First Total Synthesis of Caminoside A, an Antimicrobial Glycolipid from Sponge

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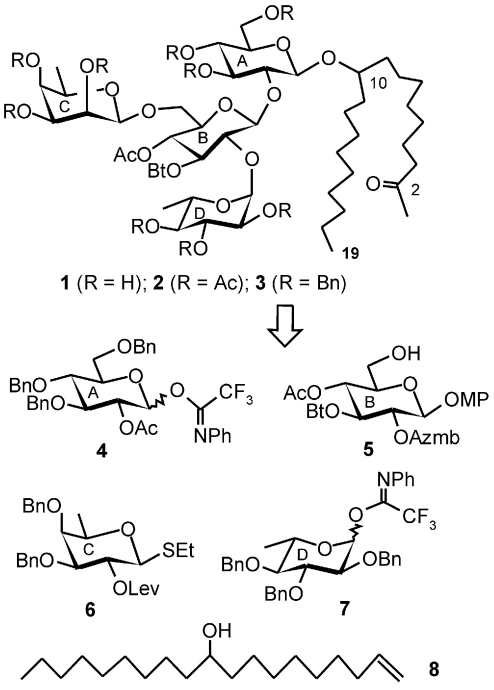
Abstract: Caminoside A, a novel antimicrobial tetrasaccharide glycolipid from the marine sponge *Caminus sphaeroconia*, which represents the first bacterial type III secretion inhibitor, is synthesized in a total of 57 steps starting from D-glucose, D-galactose, L-rhamnose, and 9-decenal.

Key words: caminoside A, D-glucose, D-galactose

Enteropathogenic *Escherichia coli* (EPEC) and enterohemorragic *E. coli* 0157:H7 (EHEC) are deadly pathogens toward children and the elderly. Infection of these pathogenic *E. coli* requires the bacterial secreted protein (Esps) and a type III secretory apparatus, which translocates secreted proteins across bacterial membranes, out of EPEC and EHEC, into the host epithelial cells. Remarkably, the type III secretory system, which is essential for the pathogenicity of EPEC and EHEC, is absent in nonpathogenic *E. coli*. Thus, selective inhibition of the type III secretory system might specifically attenuate pathogenic EPEC and EHEC without affecting the commensal *E. coli* flora.[1] Recently, a high throughput assay for inhibitors of the type III secretion of EPEC was developed. Activity-guided isolation of marine invertebrate extracts led to the discovery of caminoside A (**1**) from the marine sponge *Caminus sphaeroconia*.[1] This type III secretion inhibitor (IC_{50} = 20 μM) also displayed reasonably potent in vitro inhibition against the growth of methicillin resistant *Staphylococcus aureus* (MIC = 12 μg/mL) and vancomycin resistant *Enterococcus* (MIC = 12 μg/mL).[1] Equally attractive is the structure of caminoside A (**1**): its middle glucose residue (sugar B) is fully substituted; the terminal 6-deoxy-D-talose (sugar C) and L-quinovose (sugar D) are rare in nature; and the methyl ketone lipid aglycone is without precedent in sponge metabolites. Here we report the first total synthesis of this structurally and biologically interesting marine natural product.

Removal of the benzyl protective groups (Bn on **3**) under neutral hydrogenolysis conditions was planed as the final step to elaborate the target molecule **1**, to avoid probable cleavage or migration of the acetyl (Ac) and butyryl (Bt) moieties under acidic/basic conditions (Scheme 1). In assembling **3**, stereocontrolled construction of the four glycosidic linkages turns out to be the key. Formation of the 1,2-*trans*-β-glucopyranoside linkages (of sugars A and B) would be ensured by glycosylation with donors installed with a neighboring participating group, i.e., trifluoroacetimidate **4** bearing 2-*O*-Ac and a donor elaborated from *p*-methoxyphenyl (MP) glucoside **5** bearing 2-*O*-Azmb [2-(azidomethyl)benzoyl] group.[2] Glycosylation with the L-glucopyranose-type donor **7**, which bears a non-participating 2-*O*-Bn group, would produce the desired thermodynamically favored 1,2-*cis*-α-quinovopyranoside linkage (of sugar D) predominantly.[3] However, construction of the 1,2-*cis*-β-mannopyranoside-type linkage of the 6-deoxy-talose (sugar C) is not a trivial problem.[4] An indirect approach was thus planned: glycosylation with 1-thio-fucopyranoside **6** installed with a directing 2-*O*-Lev (levulinyl) group should afford the β-glycosidic linkage stereoselectively;[5] then selective removal of the 2-*O*-Lev group followed by an inversion of the 2-OH configuration would furnish the desired 6-deoxy-1,2-*cis*-β-talopyranosidic linkage.[4,6]



Scheme 1

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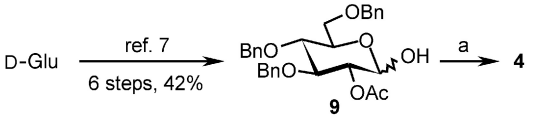
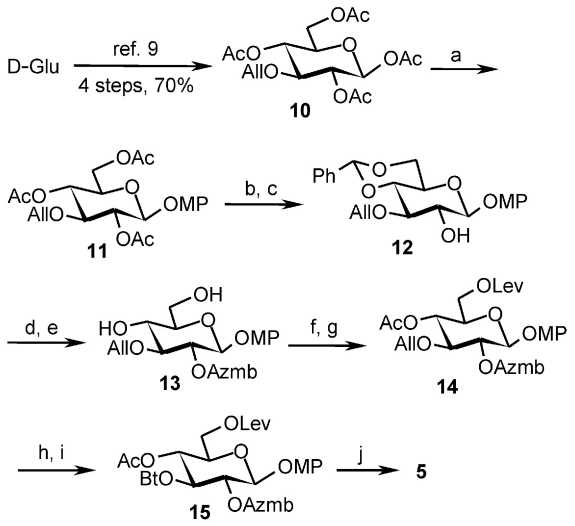
Preparation of the designed monosaccharide building blocks **4**–**7** was straightforward (Scheme 2, Scheme 3, Scheme 4, and Scheme 5). Modifying literature procedures,[7] 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-D-glucose (**9**) was conveniently synthesized in 6 steps and 42% overall yield from D-glucose. Under our reported conditions,[8] compound **9** was converted into the trifluoroacetimidate **4** in high yield (90%, Scheme 2).



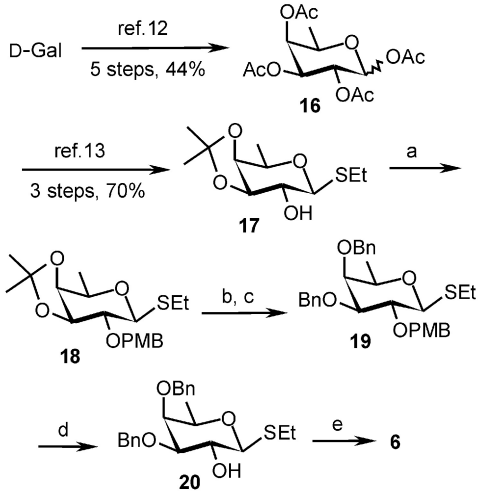
Scheme 2 (a) $CF_3C(Cl)=NPh$, K_2CO_3, acetone, r.t., 90%.

Scheme 3 shows the synthesis of *p*-methoxyphenyl 4-*O*-Ac-2-*O*-Azmb-3-*O*-Bt-β-D-glucopyranoside (**5**). 1,2,4,6-Tetra-*O*-Ac-3-*O*-Allyl-D-glucose (**10**), readily prepared from D-glucose (4 steps, 70%),[9] was converted into the *p*-methoxyphenyl β-glucoside (**11**) under glycosylation conditions (70%).[10] Removal of the 2,4,6-acetates followed by selective protection of the 4,6-OH groups with benzylidene provided **12**. The remaining 2-OH was then protected with Azmb group. Cleavage of the 4,6-benzylidene followed by selective protection of the resulting 6-OH with Lev group and the remaining 4-OH with acetate afforded orthogonally fully protected glucopyranoside **14**. Selective cleavage of the 3-*O*-All group was then achieved under the action of $PdCl_2$ in methanol,[11] albeit in a moderate yield (66%). Substitution of the resulting 3-OH with Bt group led to **15**. Finally, selective removal of the 6-*O*-Lev group using hydrazine acetate furnished **5**, which is a ready acceptor for coupling with the glycosyl donor of sugar C (**6**).



Scheme 3 (a) *p*-MeOPhOH, TMSOTf, CH_2Cl_2, 0 °C, 70%; (b) MeONa, MeOH, r.t.; (c) $PhCH(OMe)_2$, TsOH, DMF, 40 °C, 72% (for 2 steps); (d) AzmbCl, DMAP, CH_2Cl_2, r.t., 85%; (e) TsOH, CH_2Cl_2–MeOH, r.t., 76%; (f) levulinic acid, DCC, DMAP, CH_2Cl_2, 0 °C to r.t.; (g) Ac_2O, pyridine, r.t., 84% (for 2 steps); (h) $PdCl_2$, CH_2Cl_2–MeOH, r.t., 66%; (i) BtCl, pyridine, r.t., 100%; (j) H_2NNH_2–HOAc, CH_2Cl_2–HOMe, r.t., 96%.

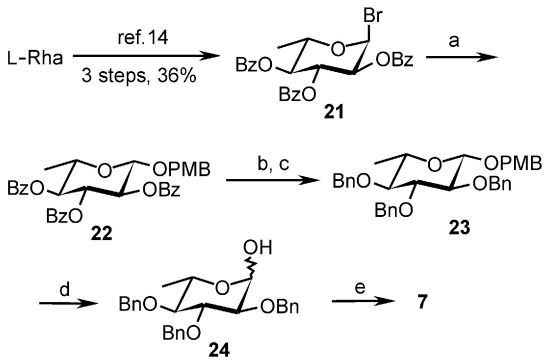
Ethyl 3,4-di-*O*-Bn-2-*O*-Lev-1-thio-β-D-fucopyranoside (**6**) was synthesized from D-galactose (Scheme 4). Known procedures were employed in the preparation of peracetyl D-fucopyranoside (**16**, 5 steps, 44% yield from D-galactose)[12] and the conversion of **16** into thioglycoside **17** (3 steps, 70%).[13] After temporary protection of the 2-OH with *p*-methoxybenzyl (PMB) group, the 3,4-*O*-isopropylidene was taken off, and the resulting 3,4-OH groups were blocked with the persistent Bn groups. Oxidative removal of the 2-*O*-PMB group followed by protection of the resulting -OH with Lev group afforded **6** in excellent yield.



Scheme 4 (a) PMBCl, NaH, DMF, 0 °C to r.t., 80%; (b) 70% HOAc, 40 °C; (c) BnBr, NaH, DMF, 0 °C to r.t., 80% (for 2 steps); (d) DDQ, CH_2Cl_2–H_2O, r.t., 88%; (e) levulinic acid, DCC, DMAP, CH_2Cl_2, r.t., 90%.

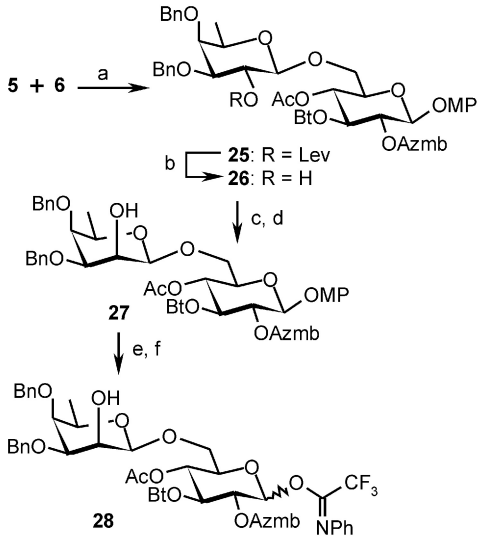
The perbenzyl L-quinovopyranosyl trifluoroacetimidate donor **7** was prepared starting from L-rhamnose (Scheme 5). The 2-OH configuration of L-rhamnose was conversed using a literature method,[14] thus leading to 2,3,4-tri-*O*-benzoyl-L-quinovopyranosyl bromide **21** in 3 steps and 36% yield. Glycosylation of **21** with *p*-methoxybenzoic alcohol under the promotion of AgOTf gave the β-*O*-PMB quinovoside **22** in quantitative yield. After changing the Bz protection into Bn protection, the anomeric PMB group was then removed under the action of trifluoroacetic acid (TFA) to give **24**, which was readily converted into the desired trifluoroacetimidate donor **7**.

The C-B disaccharide fragment was elaborated first. (Scheme 6) Coupling of thioglycoside **6** with 6-OH-glucoside **5** under the promotion of NIS/TMSOTf[15] afforded the expected (1→6)-β-disaccharide **25** in 76% yield (δ = 4.46 ppm, J = 7.8 Hz for the anomeric H-1′). Highly selective removal of the 2-*O*-Lev group, in the presence of Ac, Azmb, and Bt groups, was achieved with hydrazine acetate, giving **26** (90%). The configuration of the equatorial 2-OH was then successfully conversed into the axial one following an oxidation–reduction sequence,[6] providing **27** in a satisfactory 56% yield (for 2 steps, no equatorial isomer was detected in the reduction step). The



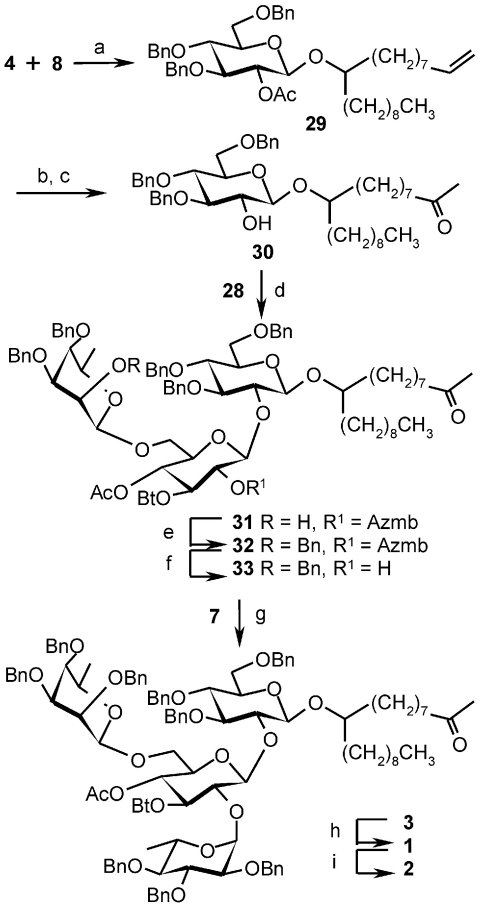
Scheme 5 (a) *p*-MeOBnOH, AgOTf, MS 4 Å, CH_2Cl_2, –10 °C, 100%; (b) MeONa–MeOH, r.t.; (c) BnBr, NaH, DMF, 0 °C to r.t., 86% (for 2 steps); (d) TFA, CH_2Cl_2, r.t., 80%; (e) $CF_3C(Cl)$=NPh, K_2CO_3, acetone, r.t.

^{1}H NMR signal of the anomeric H-1′ then showed up as a singlet (at δ = 4.29 ppm). Oxidative removal of the anomeric MP group gave the hemiacetal, which was converted into the disaccharide trifluoroacetimidate **28** in excellent yield (90% for 2 steps). No addition of the trifluoroacetimidoyl chloride with the sterically hindered axial 2-OH was detected.



Scheme 6 (a) NIS, TMSOTf, MeCN, –60 °C to r.t., 76%; (b) H_2NNH_2–HOAc, CH_2Cl_2–MeOH, r.t., 90%; (c) Dess–Martin periodane, CH_2Cl_2, r.t.; (d) $NaBH_4$, CH_2Cl_2–MeOH, 0 °C, 56% (for 2 steps); (e) CAN, toluene–MeCN–H_2O, r.t.; (f) $CF_3C(Cl)$=NPh, K_2CO_3, acetone, r.t., 90% (for 2 steps).

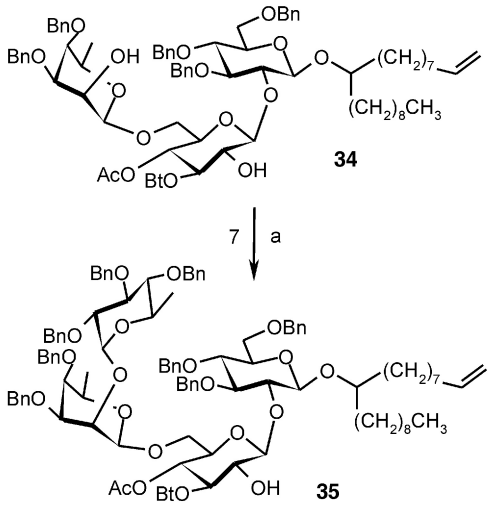
With trifluoroacetimidate donors **4**, **7**, and **28** at hand, we practiced the final assembly of the target molecule **1** (Scheme 7). 1-Nonadecen-10-ol (**8**), prepared by addition of 9-decenal[16] with nonylmagnesium bromide,[17] was glycosylated with glucopyranosyl trifluoroacetimidate (**4**) under the catalysis of TMSOTf[8] to provide the desired β-glucoside **29** in 92% yield (δ = 4.38 ppm, *J* = 7.8 Hz for the anomeric H). The 2-*O*-Ac group was then removed for coupling with disaccharide trifluoroacetimidate **28**. At this stage, the methyl ketone function in the aglycone was elaborated by a Wacker oxidation[18] of the terminal C=C double bond, providing ketone **30** in 80% yield. Glycosylation of the equatorial 2-OH of **30** with trifluoroacetimidate **28** (under conditions similar to those for **4**) afforded the desired Talβ-(1→6)-Gluβ-(1→2)-Gluβ-trisaccharide **31** in 76% yield (δ = 4.28 ppm, *J* = 7.41 Hz for the anomeric H-1″), whereupon no glycosylation took place on the free axial 2-OH of the talose moiety. Blocking this 2-OH with Bn group was found difficult: treatment of **31** with benzyl trichloroacetimidate in the presence of triflic acid provided **32** in moderate yield (61%), with 30% of the starting **32** being recovered.[19] Selective removal of the 2-*O*-Azmb group (on **32**), in the presence of Ac and Bt groups, was achieved under the action of tributylphosphine, affording **33** in 70% yield. The resulting 2-OH was then glycosylated with the newly prepared perbenzyl L-quinovopyranosyl trifluoroacetimidate **7** (under conditions similar to those used for **4** and **28**) to provide the desired tetrasacharide **3** as the major product (53%; δ = 5.23 ppm, singlet for the anomeric H of sugar D). No β-isomer was detected. Hydrogenolysis of the Bn groups in the presence of Pd/C met with no difficulty, providing the



Scheme 7 (a) TMSOTf, MS 4 Å, CH_2Cl_2, 0 °C, 92%; (b) K_2CO_3, THF–MeOH, r.t., 80%; (c) $PdCl_2$, $Cu(OAc)_2$, O_2 (1 atm), DMA–H_2O, 50 °C, 80%; (d) TMSOTf, MS 4 Å, CH_2Cl_2, 0 °C, 76%; (e) $BnOC(=NH)CCl_3$, TfOH, MS 4 Å, CH_2Cl_2–cyclohexane, r.t., 61%; (f) Bu_3P, THF–H_2O, 20 °C, 70%; (g) TMSOTf, MS 4 Å, Et_2O, 0 °C to r.t., 53%; (h) H_2 (1 atm), Pd/C, MeOH, r.t.; (i) Ac_2O, pyridine, 40 °C, 62% (for 2 steps).

target glycolipid **1**, which was transformed into peracetate **2** directly for comparison of the analytical data. The ^{1}H NMR and ^{13}C NMR spectra of **2** are identical to those from the natural compound.[1]

In a failed attempt to assemble the tetrasaccharide target, we found that glycosylation of the trisaccharide diol **34** with perbenzyl L-quinovopyranosyl trifluoroacetimidate **7** (under similar conditions as those for coupling of **7** with **33**) provided the tetrasaccharide **35** as the major product (72%, Scheme 8), whereupon the glycosylation took place unexpectedly on the axial 2-OH of the talose moiety (vs the equatorial 2-OH of the glucose moiety). While the glycosylation with glucopyranosyl trifluoroacetimidate **28** selected the equatorial 2-OH of the glucose moiety (on **30**) rather than the axial 2-OH of the talose moiety (of **28**, Scheme 7). These results reflect the importance of 'match' of the donor and acceptor for the selective glycosidic coupling.[20]



Scheme 8 (a) TMSOTf, MS 4 Å, CH_2Cl_2, 0 °C, 72%.

It should also be reported that a pair of the diastereomeric products were observed on TLC since the attachment of the sugar to the racemic alcohol aglycone (for compounds **29**–**33** and **2**, **3**, respectively). And each pair of the isolated diastereoisomers has shown little difference on their ^{1}H NMR and ^{13}C NMR signals.[21]

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References

(1) Linington, R. G.; Robertson, M.; Gauthier, A.; Finlay, B. B.; van Soest, R.; Andersen, R. J. *Org. Lett.* **2002**, *4*, 4089.
(2) (a) Wada, T.; Ohkabso, A.; Mochizuki, A.; Sekine, M. *Tetrahedron Lett.* **2001**, *42*, 1069. (b) Love, K. R.; Andradego, R. B.; Seeberger, P. H. *J. Org. Chem.* **2001**, *66*, 8165.
(3) (a) Schmidt, R. R.; Toepfer, A. *Tetrahedron Lett.* **1991**, *32*, 3353. (b) Han, X.; Schmidt, R. R. *Liebigs Ann. Chem.* **1992**, 817.
(4) (a) Fairbanks, A. J. *Synlett* **2003**, 1945. (b) Crich, D.; Sun, S. *J. Am. Chem. Soc.* **1997**, *119*, 11217.
(5) Zhu, T.; Boons, G.-J. *Org. Lett.* **2001**, *3*, 4201.
(6) (a) Liu, K. K.-C.; Danishefsky, S. J. *J. Org. Chem.* **1994**, *59*, 1892. (b) Lichtenthaler, F. W.; Schneider-Adams, T. *J. Org. Chem.* **1994**, *59*, 6728.
(7) (a) Banoub, J.; Boullanger, P.; Potier, M.; Descotes, G. *Tetrahedron Lett.* **1986**, *27*, 4145. (b) Trumtel, M.; Tavecchia, P.; Veyrieres, A.; Sinaÿ, P. *Carbohydr. Res.* **1989**, *191*, 29. (c) Wotovic, A.; Jacquinet, J.-C.; Sinaÿ, P. *Carbohydr. Res.* **1990**, *205*, 235.
(8) (a) Yu, B.; Tao, H. *Tetrahedron Lett.* **2001**, *42*, 2405. (b) Yu, B.; Tao, H. *J. Org. Chem.* **2002**, *67*, 9099. (c) Adinolfi, M.; Iadonisi, A.; Ravida, A.; Schiattarella, M. *Synlett* **2004**, 275.
(9) Takeo, K.; Nakaji, T.; Shinmitsu, K. *Carbohydr. Res.* **1984**, *133*, 275.
(10) Lee, Y. S.; Rho, E. S.; Min, Y. K.; Kim, B. T.; Kim, K. H. *J. Carbohydr. Chem.* **2001**, *20*, 503.
(11) Ogawa, T.; Yamamoto, H. *Agric. Biol. Chem.* **1985**, *49*, 475.
(12) Lerner, L. M. *Carbohydr. Res.* **1993**, *241*, 291.
(13) (a) Zegelaar-Jaarsveld, K.; van der Plas, S. C.; van der Marel, G. A.; van Boom, J. H. *J. Carbohydr. Chem.* **1996**, *15*, 591. (b) Zhang, Z.; Magnusson, G. *Carbohydr. Res.* **1994**, *262*, 79.
(14) (a) Bilik, V.; Voelter, W.; Bayer, E. *Angew. Chem., Int. Ed. Engl.* **1971**, *10*, 909. (b) Lichtenthaler, F. W.; Metz, T. *Eur. J. Org. Chem.* **2003**, 3081.
(15) (a) Konradsson, P.; Udodong, U. E.; Fraser-Reid, B. *Tetrahedron Lett.* **1990**, *31*, 4313. (b) Hunt, D. K.; Seeberger, P. H. *Org. Lett.* **2002**, *4*, 2751.
(16) Banwell, M. G.; McRae, K. J. *Org. Lett.* **2000**, *2*, 3583.
(17) Holm, T. *J. Chem. Soc., Perkin Trans. 2* **1981**, 464.
(18) (a) Smith, A. B.; Cho, Y. S.; Friestad, G. K. *Tetrahedron Lett.* **1998**, *39*, 8765. (b) Nicolaou, K. C.; Gray, D.; Tea, J. *Angew. Chem. Int. Ed.* **2001**, 3675.
(19) Wessel, H.-P.; Ivesen, T.; Bundle, D. R. *J. Chem. Soc., Perkin Trans. 1* **1985**, 2247.
(20) For a recent account, see: Fraser-Raid, B.; López, J. C.; Gómez, A. M.; Uriel, C. *Eur. J. Org. Chem.* **2004**, 1387.
(21) The stereochemistry of the aglycone was determined recently, see: MacMillan, J. B.; Linington, R. G.; Andersen, R. J.; Molinski, T. F. *Angew. Chem. Int. Ed.* **2004**, *43*, 5946.