Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron Letters
Tetrahedron Letters 47 (2006) 2087–2092

New synthesis of glycolipid immunostimulants RC-529 and CRX-524

Hélène G. Bazin, Laura S. Bess, Mark T. Livesay, Kendal T. Ryter, Craig L. Johnson, Jeffrey S. Arnold and David A. Johnson*

GlaxoSmithKline Biologicals, 553 Old Corvallis Road, Hamilton, MT 59840, USA

Received 12 October 2005; revised 25 January 2006; accepted 27 January 2006
Available online 13 February 2006

Abstract—An efficient and scalable synthesis of the potent vaccine adjuvant RC-529 (**3**) and TLR4 agonist CRX-524 (**4**) is described in eight steps from 1,3,4,6-tetra-*O*-acetyl-2-amino-2-benzyloxycarbonyl-2-deoxy-β-D-glucopyranose (**10c**) in ca. 25% overall yield. The synthesis features the strategic use of the *N*-Cbz group for β-glycosylation and the selective N,N,O-triacylation of common advanced intermediate **15** with (*R*)-3-tetradecanoyloxy or decanoyloxytetradecanoic acid (**8**, **9**) late in the synthesis. A new method for preparing and enhancing the enantiopurity of (*R*)-3-hydroxytetradecanoic acid (**6**), a key component of **3** and **4** as well as bacterial lipid A, is also described.

Lipopolysaccharide (LPS, endotoxin), a complex cell-surface component specific to Gram-negative bacteria, and its active principle, lipid A, are potent stimulators of host defense systems, both as adjuvants for vaccine antigens[1] and as inducers of non-specific resistance to infection in animal models.[2] However, the profound pyrogenicity and lethal toxicity of LPS and lipid A have limited their medicinal use.[1] As a result, considerable effort has been directed at the design and synthesis of lipid A mimetics with simplified structures and improved toxicity/bioactivity profiles.[3]

In the course of our own structural studies on the lipid A pharmacophore,[4,5] we identified a new class of monosaccharide lipid A mimetics known as aminoalkyl glucosaminide 4-phosphates (AGPs).[5] The AGPs are synthetic mimetics of compound **1** (Fig. 1), the major hexa-acyl component present in the low toxicity monophosphoryl lipid A (MLA) derived from *Salmonella minnesota* LPS, and have the general structure **2** (Fig. 1). Several members of this class, including prototypical AGPs RC-529 (**3**) and CRX-524 (**4**) (Fig. 1), have been shown to improve humoral and cell-mediated immune responses to a variety of different antigens in mice[5,6] as well as enhance non-specific resistance in mice to *Listeria monocytogenes* and influenza infections.[7,8]

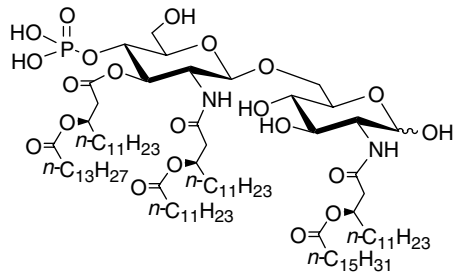
Although the precise molecular mechanism for the induction of the innate and adaptive immune responses is not fully understood, both AGP and LPS recognition is dependent upon Toll-like receptor 4 (TLR4) and accessory molecules such as MD-2, whose activation leads to pro-inflammatory cytokine and chemokine release and the mediation of immune responses.[9]

The potent immunostimulatory activity of RC-529 and CRX-524 together with their low pyrogenicity in rabbits and low toxicity in animal models make these compounds attractive candidates for clinical development. RC-529 was recently shown to be a safe and effective adjuvant in a pivotal phase III vaccine trial with a recombinant hepatitis B antigen,[10] and CRX-524 shows promise as a stand-alone therapeutic for enhancing host resistance to bacterial and viral infections.[7]

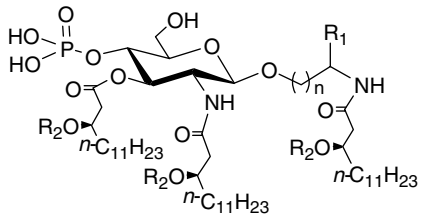
Earlier, we reported a highly convergent synthesis of RC-529 and CRX-524 in 15 linear steps from D-glucosamine.[5] However, this initial discovery synthesis suffers from a number of drawbacks. Most notably, incorporating two of the three (*R*)-3-alkanoyloxytetradecanoyl residues onto the AGP scaffold early in the synthesis leads to multiple synthetic and chromatographic steps

Keywords: Lipid A mimetics; Glycolipids; Immunostimulants; TLR4 agonist; Vaccine adjuvant; AGP.
* Corresponding author. Tel.: +1 406 375 2134; fax: +1 406 363 6129; e-mail: david.a.johnson@gskbio.com

0040-4039/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2006.01.137



1 major component in *S. minnesota* MLA



2 AGP generic structure
3 R_1= H, R_2= *n*-$C_{13}H_{27}CO$, n=1 (RC-529)
4 R_1= H, R_2= *n*-$C_9H_{19}CO$, n=1 (CRX-524)

Figure 1. Synthetic and naturally derived lipid A mimetics.

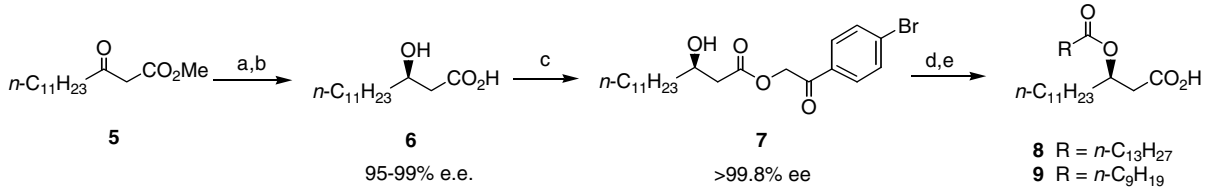
involving sensitive glycolipid intermediates. This methodology also precludes the use of a late-stage intermediate for the synthesis of AGPs, such as RC-529 and CRX-524, comprising the same sugar and aglycon units but different fatty acid substituents.

Herein, we describe a more efficient and scalable synthesis of glycolipids **3** and **4** in eight linear steps from readily available 1,3,4,6-tetra-*O*-acetyl-2-amino-2-benzyloxycarbonyl-2-deoxy-β-D-glucopyranose (**10c**)[11] and (*R*)-3-tetradecanoyloxy or decanoyloxytetradecanoic acid (**8**, **9**).[12] This new synthesis, which is convergent with respect to the AGP backbone and the alkanoyloxytetradecanoic acid moieties near the end of the synthesis, features the selective carbodiimide-mediated N,N,O-triacylation of a diamino diol common advanced intermediate (CAI) in the key step. To our knowledge, this is the first time that three (*R*)-3-alkanoyloxyalkanoic acid residues—the most common type of fatty acid present in bacterial lipid A—have been installed simultaneously in the synthesis of a lipid A derivative. Late introduction of the fatty acid chains also allows easier isolation and purification of intermediates, many of which are crystalline, and decreases the total amount of the chiral fatty acid starting material needed in comparison to other synthetic routes. As a part of the effort to make the synthesis more adaptable to large scale, a new method for preparing and enhancing the enantiopurity of the (*R*)-3-hydroxytetradecanoic acid (**6**) subunit has also been developed (Scheme 1).

Several years ago, we reported the enantioselective synthesis of several (*R*)-3-alkanoyloxytetradecanoic acids via Ru(II)–Binap-catalyzed hydrogenation of keto ester **5**[13] in the key step and subsequent enantiomeric enhancement by recrystallization of the dicyclohexylammonium (DCHA) salt of **6** from acetonitrile.[12] However, reproducibility problems with commercially available Ru(II) catalysts as well as difficulties in removing the DCHA–HBr by-product in scale-up preparations of *p*-bromophenacyl (PAc) ester **7** prompted us to examine alternate methods for the synthesis of **6** and enhancement of enantiopurity.

Application of Genet's in situ-Ru(II) asymmetric hydrogenation method[14] to keto ester **5** using anhydrous $RuCl_3$/(*R*)-Binap and base hydrolysis of the resulting hydroxy ester reproducibly gave (*R*)-3-hydroxytetradecanoic acid (**6**) in ca. 100% crude yield and 95–99% ee (determined by chiral HPLC analysis of the PAc ester derivative[12]) on up to a 200-g scale. Direct conversion of crude hydroxy acid **6** to scalemic PAc ester **7** and crystallization of the ester once or twice from toluene afforded **7** in $\geqslant$99.8% ee and >60% overall yield from keto ester **5**.[15] Compound **7** was converted to acyloxyacids **8** and **9** by O-acylation and reductive deprotection in 88% and 91% yield, respectively.[12]

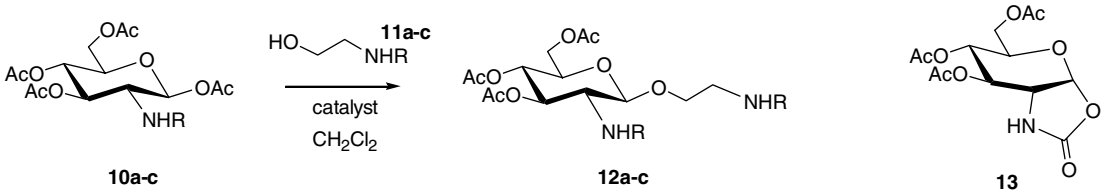
In order to develop an efficient route to common advanced intermediate **15**, we first evaluated the preparation of β-D-glucosaminides **12a–c** using the readily available *N*-alkoxycarbonyl-protected glycosyl donor/acceptor pairs **10a–c**[11]/**11a–c** (Table 1). The *N*-alkoxycarbonyl method for β-glycosylation with glucosamine-β-acetates is known to give good yields of β-glycosides with nearly exclusive 1,2-trans stereoselectivity due to participation of the carbamate protecting group.[11] The 2,2,2,-trichloroethylcarbonyl (Troc) and allyloxycarbonyl (Aoc) groups were selected for N-protection in this study because of the known utility of **10a** and **10b** in oligosaccharide synthesis[16] as well as their capacity for selective removal under mild conditions in the



5 **6** 95-99% e.e. **7** >99.8% ee **8** R = *n*-$C_{13}H_{27}$ **9** R = *n*-C_9H_{19}

Scheme 1. Reagents and conditions: (a) $RuCl_3$, (*R*)-Binap, 4.6 kg/cm^2 H_2, MeOH, 40–50 °C; (b) aq LiOH, THF, then H^+; (c) *p*-$BrC_6H_4COCH_2Br$, Et_3N, EtOAc; recrystallized twice from toluene, 64% (three steps); (d) RCO_2H, EDC–MeI, 4-pyrrolidinopyridine, CH_2Cl_2; (e) Zn, AcOH, 91% (**8**), 88% (**9**) (two steps).

Table 1. Preparation of 2-aminoethyl β-D-glucosaminides **12a–c**



10a-c **12a-c** **13**

Entry	Series	R	Glycosylation conditions[a]	Yield of **12** (%)[b]
1	a	Troc	BF_3–OEt_2, 2 equiv, rt, 2 h	77
2	b	Aoc	BF_3–OEt_2, 2 equiv, rt, 2 h	78 (<5)
3	c	Cbz	BF_3–OEt_2, 2 equiv, rt, 2 h	42 (15)
4	c	Cbz	BF_3–OEt_2, 2 equiv, 0 °C→rt, 2 h	38 (14)
5	c	Cbz	TMSOTf, 1.1 equiv, rt, 4 h	52 (10)
6	c	Cbz	TMSOTf, 1.1 equiv, −78 °C, 24 h	66 (<5)[c]
7	c	Cbz	TMSOTf, 1.1 equiv, −15 °C, 3 h	88 (<5)
8	c	Cbz	TMSOTf, 0.1 equiv, −15 °C→rt, 5 h	84 (<5)

[a] All reactions performed in CH_2Cl_2. The acceptor alcohols **11a–c** (1 equiv) were purchased commercially or prepared from ethanolamine and the appropriate chloroformate under Schotten–Baumann conditions.
[b] Yield of purified product **12**. Values in parentheses represent the amount of by-product **13** isolated or estimated by TLC in the crude product prior to purification.
[c] Crude product contained approximately 15–20% unreacted starting material **10c** by TLC.

presence of the 6-*O*-*tert*-butyldimethysilyl (TBDMS) protecting group. In contrast, the strategic use of benzyloxycarbonyl (Cbz)-protected glucosamine donors such as **10c** in natural product synthesis has not been exploited, in part because of low glycosylation yields and the formation of oxazolidinone by-products.[16]

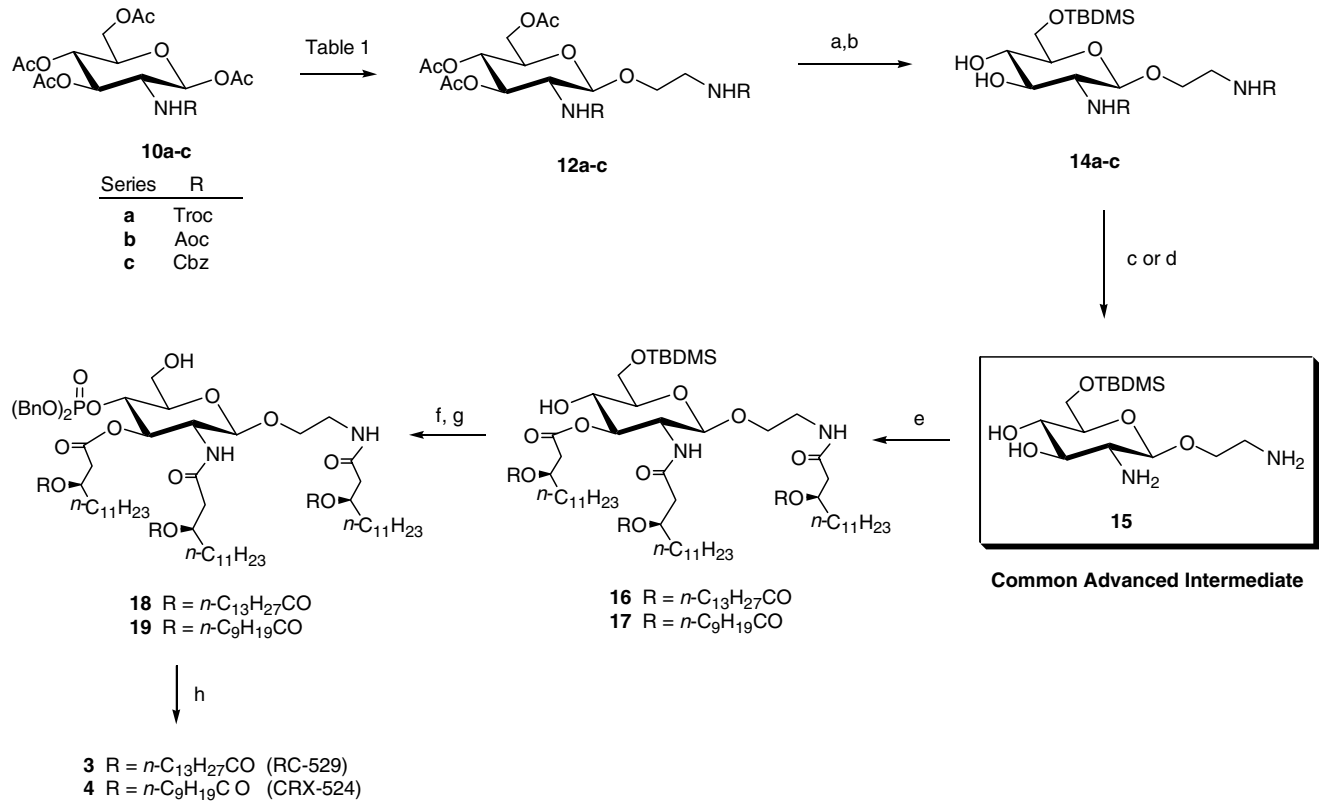
Although oxazolidinone **13** can be a significant by-product with carbamate-protected glycosyl donors that are able to accommodate a positive charge (e.g., allyl, benzyl, *tert*-butyl),[11] *N*-Aoc-protected β-acetates have been used successfully with a variety of Lewis acids.[16] As anticipated, the *N*-Troc and *N*-Aoc β-glycosides **12a** and **12b** were obtained in good yield using BF_3-etherate catalysis (entries 1 and 2). However, only moderate yields were obtained with the Cbz-protected β-acetate **12c** with BF_3-etherate due to significant oxazolidinone formation (entries 3 and 4). Substituting TMSOTf[11] for BF_3–OEt_2 and running the reaction at lower temperatures, however, mitigated against the formation of oxazolidinone **13** and led to excellent yields of the Cbz-protected β-glycoside **12c** (mp 129–130 °C) on a multi-10-g scale even in the presence of catalytic TMSOTf (entries 7 and 8). In contrast to the Troc and Aoc-protected glycosylation products **12a**,**b**, which required chromatographic purification, the Cbz-substituted β-glycoside **12c** could be purified by trituration with methyl *tert*-butyl ether (MTBE) or by crystallization from CH_2Cl_2–heptane.

The glycosylation products **12a–c** were readily converted to the 6-O-TBDMS-protected derivatives **14a–c** in good yield by O-deacetylation with NH_4OH in methanol and selective 6-O-silylation with TBDMS–Cl/pyridine (Scheme 2). Once again, the Cbz-protected product (**14c**, mp 121–122 °C) could be purified by crystallization (EtOAc–heptane) or trituration (heptane) whereas the *N*-Troc and *N*-Aoc derivatives had to be purified chromatographically.

We found that the efficiency of subsequent N-deprotection and isolation/purification of diamino diol CAI **15** were very dependent on the nature of the N-protecting group. For example, removal of the Troc groups under standard conditions (Zn/AcOH) led to the formation of complexed zinc, which was difficult to remove by aqueous workup and led to emulsions and low recoveries of diamine **15**. Likewise, deprotection of the *N*-Aoc groups of **14b** with $(Ph_3P)Pd/Bu_3SnH$[17] and aqueous workup led to low product recoveries. Omitting the aqueous workup in the Aoc case and isolating the product by evaporation and trituration (MTBE) led to a 90% yield of purified **15**, but lower acylation efficiencies in the next step (~30%), possibly because of residual tin or palladium contamination.

It was anticipated that deprotection of the Cbz groups of **14c** by catalytic hydrogenation, however, would obviate an aqueous workup and permit easy isolation of CAI **15** by simple filtration. Satisfyingly, hydrogenolysis of the Cbz derivative **14c** in methanol with 5% palladium on carbon gave the desired diamino diol **15** (143–145 °C) in high yield and purity after filtration and trituration of the crude product with MTBE or MTBE/heptane.

The one-pot carbodiimide-mediated tri-acylation of diamino diol **15** was most efficiently carried out as a single operation rather than by sequential N- and O-acylations. Accordingly, N,N,O-tri-acylation of **15** (prepared from **14c**) with (*R*)-3-alkanoyloxytetradecanoic acid **8** or **9** in the presence of ethyl 1-[3-(dimethylamino)propyl]-3-ethylcarbodimide (EDC) methiodide and catalytic 4-(dimethylamino)pyridine (DMAP, 3 mol %) in CH_2Cl_2 (0 °C→rt, 2 h) gave **16** and **17** in 59% and 66% yield, respectively, after chromatographic purification on silica gel.[18] Very little (<5%) of the (separable) tetra-acylated product was typically produced under these conditions. Higher temperatures, longer reaction times, and/or greater catalyst loadings led to significant tetra-acylation



Scheme 2. Reagents and conditions: (a) NH_4OH (10 equiv), MeOH; (b) TBDMS–Cl, py, 0.2 M, 77% (**14a**), 95% (**14b**), 91% (**14c**); (c) from **14b**: $(Ph_3P)_4Pd$ (0.02 equiv), Bu_3SnH (2 equiv), 90%; (d) from **14c**: 5% Pd/C, 1.0 kg/cm^2 H_2, MeOH, 91%; (e) **8** or **9** (3.2 equiv), EDC–MeI (3.2 equiv), DMAP (0.03 equiv), CH_2Cl_2, 0 °C→rt, 2 h, 59% (**16**), 66% (**17**); (f) (i) $(BnO)_2PN$*i*-Pr_2 (1.4 equiv), 4,5-dicyanoimidazole (1.4 equiv), CH_2Cl_2, rt; (ii) *m*-chloroperbenzoic acid (3 equiv), 0 °C; (g) TFA (10 equiv), CH_2Cl_2, rt, 77% (**18**), 65% (**19**) (two steps); (h) 20% $Pd(OH)_2$/C, 1.0 kg/cm^2 H_2, THF; 73% (**3**), 76% (**4**).

as well as the formation of other by-products. Selective acylation of the 3-hydroxyl group, which is intrinsically more nucleophilic than the 4-hydroxyl group in 2-amino sugars,[16] was confirmed by 400 MHz ^{1}H–^{1}H COSY experiments. The ^{1}H NMR spectra of **16** and **17** displayed a doublet of doublets centered at δ 4.93 ($J = 9.4$, 10.4 Hz) and δ 4.94 ($J = 9.0$, 11.0 Hz), respectively, for the H-3 protons; the H-4 protons appeared upfield at ~3.65 ppm.

Despite the report that the carbodiimide-mediated N,O-di-acylation of sugar amino alcohols with acid **8** has been performed in the presence of a stoichiometric amount of DMAP without apparent by-product formation,[19] we found that the O-acylation step (which was investigated independently with diol **14a** as well as with a related 4,6-isopropylidene derivative) was very sensitive to the level of catalyst loading. Using DMAP between 5 and 50 mol % led to progressively greater amounts of 3-*O*-tetradecanoyl or 3-*O*-decanoyl by-products with acids **8** and **9**, respectively (data not shown). Depending on the particular substrate and reaction conditions, the 3-*O*-alkanoyl side products could be the major product formed (>25%). Although the precise mechanism is not clear, these by-products do not appear to arise via β-elimination of the in situ-formed *O*-acyloxyacylisourea intermediate or cognate rearrangement[20] to a mixed anhydride as 3-*O*-alkenoyl products were not isolated or detected by LC–MS analysis of crude reaction products.

Phosphorylation of **16** and **17** using the dibenzyl phosphoramidite method and silyl deprotection with trifluoroacetic acid (TFA) gave final intermediates **18** and **19** in 77% and 65% yield, respectively, after chromatographic purification on silica gel. Deprotection of the dibenzyl phosphates **18** and **19** by hydrogenolysis in tetrahydrofuran (THF) in the presence of palladium hydroxide on carbon afforded RC-529 (**3**) and CRX-524 (**4**) as their triethylammonium salts in 73% and 76% yield, respectively, and >95% purity after silica gel chromatography and lyophilization of the free acids from aqueous *tert*-butanol containing triethylamine.[21]

In summary, an improved synthesis of the phosphoglycolipids RC-529 (**3**) and CRX-524 (**4**) has been developed in eight linear steps and ca. 25% overall yield from 1,3,4,6-tetra-*O*-acetyl-2-amino-2-benzyloxycarbonyl-2-deoxy-β-D-glucopyranose (**10c**). The strategic use of the Cbz group for amine protection leads to high β-glycosylation efficiencies and easy isolation of common advanced intermediate **15**, thereby setting the stage for the one-pot N,N,O-tri-acylation of **15** with (*R*)-3-alkanoyloxytetradecanoic acids **8** and **9**. The crystalline nature of the Cbz-protected intermediates also facilitated purification by non-chromatographic means.[22]

A new method for preparing fatty acid starting materials **8** and **9** was also developed using in situ-generated Ru(II)–Binap catalyst for asymmetric hydrogenation of keto ester **5** in the key step and enhancing the enantiopurity of scalemic PAc ester **7** by crystallization. These methodologies are currently being applied to the synthesis of other AGPs **2** and should find useful application to the preparation of glucosamine-based lipid A mimetics in general.

Acknowledgments

This work was funded in part by the Defense Advanced Research Projects Agency (DARPA) under Contract No. N66001-01-C-8007 and by the US Army Medical Research Acquisition Activity (USAMRAA) under Contract No. DAMD17-03-C-0089. Any opinions, findings, and conclusions or recommendations expressed in this article are those of the authors and do not necessarily reflect the views of DARPA or USAMRAA.

References and notes

1. Johnson, A. G. *Clin. Microbiol. Rev.* **1994**, *7*, 277–289.
2. Alexander, C.; Rietschel, E. T. *J. Endotoxin Res.* **2001**, *7*, 167–202.
3. See, for example: (a) Tamai, R.; Asai, Y.; Hashimoto, M.; Fukase, K.; Kusumoto, S.; Ishida, H.; Kiso, M.; Ogawa, T. *Immunology* **2003**, *110*, 66–72; (b) Hawkins, L. D.; Ishizaka, S. T.; McGuinness, P.; Zhang, H.; Gavin, W.; DeCosta, B.; Meng, Z.; Yang, H.; Mullarkey, M.; Young, D. W.; Yang, H.; Rossignol, D. P.; Nault, A.; Rose, J.; Przetak, M.; Chow, J. C.; Gusovsky, F. *J. Pharmacol. Exp. Ther.* **2002**, *300*, 655–661; (c) Jiang, Z.-H.; Budzynski, W. A.; Skeels, L. N.; Krantz, M. J.; Koganty, R. R. *Tetrahedron* **2002**, *58*, 8833–8842.
4. Johnson, D. A.; Keegan, D. S.; Sowell, C. G.; Livesay, M. T.; Johnson, C. L.; Taubner, L. M.; Harris, A.; Myers, K. R.; Thompson, J. D.; Gustafson, G. L.; Rhodes, M. J.; Ulrich, J. T.; Ward, J. R.; Yorgensen, Y. M.; Cantrell, J. L.; Brookshire, V. G. *J. Med. Chem.* **1999**, *42*, 4640–4649.
5. Johnson, D. A.; Sowell, C. G.; Johnson, C. L.; Livesay, M. T.; Keegan, D. S.; Rhodes, M. J.; Ulrich, J. T.; Ward, J. R.; Cantrell, J. L.; Brookshire, V. G. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 2273–2278.
6. Baldridge, J. R.; McGowan, P.; Evans, J. T.; Cluff, C.; Mossman, S.; Johnson, D.; Persing, D. *Expert Opin. Biol. Ther.* **2004**, *4*, 1129–1138, and references cited therein.
7. Baldridge, J. R.; Cluff, C. W.; Evans, J. T.; Lacy, M. J.; Stephens, J. R.; Brookshire, V. G.; Wang, R.; Ward, J. R.; Yorgensen, Y. M.; Persing, D. H.; Johnson, D. A. *J. Endotoxin Res.* **2002**, *8*, 453–458.
8. Cluff, C. W.; Baldridge, J. R.; Stöver, A.; Evans, J. T.; Johnson, D. A.; Lacy, M. J.; Clawson, V. G.; Yorgensen, V. M.; Johnson, C. L.; Livesay, M. T.; Hershberg, R. M.; Persing, D. H. *Infect. Immun.* **2005**, *73*, 3044–3052.
9. Stöver, A. G.; Correia, J. D. S.; Evans, J. T.; Cluff, C. W.; Elliott, M. W.; Jeffery, E. W.; Johnson, D. A.; Lacy, M. J.; Baldridge, J. R.; Probst, P.; Ulevitch, R. J.; Persing, D. H.; Hershberg, R. M. *J. Biol. Chem.* **2004**, *279*, 4440–4449.
10. Baldridge, J. R. Presented at the 3rd Meeting on Novel Adjuvants Currently in/close to Human Clinical Testing, World Health Organization/Tropical Disease Research Program–Fondation Mérieux, Annecy, France, 2002.
11. Boullanger, P.; Jouineau, M.; Bouammali, B.; Lafont, D.; Descotes, G. *Carbohydr. Res.* **1990**, *202*, 151–164.
12. Keegan, D. S.; Hagen, S. R.; Johnson, D. A. *Tetrahedron: Asymmetry* **1996**, *7*, 3559–3564.
13. Nakahata, K.; Imaida, M.; Ozaki, H.; Narada, T.; Tai, A. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 2186–2189.
14. Labeeuw, O.; Phansavath, P.; Genêt, J.-P. *Tetrahedron: Asymmetry* **2004**, *15*, 1899–1908.
15. General procedure for large-scale preparation of PAc ester **7**: A degassed solution of $RuCl_3$ (0.2 mol %), (*R*)-Binap (0.2 mol %), and methyl 3-oxotetradecanoate (**5**) (200 g) in MeOH (800 mL) was hydrogenated at 65 psig and 40–50 °C for 20 h, and then filtered through silica gel. The filtrate was concentrated and the hydroxy ester obtained was hydrolyzed with LiOH (1.5 equiv) in 50% aq THF (2.5 L). The resulting slurry was acidified and extracted with MTBE (2 L). The organic layer was washed with water, dried (Na_2SO_4), and concentrated. A solution of the hydroxy acid **6** was obtained and triethylamine (1.5 equiv) in EtOAc (5.5 L) was treated with 2,4-dibromoacetophenone (1 equiv) and the resulting mixture was stirred overnight at room temperature. The suspension that formed was filtered and the filtrate concentrated to give crude **7** (95.0% ee by chiral HPLC[12]), which was crystallized twice from toluene to give 219 g (64% from **5**) of **7** as a white solid: mp 111–112.5 °C (lit.[12] mp 109–109.5 °C); 99.9% ee; $[\alpha]_D$ −2.37 (*c* 2.40, $CHCl_3$) (lit.[12] $[\alpha]_D$ −2.4 (*c* 2.39, $CHCl_3$)).
16. Banoub, G.; Boullanger, P.; Lafont, D. *Chem. Rev.* **1992**, *92*, 1167–1195.
17. Dangles, O.; Guibé, F.; Balavoine, G. *J. Org. Chem.* **1987**, *52*, 4984–4993.
18. General procedure for tri-acylation of **15** with acid **9**: A solution of **15** (4.87 g), EDC–MeI (3.2 equiv), and DMAP (0.03 equiv) in CH_2Cl_2 (40 mL) at 0 °C was treated dropwise with a solution of acid **9** (3.2 equiv) in CH_2Cl_2 (20 mL) and allowed to stir and warm to room temperature over 2 h. Aqueous workup followed by flash chromatography on silica gel (gradient elution, 10→40% EtOAc–heptane containing 1% AcOH) gave 14.2 g (66%) of **17** as an amorphous solid: $[\alpha]_D$ −9.71 (*c* 2.10, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.08 (s, 3H), 0.09 (s, 3H), 0.84–0.91 (m, 27H), 1.19–1.35 (m, 90H), 1.51–1.67 (m, 12H), 2.24–2.32 (m, 6H), 2.32–2.64 (m, 6H), 3.28–3.50 (m, 3H), 3.55 (s, 1H), 3.58–3.70 (m, 2H), 3.78–3.95 (m, 4H), 4.47 (d, 1H, $J = 8.0$ Hz), 4.94 (dd, 1H, $J = 9.0$, 11.0 Hz), 5.03–5.22 (m, 3H), 6.15 (d, 1H, $J = 8.8$ Hz), 6.50 (t, 1H, $J = 5$ Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ −5.43, −5.46, 14.11, 18.26, 22.68, 24.97, 25.01, 25.04, 25.16, 25.34, 25.83, 29.15, 29.20, 29.21, 29.29, 29.31, 29.36, 29.47, 29.49, 29.53, 29.58, 29.61, 29.64, 29.66, 29.70, 31.88, 31.91, 34.34, 34.41, 34.47, 34.57, 39.34, 40.00, 41.43, 41.98, 53.63, 64.08, 67.81, 70.73, 70.82, 71.15, 71.40, 74.70, 75.82, 77.21, 101.35, 169.86, 170.20, 171.63, 173.48, 173.53, 174.14. MALDI-MS calcd for $[MNa]^+$ 1500.1850, found 1500.1860.
19. Miyajima, K.; Achiwa, K. *Chem. Pharm. Bull.* **1997**, *45*, 312–320.
20. Duynstee, H. I.; van Vliet, M. J.; van der Marel, G. A.; van Boom, J. H. *Eur. J. Org. Chem.* **1998**, 303–307.
21. Spectral data for compounds **3** and **4**: Compound **3**: ^1H NMR (400 MHz, $CDCl_3$–CD_3OD) δ 0.88 (∼t, 18H), 1.19–1.39 (m, 123H), 1.50–1.65 (m, 12H), 2.24–2.51 (m, 10H), 2.52–2.67 (m, AB type, 2H), 3.11 (q, 6H, $J = 7.2$ Hz), 3.22–3.30 (m, 1H), 3.36–3.45 (m, 2H), 3.58–3.66 (m, 1H), 3.73–3.83 (m, 3H), 3.92 (dd, 1H, $J = 2.8$, 12.8 Hz), 4.25 (∼q, 1H, $J \sim 10$ Hz), 4.53 (d, 1H, $J = 8.4$ Hz), 5.07–5.24 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 8.57, 14.10, 22.68, 25.05, 25.34, 29.21, 29.24, 29.29, 29.37, 29.40, 29.42, 29.44, 29.51, 29.55, 29.57, 29.62, 29.64, 29.67, 29.68, 29.73,

29.77, 31.91, 31.93, 34.33, 34.41, 34.47, 34.50, 34.56, 39.47, 41.23, 41.59, 45.79, 54.77, 60.69, 68.26, 70.63, 70.89, 71.32, 71.47, 73.04, 75.37, 77.20, 100.93, 170.06, 170.33, 170.72, 173.39, 173.50, 174.02; ^{31}P NMR (162 MHz, $CDCl_3$) δ 1.60; MALDI-MS calcd for $[MNa]^+$ 1634.2526, found 1634.2575. Compound **4**: ^{1}H NMR ($CDCl_3$–CD_3OD) δ 0.88 (∼t, 18H), 1.19–1.35 (m, 99H), 1.50–1.65 (m, 12H), 2.26–2.51 (m, 10H), 2.54–2.67 (m, AB type, 2H), 3.07 (q, 6H, $J = 7.6$ Hz), 3.25–3.32 (m, 1H), 3.35–3.45 (m, 2H), 3.58–3.64 (m, 1H), 3.72–3.82 (m, 3H), 3.95 (d, 1H, $J = 11.2$ Hz), 4.25 (∼q, 1H, $J \sim 10$ Hz), 4.57 (d, 1H, $J = 8.4$ Hz), 5.07–5.24 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 8.54, 14.11, 22.68, 25.04, 25.36, 29.20, 29.23, 29.28, 29.31, 29.34, 29.37, 29.42, 29.50, 29.52, 29.55, 29.64, 29.65, 29.67, 29.70, 29.71, 29.73, 29.75, 31.88, 31.89, 31.92, 31.94, 34.35, 34.43, 34.47, 34.50, 34.57, 39.46, 41.23, 41.59, 45.79, 54.88, 60.67, 68.30, 70.61, 70.89, 71.32, 73.01, 75.54, 77.21, 100.95, 170.05, 170.34, 170.72, 173.40, 173.49, 173.93; ^{31}P NMR ($CDCl_3$) δ 1.55; MALDI-MS calcd for $[MNa]^+$ 1466.0648, found 1466.0683.

22. All compounds were characterized by ^{1}H and ^{13}C NMR; the amide and hydroxyl protons of compounds **3** and **4** were pre-exchanged with deuterium (methanol-d_4) prior to obtaining the ^{1}H NMR spectra; new compounds gave satisfactory elemental analyses and/or mass spectral data.