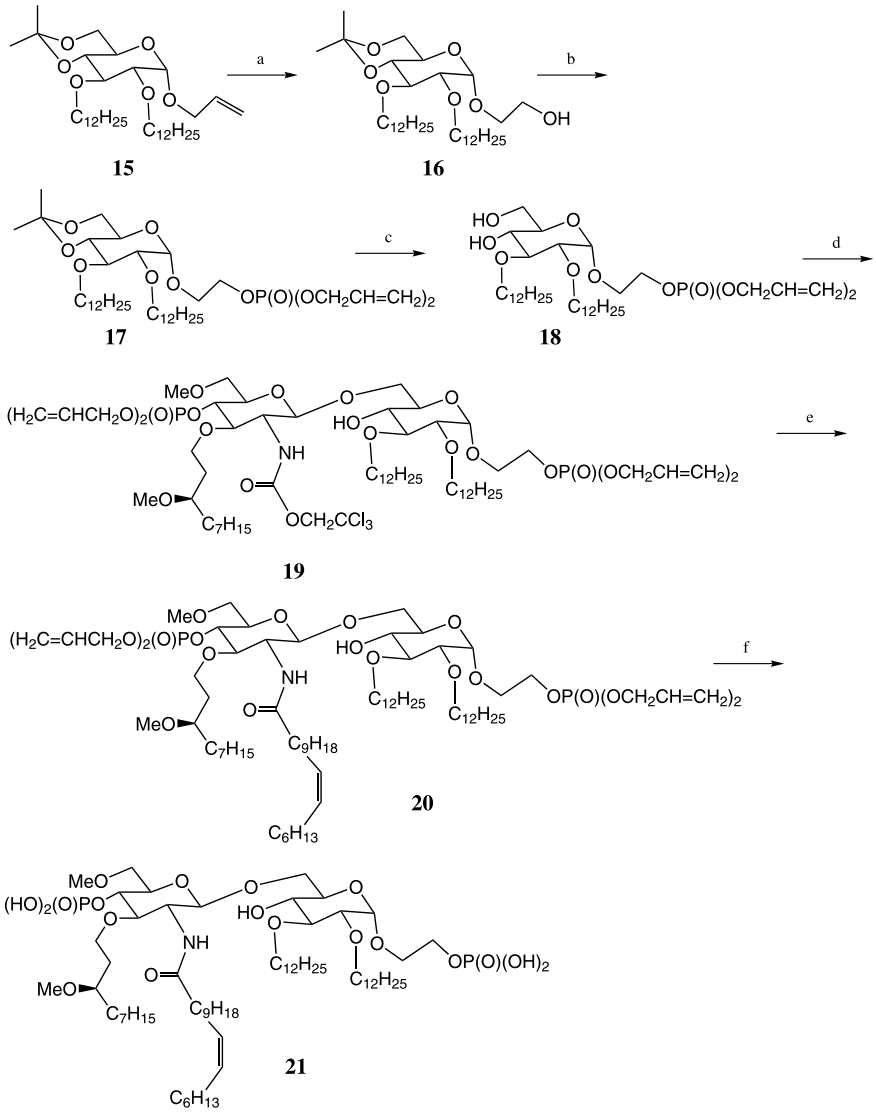


Scheme 1. Reagents and conditions: (a) tetradecyl methanesulfonate, NaH, DMF, rt, 16 h, 75%; (b) $RuO_2.xH_2O$, $NaIO_4$, $MeCN–CCl_4–H_2O$ (2:2:3), rt, 3 h, then allyl bromide, Et_3N, DMF, rt, 16 h, 72%; (c) $AcOH–H_2O$ (4:1), 65 °C, 2 h, 71%; (d) TMSOTf, MS 4A, CH_2Cl_2, −40 °C, 1 h, 43%; (e) (1) Zn, AcOH–THF (1:1), rt, 4 h; (2) (*Z*)-11-octadecenoyl chloride, $NaHCO_3$, $THF–H_2O$ (5:1), rt, 30 min, two steps 77%; (f) $(PPh_3)_4Pd$, PPh_3, Et_3N, HCOOH, THF, under N_2, 55 °C, 4 h, 95%.



Scheme 2. Reagents and conditions: (a) RuO_2 hydrate, $NaIO_4$, $MeCN–CCl_4–H_2O$ (2:2:3), rt, 3 h, then allyl bromide, Et_3N, DMF, rt, 16 h, 69%; (b) $AcOH–H_2O$ (4:1), 65 °C, 2 h, 60%; (c) **5**, TMSOTf, MS 4A, CH_2Cl_2, −40 °C, 1 h, 45%; (d) (1) Zn, AcOH–THF (1:1), rt, 4 h; (2) (*Z*)-11-octadecenoyl chloride, $NaHCO_3$, $THF–H_2O$ (5:1), rt, 30 min, 67%; (e) $(PPh_3)_4Pd$, PPh_3, Et_3N–HCOOH, THF, under N_2, 55 °C, 4 h, 55%.



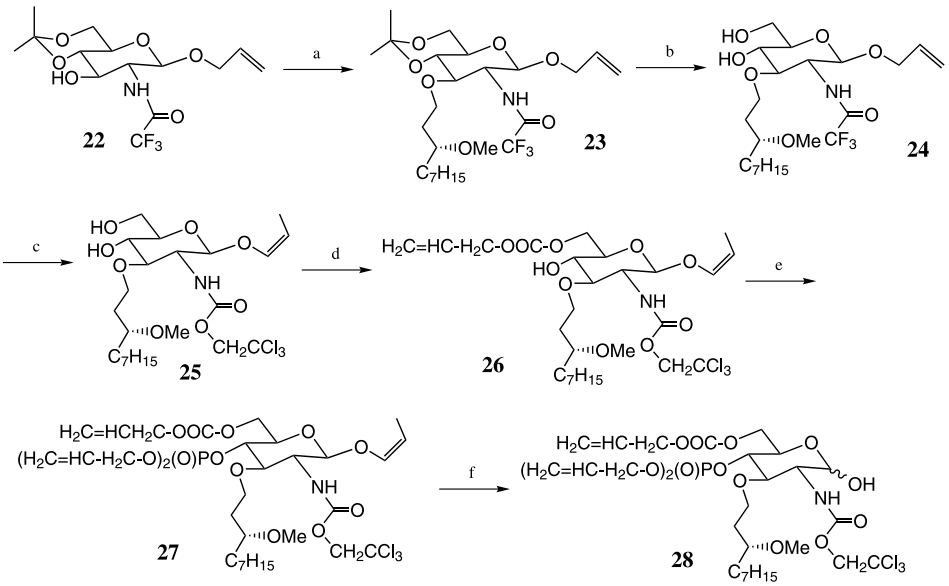
Scheme 3. Reagents and conditions: (a) OsO_4, $NaIO_4$, THF–H_2O (7:2), rt, 3 h, then $NaBH_4$, EtOH, rt, 20 min, quenched with AcOH, 73%; (b) *i*-$Pr_2NP(OCH_2CH{=}CH_2)_2$, 1*H*-tetrazole, CH_2Cl_2, rt, 30 min, then, aq 30% H_2O_2, THF–CH_2Cl_2, rt, 15 min, 99%; (c) *p*-TsOH, MeOH, rt, 2 h, 96%; (d) **5**, AgOTf, TMSOTf, MS 4A, under N_2, CH_2Cl_2, rt, 16 h, 60%; (e) (1) Zn, AcOH–THF (1:1), rt, 3.5 h; (2) (*Z*)-11-octadecenoyl chloride, $NaHCO_3$, THF–H_2O (5:1), rt, 2 h, two steps 75%; (f) $(PPh_3)_4Pd$, PPh_3, Et_3N–HCOOH, THF, under N_2, 55 °C, 16 h, 45%.

of the reducing glucosamine part is α without exception. We were interested in the structures of RsDPLA and E5564, and synthesized some E5564-related compounds, which were replaced with glucose analogues instead of the glucosamine at the reducing end, to examine the LPS-antagonistic activity. At this time, we fixed the nonreducing glucosamine end to that of E5564 except for compound **31**, and also we restricted the anomeric substituents of the reducing glucose end to five structural groups as the α-carboxymethyl, α-2-(phosphonooxy)ethyl, α-2-(phosphono)ethyl, α-3-(phosphonooxy)propyl and α-3-(phosphono)-propyl groups such as compounds **8** and **14**, and **21**, **31**, **40**, **51** and **57**, and **62**, and **67**, and **72**, respectively.

2. Results and discussion

2.1. Synthesis

Firstly, we synthesized compounds **8** and **14** having an α-carboxymethyl group at the anomeric position. For synthesis of **8**, known alcohol **1**[6] was used as a starting material. Compound **1** was treated with tetradecyl methanesulfonate using sodium hydride as a base to give **2**. Oxidative cleavage of the allyl group of **2** with ruthenium oxide hydrate and sodium periodate gave carboxylic acid, which was esterified with allyl bromide using triethyl amine in *N*,*N*-dimethylformamide (DMF) to yield allyl ester **3**. The 4,6-*O*-isopropylidene group of **3** was deprotected with aqueous 80% acetic acid at 65 °C to yield diol **4**, which was coupled with already reported trichloromethylimidoyl 2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-(trichloroethoxycarbonylamino)-D-glucopyranoside **5**[5] at −40°C using trimethylsilyl trifluoromethanesulfonate (TMSOTf) as a catalyst to afford β(1-6)-linked[5] disaccharide **6**. The 2,2,2-trichloroethoxycarbonyl group of **6** was deprotected with zinc powder in AcOH–THF (1:1) to give amine, which was treated with (*Z*)-11-octadecenoyl chloride in THF–H_2O (5:1) using $NaHCO_3$ to afford amide **7**. The three allyl groups of **7** were deprotected with

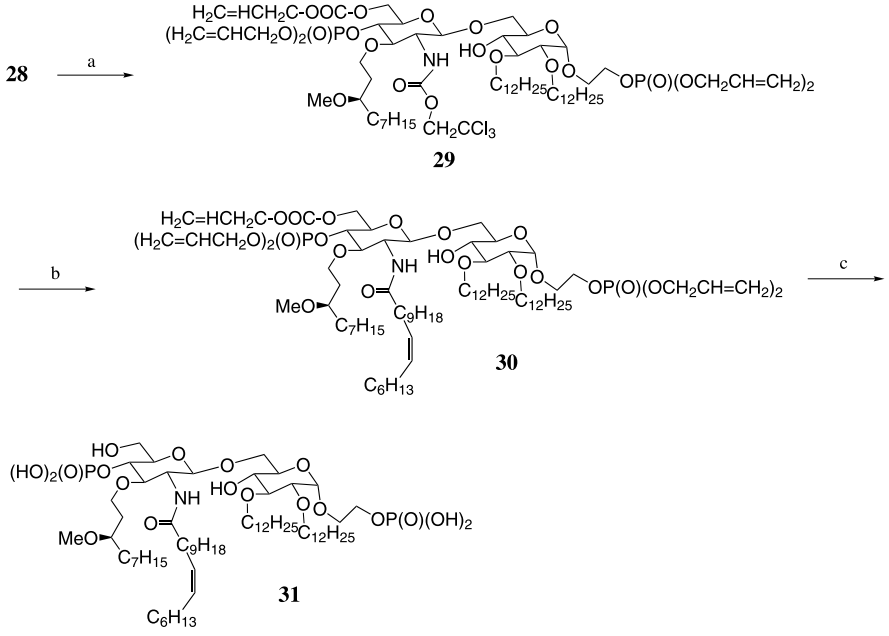


Scheme 4. Reagents and conditions: (a) (*R*)-3-methoxydecyl *p*-tolouenesulfonate, NaH, DMF, rt, 5 h, 77%; (b) *p*-TsOH, MeOH, rt, 2 h, 92%; (c) (1) *t*-BuOK, DMSO, 85 °C, N_2, 2 h, then H_2O, 85 °C, 2 h, (2) $ClCOOCH_2CCl_3$, aq satd $NaHCO_3$, THF, 0 °C, 30 min, 76%; (d) $ClCOOCH_2CH{=}CH_2$, pyridine, 0 °C, 30 min, 94%; (e) *i*-$Pr_2NP(OCH_2CH{=}CH_2)_2$, 1*H*-tetrazole, CH_2Cl_2, rt, 20 min, then aq. 30% H_2O_2, THF–CH_2Cl_2, 0 °C, 30 min, 86%; (f) I_2, THF–H_2O (5:1), rt, 30 min, 94%.

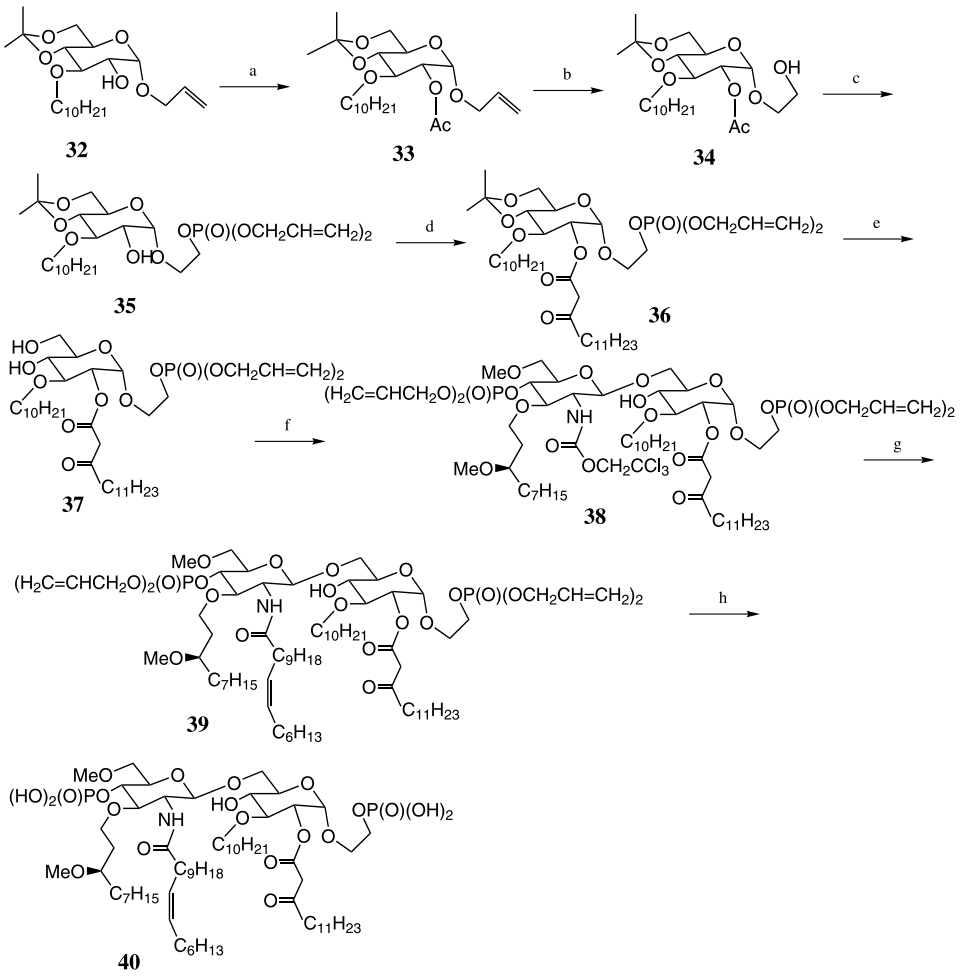
tetrakis(triphenylphosphine)palladium(0) [$Pd(PPh_3)_4$], triphenylphosphine (PPh_3) and triethylamine-formic acid (Et_3N–HCOOH) in THF to yield **8** (Scheme 1).

For the synthesis of **14**, known alcohol **9**[6] was used as a starting material under almost the same conditions as for the synthesis of **8** from **1**. Oxidative cleavage of the allyl group of **9** with ruthenium oxide hydrate and sodium periodate gave carboxylic acid, which was esterified with allyl bromide using triethyl amine in DMF to yield allyl ester **10**, and the successive cleavage of 4,6-*O*-isopropylidene and *tert*-butyldimethylsilyl groups from **10** was performed with aqueous 80% acetic acid at 65 °C to yield triol **11**. Compound **11** was coupled with imidate **5** at −40 °C using TMSOTf to afford β(1-6)-linked disaccharide **12**. The 2,2,2-trichloroethoxycarbonyl group of **12** was deprotected with zinc powder in AcOH-THF (1:1) to give amine, which was treated with (*Z*)-11-octadecenoyl chloride in THF–H_2O (5:1) using $NaHCO_3$ to afford amide **13**. The three allyl groups of **13** were deprotected with $Pd(PPh_3)_4$, PPh_3 and Et_3N–HCOOH in THF to yield **14** as mentioned above (Scheme 2).

Secondly, we synthesized compounds **21**, **31**, **40**, **51** and **57** having an α-2-(phosphonooxy)ethyl group at the anomeric position. Known compound **15**[6] was used as a starting



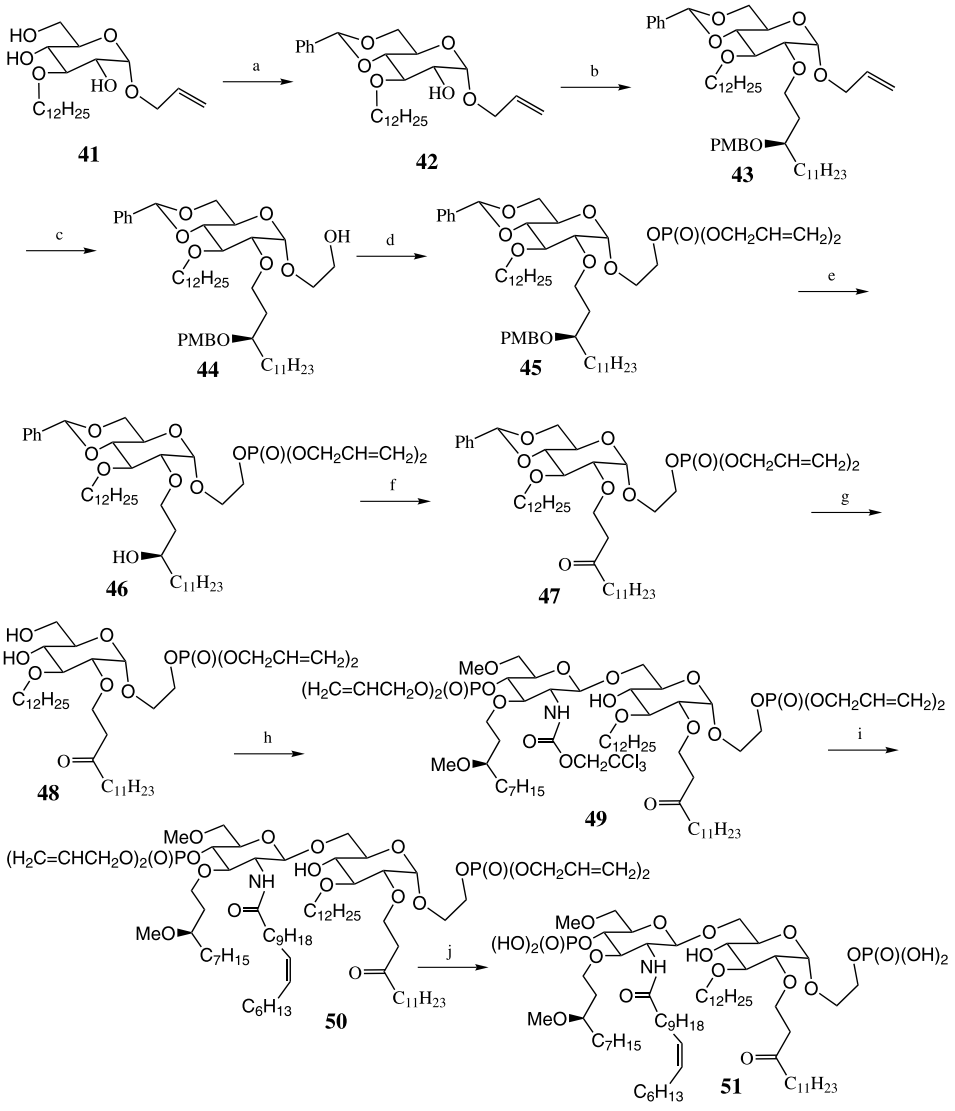
Scheme 5. Reagents and conditions: (a) CCl_3CN, Cs_2CO_3, CH_2Cl_2, rt, 1 h, then **18**, TMSOTf, MS 4A, CH_2Cl_2, 0 °C, then rt, 16 h, 52%; (b) (1) Zn, AcOH–THF (1:9), 24 °C, 3.5 h; (2) (*Z*)-11-octadecenoic acid, WSC.HCl, CH_2Cl_2, rt, 10 h, two steps 47%; (c) $(PPh_3)_4Pd$, PPh_3, Et_3N–HCOOH, THF, under N_2, 55 °C, 16 h, 76%.



Scheme 6. Reagents and conditions: (a) Ac_2O, pyridine, rt, 5 h, 99%; (b) (1) OsO_4, $NaIO_4$, THF–H_2O (3:1), rt, 2 h; (2) $NaBH_4$, EtOH, rt, 10 min, 71%; (c) (1) KOH, EtOH, rt, 1.5 h; (2) *i*-$Pr_2NP(OCH_2CH{=}CH_2)_2$, 1*H*-tetrazole, CH_2Cl_2, rt, 20 min, then aq 30% H_2O_2, CH_2Cl_2–THF (1:1), rt, 10 min, 80%; (d) 3-oxotetradecanoic acid, WSC HCl, CH_2Cl_2, rt, 40 min, 75%; (e) AcOH–H_2O (v/v, 4:1), 85 °C, 1.5 h, 86%; (f) **5**, AgOTf, TMSOTf, MS 4A, CH_2Cl_2, under N_2, rt, 16 h, 68%; (g) (1) Zn, AcOH–THF (1:1), rt, 4 h; (2) (*Z*)-11-octadecenoyl chloride, $NaHCO_3$, THF–H_2O (5:1), rt, 30 min, two steps 81%; (h) $(PPh_3)_4Pd$, PPh_3, Et_3N–HCOOH, THF, under N_2, 55 °C, 16 h, 84%.

material for the synthesis of **21**. The allyl group of **15** was converted to aldehyde by OsO_4–$NaIO_4$ oxidation in THF–H_2O (7:2), and the aldehyde was successively reduced to alcohol **16** using $NaBH_4$ in EtOH. Treatment of **16** with diallyl diisopropylphosphoramidite and 1*H*-tetrazole in THF yielded phosphite, which was contiguously oxidized to phosphate **17** by use of H_2O_2. The 4,6-*O*-isopropylidene protecting group of **17** was cleaved using *p*-toluenesulfonic acid monohydrate in MeOH to give diol **18**. Compound **18** was coupled with imidate **5** at room temperature using AgOTf and TMSOTf to afford β(1-6)-linked disaccharide **19**. In this coupling, we found that the use of one equivalent of AgOTf and catalytic amount of TMSOTf at room temperature improved the yield comparing with the known method of single use of TMSOTf at −78∼−40 °C. When TMSOTf reagent was not used as a catalyst, excess AgOTf (about 2 equiv) was needed in the formation of **60** from **5** and **59** as described later. The 2,2,2-trichloroethoxycarbonyl group of **19** was deprotected with zinc powder in AcOH–THF (1:1) to give amine, which was treated with (*Z*)-11-octadecenoyl chloride in THF–H_2O (5:1) using $NaHCO_3$ to afford amide **20** according to almost the same procedure as for the synthesis of **7** from **6**. The three allyl groups of **20** were deprotected with $Pd(PPh_3)_4$, PPh_3 and Et_3N–HCOOH in THF to yield **21** as mentioned above (Scheme 3).

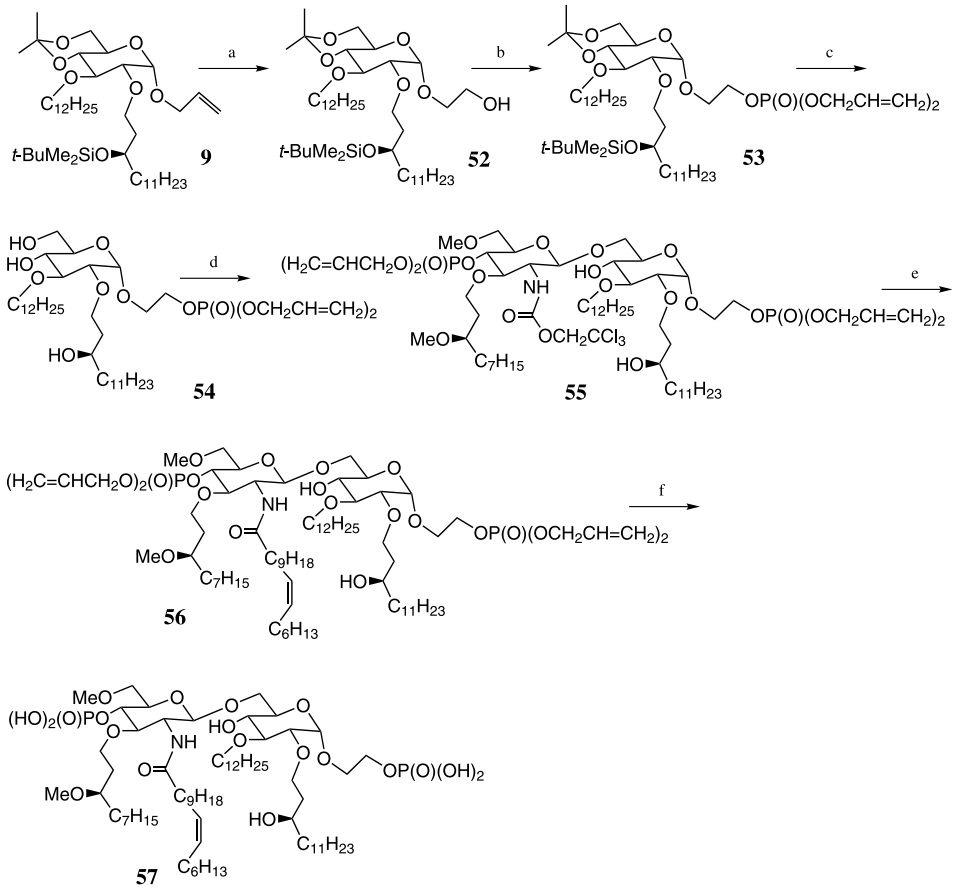
Compound **22**[7] was used as a starting material for the synthesis of **31**. Alcohol **22** was reacted with (*R*)-3-methoxydecyl *p*-toluenesulfonate in DMF using NaH to yield **23**, which was treated with *p*-toluenesulfonic acid in methanol to afford diol **24**. Double bond isomerization[5] of **24** was performed with potassium *tert*-butoxide in dimethylsulfoxide (DMSO) at 85 °C for 2 h, and hydrolysis of C2 trifluoroacetamide to yield amino-free (*Z*)-vinyl ether, which was successively protected with 2,2,2-trichloroethyl chloroformate in aqueous THF using $NaHCO_3$ to give **25**. Treatment of diol **25** with allyl chloroformate using pyridine gave **26** being protected by the 6-*O*-allyloxycarbonyl group. The remaining C5 secondary alcohol of **26** was treated with diallyl diisopropylphosphoramidite and 1*H*-tetrazole, and subsequent oxidation of the generated phosphite with aq 30% H_2O_2 yielded phosphate **27**. Oxidative cleavage of the vinyl ether group of **27** was accomplished by iodine in THF–H_2O to yield **28**. After the activation of the anomeric alcohol of **28** with trichloroacetonitrile using cesium carbonate the generated imidoyl compound was coupled with diol **18** using TMSOTf as a condensing catalyst to



Scheme 7. Reagents and conditions: PMB = 4-methoxybenzyl; (a) $PhCH(OMe)_2$, cat. *p*-TsOH, DMF, rt, 16 h, 69%; (b) (*R*)-3-(4-methoxybenzyloxy)tetradecyl methanesulfonate, NaH, DMF, rt, 6 h, 67%; (c) (1) OsO_4, $NaIO_4$, acetone–H_2O (4:1), rt, 4 h, (2) $NaBH_4$, EtOH, rt, 1 h, 64%; (d) *i*-$Pr_2NP(OCH_2CH{=}CH_2)_2$, 1*H*-tetrazole, THF, rt, 4 h, then aq 30% H_2O_2, 0 °C, 1 h, 96%; (e) DDQ, CH_2Cl_2–H_2O (10:1), rt, 1.5 h, 90%; (f) PCC, CH_2Cl_2, rt, 5 h, 93%; (g) aq 80% AcOH, 65 °C, 2 h, 92%; (h) **5**, cat. TMSOTf, MS 4A, CH_2Cl_2, N_2, 0 °C, 1 h, 59%; (i) Zn, AcOH–THF (1:9), rt, 4 h, then (*Z*)-11-octadecenoic acid, WSC.HCl, CH_2Cl_2, rt, 10 h, 56%; (j) $(PPh_3)_4Pd$, PPh_3, Et_3N–HCOOH, THF, N_2, 55 °C, 4 h, 90%.

afford disaccharide **29**. The 2,2,2-trichloroethoxycarbonyl group of **29** was deprotected with zinc powder in AcOH–THF (1:9) to give amine, which was treated with (*Z*)-11-octadecenoic acid in CH_2Cl_2 using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (WSC·HCl) as a dehydrating agent to afford amide **30** in accordance with the same procedure as that to convert **49** to **50** as described later. The five allyl groups of **30** were deprotected with $Pd(PPh_3)_4$, PPh_3 and Et_3N–HCOOH in THF to yield **31** as mentioned above (Schemes 4 and 5).

Compound **32**[6] was used as a starting material for the synthesis of **40**. The C2 alcohol of **32** was acetylated with acetic anhydride–pyridine to give **33**. This protection was necessary for OsO_4 oxidation, because alcohol **32** did not react with OsO_4 under the same conditions as for the synthesis of **34** from **33**. The allyl group of **33** was treated with OsO_4–$NaIO_4$ in THF–H_2O, and subsequent reduction with $NaBH_4$ in EtOH to give alcohol **34**, which was deacetylated by a catalytic amount of KOH in EtOH and the primary alcohol was selectively reacted with diallyl diisopropylphosphoramidite and 1*H*-tetrazole, and continuous oxidation of generated phosphite with aq 30% H_2O_2 to yield phosphate **35**. Esterification of C2 alcohol **35** with 3-oxotetradecanoic using WSC·HCl as a dehydrating agent gave **36**. Treatment of **36** with aq 80% AcOH at 85 °C for 1.5 h yielded diol **37**, which was coupled with imidate **5** using silver trifluoromethanesulfonate (AgOTf) and TMSOTf as condensing catalysts to afford disaccharide **38**. The 2,2,2-trichloroethoxycarbonyl group of **38** was replaced with (*Z*)-11-octadecenoyl group using zinc powder in AcOH–THF (1:1) and then (*Z*)-11-octadecenoyl chloride in THF–H_2O (5:1) using $NaHCO_3$ to afford amide **39** in accordance with the same procedure as that to convert **6** to **7**. Five allyl groups of **39** were deprotected with $Pd(PPh_3)_4$, PPh_3 and Et_3N–HCOOH in THF to yield **40** as mentioned above (Scheme 6).

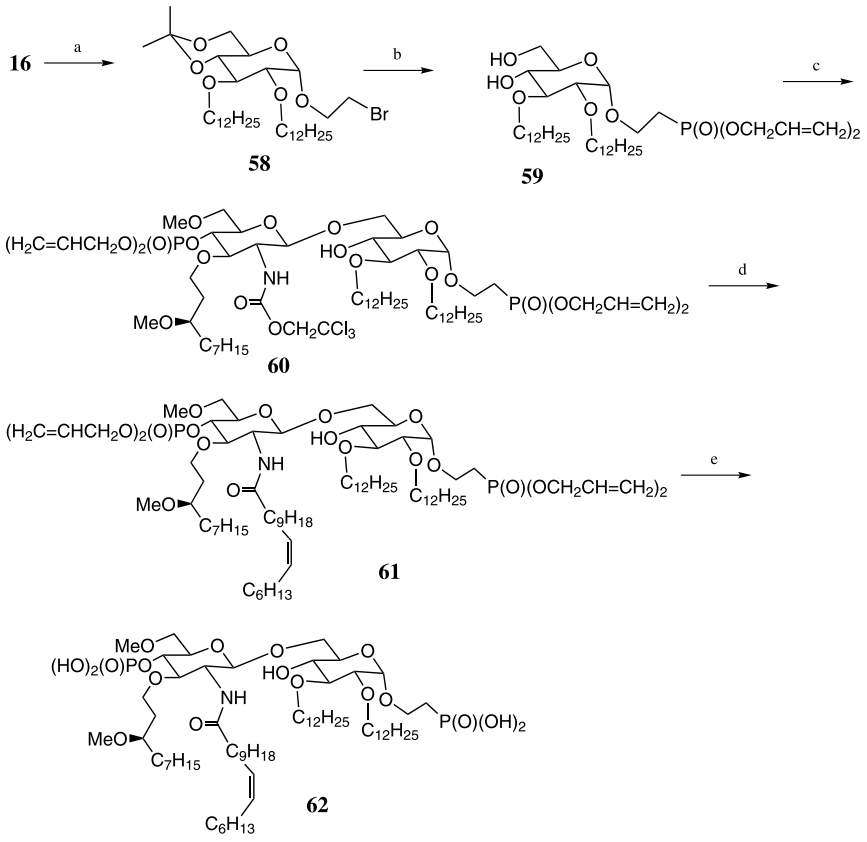


Scheme 8. Reagents and conditions: (a) OsO_4, $NaIO_4$, THF–H_2O (7:2), rt, 2 h, and then $NaBH_4$, EtOH, 0 °C, 20 min, 41%; (b) *i*-$Pr_2NP(OCH_2CH{=}CH_2)_2$, 1*H*-tetrazole, CH_2Cl_2, rt, 30 min, then aq 31% H_2O_2, rt, 15 min, 97%; (c) *p*-TsOH.HCl, MeOH, rt, 2 h, 84%; (d) **5**, AgOTf, TMSOTf, MS 4A, N_2, rt, 16 h, 67%; (e) Zn, AcOH–THF (1:1), rt, 4 h, and then (*Z*)-11-octadecenoyl chloride, $NaHCO_3$, THF–H_2O (5:1), rt, 2 h, 82%; (f) $Pd(PPh_3)_4$, PPh_3, Et_3N–HCOOH, N_2, 55 °C, 16 h, 67%.

Alcohol **41**[6] was used as a starting material for the synthesis of **51**. Treatment of **41** with benzaldehyde dimethyl acetal using *p*-TsOH monohydrate gave benzylidene **42**, which was treated with (*R*)-3-(4-methoxybenzyloxy)tetradecyl methanesulfonate using sodium hydride to give **43**. Oxidative cleavage of the allyl group of **43** with OsO_4–$NaIO_4$ in acetone–H_2O, and subsequent reduction of the generated aldehyde with $NaBH_4$ gave alcohol **44**, which reacted with diallyl diisopropylphosphoramidite using 1*H*-tetrazole as an acid catalyst, and continuous oxidation of generated phosphite with aq 30% H_2O_2 to yield phosphate **45**. Oxidative cleavage of the *p*-methoxybenzyl group of the alcohol protective group of **45** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in CH_2Cl_2–H_2O gave alcohol **46**. The alcohol on the branched chain of **46** was oxidized with pyridinium chlorochromate (PCC) to afford ketone **47**. The 4,6-*O*-benzylidene group of **47** was deprotected with aq 80% AcOH at 60 °C to yield diol **48**. Treatment of **48** and imidate **5** at 0 °C using TMSOTf afforded β(1-6)-linked disaccharide **49**. The 2,2,2-trichloroethoxycarbonyl group of **49** was deprotected with zinc powder in AcOH–THF (1:9) to give amine, which was treated with (*Z*)-11-octadecenoic acid using WSC·HCl as a dehydrating agent to afford amide **50**. The four allyl groups of phosphate **50** were deprotected with $Pd(PPh_3)_4$, PPh_3 and Et_3N–HCOOH in THF to yield **51** as mentioned in the formation of **8** from **7** (Scheme 7).

Compound **9**[6] was used as a starting material for the synthesis of **57**. The allyl group of **9** was converted to aldehyde by OsO_4–$NaIO_4$ oxidation in THF–H_2O (7:2), and the aldehyde was continuously reduced to alcohol **52** using $NaBH_4$ in EtOH. Treatment of **52** with diallyl diisopropylphosphoramidite and 1*H*-tetrazole in THF yielded phosphite, which was immediately oxidized to phosphate **53** using aq 31% H_2O_2. The 4,6-*O*-isopropylidene and *tert*-butyldimethylsilyl protecting groups of **53** were cleaved by using *p*-TsOH monohydrate in MeOH to give triol **54**. Compound **54** was coupled with imidate **5** at room temperature using AgOTf and catalytic amount of TMSOTf to afford β(1-6)-linked disaccharide **55**. The 2,2,2-trichloroethoxycarbonyl group of **55** was deprotected with zinc powder in AcOH–THF (1:1) to give amine, which was treated with (*Z*)-11-octadecenoyl chloride in THF–H_2O (5:1) using $NaHCO_3$ to afford amide **56**. Three allyl groups of **56** were deprotected with $Pd(PPh_3)_4$, PPh_3 and Et_3N–HCOOH in THF to yield **57** according to almost the same procedure as for the synthesis of **21** from **20** (Scheme 8).

Thirdly, we synthesized compound **62** having an α-2-(phosphono)ethyl group at the anomeric position.



Scheme 9. Reagents and conditions: (a) (1) CBr_4, PPh_3, CH_2Cl_2, rt, 2 h, 99%; (b) (1) $P(OCH_2CH{=}CH_2)_3$, 180 °C, 3 h, (2) aq. 80% AcOH, 60 °C, 1 h, two steps 60%; (c) **5**, AgOTf, CH_2Cl_2, rt, 1 h, 74%; (d) (1) Zn, AcOH–THF (1:8), rt, 3 h; (2) (*Z*)-11-octadecenoic acid, WSC.HCl , rt, 20 h, two steps 52%; (e) $(PPh_3)_4Pd$, PPh_3, Et_3N–HCOOH, THF, under N_2, 55 °C, 4 h, 82%.

Compound **16** was used as a starting material for the synthesis of **62**. The alcohol of **16** was brominated with carbon tetrabromide and triphenylphosphine to give **58**. Treatment of **58** with triallylphosphite at 180 °C for 3 h, and then aq 80% AcOH at 60 °C for 1 h gave diol **59**, which was coupled with imidate **5** at room temperature using two equivalents of AgOTf to afford β(1-6)-linked disaccharide **60**. The 2,2,2-trichloroethoxycarbonyl group of **60** was deprotected with zinc powder in AcOH–THF (1:8) to give amine, which was treated with (*Z*)-11-octadecenoic acid in CH_2Cl_2 using WSC·HCl as a dehydrating agent to afford amide **61** as mentioned in the formation of **50** from **49**. The four allyl groups of **61** were deprotected with $Pd(PPh_3)_4$, PPh_3 and Et_3N–HCOOH in THF to yield **62** (Scheme 9).

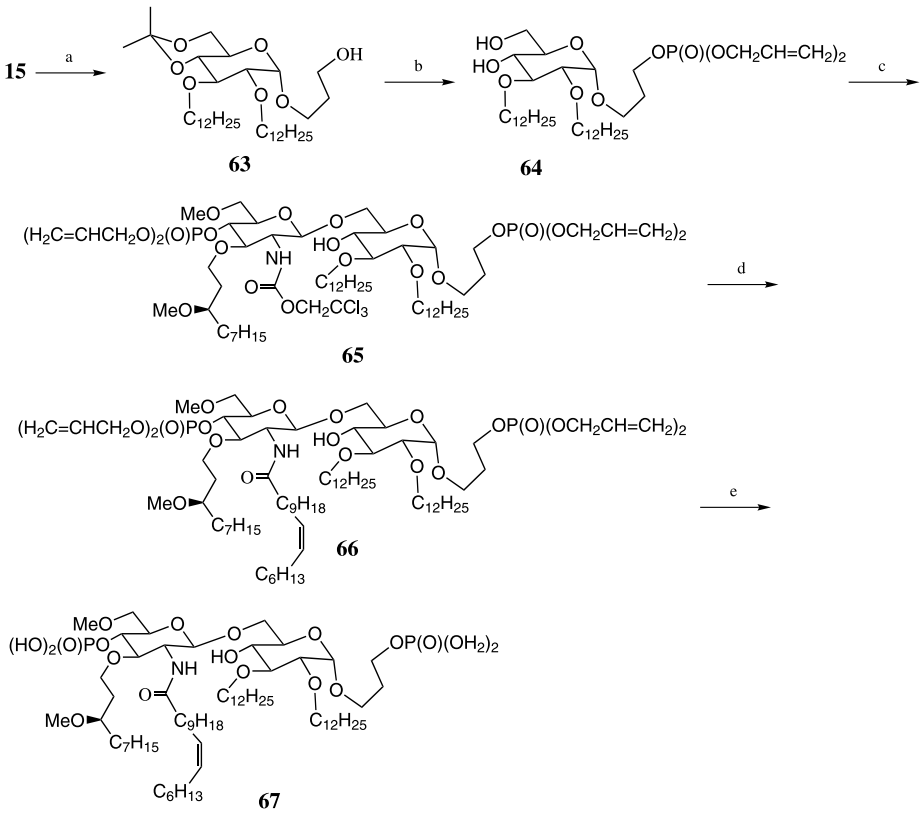
Fourthly, we synthesized compounds **67** having an α-3-(phosphonooxy)propyl group at the anomeric position, and **15**[6] was used as a starting material. Hydroboration of the allyl double bond of **15** with 9-borabicyclo[3.3.1]nonane (9-BBN), and subsequent oxidation of the borane compound with aq 30% H_2O_2 and aq 3 M NaOH gave alcohol **63**. After the alcohol of **63** was converted diallyl phosphate, the 4,6-*O*-isopropylidene group of **63** was deprotected with aq 80% acetic acid at 60 °C to yield diol **64**, which was coupled with imidate **5** at 0 °C using TMSOTf to afford β(1-6)-linked disaccharide **65** as mentioned for the formation of **49** from **48**. The 2,2,2-tri-chloroethoxycarbonyl group of **65** was deprotected with zinc powder in AcOH–THF (1:1) to give amine, which was treated with (*Z*)-11-octadecenoic acid using WSC·HCl as a dehydrating agent to afford amide **66**. The four allyl groups of **66** were deprotected with $Pd(PPh_3)_4$ and Et_3N–HCOOH in THF to yield **67** (Scheme 10).

Finally, we synthesized compound **72** having an α-3-(phosphono)propyl group at the anomeric position. The alcohol of **63** was brominated with CBr_4 and PPh_3 to give **68**. Treatment of **68** with triallylphosphite at 180 °C for 3 h, and then aq 80% AcOH at 60 °C for 1 h gave diol **69**, which was coupled with imidate **5** at 0 °C using TMSOTf to afford β(1-6)-linked disaccharide **70** as mentioned for the formation of **49** from **48**. The 2,2,2-trichloroethoxycarbonyl group of **70** was deprotected with zinc powder in AcOH–THF (1:1) to give amine, which was treated with (*Z*)-11-octadecenoyl chloride in THF–H_2O (5:1) using $NaHCO_3$ to afford amide **71** as mentioned in the formation of **7** from **6**. The four allyl groups of **71** were deprotected with $Pd(PPh_3)_4$, PPh_3 and Et_3N–HCOOH in THF to yield **72** (Scheme 11).

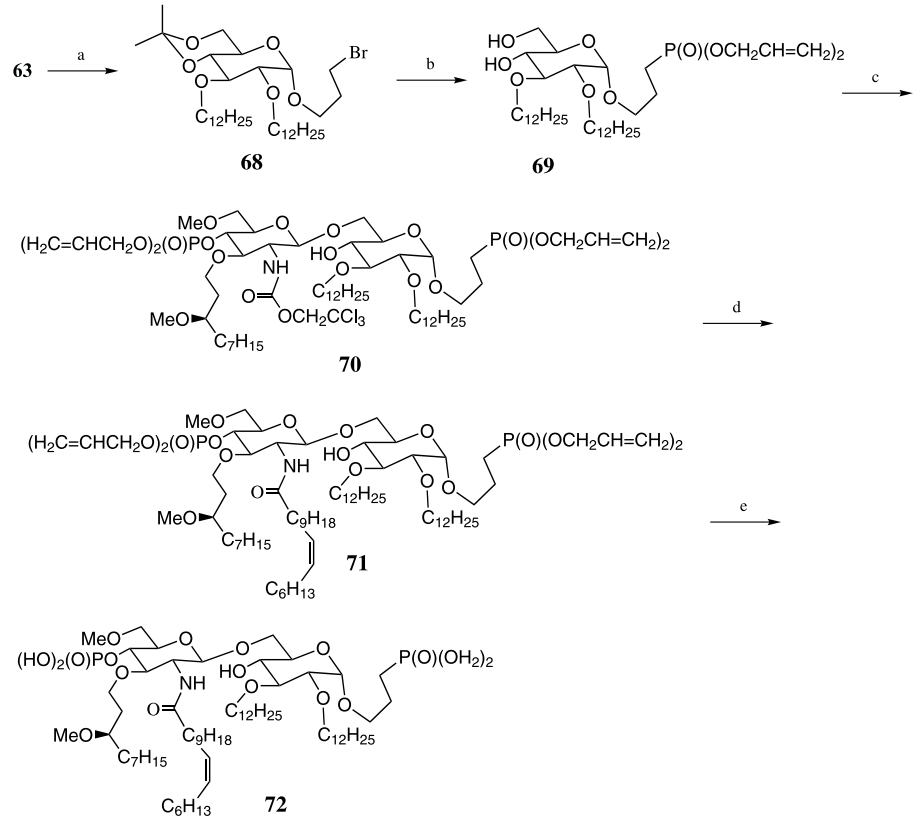
Thus, we could synthesize ten disaccharides (**8**, **14**, **21**, **31**, **40**, **51**, **57**, **62**, **67** and **72**).

2.2. Biological activity

The inhibitory activities on LPS-induced TNFα production in vitro (LPS-antagonistic activity) of ten synthetic compounds were investigated using human whole blood cells.[8] The IC_{50} values (nM) of these ten compounds, **8**, **14**, **21**, **31**, **40**, **51**, **57**, **62**, **67** and **72**, toward human whole blood cells were 11.2, 15.4, 2.7, 0.1, 0.4, 1.3, 3.2, 3.2, 1.4 and 14.4,



Scheme 10. Reagents and conditions: (a) 9-BBN, THF, rt, 18 h, then aq 3 M NaOH and aq 30% H_2O_2, rt, 3 h, 89%; (b) (1) *i*-$Pr_2NP(OCH_2CH{=}CH_2)_2$, 1*H*-tetrazole, THF, rt, 4 h, then aq 30% H_2O_2, THF, 0 °C, 1 h; (2) aq 80% AcOH, 60 °C, 1 h, 71%; (c) **5**, TMSOTf, MS 4A, CH_2Cl_2, under N_2, 0 °C, 1 h, 53%; (d) (1) Zn, AcOH–THF (1:1), rt, 4 h; (2) (*Z*)-11-octadecenoic acid, WSC.HCl, CH_2Cl_2, rt, 10 h, two steps 58%; (e) $(PPh_3)_4Pd$, PPh_3, Et_3N–HCOOH, THF, N_2, 55 °C, 4 h, 84%.



Scheme 11. Reagents and conditions: (a) CBr_4, PPh_3, CH_2Cl_2, rt, 1 h, 72%; (b) (1) triallylphosphite, 180 °C, 3 h; (2) aq 80% AcOH, 60 °C, 1 h, two steps 40%; (c) **5**, AgOTf, TMSOTf, MS 4A, CH_2Cl_2, N_2, rt, 16 h, 58%; (d) (1) Zn, AcOH–THF (1:1), rt, 4 h; (2) (*Z*)-11-octadecanoyl chloride, $NaHCO_3$, THF–H_2O (5:1), rt, 30 min, two steps 52%; (e) $(PPh_3)_4Pd$, PPh_3, Et_3N–HCOOH, THF, N_2, 55 °C, 4 h, 80%.

respectively. The activities of the α-carboxymethyl compounds (**8** and **14**) in the anomeric position were relatively weak, and those of compounds **21**, **31**, **40**, **51** and **57** having an α-2-(phosphonooxy)ethyl group, **62** having an α-2-(phosphono)ethyl group and **67** having an α-3-(phosphonooxy)propyl group at the anomeric position were sufficiently strong. Above all, the activity (IC_{50}=0.1 nM) of compounds **31** was strongest. The difference between the C6′ methoxyl group of **21** and the C6′ hydroxyl group of **31** largely affected the activity. Compounds **21** (IC_{50}=2.7 nM) possessing an α-2-(phosphonooxy)ethyl group and **62** (IC_{50}=3.2 nM) possessing an α-2-(phosphono)ethyl group at the anomeric position showed almost the same level of activities. On the contrary, the activity of 3-(phosphono)-propyl **72** (IC_{50}=14.4 nM) was much weaker than that of 3-(phosphonooxy)propyl **67** (IC_{50}=1.4 nM). It is difficult to understand that the difference of one methylene length between compounds **62** and **72** or one oxygen existence between compounds **67** and **72** has such an influence on the LPS-antagonistic activity.

In addition, inhibitory activity (ID_{50}) on TNFα production toward galactosamine loaded C3H/HeN mice in vivo of compounds **21**, **31**, **57**, **62** and **67** was measured.[9] The values were 0.29, 0.50, 0.61, not dose-dependent and 0.33 mg/kg, respectively. These compounds were sufficiently strong except for anomeric 2-(phosphono)ethyl compound **62** toward C3H/HeN mice. However, judging from the result of C6′ methoxy compound **21** and C6′ hydroxy compound **31**, methoxy compound **21** was a little stronger than hydroxyl compound **31** toward C3H/HeN mice in spite of compound **31** having been much stronger activity than compound **21** toward human whole blood cells. The difference of one methylene length between compounds 2-(phosphonooxy)ethyl **21** and 3-(phosphonooxy)propyl **67** did not influence largely the inhibitory activities on LPS-induced TNFα production.

Usually, lipid A analogs having six fatty acids chains[10] show LPS-agonistic (endotoxic) activity toward both human and mouse macrophages, and lipid IVa (biosynthetic precur of lipid A)[11] having four fatty acid chains shows LPS-antagonistic activity toward human blood cells and adversely endotoxic activity toward mouse macrophages.[12] However, the synthetic compounds, this time, showed LPS-antagonistic activity toward both human whole blood cells and galactosamine loaded C3H/HeN mice. This tendency was the same as that for nontoxic natural RsDPLA[3] and synthetic E5564[5] containing a (*Z*)-double bond in one of the fatty acids.

3. Conclusion

Thus, we could synthesize ten E5564-related disaccharides containing a glucose instead of the glucosamine at the reducing end. As a result, it was proved that these novel synthetic compounds had almost the same or stronger activities towards both human blood cells and murine macrophages than against classic lipid A-type disaccharides having the glucosamine-glucosamine moieties.[13]

4. Experimental

4.1. General procedure

^{1}H NMR spectra were recorded with a JEOL-GSX 400 or a JNM-ECT 500 spectrometer using tetramethylsilane (TMS) as an internal standard. IR absorption spectra were measured with an IR A-2 spectrophotometer, and mass spectra were obtained with a JMS-700 mass spectrometer. Separation of compounds by column chromatography was done with silica gel 60 (230–400 mesh ASTM) under a slightly elevated pressure (111–182 kPa) for easy elution. Commercially available anhydrous THF and dichloromethane were used for the reactions. DMF and pyridine were dried by storage over 4 Å molecular sieves.

4.1.1. Allyl 3-*O*-dodecyl-4,6-*O*-isopropylidene-2-*O*-tetradecyl-α-D-glucopyranoside (2). To a solution of allyl 3-*O*-docecyl-α-D-glucopyranoside **1** (2.84 g, 6.63 mmol) and tetradecyl methanesulfonate (2.33 g, 7.95 mmol) in DMF (20 ml) was added NaH (55% oil dispersion 347 mg, 7.95 mmol). After stirring for 16 h at room temperature, the reaction mixture was diluted with EtOAc, which was washed with ice water and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (9:1) gave **2** (3.10 g, 75%) as an oil. 400 MHz ^{1}H NMR ($CDCl_3$) 0.88 (6H, t, J=6.6 Hz), 1.26 (40H, bs), 1.41 (3H, s), 1.48 (3H, s), 1.50–1.61 (4H, m), 3.30 (1H, m), 3.50–3.74 (8H, m), 3.84 (1H, m), 4.07 (1H, dd, J=6.6, 13.2 Hz), 4.18 (1H, dd, J=5.1, 13.2 Hz), 4.91 (1H, d, J=3.7 Hz), 5.22 (1H, m), 5.32 (1H, m), 5.92 (1H, m)). FABMS (positive-ion) *m/z*, 625 $[M+H]^+$, 647 $[M+Na]^+$.

4.1.2. (Allyloxycarbonyl)methyl 3-*O*-dodecyl-4,6-*O*-isopropylidene-2-*O*-tetradecyl-α-D-glucopyranoside (3). To a solution of **2** (625 mg, 1.00 mmol) in MeCN–CCl_4–H_2O (2:2:3, 35 ml) were added RuO_2xH_2O (16 mg) and $NaIO_4$ (4.0 g). The mixture was stirred for 3 h at room temperature, and diluted with EtOAc, which was washed with water and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give a carboxylic acid, which was dissolved in DMF (10 ml). Allyl bromide (1.2 ml) and Et_3N (0.8 ml) were added to this solution, and this mixture was stirred for 16 h at room temperature, and diluted with EtOAc, which was washed with water and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrate in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with cyclohexane–EtOAc (9:1) gave **3** (490 mg, 72%) as an oil. 400 MHz ^{1}H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.6 Hz), 1.25 (40H, bs), 1.40 (3H, s), 1.48 (3H, s), 1.48–1.65 (4H, m), 3.18 (1H, m), 3.33 (1H, m), 3.50–3.89 (8H, m), 4.18, 4.34 (2H, AB-q, J=16.2 Hz), 4.62–4.67 (2H, m), 5.08 (1H, m), 5.22 (1H, m), 5.24–5.36 (2H, m), 5.91 (1H, m). FABMS (positive-ion) *m/z*, 683 $[M+H]^+$, 705 $[M+Na]^+$.

4.1.3. (Allyloxycarbonyl)methyl 3-*O*-dodecyl-2-*O*-tetradecyl-α-D-glucopyranoside (4). A solution of **3** (480 mg, 0.703 mmol) in AcOH–H_2O (4:1, 5 ml) was stirred for 2 h at 65 °C, and concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with

cyclohexane–EtOAc (3:2, then 2:3) gave triol **4** (319 mg, 71%) as a wax. IR ν_{max}(film) 3410, 2923, 2852, 1758, 1466 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3+D_2O$) δ 0.88 (6H, t, $J=6.6$ Hz), 1.26 (40H, bs), 1.54–1.68 (4H, m), 3.19 (1H, m), 3.33 (1H, m), 3.47 (1H, m), 3.51–3.67 (2H, m), 3.72–3.88 (4H, m), 3.91–3.98 (2H, m), 4.21, 4.33 (2H, AB-q, $J=16.8$ Hz), 4.62–4.67 (2H, m), 5.12 (1H, m), 5.25–5.37 (2H, m), 5.91 (1H, m). FABMS (positive-ion) *m/z*, 665 $[M+Na]^+$. Anal. Calcd for $C_{37}H_{70}O_8$,: C, 69.12; H, 10.97. Found: C, 69.09; H, 10.81.

4.1.4. (Allyloxycarbonyl)methyl 6-*O*-{2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl}-3-*O*-dodecyl-2-*O*-tetradecyl-α-D-glucopyranoside (6). To a solution of imidate **5** (249.2 mg, 0.296 mmol) and **4** (190 mg, 0.296 mmol) in CH_2Cl_2 (7 ml) was added MS 4 Å (400 mg). After stirring for 30 min at room temperature, the mixture was cooled at −40 °C, and TMSOTf (10 mg, 0.045 mmol) was added to this mixture. The mixture was stirred for 2 h at −40 °C under nitrogen, and diluted with EtOAc, which was washed with satd $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with cyclohexane–EtOAc (1:1, then 1:2) gave **6** (169 mg, 43%) as a gum. IR ν_{max}(film) 3448, 3291, 3084, 2925, 2855, 1751, 1650 (w), 1546, 1465 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.89 (9H, t, $J=$ 6.6 Hz), 1.26 (50H, bs), 1.40–1.60 (8H, m), 3.25–3.91 (23H, m, containing 3H, s, at δ 3.28 ppm, and 3H, s, at δ 3.39 ppm), 4.05–4.19 (2H, m), 4.28–4.35 (3H, m), 4.55–4.81 (8H, m), 5.12 (1H, m), 5.09 (1H, d, $J=3.7$ Hz), 5.24–5.39 (6H, m), 5.90–5.99 (3H, m). FABMS (positive-ion) *m/z*, 1346 $[M+Na]^+$, 1344 $[M+Na, {}^{35}Cl]^+$. HRFABMS, calcd for $C_{64}H_{115}NCl_3O_{18}PNa$: 1344.6818. Found: 1344.6826.

4.1.5. (Allyloxycarbonyl)methyl 6-*O*-{2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-[(*Z*)-11-octadecenoylamino]-β-D-glucopyranosyl}-3-*O*-dodecyl-2-*O*-tetradecyl-α-D-glucopyranoside (7). To a solution of **6** (154 mg, 0.116 mmol) in THF–AcOH (1:1, 6 ml) was added Zn powder (150 mg). The mixture was stirred vigorously with a magnetic stirrer at 25 °C for 4 h, and filtered. The filtrate was concentrated in vacuo below 30 °C, and diluted with EtOAc, which was washed with aq satd $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give an amine, which was dissolved in THF (1.0 ml)–H_2O (0.3 ml) containing $NaHCO_3$ (30 mg, 0.357 mmol). To this solution was added a solution of (*Z*)-11-octadecenoyl chloride [obtained from (*Z*)-11-octadecenoic acid (40 mg, 0.142 mmol) by treatment of the excess oxalyl chloride in benzene at 25 °C for 2 h] in THF (0.5 ml) with vigorous stirring at 25 °C. After stirring for 0.5 h, the reaction mixture was diluted with EtOAc, which was washed with aq satd $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with cyclohexane–EtOAc (1:2) gave **7** (125 mg, 77%) as a gum. IR ν_{max}(KBr) 3306, 3082 (w), 2925, 2854, 1760, 1659, 1634, 1545 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (12H, t, $J=6.4$ Hz), 1.25 (62H, bs), 1.35–1.80 (14H, m), 2.00–2.02 (6H, m), 2.10–2.25 (4H, m), 3.10–4.40 (29H, m, containing two 3H, s, at 3.28 and 3.38 ppm), 4.54–4.66 (6H, m), 5.09–5.39 (10H, m), 5.89–5.98 (3H, m), 5.96–6.06 (1H, m). FABMS (positive-ion) *m/z*, 1434 $[M+Na]^+$. HRFABMS, calcd for $C_{79}H_{146}NO_{17}$-PNa: 1435.0226. Found: 1435.0234.

4.1.6. Carboxymethyl 6-*O*-{2-deoxy-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-[(*Z*)-11-octadecenoylamino]-4-*O*-phosphono-β-D-glucopyranosyl}-3-*O*-dodecyl-2-*O*-tetradecyl-α-D-glucopyranoside (8). To a solution of **7** (104 mg, 0.074 mmol) in dry THF (6 ml) were added PPh_3 (10 mg, 0.034 mmol), Et_3N (37 mg, 0.365 mmol), HCOOH (34 mg, 0.728 mmol) and $Pd(PPh_3)_4$ (5 mg, 0.004 mmol) in this sequence. The solution was stirred for 4 h at 55 °C under nitrogen, and concentrated in vacuo to give a mixture, which was chromatographed on a DEAE-cellulose (Whatman ion-exchange cellulose, wet 3 g) column. The column was prepared by preliminary consecutive washing with 30 ml each of 0.5 M HCl, H_2O, 0.5 M NaOH, and H_2O, and 12 ml each of 1 M AcOH and H_2O, and 30 ml of 0.05 M $AcONH_4$, 30 ml each of $CHCl_3$–MeOH–H_2O (2:3:1) and finally $CHCl_3$–MeOH (2:1). The column was eluted with 3 ml each of $CHCl_3$–MeOH (2:1), then 0.05 M $AcONH_4$ in $CHCl_3$–MeOH–H_2O (2:3:1). The fractions containing **8** were collected. To this solution were added another volume of $CHCl_3$ and aq 0.15 M HCl to adjust the ratio of $CHCl_3$–H_2O–MeOH to 1:1:1, and the mixture was shaken well. The lower $CHCl_3$ layer was separated, and concentrated in vacuo to give **8** (83 mg, 95%) as a powder. [The fractions were analyzed by silica-gel TLC and a solvent system of $CHCl_3$–MeOH–AcOH–H_2O (8:4:1:1).] IR ν_{max}(KBr) 3500–3000 (br), 2924, 2853, 1735, 1655, 1630, 1549, 1466 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3+D_2O$) δ 0.88 (12H, t, $J=$ 6.6 Hz), 1.26 (72H, bs), 1.40–1.80 (8H, m), 1.99–2.02 (4H, m), 2.20–2.40 (2H, m), 3.10–5.10 (29H, m, containing two 3H, s at δ 3.25 and 3.38 ppm), 5.31–5.38 (2H, m). FABMS (negative-ion) *m/z*, 1291 $[M-H]^-$. HRFABMS, calcd for $C_{70}H_{133}NO_{17}P$: 1290.9336. Found: 1290.9317. Anal. Calcd for $C_{70}H_{134}NO_{17}P$: C, 62.35; H, 10.09; N, 1.70; P, 2.08. Found: C, 62.78; H, 10.41; N, 1.10; P, 2.40.

4.1.7. (Allyloxycarbonyl)methyl 2-*O*-[(*R*)-3-*tert*-butyldimethylsilyloxytetradecyl]-3-*O*-dodecyl-4,6-*O*-isopropylidene-α-D-glucopyranoside (10). Compound **9** was treated as described for the formation of **3** from **2** to give **10** (72% yield) as an oil. IR ν_{max}(film) 2926, 2856, 1759, 1745 (shoulder) cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.04 (3H, s), 0.05 (3H, s), 0.88 (9H, s, and 6H, t, $J=6.6$ Hz), 1.26 (38H, bs), 1.40 (3H, s), 1.48 (3H, s), 1.48–1.55 (2H, m), 1.74–1.79 (2H, m), 3.33 (1H, m), 3.50–3.86 (10H, m), 4.17, 4.30 (2H, AB-q, $J=12.8$ Hz), 4.64–4.65 (2H, m), 5.07 (1H, d, $J=3.7$ Hz), 5.24–5.36 (2H, m), 5.92 (1H, m). FABMS (positive-ion) *m/z*, 813 $[M+H]^+$, 835 $[M+Na]^+$. HRFABMS, calcd for $C_{46}H_{88}O_9SiNa$: 835.6095. Found: 835.6084.

4.1.8. (Allyloxycarbonyl)methyl 2-*O*-[(*R*)-3-hydroxytetradecyl]-3-*O*-dodecyl-α-D-glucopyranoside (11). Compound **10** was treated as described for the formation of **4** from **3** to give triol **11** (60%) as a solid, mp 55–56 °C (recrystallized from EtOAc/hexane=1/4). IR ν_{max}(KBr) 3426, 2919, 2850, 1753, 1468 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, $J=6.6$ Hz), 1.26 (36H, bs), 1.40–1.78

(8H, m), 2.10 (1H, bs, OH)), 2.62 (1H, bs, OH), 3.34 (1H, dd, J=3.7, 9.5 Hz), 3.49–4.00 (10H, m), 4.20, 4.34 (2H, AB-q, J=16.8 Hz), 4.62–4.66 (2H, m), 5.21 (1H, d, J=2.9 Hz), 5.25–5.36 (2H, m), 5.91 (1H, m). Anal. Calcd for $C_{37}H_{70}O_9$: C, 67.44; H, 10.71. Found: C, 67.41; H, 10.70.

4.1.9. (Allyloxycarbonyl)methyl 6-*O*-{2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl}-3-*O*-dodecyl-2-*O*-[(R)-3-hydroxytetradecyl]-α-D-glucopyranoside (12). Compound **11** and imidate **5** were treated as described for the formation of **6** from **5** to give **12** (45%) as a gum, and imidate **5** (85 mg, 34%) and starting triol **11** (80 mg, 42%) were recovered. IR ν_{max}(film) 3457 (br), 3325 (br), 3085 (w), 2926, 2855, 1748, 1650 (w), 1545 cm^{-1}. 400 MHz ^{1}H NMR ($CDCl_3$) δ 0.88 (9H, t, J=6.6 Hz), 1.26 (46H, bs), 1.36–1.80 (10H, m), 3.22–4.90 (37H, m, containing 3H, s, at δ 3.27 ppm, and 3H, s, at δ 3.39 ppm), 5.07–5.40 (7H, m), 5.87–5.99 (3H, m). FABMS (positive-ion) m/z, 1360 $[M+Na, {}^{35}Cl]^+$. HRFABMS, calcd for $C_{64}H_{115}NCl_3O_{19}PNa$,: 1360.6764. Found: 1360.6764.

4.1.10. (Allyloxycarbonyl)methyl 6-*O*-{2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-[(*Z*)-11-octadecenoylamino]-β-D-glucopyranosyl}-3-*O*-dodecyl-2-*O*-[(R)-3-hydroxytetradecyl]-α-D-glucopyranoside (13). Compound **12** was treated as described for the formation of **7** from **6** to give **13** (67%) as a gum. IR ν_{max}(film) 3500–3000 (br), 2926, 2855, 1752, 1652, 1549 cm^{-1}. 400 MHz ^{1}H NMR ($CDCl_3$) δ 0.88 (12H, t, J=6.6 Hz), 1.26 (72H, bs), 1.37–1.85 (6H, m), 1.97–2.02 (4H, m), 2.12–2.24 (2H, m), 2.97–4.32 (30H, m, containing two 3H, s, at 3.27 and 3.38 ppm), 4.53–4.66 (6H, m), 5.02–5.40 (10H, m), 5.85–5.98 (3H, m), 6.09 (1H, d, J=6.6 Hz). FABMS (positive-ion) m/z, 1450 $[M+Na]^+$. HRFABMS, calcd for $C_{79}H_{146}NO_{18}PNa$: 1451.0174. Found: 1451.0171.

4.1.11. Carboxymethyl 6-*O*-{2-deoxy-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-[(*Z*)-11-octadecenoylamino]-4-*O*-phosphono-β-D-glucopyranosyl}-3-*O*-dodecyl-2-*O*-[(R)-3-hydroxytetradecyl]-α-D-glucopyranoside (14). Compound **13** (110 mg, 0.077 mmol) was treated as described for the formation of **8** from **7** to give **14** (55 mg, 55%) as a powder. IR ν_{max}(KBr) 3292 (br), 2925, 2854, 1737, 1654, 1631, 1552, 1466 cm^{-1}. 400 MHz ^{1}H NMR ($CDCl_3+CD_3OD$, 5:1) δ 0.88 (12H, t, J=6.6 Hz), 1.27 (70H, bs), 1.40–1.80 (8H, m), 2.00–2.03 (4H, m), 2.15–2.23 (2H, m), 3.26–4.26 (28H, m, containing two 3H, s at δ 3.31 and 3.41 ppm), 4.65 (1H, d, J=6.5 Hz), 5.08 (1H, d, J=2.9 Hz), 5.35 (2H, m). FABMS (negative-ion) m/z, 1306 $[M-H]^-$, 1328 $[M-2H+Na]^-$. HRFABMS, calcd for $C_{70}H_{133}NO_{18}P$: 1306.9260. Found: 1306.9288. Anal. Calcd for $C_{70}H_{134}NO_{18}P$: C, 64.24; H, 10.32; N, 1.07; P, 2.37. Found: C, 64.46; H, 10.46; N, 1.27; P, 2.29.

4.1.12. 2-Hydroxyethyl 2,3-di-*O*-dodecyl-4,6-*O*-isopropylidene α-D-glucopyranoside (16). To a solution of **15** (4.00 g, 6.70 mmol) in THF–H_2O (7:2, 66 ml) were added $NaIO_4$ (6.7 g) and a 2.5% solution of OsO_4 in *tert*-BuOH (1.4 ml). This mixture was stirred for 3 h at room temperature, and diluted with EtOAc, which was washed with aq satd $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrate in vacuo to give an aldehyde (4 g), which was dissolved in EtOH (50 ml). To this solution was added $NaBH_4$ (270 mg), and the solution was stirred for 20 min, quenched with AcOH (200 mg), diluted with EtOAc, which was washed with aq satd $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (3:1) gave alcohol **16** (3.00 g, 73%) as an oil. 400 MHz ^{1}H NMR ($CDCl_3$) δ 0.88 (9H, t, J=6.6 Hz), 1.26 (36H, bs), 1.40 (3H, s), 1.41–1.43 (4H, m), 1.48 (3H, s), 2.82 (1H, bs, OH), 3.30 (1H, m), 3.51–3.85 (14H, m), 4.89 (1H, d, J=3.7 Hz). FABMS (positive-ion) m/z, 601 $[M+H]^+$, 623 $[M+Na]^+$.

4.1.13. 2-(Diallylphosphonooxy)ethyl 2,3-di-*O*-dodecyl-4,6-*O*-isopropylidene-α-D-glucopyranoside (17). To a solution of **16** (5.65 g, 9.2 mmol) in CH_2Cl_2 (70 ml) were added 1*H*-tetrazole (1.54 g, 22 mmol), diallyl diisopropylphosphoramidite (3.50 g, 1.128 mmol) and Na_2SO_4 (6 g). After stirring for 30 min at room temperature, to this reaction mixture were added THF (70 ml) and aq 30% H_2O_2 solution (2 ml). The mixture was stirred for 15 min at room temperature, and diluted with EtOAc, which was washed with aq 10% $Na_2S_2O_3$, aq satd $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (2:1) gave alcohol **17** (6.60 g, 99%) as an oil. 400 MHz ^{1}H NMR ($CDCl_3$) δ 0.88 (6H, J=6.6 Hz), 1.21–1.38 (36H, m), 1.39 (3H, s), 1.47 (3H, s), 1.50–1.58 (4H, m), 3.25–3.3 (1H, m), 3.51–3.88 (11H, m) 4.23–4.27 (2H, m) 4.56–4.60 (4H, m), 4.89 (1H, d, J=3.7 Hz), 5.25–5.41 (4H, m), 5.91–6.00 (2H, m). FABMS (positive-ion) m/z, 761 $[M+H]^+$, 783 $[M+Na]^+$.

4.1.14. 2-(Diallylphosphonooxy)ethyl 2,3-di-*O*-dodecyl-α-D-glucopyranoside (18). To a solution of **17** (6.60 g, 8.67 mmol) in MeOH (60 ml) was added *p*-TsOH·H_2O (412 mg, 2.17 mmol). After stirring for 2 h at room temperature, the reaction mixture was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with EtOAc and then 10% MeOH in EtOAc gave **18** (6.01 g, 96%) as a wax. 400 MHz ^{1}H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.6 Hz), 1.26 (36H,bs), 1.5–1.71 (4H,m), 3.27 (1H, dd, J=3.7, 8.8 Hz), 3.41–3.91 (11H, m), 4.24–4.28 (2H, m), 4.48–4.59 (4H, m), 4.95 (1H, d, J=3.7 Hz), 5.19–5.4 (4H,m), 5.9–6.00 (2H, m). FABMS (positive-ion) m/z, 721. $[M+H]^+$, 743 $[M+Na]^+$. HRFABMS, calcd for $C_{38}H_{74}O_{10}P$: 721.4941. Found: 721.4950.

4.1.15. 2-(Diallylphosphonooxy)ethyl 6-*O*-{2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl}-2,3-di-*O*-dodecyl-α-D-glucopyranoside (19). To a solution of **5** (1.00 g, 1.18 mmol) and **18** (1.02 g, 1.49 mmol) in CH_2Cl_2 (30 ml) were added MS 4 Å (1.5 g), AgOTf (350 mg, 1.36 mmol) and TMSOTf (20 mg, 0.090 mmol). The mixture was stirred for 16 h at room temperature under nitrogen, and diluted with EtOAc, which was washed with aq satd $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in

vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with EtOAc–Hexane (2:1) gave **19** (1.01 g, 60%) as a gum. 400 MHz ^{1}H NMR ($CDCl_3$) δ 0.88 (9H, t, J=6.6 Hz), 1.26 (48H, bs), 1.38–1.46 (2H, m), 1.53–1.58 (4H, m), 1.69–1.77 (2H, m), 2.60 (1H, bs, OH), 3.21–3.89 (23H, m, containing two 3H at δ 3.28 and 3.39 ppm), 4.09–4.32 (4H, m), 4.52–4.60 (8H, m), 4.95 (1H, d, J=3.7 Hz), 5.24–5.40 (8H, m), 5.89–5.99 (4H, m), 6.41 (1H, broad, NH). FABMS (positive-ion) *m/z*, 1422 $[M+Na, {}^{35}Cl]^+$, 1424. HRFABMS, calcd for $C_{65}H_{118}Cl_3NO_{20}P_2Na$: 1422.6468. Found: 1422.6473.

4.1.16. 2-(Diallylphosphonooxy)ethyl 6-*O*-{2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-[(*Z*)-11-octadecenoylamino]-β-D-glucopyranosyl}-2,3-di-*O*-dodecyl-α-D-glucopyranoside (20). Compound **19** was treated as described for the formation of **7** from **6** to give **20** (75%) as a gum. 400 MHz ^{1}H NMR ($CDCl_3$) δ 0.88 (12H, t, J=6.6 Hz), 1.26 (66H, bs), 1.40–1.79 (10H, m), 1.99–2.04 (4H, m), 2.17–2.25 (2H, m), 3.01(1H, bs, OH), 3.17–3.82 (24H, m, containing two 3H, s, at 3.28 and 3.38 ppm), 3.97–4.31 (5H, m), 4.52–4.58 (8H, m), 4.88 (1H, d, J=3.7 Hz), 5.13 (1H, d, J=8.1 Hz), 5.23–5.38 (10H, m), 5.90–6.00 (4H, m), 6.62 (1H, d, J=6.6 Hz, NH). FABMS (positive-ion) *m/z*, 1512 $[M+Na]^+$. HRFABMS, calcd for $C_{80}H_{149}NO_{19}P_2Na$: 1513.0097. Found: 1513.0121.

4.1.17. 2-(Phosphonooxy)ethyl 6-*O*-{2-deoxy-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-[(*Z*)-11-octadecenoylamino]-4-*O*-phosphono-β-D-glucopyranosyl}-2,3-di-*O*-dodecyl-α-D-glucopyranoside (21). Compound **20** was treated for 16 h as described for the formation of **8** from **7** to give **21** (45%) as a wax. IR ν_{max}(KBr) 3292 (br), 2954, 2853, 1630 cm^{-1}. 400 MHz ^{1}H NMR ($CDCl_3+CD_3OD$) δ 0.88 (12H, t, J=6.6 Hz), 1.26 (66H, bs), 1.40–1.79 (10H, m), 1.99–2.07 (4H, m), 2.17–2.25(2H, m), 3.20–3.90 (24H, m, containing two 3H, s at 3.30 and 3.40 ppm), 4.00–4.21 (5H, m), 4.65(1H, d, J=8.1 Hz), 4.88 (1H, d, J=3.7 Hz), 5.37–5.40 (2H, m). FABMS (negative-ion) *m/z*, 1328 $[M-H]^-$, 1350 $[M+Na\text{-}2H]^-$. Anal. Calcd for $C_{68}H_{133}NO_{19}P_2$.: C, 61.37; H, 10.07; N, 1.05; P, 4.66. Found: C, 60.89; H, 10.11; N, 1.13; P, 4.42.

4.1.18. Allyl 2-deoxy-4,6-*O*-isopropylidene-3-*O*-[(*R*)-3-methoxydecyl]-2-(trifluoroacetylamino)-β-D-glucopyranoside (23). Compound **22** and (*R*)-3-methoxydecyl *p*-toluenesulfonate were treated as described for the formation of **2** from **1** to give **23** (77%) as an amorphous solid. IR ν_{max}(KBr) 3304, 3114,2995, 2930,2877, 2858, 2825, 1705, 1674 cm^{-1} 500 MHz ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, t, J=6.8 Hz), 1.27–1.47 (12H, m), 1.41 (3H, s), 1.50 (3H, s), 1.63–1.67 (2H, m), 3.25 (1H, m), 3.28 (3H, s), 3.32 (1H, td, J=9.8, 4.9 Hz), 3.43 (1H, m), 3.58–3.64 (2H, m), 3.77–3.87 (3H, m), 3.93 (1H, dd, J=4.9, 10.7 Hz), 4.06 (1H, dd, J=5.9, 12.7 Hz), 4.31 (1H, dd, J=4.9, 12.7 Hz), 4.88 (1H, d, J=7.8 Hz), 5.19–5.28 (2H, m), 5.83 (1H, m), 6.56 (1H, d, J=7.8 Hz, NH). FABMS (positive-ion) *m/z*, 548 $(M+Na)^+$, 526 $(M+H)^+$. HRFABMS, calcd for $C_{25}H_{42}F_3NO_7Na$: 548.2808. Found: 548.2815.

4.1.19. Allyl 2-deoxy-3-*O*-[(*R*)-3-methoxydecyl]-2-(trifluoroacetylamino)-β-D-glucopyranoside (24). Compound **23** was treated as described for the formation of **18** from **17** to give **24** (92%) as a powder. IR ν_{max}(KBr) 3548, 3375, 3265, 3116, 2927, 2874, 2858, 1702, 1672 cm^{-1}. 400 MHz ^{1}H NMR ($CDCl_3$) δ 0.88 (3H, t, J=6.6 Hz), 1.27–1.77 (14H, m), 3.01 (2H, bs, OH), 3.29 (3H, s), 3.34–3.53 (3H, m), 3.59 (1H, t, J=9.5, 8.8 Hz), 3.67 (1H, m), 3.75–3.85 (3H, m), 3.92 (1H, dd, J=3.7, 11.7 Hz), 4.07 (1H, m), 4.31 (1H, m), 4.84 (1H, d, J=8.8 Hz), 5.19–5.29 (2H, m). FABMS (positive-ion) *m/z*, 508 $[M+Na]^+$, 486 $[M+H]^+$. HRFABMS, calcd for $C_{22}H_{38}F_3NO_7Na$: 508.2494. Found: 508.2503.

4.1.20. (*Z*)-1-Propenyl 2-deoxy-3-*O*-[(*R*)-3-methoxydecyl]-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (25). A solution of **24** (5.34 g, 11.0 mmol) in DMSO (30 ml) containing *tert*-BuOK (3.10 g, 27.6 mmol) was stirred for 85 °C for 2 h under nitrogen. To this solution was added H_2O (10 ml), and this solution was stirred at 85 °C for 6 h. After cooling, the aqueous solution was extracted with CH_2Cl_2 (three times), washed with H_2O and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give an amine, which was dissolved in THF (40 ml), and aq satd $NaHCO_3$ (20 ml) was added. To this mixture was added 2,2,2-trichloroethyl chloroformate (2.58 g, 12.2 mmol), and this mixture was stirred at 0 °C for 30 min. After another amount of aq satd $NaHCO_3$ was added, the reaction mixture was extracted with CH_2Cl_2, which was washed with brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (2:3) gave **25** (4.70 g, 76%) as a solid. IR ν_{max}(KBr) 3323, 3054, 2927, 2873, 2857, 1716, 1672, 1642 cm^{-1}. 400 MHz ^{1}H NMR ($CDCl_3$) δ 0.89 (3H, t, J=6.8 Hz), 1.28–1.62 (15H, m), 1.74–1.79 (2H, m), 2.36 (1H, s, OH), 3.30 (3H, s), 3.36–3.46 (3H, m), 3.58–3.71 (3H, m), 3.81 (1H, m), 3.90–3.94 (2H, m), 4.06 (1H, s, OH), 4.57 1H, m), 4.74 (2H, s), 4.86 (1H, d, J=6.8 Hz), 5.37 (1H, br, NH), 6.15 (1H, m). FABMS (positive-ion) *m/z*, 586 $[M+Na]^+$, 564 $[M+H]^+$. HRFABMS, calcd for $C_{23}H_{40}Cl_3NO_8Na$: 586.1726. Found: 586.1703.

4.1.21. (*Z*)-1-Propenyl 6-*O*-allyloxycarbonyl-2-deoxy-3-*O*-[(*R*)-3-methoxydecyl]-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (26). To a solution of **25** (4.60 g, 8.14 mmol) in CH_2Cl_2 (30 ml) were added pyridine (1.4 ml, 17.3 mmol) and allyl chloroformate (1.1 ml, 10.4 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h, diluted with CH_2Cl_2, washed with aq satd $NaHCO_3$, H_2O and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (3:2) gave **26** (4.95 g, 94%) as a wax. IR ν_{max}(KBr) 3518, 3310, 3086, 3059, 2932, 2885, 2857, 1728, 1709, 1674, 1652 cm^{-1}. 400 MHz ^{1}H NMR ($CDCl_3$) δ 0.89 (3H, t, J=6.8 Hz), 1.25–1.62 (15H, m), 1.70–1.79 (2H, m), 3.30 (3H, s), 3.36–3.44 (2H, m), 3.50–3.71 (4H, m), 3.91–3.94 (2H, m, containing OH), 4.38 (1H, dd, J=4.9, 11.7 Hz), 4.50 (1H, dd, J=2.0, 11.7 Hz), 4.56 (1H, m), 4.63 (2H, d, J=5.9 Hz), 4.74 (2H, s), 4.85 (1H, m), 5.26–5.38 (3H, m, containing NH), 5.93 (1H, m), 6.16 (1H, dd, J=2.0, 5.9 Hz). FABMS (positive-ion) *m/z*, 670 $[M+Na]^+$, 648 $[M+H]^+$. HRFABMS, calcd for $C_{27}H_{44}Cl_3NO_{10}Na$: 670.1914. Found: 670.1959.

4.1.22. (*Z*)-1-Propenyl 6-*O*-allyloxycarbonyl-2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (27). To a solution of **26** (4.80 g, 7.40 mmol) in THF (30 ml) were added 1*H*-tetrazole (830 mg, 11.8 mmol) and diallyl diisopropylphosphoramidite (2.50 g, 10.2 mmol) under nitrogen at room temperature. After stirring for 20 min, the mixture was cooled to 0 °C, and aq 30% H_2O_2 (10 ml) was added to this phosphite solution. The mixture was stirred for 30 min, and diluted with EtOAc, which was washed with aq satd $Na_2S_2O_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (3:2) gave **27** (5.17 g, 86%) as a gum. IR ν_{max}($CHCl_3$) 3450, 3089, 2955, 2873, 2859, 2829, 1746, 1674, 1650 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (3H, t, J=6.8 Hz), 1.27–1.56 (15H, m), 1.68–1.81 (2H, m), 3.29 (3H, s), 3.31 (1H, m), 3.47 (1H, m), 3.72–3.82 (3H, m), 3.90 (1H, m), 4.32–4.38 (2H, m), 4.52–4.63 (8H, m), 4.73 (2H, s), 4.99 (1H, m), 5.19–5.38 (6H, m), 5.56 (1H, m, NH), 5.89–5.99 (3H, m), 6.13 (1H, m). FABMS (positive-ion) *m*/*z*, 830 $[M+Na]^+$, 808 $[M+H]^+$. HRFABMS, calcd for $C_{33}H_{53}Cl_3NO_{13}PNa$: 830.2215. Found: 830.2231.

4.1.23. 6-*O*-Allyloxycarbonyl-2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-(methoxy)decyl]-2-(2,2,2-trichloroethoxycarbonylamino)-D-glucopyranose (28). To a solution of **27** (4.80 g, 5.93 mmol) in THF (30 ml) were added I_2 (3.08 g, 12.1 mmol) and H_2O (6 ml). After stirring for 30 min at room temperature, the reaction mixture was diluted with EtOAc, washed with aq satd $Na_2S_2O_3$, aq satd $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (1:1) gave **28** (4.30 g, 94%) as a gum. IR ν_{max}($CHCl_3$) 3598, 3435, 3317, 3089, 2955, 2931, 2873, 2858, 1746, 1651 cm^{-1}. 500 MHz ^1H NMR ($CDCl_3$) δ 0.88 (3H, t, J=6.8 Hz), 1.26–1.50 (12H, m), 1.67–1.78 (2H, m), 3.26 (3H, s), 3.32 (1H, m), 3.63–3.73 (2H, m), 3.86–3.94 (2H, m), 4.18 (1H, m), 4.30–4.38 (3H, m), 4.51–4.63 (7H, m), 4.67, 4.74 (2H, AB-q, J=11.7 Hz), 5.24–5.40 (7H, m), 5.81 (1H, d, J=8.8 Hz, NH), 5.89–5.98 (3H, m). FABMS (positive-ion) *m*/*z*, 790 $[M+Na]^+$, 768 $[M+H]^+$. HRFABMS, calcd for $C_{30}H_{50}Cl_3NO_{13}P$: 768.2085. Found: 768.2089.

4.1.24. 2-(Diallylphosphonooxy)ethyl 6-*O*-{6-*O*-allyloxycarbonyl-2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosy}-2,3-di-*O*-dodecyl-α-D-glucopyranoside (29). To a solution of **28** (164 mg, 0.213 mmol) in CH_2Cl_2 (5 ml) were added trichloroacetonitrile (0.15 ml, 1.4 mmol) and Cs_2CO_3 (15 mg). After stirring for 1 h at room temperature, this solution was diluted with CH_2Cl_2, and washed with aq satd $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give an imidate, which was dissolved in CH_2Cl_2 (5 ml). To this solution were added a solution of **18** (154 mg, 0.213 mol) and molecular sieves 4 Å (150 mg) under nitrogen. After stirring for 30 min at room temperature, to this mixture was added TMSOTf (10 mg) at 0 °C. After stirring for 16 h at rt, the reaction mixture was quenched with aq satd $NaHCO_3$, extracted with CH_2Cl_2, and concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (1:4) gave **29** (125 mg, 52%) as a gum. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (9H, t, J=6.6 Hz), 1.26–1.44 (39H, m), 1.53–1.60 (6H, m), 1.64–1.79 (3H, m), 2.50 (1H, bs), 3.23–3.36 (8H, m, containing 3H, s, at δ 3.28 ppm), 3.45–3.92 (15H, m), 4.10 (1H, br d, J= 8.1 Hz), 4.19–4.36 (5H, m), 4.53–4.59 (10H, m), 4.63 (2H, d, J=5.9 Hz), 4.68 (1H, br.d, J=11.7 Hz), 4.78 (1H, d, J=11.7 Hz), 4.88–4.91 (2H, m), 5.23–5.40 (10H, m), 5.88–5.98 (5H, m), 6.54 (1H, m). FABMS (positive-ion) *m*/*z*, 1492 $[M+Na]^+$.

4.1.25. 2-(Diallylphosphonooxy)ethyl 6-*O*-{6-*O*-allyloxycarbonyl-2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-2-[(*Z*)-11-octadecenoylamino]-β-D-glucopyranosyl}-2,3-di-*O*-dodecyl-α-D-glucopyranoside (30). Compound **29** (125 mg, 0.085 mmol) was treated as described later in the formation of **50** from **49** to give **30** (62 mg, 47%) as an oil. IR ν_{max}(film) 3300, 3085, 2925, 2855, 1752, 1652 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (12H, t, J=6.8 Hz), 1.25–1.79 (77H, m), 1.99–2.01 (3H, m), 2.16–2.24 (2H, m), 2.87 (1H, d, J=3.9 Hz), 3.12–3.17 (1H, m), 3.24 (1H, dd, J=9.8, 2.9 Hz), 3.26–3.38 (5H, m, containing 3H, s, at δ 3.28 ppm), 3.45–3.55 (3H, m), 3.57–3.85 (8H, m), 4.02–4.06 (1H, m), 4.10 (1H, d, J=10.7 Hz), 4.16–4.33 (3H, m), 4.51–4.64 (12H, m), 4.88 (1H, d, J= 3.9 Hz), 5.19 (1H, d, J=7.8 Hz), 5.23–5.40 (12H, m), 5.89–5.98 (5H, m), 6.74 (1H, d, J=6.8 Hz). FABMS (positive-ion) *m*/*z*, 1582 $[M+Na]^+$. HRFABMS, calcd for $C_{83}H_{151}O_{21}NP_2Na$: 1583.0161. Found: 1583.0179.

4.1.26. 2-(Phosphonooxy)ethyl 6-*O*-{2-deoxy-3-*O*-[(*R*)-3-methoxydecyl]-2-[(*Z*)-11-(octadecenoyl)amino-4-*O*-phosphono]-β-D-glucopyranosyl}-2,3-di-*O*-dodecyl-α-D-glucopyranoside (31). Compound **30** (136 mg, 0.087 mmol) was treated as described for the formation of **8** from **7** to give **31** (67 mg, 76%) as a powder. IR ν_{max}(KBr) 3285, 3064, 3005, 2955, 2923, 2853, 2327, 1716, 1657, 1632 cm^{-1}. 500 MHz ^1H NMR ($CDCl_3+CD_3OD$) δ 0.90 (12H, t, J=6.8 Hz), 1.29–1.46 (70H, m), 1.56–1.74 (8H, m), 2.01–2.04 (4H, m), 2.24–2.28 (2H, m), 3.20 (1H, dd, J= 3.9, 9.8 Hz), 3.30 (3H, s), 3.31–3.40 (2H, m), 3.45–3.49 (2H, m), 3.53–3.56 (1H, m), 3.62–3.88 (12H, m), 4.07–4.17 (4H, m), 4.49 (1H, d, J=8.8 Hz), 4.92 (1H, d, J=3.9 Hz), 5.33–5.35 (2H, m). ESIMS (negative-ion) *m*/*z*, 1314 $[M-H]^-$. HRESIMS, calcd for $C_{67}H_{130}NO_{19}P_2$: 1314.8708. Found: 1314.8694. Anal. Calcd for $C_{67}H_{131}NO_{19}P_2$: C, 61.12; H, 10.03; N, 1.06; P, 4.70. Found: C, 60.98; H, 10.01; N, 1.10; P, 4.62.

4.1.27. Allyl 2-*O*-acetyl-3-*O*-decyl-4,6-*O*-isopropylidene-α-D-glucopyranoside (33). A solution of **32** (1.50 g, 4.244 mmol) in pyridine (5 ml) was allowed to stand for 5 h at room temperature, and concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with cyclohexane–EtOAc (3:1) gave **33** (1.50 g, 99%) as an oil. IR ν_{max}(film) 2995, 2926, 2857, 1748, 1647 (w) cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (3H, t, J= 6.9 Hz), 1.26 (16H, bs), 1.41 (3H, s), 1.50 (3H, s), 2.11 (3H, s), 3.54 (1H, m), 3.60–3.77 (5H, m), 3.84 (1H, m), 3.98 (1H, dd, J=6.6, 13.2 Hz), 4.16 (1H, dd, J=5.5, 13.5 Hz), 4.77

(1H, dd, J=4.5, 9.5 Hz), 5.00 (1H, d, J=3.7 Hz), 5.20–5.32 (2H, m), 5.88 (1H, m). FABMS (positive-ion) *m/z*, 443 $[M+H]^+$, 465 $[M+Na]^+$. HRFABMS, calcd for $C_{24}H_{42}O_7Na$: 465.2828. Found: 465.2810.

4.1.28. 2-Hydroxyethyl 2-*O*-acetyl-3-*O*-decyl-4,6-*O*-isopropylidene-α-D-glucopyranoside (34). To a solution of **33** (2.00 g, 4.519 mmol) in THF–H_2O (3:1, 40 ml) were added $NaIO_4$ (5.70 g, 26.65 mmol) and a 2.5% solution of OsO_4 in *tert*-BuOH (900 mg). This mixture was stirred for 2 h at room temperature, and diluted with EtOAc, washed with aq satd $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give an aldehyde, which was dissolved in EtOH (40 ml). To this solution was added $NaBH_4$ (200 mg, 5.286 mmol), and the solution was stirred for 10 min, quenched with AcOH (300 mg), diluted with EtOAc, washed with aq satd $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (3:2) gave alcohol **34** (1.43 g, 71%) as an oil. IR ν_{max}(film) 3484, 2995, 2926, 2857, 1748 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3+D_2O$) δ 0.88 (3H, t, J=6.8 Hz), 1.26 (16H, bs), 1.42 (3H, s), 1.48–1.52 (5H, m, containing 3H, s, at δ 1.50 ppm), 2.11 (3H, s), 3.53–3.87 (11H, m), 4.80 (1H, dd, J=3.9, 9.8 Hz), 5.00 (1H, d, J=3.9 Hz). FABMS (positive-ion) *m/z*, 447 $[M+H]^+$, 469 $[M+Na]^+$. HRFABMS, calcd for $C_{23}H_{42}O_8Na$: 469.2777. Found: 469.2778.

4.1.29. 2-(Diallylphosphonooxy)ethyl 3-*O*-decyl-4,6-*O*-isopropylidene-α-D-glucopyranoside (35). A solution of **34** (430 mg, 0.972 mmol) in EtOH (99.5%, 15 ml) containing KOH (10 mg) was stirred for 5 h at room temperature, concentrated in vacuo and diluted with EtOAc, which was washed with brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give a residue, which was dissolved in CH_2Cl_2 (10 ml). To this solution Na_2SO_4 (300 mg), 1*H*-tetrazole (102 mg, 1.457 mmol) and diallyl diisopropylphosphoramidite (262 mg, 1.069 mmol) were added at room temperature. After stirring for 20 min, THF (10 ml) and aq 30% H_2O_2 (ca. 200 mg) were added. After stirring for 15 min at room temperature, the reaction mixture was diluted with EtOAc, washed with aq 10% $Na_2S_2O_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with hexane–EtOAc gave alcohol **35** (430 mg, 79%) as an oil. IR ν_{max}(film) 3409, 2994, 2926, 2857, 1744 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (3H, J=6.6 Hz), 1.26 (14H, bs), 1.40 (3H, s), 1.49 (3H, s), 1.55–1.59 (2H, m), 3.09 (1H, d, J=8.1 Hz, OH), 3.44–3.94 (10H, m), 4.23–4.28 (2H, m) 4.55–4.60 (4H, m), 4.88 (1H, d, J=3.7 Hz), 5.25–5.30 (2H, m), 5.36–5.43 (2H, m), 5.91–6.02 (2H, m).

4.1.30. 2-(Diallylphosphonooxy)ethyl 3-*O*-decyl-4,6-*O*-isopropylidene-2-*O*-(3-oxotetradecanoyl)-α-D-glucopyranoside (36). To a solution of **35** (440 mg, 0.779 mmol) in CH_2Cl_2 (30 ml) were added 3-oxotetradecanoic acid (208 mg, 0.858 mmol) and WSC·HCl (179 mg, 0.935 mmol). The mixture was stirred for 40 min, and concentrated in vacuo to give a residue, which was diluted with EtOAc. The solution was washed with aq satd $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with cyclohexane-EtOAc (1:1) gave **36** (464 mg, 75%) as a gum. IR ν_{max}(film) 2926, 2856, 1750, 1719, 1650 (w), 1627 (w) cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, J=6.6 Hz), 1.26 (30H, bs), 1.39 (3H, s), 1.40–1.63 (7H, m, containing 3H, s, at δ 1.49 ppm), 2.53 (2H, t, J=7.3 Hz), 3.40–3.54 (3H, m), 3.61–3.75 (6H, m), 3.82–3.88 (2H, m), 4.19–4.23 (2H, m) 4.56–4.59 (4H, m), 4.77 (1H, dd, J=3.7, 9.5 Hz), 5.03 (1H, d, J=4.4 Hz), 5.26–5.41 (4H, m), 5.93–6.00 (2H, m). FABMS (positive-ion) *m/z*, 789 $[M+H]^+$, 811 $[M+Na]^+$. HRFABMS, calcd for $C_{41}H_{73}O_{12}PNa$: 811.4737. Found: 811.4724.

4.1.31. 2-(Diallylphosphonooxy)ethyl 3-*O*-decyl-2-*O*-(3-oxotetradecanoyl)-α-D-glucopyranoside (37). A solution of **36** (460 mg, 0.583 mmol) in aq 80% AcOH (46 ml) was heated at 85 °C for 1.5 h, and concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with EtOAc–MeOH (19:1) gave **37** (374 mg, 86%) as a gum. IR ν_{max}(film) 3407 (br), 2925, 2855, 1747, 1718, 1650 (w), 1465 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, J=6.6 Hz), 1.26 (30H, bs), 1.53–1.60 (4H, m), 2.51–2.55 (3H, m, containing OH), 2.66 (1H, d, J=2.9 Hz, OH), 3.46–3.90 (11H, m, containing 2H, s at δ 3.50 ppm), 4.20–4.25 (2H, m) 4.55–4.59 (4H, m), 4.72 (1H, dd, J=3.7, 9.5 Hz), 5.08 (1H, d, J=3.7 Hz), 5.26–5.41 (4H, m), 5.91–5.99 (2H, m). FABMS (positive-ion) *m/z*, 749 $[M+H]^+$, 771 $[M+Na]^+$. HRFABMS, calcd for $C_{38}H_{69}O_{12}PNa$: 771.4425. Found: 771.4418.

4.1.32. 2-(Diallylphosphonooxy)ethyl 3-*O*-decyl-6-*O*-{2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl}-2-*O*-(3-oxotetradecanoyl)-α-D-glucopyranoside (38). Compound **37** (374 mg, 0.499 mmol) was treated as described for the formation of **19** from **5** and **18** to give **38** (486 mg, 68%) as a gum after silica gel column chromathography with EtOAc. IR ν_{max}(film) 3292 (br), 3085 (w), 2927, 2856, 1748, 1721, 1650 (w), 1545, 1460 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (9H, t, J=6.6 Hz), 1.26 (42H, bs), 1.40–1.80 (8H, m), 2.52 (1H, t, J=7.7 Hz), 2.80 (1H, bs, OH), 3.24–3.84 (24H, m, containing two 3H at δ 3.29 and 3.39 ppm), 4.09–4.32 (4H, m), 4.53–4.59 (8H, m), 4.69–4.71 (3H, m), 4.87 (1H, m), 5.03 (1H, d, J=3.7 Hz), 5.24–5.41 (8H, m), 5.90–5.99 (4H, m), 6.36 (1H, bs, NH). FABMS (positive-ion) *m/z*, 1428 $[M+H]^+$, 1450 $[M+Na, {}^{35}Cl]^+$. HRFABMS, calcd for $C_{65}H_{114}Cl_3NO_{22}P_2Na$: 1450.6298. Found: 1450.6301.

4.1.33. 2-(Diallylphosphonooxy)ethyl 3-*O*-decyl-6-*O*-{2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-[(*Z*)-11-octadecenoylamino]-β-D-glucopyranosyl}-2-*O*-(3-oxotetradecanoyl)-α-D-glucopyranoside (39). Compound **38** was treated as described for the formation of **7** from **6** to give **39** (81%) as a gum. IR ν_{max}(film) 3302, 3086 (w), 2926, 2855, 1746, 1719, 1654, 1553, 1465 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (12H, t, J=6.6 Hz), 1.26 (60H, bs), 1.40–1.80 (10H, m), 1.99–2.02 (4H, m), 2.17–2.27 (2H, m), 2.52 (2H, m), 3.20–3.91 (26H, m, containing two 3H, s, at 3.29 and 3.38 ppm), 4.07–4.27 (6H, m), 4.54–4.57 (8H, m), 4.70 (1H, dd, J=3.7,

9.5 Hz), 5.02 (1H, d, J=3.7 Hz), 5.19 (1H, d, J=8.1 Hz), 5.23–5.40 (10H, m), 5.88–5.99 (4H, m), 6.51 (1H, d, J=6.6 Hz, NH). FABMS (positive-ion) m/z, 1518 $[M+H]^+$, 1540 $[M+Na]^+$. HRFABMS, calcd for $C_{80}H_{145}NO_{21}P_2Na$: 1540.9682. Found: 1540.9653.

4.1.34. 2-(Phosphonooxy)ethyl 3-*O*-decyl-6-*O*-{2-deoxy-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-[(*Z*)-11-octadecenoylamino]-4-*O*-phosphono-β-D-glucopyranosyl}-2-*O*-(3-oxotetradecanoyl)-α-D-glucopyranoside (40). Compound **39** (200 mg, 0.132 mmol) was treated for 16 h at 55 °C as described for the formation of **8** from **7** to give **40** (150 mg, 84%) as a wax. IR ν_{max}(KBr) 3286 (br), 2825, 2854, 1742, 1716, 1629, 1552, 1466 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$–CD_3OD, 5:1) δ 0.88 (12H, t, J=6.6 Hz), 1.26 (60H, bs), 1.40–1.63 (8H, m), 1.72–1.77 (2H, m), 1.99–2.04 (4H, m), 2.18–2.22 (2H, m), 2.53–2.57 (2H, m), 3.30–3.83 (24H, m, containing two 3H, s at 3.30 and 3.41 ppm), 4.01–4.14 (4H, m), 4.65–4.72 (2H, m), 4.98 (1H, d, J=3.7 Hz), 5.30–5.36 (2H, m). FABMS (negative-ion) m/z, 1356 $[M-H]^-$. Anal. Calcd for $C_{68}H_{129}NO_{21}P_2H_2O$: C, 59.33; H, 9.59; N, 1.02; P, 4.50. Found: C, 59.24; H, 9.66; N, 1.20; P, 4.32.

4.1.35. Allyl 4,6-*O*-benzylidene-3-*O*-dodecyl-α-D-glucopyranoside (42). To a solution of **41** (2.85 g, 7.33 mmol) in DMF (30 ml) were added benzaldehyde dimethyl acetal (3.3 ml) and *p*-toluenesulfonic acid monohydrate (140 mg). The mixture was stirred for 16 h at room temperature, diluted with ether, washed with aq. sat. $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (5:1) gave **42** (3.05 g, 87%) as a solid. IR ν_{max}(KBr) 3441, 1371, 1072 cm^{-1}. 500 MHz ^1H NMR ($CDCl_3$) δ 0.88 (3H, t, J=6.8 Hz), 1.23–1.33 (18H, m), 1.57–1.61 (2H, m), 2.29 (1H, d, J=6.8 Hz, OH), 3.52–3.56 (2H, m), 3.66–3.75 (3H, m), 3.84–3.90 (2H, m), 4.07 (1H, dd, J=6.8, 12.7 Hz), 4.24 (1H, dd, J=4.9, 12.7 Hz), 4.27 (1H, dd, J=4.9, 9.8 Hz), 4.96 (1H, d, J=2.9 Hz), 5.24 (1H, d, J=10.7 Hz), 5.34 (1H, dd, J=2.0, 15.6 Hz), 5.55 (1H, s), 5.94 (1H, m), 7.33–7.39 (3H, m), 7.47–7.49 (2H, m). FABMS (positive-ion): m/z 477 $(M+H)^+$, 499 $(M+Na)^+$. HRFABMS, calcd for $C_{28}H_{44}O_6Na$: 499.3036. Found: 499.3040. Anal. Calcd for $C_{28}H_{44}O_6$: C, 70.56; H, 9.30. Found: C, 70.21; H, 9.00.

4.1.36. Allyl 4,6-*O*-benzylidene-3-*O*-dodecyl-2-*O*-[(*R*)-3-(4-methoxybenzyloxy)tetradecyl]-α-D-glucopyranoside (43). To a solution of **42** (3.54 g, 7.43 mmol) in DMF (100 ml) was gradually added NaH (60% oil dispersion, 1.62 g, 40.5 mmol) at 0 °C with stirring. After 15 min at 0 °C, (*R*)-3-(4-methoxybenzyloxy)tetradecyl methanesulfonate (4.02 g, 9.38 mmol) was added to the mixture. After stirring for 6 h at room temperature, the mixture was quenched with water, extracted with EtOAc, washed with water and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo and chromatographed on a silica gel column. Elution with hexane–EtOAc (4:1) gave **43** (4.01 g, 67%) as a white solid. IR ν_{max}(KBr) 3067, 3038, 2920, 2851, 1615 cm^{-1}. 500 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.8 Hz), 1.21–1.59 (40H, m), 1.76–1.87 (2H, m), 3.34 (1H, dd, J=3.9, 8.8 Hz), 3.51 (1H, dd, J=8.8, 9.8 Hz), 3.56 (1H, m), 3.68–3.82 (9H, m, containing 3H, s, at 3.80 ppm), 3.86 (1H, dt, J=4.9, 9.8 Hz), 4.07 (1H, dd, J=6.8, 12.7 Hz), 4.20 (1H, dd, J=5.9, 12.7 Hz), 4.26 (1H, dd, J=4.9, 9.8 Hz), 4.43, 4.47 (2H, ABq, J=11.7 Hz), 4.96 (1H, d, J=3.9 Hz), 5.21–5.35 (2H, m), 5.55 (1H, s), 5.92 (1H, m), 6.87 (2H, d, J=7.8 Hz), 7.25–7.27 (2H, m), 7.33–7.38 (3H, m), 7.59–7.50 (2H, m). FABMS (positive-ion): m/z 831 $(M+Na)^+$. HRFABMS, calcd for $C_{50}H_{80}O_8Na$: 831.5755. Found: 831.5731.

4.1.37. 2-Hydroxyethyl 4,6-*O*-benzylidene-3-*O*-dodecyl-2-*O*-[(*R*)-3-(4-methoxybenzyloxy)tetradecyl]-α-D-glucopyranoside (44). To a solution of **43** (1.63 g, 2.01 mmol) in acetone (16 ml) and H_2O (4 ml) were added $NaIO_4$ (1.28 g, 5.98 mmol) and OsO_4 (2.5 wt% solution in *t*-BuOH, 1.3 ml, 0.104 mmol). After stirring for 4 h at room temperature, the reaction mixture was quenched with aq satd $Na_2S_2O_3$, extracted with EtOAc, washed with water and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give a residue, which was dissolved in EtOH (15 ml). To this solution was added $NaBH_4$ (102 mg, 2.71 mmol) at 0 °C. After stirring for 1 h at room temperature, the mixture was concentrated in vacuo, diluted with EtOAc, washed with water and brine, dried over $MgSO_4$, filtered, concentrated in vacuo and chromatographed on a silica gel column. Elution with hexane–EtOAc (3:2) gave **44** (1.05 g, 64%) as a gum. IR ν_{max}($CHCl_3$) 3516, 2928, 2856 cm^{-1}. 500 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.8 Hz), 1.22–1.62 (40H, m), 1.77–1.83 (2H, m), 2.82 (1H, dd, J=5.9, 6.8 Hz), 3.35 (1H, d, J=3.9, 9.8 Hz), 3.50 (1H, dd, J=8.8, 9.8 Hz), 3.53 (1H, m), 3.63 (1H, m), 3.68–3.91 (13H, m, containing 3H, s, at 3.80 ppm), 4.27 (1H, dd, J=4.9, 9.8 Hz), 4.42, 4.47 (2H, ABq, J=11.7 Hz), 4.95 (1H, d, J=3.9 Hz), 5.54 (1H, s), 6.87 (2H, d, J=7.8 Hz), 7.25–7.27 (2H, m), 7.33–7.38 (3H, m), 7.48–7.50 (2H, m). FABMS (positive-ion): m/z 835 $(M+Na)^+$.

4.1.38. 2-(Diallylphosphonoxy)ethyl 4,6-*O*-benzylidene-3-*O*-dodecyl-2-*O*-[(*R*)-3-(4-methoxybenzyloxy)tetradecyl]-α-D-glucopyranoside (45). To a solution of **44** (930 mg, 1.14 mmol) and 1*H*-tetrazole (157 mg, 2.25 mmol) in THF (10 ml) was added diallyl diisopropylphosphoramidite (379 mg, 1.54 mmol). After stirring for 4 h at room temperature, aq 30% H_2O_2 (1 ml) was added to the reaction mixture at 0 °C. After stirring for 1 h at 0 °C, the mixture was quenched with aq satd $Na_2S_2O_3$, extracted with EtOAc, washed with water, aq satd $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo and chromatographed on a silica gel column. Elution with hexane–EtOAc (1:1) gave **45** (1.07 g, 96%) as a gum. 500 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.8 Hz), 1.21–1.59 (40H, m), 1.73–1.85 (2H, m), 3.33 (1H, dd, J=3.9, 8.8 Hz), 3.50 (1H, t, J=9.8 Hz), 3.55 (1H, m), 3.66–3.91 (12H, m, containing 3H, s, at 3.80 ppm), 4.22–4.29 (3H, m), 4.42, 4.47 (2H, AB-q, J=11.7 Hz), 4.54–4.57 (4H, m), 4.94 (1H, d, J=3.9 Hz), 5.21–5.39 (4H, m), 5.54 (1H, s), 5.87–5.98 (2H, m), 6.86–6.91 (2H, m), 7.25–7.27 (2H, m), 7.32–7.38 (3H, m), 7.47–7.49 (2H, m). FABMS (positive-ion): m/z 995 $(M+Na)^+$, 973 $(M+H)^+$.

4.1.39. 2-(Diallylphosphonoxy)ethyl 4,6-*O*-benzylidene-3-*O*-dodecyl-2-*O*-[(*R*)-3-hydroxytetradecyl]-α-D-glucopyranoside (46). To a solution of **45** (1.04 g, 1.07 mmol) in CH_2Cl_2 (10 ml) and H_2O (1 ml) was added DDQ (291 mg,

1.28 mmol). After stirring for 1.5 h at room temperature, the solution was diluted with CH_2Cl_2, washed with aq satd $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo, and chromatographed on a silica gel column. Elution with hexane-EtOAc (3:7) gave **46** (821 mg, 90%) as a gum. IR $\nu_{max}(CHCl_3)$ 3502, 2927, 2855, 1732 cm $^{-1}$. 500 MHz ^{1}H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.8 Hz), 1.23–1.59 (40H, m), 1.65–1.75 (2H, m), 3.19 (1H, d, J=2.9 Hz, OH), 3.37 (1H, dd, J=3.9, 9.8 Hz), 3.52 (1H, t, J=9.8 Hz), 3.66–3.92 (10H, m), 4.23–4.29 (3H, m), 4.55–4.59 (4H, m), 5.01 (1H, d, J=3.9 Hz), 5.22–5.24 (2H, m), 5.33–5.37 (2H, m), 5.53 (1H, s), 5.89–5.97 (2H, m), 7.33–7.38 (3H, m), 7.46–7.48 (2H, m). FABMS (positive-ion): m/z 875 $(M+Na)^+$, 853 $(M+H)^+$. HRFABMS, calcd for $C_{47}H_{81}O_{11}PNa$: 875.5413. Found: 875.5426.

4.1.40. 2-(Diallylphosphonoxy)ethyl 4,6-*O*-benzylidene-3-*O*-dodecyl-2-*O*-(3-oxotetradecyl)-α-D-glucopyranoside (47). To a solution of **46** (810 mg, 0.950 mmol) in CH_2Cl_2 (10 ml) was added PCC (413 mg, 1.92 mmol). After stirring for 5 h at room temperature, the solution was filtered through Celite. The filtrate was concentrated in vacuo and chromatographed on a silica gel column. Elution with hexane–EtOAc (3:7) gave **47** (753 mg, 93%) as a gum. IR $\nu_{max}(CHCl_3)$ 2927, 2855, 1713 cm^{-1}. 500 MHz ^{1}H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.8 Hz), 1.23–1.30 (34H, m), 1.52–1.56 (4H, m), 2.43 (2H, dd, J=6.8, 7.8 Hz), 2.64, 2.74 (2H, ABqt, J=16.6, 6.8 Hz), 3.38 (1H, dd, J=3.9, 9.8 Hz), 3.49 (1H, dd, J=8.8, 9.8 Hz), 3.63–3.71 (3H, m), 3.75–3.78 (2H, m), 3.83–3.92 (4H, m), 4.22–4.28 (3H, m), 4.55–4.57 (4H, m), 4.95 (1H, d, J=3.9 Hz), 5.22–5.24 (2H, m), 5.33–5.37 (2H, m), 5.52 (1H, s), 5.89–5.97 (2H, m), 7.34–7.37 (3H, m), 7.46–7.48 (2H, m). FABMS (positive-ion): m/z 873 $(M+Na)^+$, 851 $(M+H)^+$. HRFABMS, calcd for $C_{47}H_{79}O_{11}PNa$: 873.5257. Found: 873.5265.

4.1.41. 2-(Diallylphosphonoxy)ethyl 3-*O*-dodecyl-2-*O*-(3-oxotetradecyl)-α-D-glucopyranoside (48). Compound **47** was treated as described for the formation of **4** from **3** to give diol **48** (92%) as an amorphous solid. IR $\nu_{max}(CHCl_3)$ 3599, 3409, 2927, 2872, 2855, 1713 cm^{-1}. 500 MHz ^{1}H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.8 Hz), 1.26–1.31 (34H, m), 1.51–1.56 (4H, m), 1.72 (1H, bs, OH), 2.43 (2H, t, J=6.8, 7.8 Hz), 2.62 (1H, bs, OH), 2.64, 2.71 (2H, ABqt, J=16.6, 6.8 Hz), 3.30 (1H, dd, J=2.9, 9.8 Hz), 3.44 (1H, t, J=8.8 Hz), 3.50 (1H, t, J=8.8 Hz), 3.58 (1H, m), 3.72–3.91 (8H, m), 4.20–4.30 (2H, m), 4.55–4.59 (4H, m), 4.97 (1H, d, J=2.9 Hz), 5.25–5.40 (4H, m), 5.91–5.99 (2H, m). FABMS (positive-ion): m/z 785$(M+Na)^+$. HRFABMS, calcd for $C_{40}H_{75}O_{11}PNa$: 785.4943. Found: 785.4951.

4.1.42. 2-(Diallylphosphonoxy)ethyl 6-*O*-{2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl}-3-*O*-dodecyl-2-*O*-(3-oxotetradecyl)-α-D-glucopyranoside (49). A solution of imidate **5** (844 mg, 1.00 mmol), diol **48** (626 mg, 0.821 mmol) and molecular sieves 4 Å (590 mg) in CH_2Cl_2 (10 ml) was stirred at room temperature. After stirring for 1 h, cat. TMSOTf (6 μl, 0.033 mmol) was added to the mixture at 0 °C. After stirring for 1 h at 0 °C, the mixture was quenched with aq satd $NaHCO_3$, diluted with CH_2Cl_2, washed with water and brine, dried over $MgSO_4$, filtered and concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (1:3) gave **49** (627 mg, 53%) as an amorphous solid. IR $\nu_{max}(CHCl_3)$ 3588, 3450, 3272, 3088, 2928, 2873, 2856, 1732, 1651 cm^{-1}. 500 MHz ^{1}H NMR ($CDCl_3$) δ 0.88 (9H, t, J=6.8 Hz), 1.25–1.78 (52H, m), 2.42 (2H, dd, J=6.8, 7.8 Hz), 2.60–2.73 (3H, m, containing 1H, OH), 3.26–3.36 (8H, m, containing 3H, s, at 3.28 ppm), 3.39 (3H, s), 3.44 (1H, t, J=8.8 Hz), 3.54 (1H, m), 3.60–3.64 (2H, m), 3.68–3.89 (11H, m), 4.10 (1H, d, J=11.7 Hz), 4.20 (1H, m), 4.25–4.31 (2H, m), 4.53–4.59 (8H, m), 4.70, 4.77 (2H, AB-q, J=12.8 Hz), 4.85 (1H, d, J=7.8 Hz), 4.92 (1H, d, J=2.9 Hz), 5.24–5.39 (8H, m), 5.90–5.99 (4H, m), 6.45 (1H, bs, NH). FABMS (positive-ion): m/z 1464 $(M+Na)^+$. HRFABMS, calcd for $C_{67}H_{120}Cl_3NO_{21}P_2Na$: 1464.6798. Found: 1464.6812.

4.1.43. 2-(Diallylphosphonoxy)ethyl 6-*O*-{2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-[(*Z*)-11-octadecenoylamino]-β-D-glucopyranosyl}-3-*O*-dodecyl-2-*O*-(3-oxotetradecyl)-α-D-glucopyranoside (50). To a solution of **49** (432 mg, 0.300 mmol) in THF (9 ml) and acetic acid (1 ml) was added zinc dust (776 mg, 11.872 mmol). After vigorously stirring for 4 h at room temperature, the solution was filtered to remove the Zn powder and concentrated in vacuo to give a crude product. The product was diluted with EtOAc, washed with aq satd $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated in vacuo to give a crude product, which was dissolved in CH_2Cl_2 (10 ml). (*Z*)-11-Octadecenoic acid (198 mg, 0.697 mmol) and WSC·HCl (167 mg, 0.873 mmol) were added to this solution. After stirring for 10 h at room temperature, the mixture was diluted with CH_2Cl_2, washed with water and brine, dried over $MgSO_4$, filtered and concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (1:9) gave **50** (256 mg, 56%) as an amorphous solid. IR $\nu_{max}(CHCl_3)$ 3605, 3453, 3316, 2928, 2856, 1712, 1662 cm^{-1}. 500 MHz ^{1}H NMR ($CDCl_3$) δ 0.88 (12H, t, J=6.8 Hz), 1.18–1.80 (74H, m), 1.99–2.01 (4H, m), 2.14–2.26 (2H, m), 2.41 (2H, dd, J=6.8, 7.8 Hz), 2.62, 2.70 (2H, AB-q, t, J=16.6, 6.8 Hz), 3.07 (1H, d, J=2.9 Hz, OH), 3.20 (1H, m), 3.25–3.32 (5H, m, containing 3H, s, at 3.28 ppm), 3.35–3.40 (4H, m, containing 3H, s, at 3.38 ppm), 3.43 (1H, dd, J=8.8, 9.8 Hz), 3.56–3.88 (13H, m), 3.96 (1H, dd, J=8.8, 9.8 Hz), 4.09 (1H, d, J=9.8 Hz), 4.17 (1H, m), 4.22–4.29 (2H, m), 4.53–4.58 (8H, m), 4.90 (1H, d, J=2.9 Hz), 5.15 (1H, d, J=7.8 Hz), 5.23–5.40 (10H, m). FABMS (positive-ion): m/z 1554 $(M+Na)^+$. HRFABMS, calcd for $C_{82}H_{151}NO_{20}P_2Na$: 1555.0201. Found: 1555.0200.

4.1.44. 2-(Phosphonoxy)ethyl 6-*O*-{2-deoxy-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-[(*Z*)-11-octadecenoylamino]-4-*O*-phosphono-β-D-glucopyranosyl}-3-*O*-dodecyl-2-*O*-(3-oxotetradecyl)-α-D-glucopyranoside (51). Compound **50** (204 mg, 0.133 mmol) was treated as described for the formation of **8** from **7** to give **51** (164 mg, 90%) as a white powder. IR ν_{max}(KBr) 3287, 3070, 2954, 2925, 2854, 2324, 1714, 1629 cm^{-1}. 500 MHz ^{1}H NMR ($CDCl_3$) δ 0.90 (12H, t, J=6.8 Hz), 1.30–1.67 (72H, m), 1.72–1.76 (2H, m), 1.99–2.04 (4H, m), 2.20–2.30 (2H, m), 2.50 (2H, t, J=7.3 Hz), 2.67–2.69 (2H, m), 3.23 (1H, dd, J=3.9, 9.8 Hz), 3.29–3.31 (4H, m, containing 3H, s, at 3.29 ppm), 3.35 (1H, t, J=8.8 Hz), 3.39 (3H, s), 3.42

(1H, dd, J=8.8, 9.8 Hz), 3.52–3.77 (11H, m), 3.79–3.91 (5H, m), 4.04–4.15 (4H, m), 4.48 (1H, d, J=8.8 Hz), 4.93 (1H, d, J=3.9 Hz), 5.31–5.37 (2H, m). ESIMS (negative-ion): m/z 1370 $(M-H)^-$. HRESIMS, calcd for $C_{70}H_{134}NO_{20}P_2$: 1370.8966. Found: 1370.8944.

4.1.45. 2-Hydroxyethyl 2-*O*-[(*R*)-3-(*tert*-butyldimethylsilyloxy)tetradecyl]-3-*O*-dodecyl-4,6-*O*-isopropylidene-α-D-glucopyranoside (52). To a solution of **9** (1.45 g, 1.925 mmol) in THF (35 ml) and H_2O (10 ml) were added $NaIO_4$ (3.00 g, 14.03 mmol) and OsO_4 (2.5 wt% solution in *t*-BuOH, 1.3 ml, 1.04 mmol). After stirring for 2 h at room temperature, the reaction mixture was diluted with ether, washed with aq satd $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give a residue, which was dissolved in EtOH (40 ml). To this solution was added $NaBH_4$ (150 mg, 3.965 mmol) at 0 °C. After stirring for 20 min at 0 °C, diluted with EtOAc, washed with aq satd $NaHCO_3$ and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo to give a residue, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (4:1) gave **52** (598 mg, 41%) as a gum. IR ν_{max}(film) 3468 (br), 2926, 2856, 1465, 1380, 1370 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.04 (6H, s), 0.88 (9H, s), 0.89 (6H, t, J=6.3 Hz), 1.26 (36H, bs), 1.40 (3H, s), 1.41–1.43 (2H, m), 1.48 (3H, s), 1.52–1.56 (2H, m), 1.71–1.75 (2H, m), 2.82 (1H, br, OH), 3.30 (1H, m), 3.51–3.85 (14H, m), 4.89 (1H, d, J=3.7 Hz). FABMS (positive-ion): m/z 759 $(M+H)^+$, 781 $(M+Na)^+$. HRFABMS, calcd for $C_{43}H_{86}O_8SiNa$: 781.5990. Found: 781.5977.

4.1.46. 2-(Diallylphosphonooxy)ethyl 2-*O*-[(*R*)-3-(*tert*-butyldimethylsilyloxy)tetradecyl]-3-*O*-dodecyl-4,6-*O*-isopropylidene-α-D-glucopyranoside (53). Compound **52** (428 mg, 0.654 mmol) was treated as described for the formation of **17** from **16** to give **53** (504 mg, 97%) as a gum. IR ν_{max}(film) 2927, 2856, 1464 cm^{-1}.400 MHz ^1H NMR ($CDCl_3$) δ 0.04 (6H, s), 0.88 (15H, bs), 1.26 (36H, bs), 1.39 (3H, s), 1.41–1.43 (2H, m), 1.48 (3H, s), 1.51–1.55 (2H, m), 1.64–1.76 (2H, m), 3.28 (1H, m), 3.41–3.89 (12H, m), 4.22–4.27 (2H, m), 4.56–4.59 (4H, m), 4.88 (1H, d, J=3.7 Hz), 5.21–5.41 (4H, m), 5.91–6.01 (2H, m). FABMS (positive-ion): m/z 919 $(M+H)^+$, 941 $(M+Na)^+$. HRFABMS, calcd for $C_{49}H_{96}O_{11}PSi$: 919.6459. Found: 919.6484.

4.1.47. 2–(Diallylphosphonooxy)ethyl 3-*O*-dodecyl-2-*O*-[(*R*)-3-hydroxytetradecyl]-α-D-glucopyranoside (54). Compound **53** (489 mg, 0.532 mmol) was treated as described for the formation of **18** from **17** to give triol **54** (340 mg, 84%) as a gum. IR ν_{max}(film) 3388 (br), 2918, 2850, 1467 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.6 Hz), 1.26 (36H, bs), 1.41–1.73 (6H, m), 2.56 (1H, m, OH), 2.68 (1H, d, J=2.9 Hz, OH), 3.12 (1H, bs, OH), 3.29 (1H, m), 3.44–3.92 (12H, m), 4.22–4.28 (2H, m), 4.55–4.59 (4H, m), 5.00 (1H, d, J=3.7 Hz), 5.25–5.28 (2H, m), 5.35–5.41 (2H, m), 5.90–6.00 (2H, m). FABMS (positive-ion): m/z 765 $(M+H)^+$, 787 $(M+Na)^+$. HRFABMS, calcd for $C_{40}H_{77}O_{11}PNa$: 787.5101. Found: 787.5118.

4.1.48. 2-(Diallylphosphonooxy)ethyl 6-*O*-{2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl}-3-*O*-dodecyl-2-*O*-[(*R*)-3-hydroxytetradecyl]-α-D-glucopyranoside (55). Compound **54** was treated as described for the formation of **19** from imidate **5** and **18** to give compound **55** as a gum in 67% yield. IR ν_{max}(film) 3500–3250, 2926, 2856, 1746, 1547, 1464 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (9H, t, J=6.6 Hz), 1.26 (51H, bs), 1.38–1.77 (8H, m), 2.72 (1H, bs, OH), 3.13 (1H, bs, OH), 3.21–3.90 (24H, m, containing two 3H, s, at δ 3.28 and 3.39 ppm), 4.07–4.30 (4H, m), 4.53–4.60 (8H, m), 4.65–4.90 (3H, m), 4.95 (1H, d, J=3.7 Hz), 5.24–5.40 (8H, m), 5.89–5.99 (4H, m), 6.40 (1H, bs, NH). FABMS (positive-ion): m/z 1466 $(M+Na)^+$, 1468. HRFABMS, calcd for $C_{67}H_{122}Cl_3NO_{21}P_2Na$: 1466.6984. Found: 1466.6962.

4.1.49. 2-(Diallylphosphonooxy)ethyl 6-*O*-{2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-[(*Z*)-11-octadecenoylamino]-β-D-glucopyranosyl}-3-*O*-dodecyl-2-*O*-[(*R*)-3-hydroxytetradecyl]-α-D-glucopyranoside (56). Compound **55** was treated as described for the formation of **7** from **6** to give **56** (82%) as a gum. IR ν_{max}(film) 3398 (br), 2925, 2855, 1640, 1555, 1465 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.88 (12H, t, J=6.6 Hz), 1.26 (68H, bs), 1.40–1.79 (10H, m), 1.99–2.04 (4H, m), 2.17–2.21 (2H, m), 3.18 (1H, bs, OH), 3.20 (1H, m), 3.27–3.95 (26H, m, containing two 3H, s, at δ 3.28 and 3.38 ppm), 4.07–4.27 (4H, m), 4.55–4.65 (8H, m), 4.94 (1H, d, J=3.7 Hz), 5.13 (1H, d, J=8.1 Hz), 5.25–5.39 (10H, m), 5.90–5.99 (4H, m), 6.58 (1H, d, J=7.3 Hz, NH). FABMS (positive-ion): m/z 1556 $(M+Na)^+$. HRFABMS, calcd for $C_{82}H_{153}NO_{20}P_2Na$: 1557.0359. Found: 1557.0341.

4.1.50. 2-(Phosphonoxy)ethyl 6-*O*-{2-deoxy-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-[(*Z*)-11-octadecenoylamino]-4-*O*-phosphono-β-D-glucopyranosyl}-3-*O*-dodecyl-2-*O*-[(*R*)-3-hydroxytetradecyl]-α-D-glucopyranoside (57). Compound **56** was treated as described for the formation of **21** from **20** to give **57** (67%) as a powder. IR ν_{max}(KBr) 3289 (br), 2924, 2853, 1629, 1554 cm^{-1}. 400 MHz ^1H NMR ($CDCl_3$) δ 0.90 (12H, t, J=6.6 Hz), 1.26 (68H, bs), 1.40–1.77 (10H, m), 1.98–2.04 (4H, m), 2.17–2.22 (2H, m), 3.23–3.85 (26H, m, containing two 3H, s, at δ 3.30 and 3.40 ppm), 4.00–4.18 (4H, m), 4.69 (1H, d, J=8.1 Hz), 4.94 (1H, d, J=3.7 Hz), 5.33–5.38 (2H, m). FABMS (negative-ion): m/z 1372 $(M-H)^-$. HRFABMS, calcd for $C_{70}H_{136}NO_{20}P_2$: 1372.9131. Found: 1372.9121. Anal. Calcd for $C_{70}H_{137}NO_{20}P_2$: C, 60.36; H, 10.06; N, 1.01; P, 4.45. Found: C, 60.11; H, 10.15; N, 1.28; P, 4.34.

4.1.51. 2-Bromoethyl 2,3-di-*O*-dodecyl-4,6-*O*-isopropylidene-α-D-glucopyranoside (58). To a solution of **16** (1.10 g, 1.83 mmol) in CH_2Cl_2 (10 mL) was added CBr_4 (731 mg, 2.20 mmol) and PPh_3 (671 mg, 2.56 mmol). After stirring for 2 h at room temperature, the solution was diluted with CH_2Cl_2, washed with water and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo, and chromatographed on a silica gel column. Elution with hexane–EtOAc (9:1) gave **58** (1.20 g, 99%) as a gum. IR ν_{max}($CHCl_3$) 2927, 2855 cm^{-1}. 500 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, J=6.8 Hz), 1.25–1.60 (46H, m, containing two 3H, s, δ 1.41 and 1.48 ppm), 3.30 (1H, dd, J=2.9, 8.8 Hz), 3.50–3.57 (4H, m), 3.59–3.75 (6H, m), 3.83–3.96 (3H, m), 4.91 (1H, d, J=3.9 Hz). FABMS (positive-ion) m/z 685 $(M+Na)^+$. HRFABMS, calcd for $C_{35}H_{67}BrO_6Na$: 685.4002. Found: 685.4056.

4.1.52. 2-(Diallylphosphono)ethyl 2,3-di-*O*-dodecyl-α-D-glucopyranoside (59). A mixture of **58** (1.20 g, 1.81 mmol) and triallylphosphite (5 mL) was stirred at 180 °C for 3 h. The mixture was cooled to room temperature, and chromatographed on a silica gel short column. Elution with hexane-EtOAc (2:3) gave a compound, which was dissolved in aq 80% AcOH (15 mL) and stirred at 60 °C for 1 h. The solution was diluted with EtOAc, washed with aq satd $NaHCO_3$ and brine, dried over $MgSO_4$, filtered. The filtrate was concentrated in vacuo and chromatographed on a silica gel column. Elution with $CHCl_3$–EtOH (20:1) gave **59** (760 mg, 60%) as a white powder. IR $\nu_{max}(CHCl_3)$ 3598, 3420, 3089, 2927, 2855 cm^{-1}. 500 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, $J=6.8$ Hz), 1.26–1.61 (40 H, m), 2.28(1H, bs, OH), 2.58 (1H, bs, OH), 3.27 (1H, dd, $J=2.9$, 9.8 Hz), 3.44–3.53 (3 H, m), 3.57–3.62 (2H, m), 3.70–3.80 (3H, m), 3.84–3.92 (2H, m), 3.98 (1H, m), 4.51–4.60 (4H, m), 4.90 (1H, d, $J=2.9$ Hz), 5.24–5.38 (4H, m), 5.90–5.98 (2H, m). FABMS (positive-ion) *m/z* 727 $(M+Na)^+$; HRFABMS, calcd for $C_{38}H_{73}O_9PNa$: 727.4889. Found: 727.4892.

4.1.53. 2-(Diallylphosphono)ethyl 6-*O*-{2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl}-2,3-di-*O*-dodecyl-α-D-glucopyranoside (60). To a solution of imidate **5** (338 mg, 0.401 mmol) and diol **59** (189 mg, 0.268 mmol) were dissolved in CH_2Cl_2 (5 mL). To this solution was added AgOTf (155 mg, 0.604 mmol) at room temperature. After stirring for 1 h at room temperature, the mixture was quenched with aq. sat. $NaHCO_3$, diluted with CH_2Cl_2, washed with water and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with hexane–EtOAc (1:9) gave **60** (273 mg, 74%) as an amorphous compound. IR $\nu_{max}(CHCl_3)$ 3449, 3255, 3088, 2928, 2873, 2856, 1734 cm^{-1}. 500 MHz ^1H NMR ($CDCl_3$) δ 0.88 (9H, t, $J=6.8$ Hz), 1.26–1.80 (54H, m), 2.24–2.32 (2H, m), 2.55 (1H, s, OH), 3.21–3.36 (7H, m, containing 3H, s, δ 3.29), 3.39 (3H, s), 3.42–3.66 (7H, m), 3.71–3.88 (6H, m), 3.92–3.99 (2H, m), 4.16 (1H, d, $J=10.7$ Hz), 4.30 (1H, q, $J=8.8$, 9.8 Hz), 4.53–4.59 (8H, m), 4.70, 4.78 (2H, AB-q, $J=11.7$ Hz), 4.87 (1H, d, $J=2.9$ Hz), 4.93 (1H, d, $J=7.8$ Hz), 5.23–5.39 (8H, m), 5.88–5.98 (4H, m), 6.75 (1H, d, $J=6.8$ Hz, NH). FABMS (positive-ion) *m/z* 1406 $(M+Na)^+$. HRFABMS, calcd for $C_{65}H_{118}Cl_3NO_{19}P_2Na$: 1406.6732. Found: 1406.6720.

4.1.54. 2-(Diallylphosphono)ethyl 6-*O*-{2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-[(*Z*)-11-octadecenoylamino]-β-D-glucopyranosyl}-2,3-di-*O*-dodecyl-α-D-glucopyranoside (61). To a solution of **60** (220 mg, 0.159 mmol) in THF (4 mL) and acetic acid (0.5 mL) was added zinc dust (210 mg). After vigorously stirring for 3 h at room temperature, the solution was filtered to remove the Zn dust, and concentrated in vacuo to give a crude product. The product was diluted with EtOAc, washed with aq satd $NaHCO_3$ and brine, dried over Na_2SO_4, filtered and concentrated in vacuo to give a crude product, which was dissolved in CH_2Cl_2 (5 mL), and (*Z*)-11-octadecenoic acid (68 mg, 0.239 mmol) and WSC·HCl (55 mg, 0.287 mmol) were added to this solution. After stirring for 20 h at room temperature, the mixture was diluted with CH_2Cl_2, washed with water and brine, dried over $MgSO_4$, filtered and concentrated in vacuo to give a mixture, which was chromatographed on a silica gel column. Elution with EtOAc gave **61** (122 mg, 52%) as an amorphous compound. IR $\nu_{max}(CHCl_3)$ 3004, 2928, 2856, 1662 cm^{-1}. 500 MHz ^1H NMR ($CDCl_3$) δ 0.88 (12H, t, $J=6.8$ Hz), 1.25–1.80 (76H, m), 1.99–2.01 (4H, m), 2.16–2.33 (4H, m), 2.85 (1H, d, $J=3.9$ Hz, OH), 3.07 (1H, m), 3.23–3,25 (2H,m), 3.29 (3H, s), 3.36 (1H, m), 3.38 (3H, s), 3.44 (1H, t, $J=8.8$ Hz), 3.48–3.84 (12H, m), 3.91 (1H, m), 4.09 (1H, dd, $J=9.8$, 8.8 Hz), 4.17 (1H, d, $J=10.7$ Hz), 4.25 (1H, dd, $J=9.8$,8.8 Hz), 4.50–4.58 (8H, m), 4.85 (1H, d, $J=3.9$ Hz), 5.19 (1H, d, $J=7.8$ Hz), 5.23–5.38 (10H, m), 5.89–5.98 (4H,m), 6.91 (1H, d, $J=6.8$ Hz, NH). FABMS (positive-ion) *m/z* 1496 $(M+Na)^+$; HRFABMS, calcd for $C_{80}H_{149}NO_{18}P_2Na$: 1497.0139. Found: 1497.0123.

4.1.55. 2-(Phosphono)ethyl 6-*O*-{2-deoxy-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-[(*Z*)-11-octadecenoylamino]-4-*O*-phosphono-β-D-glucopyranosyl}-2,3-di-*O*-dodecyl-α-D-glucopyranoside (62). Compound **61** (110 mg, 0.075 mmol) was treated as described in the formation of **8** from **7** to give **62** (80 mg, 82%) as a white powder. IR ν_{max}(KBr) 3284, 3218, 3178, 3116, 2955, 2924, 2853, 1655, 1630 cm^{-1}. 500 MHz ^1H NMR (CD_3OD:$CDCl_3=5:1$) δ 0.90 (12H, t, $J=6.8$ Hz), 1.28–1.67 (74H, m), 1.73–1.77 (2H, m), 2.01–2.03 (4H, m), 2.16 (2H, dt, $J=18.6$, 7.8 Hz), 2.25 (2H, t, $J=6.8$ Hz), 3.22 (1H, dd, $J=2.9$, 9.8 Hz), 3.31 (3H, s), 3.34 (1H, m), 3.41 (3H, s), 3.46 (1H, dd, $J=9.8$, 8.8 Hz), 3.54–3.85 (15H, m), 3.96 (1H, m), 4.07–4.12 (2H, m), 4.54 (1H, d, $J=8.8$ Hz). 4.87 (1H, d, $J=3.9$ Hz), 5.31–5.37 (2H, m).

4.1.56. 3-Hydroxypropyl 2,3-di-*O*-dodecyl-4,6-*O*-isopropylidene-α-D-glucopyranoside (63). To a solution of **15** (3.30 g, 5.53 mmol) in THF (25 ml) was added 9-BBN (0.5 M solution in THF, 28 ml, 14.0 mmol). After stirring for 18 h at room temperature, aq 3 M NaOH (50 ml) and aq 30% H_2O_2 (18 ml) were added to the reaction mixture at 0 °C. After stirring for 3 h at room temperature, the mixture was extracted with EtOAc, washed with satd NH_4Cl and brine, dried over $MgSO_4$ and filtered. The filtrate was concentrated in vacuo, and chromatographed on a silica gel column. Elution with hexane–EtOAc (7:3) gave **63** (3.03 g, 89%) as a gum. IR $\nu_{max}(CHCl_3)$ 3506, 2927, 2855 cm^{-1}. 500 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, $J=6.8$ Hz), 1.25–1.94 (48H, m, containing two 3H, s, at 1.40 and 1.48 ppm), 2.88 (1H, dd, $J=4.9$, 6.8 Hz, OH), 3.28 (1H, dd, $J=3.9$, 7.8 Hz), 3.49–3.85 (12H, m), 3.96 (1H, m), 4.87 (1H, d, $J=3.9$ Hz). FABMS (positive-ion): *m/z* 637 $(M+Na)^+$, 615 $(M+H)^+$. HRFABMS, calcd for $C_{36}H_{70}O_7Na$: 637.5011. Found: 637.5034.

4.1.57. 3-(Diallylphosphonooxy)propyl 2,3-di-*O*-dodecyl-α-D-glucopyranoside (64). To a solution of **63** (981 mg, 1.59 mmol) and 1*H*-tetrazole (168 mg, 2.40 mmol) in THF (8 ml) was added diallyl diisopropylphosphoramidite (512 mg, 2.09 mmol). After stirring for 4 h at room temperature, aq 30% H_2O_2 (1 ml) was added to the reaction mixture at 0 °C. After stirring for 1 h at 0 °C, the mixture was quenched with aq 10% $Na_2S_2O_3$, diluted with EtOAc, washed with water, aq satd $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, and concentrated in vacuo to give a

residue, which was dissolved in aq 80% AcOH (10 ml) and stirred at 60 °C for 1 h. The solution was diluted with EtOAc, washed with aq satd $NaHCO_3$ and brine, dried over $MgSO_4$, filtered, concentrated in vacuo, and chromatographed on a silica gel column. Elution with hexane–EtOAc (1:9) gave **64** (828 mg, 71%) as a white powder. IR $\nu_{max}(CHCl_3)$ 3600, 3393, 2927, 2855 cm^{-1}. 500 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, $J=6.8$ Hz), 1.25–1.33 (36H, m), 1.53–1.59 (4H, m), 1.71 (1H, bs, OH), 1.97–2.09 (3H, m, containing OH), 3.26 (1H, dd, $J=3.9$, 9.8 Hz), 3.43 (1H, t, $J=8.8$ Hz), 3.51–3.75 (7H, m), 3.83–3.91 (3H, m), 4.13 (1H, m), 4.27 (1H, m), 4.53–4.57 (4H, m), 4.89 (1H, d, $J=3.9$ Hz), 5.25–5.39 (4H, m), 5.90–5.98 (2H, m). FABMS (positive-ion): *m/z* 757 $(M+Na)^+$, 735 $(M+H)^+$. HRFABMS, calcd for $C_{39}H_{76}O_{10}P$: 735.5175. Found: 735.5180.

4.1.58. 3-(Diallylphosphonoxy)propyl 6-*O*-{2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl}-2,3-di-*O*-dodecyl-α-D-glucopyranoside (65). Compound **64** was treated as described for the formation of **49** from **48** to give **65** (53%) as an amorphous solid. IR ν_{max} ($CHCl_3$) 3450, 3088, 2928, 2873, 2856, 1737 cm^{-1}. 500 MHz ^1H NMR ($CDCl_3$) δ 0.88 (9H, t, $J=6.8$ Hz), 1.25–1.79 (54H, m), 1.95–2.06 (2H, m), 2.78 (1H, d, $J=2.9$ Hz, OH), 3.24 (1H, dd, $J=3.9$, 9.8 Hz), 3.28 (3H, s), 3.30 (1H, m), 3.39 (3H, s), 3.41–3.86 (17H, m), 4.08 (1H, d, $J=8.8$ Hz), 4.12–4.24 (2H, m), 4.29 (1H, m), 4.52–4.60 (8H, m), 4.72, 4.76 (2H, AB-q, $J=11.7$ Hz), 4.84 (1H, d, $J=3.9$ Hz), 4.87 (1H, d, $J=7.8$ Hz), 5.24–5.39 (8H, m), 5.90–5.98 (4H, m), 6.03 (1H, bs, NH). FABMS (positive-ion): *m/z* 1436 $(M+Na)^+$. HRFABMS, calcd for $C_{66}H_{120}Cl_3NO_{20}P_2Na$: 1436.6844. Found: 1436.6847.

4.1.59. 3-(Diallylphosphonoxy)propyl 6-*O*-{2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-[(*Z*)-11-octadecenoylamino]-β-D-glucopyranosyl}-2,3-di-*O*-dodecyl-α-D-glucopyranoside (66). Compound **65** was treated as described for the formation of **50** from **49** to give **66** as an amorphous solid. IR $\nu_{max}(CHCl_3)$ 3454, 3319, 3089, 2928, 2856, 1665 cm^{-1}. 500 MHz ^1H NMR ($CDCl_3$) δ 0.88 (12H, t, $J=6.8$ Hz), 1.25–1.80 (76H, m), 1.95–2.05 (6H, m), 2.14–2.27 (2H, m), 3.17 (1H, d, $J=3.9$ Hz, OH), 3.21–3.24 (2H, m), 3.28 (3H, s), 3.30 (1H, m), 3.38 (3H, s), 3.41–3.82 (15H, m), 3.94 (1H, t, $J=9.8$, 8.8 Hz), 4.07 (1H, d, $J=8.8$ Hz), 4.11–4.20 (2H, m), 4.25 (1H, q, $J=8.8$, 9.8 Hz), 4.52–4.58 (8H, m), 4.84 (1H, d, $J=3.9$ Hz), 5.16 (1H, d, $J=7.8$ Hz), 5.23–5.39 (10H, m), 5.90–5.98 (4H, m), 6.36 (1H, d, $J=6.8$ Hz, NH). FABMS (positive-ion): *m/z* 1526 $(M+Na)^+$. HRFABMS, calcd for $C_{81}H_{151}NO_{19}P_2Na$: 1527.0261. Found: 1527.0277.

4.1.60. 3-(Phosphonoxy)propyl 6-*O*-{2-deoxy-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-[(*Z*)-11-octadecenoylamino]-4-*O*-phosphono-β-D-glucopyranosyl}-2,3-di-*O*-dodecyl-α-D-glucopyranoside (67). Compound **66** was treated as described for the formation of **8** from **7** to give **67** (84%) as a white powder. IR ν_{max}(KBr) 3285, 3228, 3069, 3004, 2955, 2924, 2853, 2318, 1656, 1630 cm^{-1}. 500 MHz ^1H NMR ($CD_3OD+CDCl_3$) δ 0.89 (12H, t, $J=6.8$ Hz), 1.30–1.67 (74H, m), 1.72–1.75 (2H, m), 1.93–1.99 (2H, m), 2.01–2.04 (4H, m), 2.20–2.30 (2H, m), 3.17 (1H, dd, $J=3.9$, 9.8 Hz), 3.29 (3H, s), 3.33 (1H, m), 3.39 (3H, s), 3.44 (1H, dd, $J=9.8$, 8.8 Hz), 3.48–3.67 (10H, m), 3.73–3.85 (6H, m), 4.04–4.12 (4H, m), 4.48 (1H, d, $J=8.8$ Hz), 4.85 (1H, d, $J=2.9$ Hz), 5.31–5.38 (2H, m). ESIMS (negative-ion): *m/z* 1342 $(M-H)^-$. HRESIMS (negative-ion): calcd for $C_{69}H_{134}NO_{19}P_2$: 1342.9031. Found: 1342.9047.

4.1.61. 3-Bromopropyl 2,3-di-*O*-dodecyl-4,6-*O*-isopropylidine-α-D-glucopyranoside (68). Compound **63** was treated as described for the formation of **58** from **16** to give **68** (72%) as a gum. IR $\nu_{max}(CHCl_3)$ 2927, 2855 cm^{-1}. 500 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, $J=6.6$ Hz), 1.21–1.32 (36H, m), 1.41 (3H, s), 1.48 (3H, s), 1.50–1.60 (4H, m), 2.13–2.19 (2H, m), 3.29 (1H, m), 3.49–3.75 (11H, m), 3.81–3.87 (2H, m), 4.86 (1H, d, $J=4.4$ Hz). FABMS (positive-ion): *m/z* 699 $(M+Na)^+$. HRFABMS, calcd for $C_{36}H_{69}BrO_6Na$: 699.4173. Found: 699.4180.

4.1.62. 3-(Diallylphosphono)propyl 2,3-di-*O*-dodecyl-α-D-glucopyranoside (69). Compound **68** was treated as described for the formation of **59** from **58** to give **69** (40%) as a white powder. IR $\nu_{max}(CHCl_3)$ 3600, 2927, 2856 cm^{-1}. 500 MHz ^1H NMR ($CDCl_3$) δ 0.88 (6H, t, $J=6.8$ Hz), 1.26–1.33 (36H, m), 1.53–1.60 (4H, m), 1.83–2.00 (4H, m), 2.64–2.75 (2H, bs, 2OH), 3.26 (1H, dd, $J=3.9$, 9.8 Hz), 3.44 (1H, dd, $J=8.8$, 9.8 Hz), 3.48–3.68 (6H, m), 3.72–3.80 (2H, m), 3.84–3.93 (2H, m), 4.49–4.58 (4H, m), 4.90 (1H, d, $J=3.9$ Hz), 5.23–5.38 (4H, m), 5.90–5.98 (2H, m). FABMS (positive-ion): *m/z* 741 $(M+Na)^+$, 719 $(M+H)^+$. HRFABMS, calcd for $C_{39}H_{76}O_9P$: 719.5222. Found: 719.5242.

4.1.63. 3-(Diallylphosphono)propyl 6-*O*-{2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranosyl}-2,3-di-*O*-dodecyl-α-D-glucopyranoside (70). Compound **69** (470 mg, 0.654 mmol) was treated as described in the formation of **19** from **18** to give **70** (533 mg, 58%) as an amorphous solid. IR $\nu_{max}(CHCl_3)$ 3588, 3450, 3253, 3088, 2928, 2873, 2856, 1733 cm^{-1}. 500 MHz ^1H NMR ($CDCl_3$) δ 0.88 (9H, t, $J=6$,8 Hz), 1.26–1.79 (54H, m), 1.83–2.03 (4H, m), 2.64 (1H, s, OH), 3.22–3.27 (3H, m, containing 1H, dd, $J=3.9$, 9.8 Hz, at 3.23 ppm), 3.28 (3H, s), 3.36 (1H, m), 3.39 (3H, s), 3.44–3.88 (15H, m), 4.11 (1H, d, $J=9.8$ Hz), 4.29 (1H, m), 4.48–4.59 (8H, m), 4.73, 4.75 (2H, ABq, $J=12.7$ Hz), 4.84 (1H, d, $J=2.9$ Hz), 4.90 (1H, d, $J=7.8$ Hz), 5.24–5.39 (8H, m), 5.90–5.98 (4H, m), 6.36 (1H, d, $J=5.9$ Hz). FABMS (positive-ion): *m/z* 1420 $(M+Na)^+$. HRFABMS, calcd for $C_{66}H_{120}NO_{19}Cl_3P_2Na$: 1420.6896. Found: 1420.6904.

4.1.64. 3-(Diallylphosphono)propyl 6-*O*-{2-deoxy-4-*O*-diallylphosphono-3-*O*-[(*R*)-3-(methoxy)decyl]-6-*O*-methyl-2-[(*Z*)-11-octadecenoylamino]-β-D-glucopyranosyl}-2,3-di-*O*-dodecyl-α-D-glucopyranoside (71). Compound **70** (480 mg, 0.343 mmol) was treated as described in the formation of **7** from **6** to give **71** (266 mg, 52%) as an amorphous solid. IR $\nu_{max}(CHCl_3)$ 3454, 3300, 3088, 2928, 2856, 1662 cm^{-1}. 500 MHz ^1H NMR ($CDCl_3$) δ 0.88 (12H, t, $J=6.6$ Hz), 1.20-1.96 (80H, m), 1.99-2.03 (4H, m), 2.14–2.26 (2H, m), 3.08 (1H, d, $J=3.9$ Hz, OH), 3.18 (1H, m), 3.22 (1H, dd, $J=3.9$, 9.8 Hz), 3.26–3.32 (4H, m, containing

3H, s, at 3.28 ppm), 3.35–3.42 (4H, m, containing 3H, s, at 3.38 ppm), 3.46 (1H, dd, $J=8.8$, 9.8 Hz), 3.48–3.83 (13H, m), 3.98 (1H, dd, $J=7.8$, 8.8 Hz), 4.09 (1H, d, $J=9.8$ Hz), 4.25 (1H, t, $J=8.8$ Hz), 4.48–4.58 (8H, m), 4.83 (1H, d, $J=3.9$ Hz), 5.17 (1H, d, $J=7.8$ Hz), 5.23–5.38 (10H, m), 5.90–5.98 (4H, m), 6.52 (1H, d, $J=6.8$ Hz, NH). FABMS (positive-ion): *m/z* 1510 $(M+Na)^+$. HRFABMS, calcd for $C_{81}H_{151}NO_{18}P_2Na$: 1511.0304. Found: 1511.0305.

4.1.65. 3-(Phosphono)propyl 6-*O*-{2-deoxy-3-*O*-[(*R*)-3-methoxydecyl]-6-*O*-methyl-2-[(*Z*)-11-octadecenoylamino]-4-*O*-phosphono-β-D-glucopyranosyl}-2,3-di-*O*-dodecyl-α-D-glucopyranoside. (72). Compound **71** (251 mg, 0.168 mmol) was treated as described in the formation of **8** from **7** to give **72** (177 mg, 80%) as a white powder. IR ν_{max}(KBr) 3286, 3069, 3004, 2954, 2925, 2854, 1630 cm^{-1}. 500 MHz ^1H NMR (CD_3OD) δ 0.89 (12H, t, $J=6.8$ Hz), 1.30–1.92 (80H, m), 2.01–2.05 (4H, m), 2.20–2.29 (2H, m), 3.18 (1H, dd, $J=3.9$, 9.8 Hz), 3.29 (3H, s), 3.33 (1H, m), 3.39 (3H, s), 3.43 (1H, dd, $J=3.3$, 9.8 Hz), 3.46 (1H, m), 3.50–3.67 (9H, m), 3.71–3.85 (6H, m), 4.04–4.12 (2H, m), 4.48 (1H, d, $J=7.8$ Hz), 4.85 (1H, d, $J=3.9$ Hz), 5.31–5.38 (2H, m). ESIMS (negative-ion): *m/z* 1326 $(M-H)^-$. HRESIMS: calcd for $C_{69}H_{134}NO_{18}P_2$: 1326.9069. Found: 1326.9047.

4.2. Methods for measurement of biological activity

The sources of the materials used in the study are as follows: lipopolysaccharide (LPS) from *E. coli* serotype 026:B6 and 12-*O*-tetradecanoylphorbol acetate (TPA) were from Sigma, St. Louis, MO; RPMI-1640 medium, fetal bovine serum (FBS) and newborn calf serum (NBCS) were from Gibco, Grand Island, NY; and human TNFα ELISA kit and mouse TNFα ELISA kit were from Genzyme-Techne, Minneapolis, MN.

4.2.1. Production of TNFα by human whole blood.[8] *Materials*: Lipopolysaccharide (LPS, lot 50K4117, *E. coli* 026:B6), human tumor necrosis factor alpha (TNFα) immunoassay kit and 96-well assay plates were purchased from Sigma, BioSource International, Inc. and Corning Inc. (Cat. No. 3956), respectively.

Whole blood TNFα production: Fresh blood was collected aseptically in the presence of heparin by venipuncture from healthy adult volunteers. The subjects did not have any apparent inflammatory conditions and had taken no drugs for at least 7 days prior to blood collection. Written informed consent was obtained from all volunteers before the experiment. In each well of the plates, 360 μL aliquots of blood were mixed with 20 μL of LPS solution (200 ng/mL, final concentration: 10 ng/mL) dissolved in PBS in the presence (for test sample) or absence (for positive control sample) of the test compounds solution (dissolved in DMSO/PBS solution). For the negative control samples, the same amount of blood was cultured without either LPS or a test compound solution. After 6 h of incubation at 37 °C, the plates were centrifuged at 490×*g* for 15 min, and the plasma was collected and stored at −20 °C. The concentrations of TNFα in the plasma were measured with commercially available immunoassay kits.

Statistical analysis: The percentage of inhibition of TNFα production was calculated by the following formula: 1−(concentration of TNFα in the test sample−concentration of TNFα in the negative control sample)/(concentration of TNFα in the positive control sample−concentration of TNFα in the negative control sample)×100. The suppressive activities of test compounds are expressed as the fifty percent inhibitory concentration (IC_{50}) of the test compound, the concentration at which the test compound suppresses TNFα production by 50%. The IC_{50} was calculated from the percentage of inhibition using the SAS System for Windows. The results are expressed as the mean IC_{50} of triplicate experiments.

4.2.2. Production of TNFα by galactosamine loaded C3H/HeN mice in vivo.[9] *Materials: Animals*: Male C3H/HeN mice were purchased from Charles River Japan (Tokyo, Japan). All mice were used at the age of 7 weeks, and housed at Sankyo Laboratories (Tokyo, Japan) with free access to standard rodent chow diet.

Reagents: Lipopolysaccharide (LPS, from *Escherichia coli* O26:B6) and D-Galactosamine (GalN) were purchased from Sigma (St Louis, MO). Enzyme-linked immunosorbent assay (ELISA) kits of murine TNFα were from R&D Systems (Minneapolis, MN).

TNFα production: Naïve C3H/HeN mice (five per group) were intravenously injected with the test compound solution (10 ml/kg; dissolved in 0.1% triethylamine /saline solution), and immediately after, mice were intravenously injected with a mixture of LPS (0.05 mg/10 ml saline/kg) and GalN (1 g/10 ml saline/kg). Mice were injected with vehicle (0.1% triethylamine/saline solution) and saline for negative control samples, and with vehicle and LPS/GalN for positive control samples. One hour after injection, venous blood was collected under ether anesthesia with heparinized syringes fitted with 23-gauge needles from the abdominal vena, and was centrifuged at 4 °C for 3 min at 13,230×*g* to obtain the plasma. Plasma was stored at −30 °C before measuring TNFα levels by ELISA. The concentrations of TNFα of mouse plasma were measured using ELISA analysis according to the manufacturer's instructions.

Statistical analysis: The percentage of inhibition of TNFα production was calculated by the following formula: [1−(concentration of TNFα in the test sample−concentration of TNFα in the negative control sample)/(concentration of TNFα in the positive control sample −concentration of TNFα in the negative control sample)]×100. The suppressive activities of test compounds are expressed as the mean of fifty percent inhibitory dose (ID_{50}) of the test compound, at which the test compound suppresses TNFα production by 50%. The ID_{50} was calculated from the percentage of inhibition using the SAS System for Windows (version 5).

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