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Total Synthesis and Absolute Stereochemistry of Integric Acid

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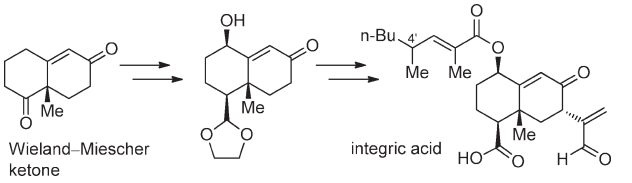
Received August 26, 2009



An efficient total synthesis of integric acid is described starting from the Wieland−Miescher ketone. Key steps involve a one-step orthogonal deprotection/protection strategy of a thioacetal/aldehyde and the selective oxidative cleavage of a prenyl group in the presence of two other unsaturated moieties. The synthesis of both C4′ diastereoisomers of integric acid delivered unambiguous evidence for (*S*)-stereochemistry at the C4′ position.

In 1999 integric acid (**1**) was isolated from the fermentation broth of *Xylaria* sp.[1] It inhibits 3′-end processing, strand transfer, and disintegration reactions catalyzed by HIV-1 integrase with IC_{50} values of 3−10 μM. The interest for HIV-1 integrase inhibitors has steadily increased over the past few years and efforts in this field have recently been rewarded with FDA approval of the first HIV-1 integrase inhibitor Raltegravir (2007).[2,3] The eremophilane sesquiterpenoid structure of **1** is also encountered in natural products such as the NPY receptor inhibitors xylarenals A (**4a**) and B (**4b**),[4] the cytotoxic 07H239-A (**3**),[5] and eremoxylarins A (**2b**) and B (**2a**) showing antimicrobial activity (Figure 1).[6] Except for

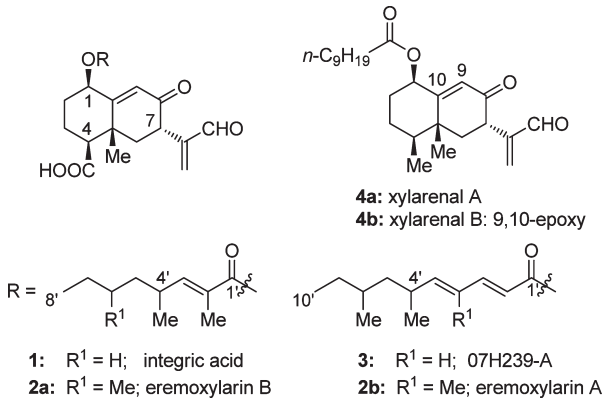


FIGURE 1. Structures of integric acid (**1**), eremoxylarins A (**2b**) and B (**2a**), 07H239-A (**3**), and xylarenals A (**4a**) and B (**4b**).

xylarenal A,[7] no total syntheses have been developed to access this valuable compound class. In addition, the absolute configuration of integric acid was determined by the synthesis of the corresponding PGME amides. However, the stereochemistry of the chiral center at C4′ in the side chain could not be assigned. Basic hydrolysis of the ester afforded (*E*)-2,4-dimethyloct-2-enoic acid (**8**) with an optical rotation of $[\alpha]_D^{22}$ +9.5 (*c* 0.42, MeOH), which is only indicative of (*S*)-stereochemistry at C4′.[8]

Preliminary structure−activity relationship (SAR) studies through chemical modification of the natural product itself have been reported, but these efforts were severely hampered by the instability of the α,β-unsaturated aldehyde.[9] Clearly, a total synthesis of integric acid, which allows for the incorporation of the unsaturated aldehyde moiety at a late stage, would give access to more relevant derivatives of **1** for biological studies. Herein we describe a total synthesis of integric acid and the determination of the absolute stereochemistry at C4′.

Inspired by the synthesis of xylarenal A by Bonjoch and co-workers,[7] we envisioned to synthesize integric acid (**1**) as shown in Scheme 1. Cleavage of the ester bond, protection of the carboxylic acid as the dioxolane, and masking of the α,β-unsaturated aldehyde moiety as an allyl group reveal the advanced intermediates **6** and **8**. To unambiguously assign the side chain stereochemistry, both C4′ diastereoisomers of **1** had to be synthesized and consequently both enantiomers of **8** starting from hexanal. Intermediate **6** can be traced back to the Wieland−Miescher ketone (**7**) via oxidation of the corresponding dienyl acetate, a Wittig homologation, and allylation as the key steps.

The enantiomers of **8** were prepared in five steps from hexanal (Scheme 2).[10] The RAMP-hydrazone of hexanal was diastereoselectively alkylated with MeI yielding **9**, which was subsequently converted to the aldehyde by oxidative

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(2) (a) Flexner, C. *Nat. Rev. Drug Discovery* **2007**, *6*, 959–966. (b) Craigie, R. *J. Biol. Chem.* **2001**, *276*, 23213–23216.

(3) Deeks, S. G.; Kar, S.; Gubernick, S. I.; Kirkpatrick, P. *Nat. Rev. Drug Discovery* **2008**, *7*, 117–118.

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(7) Diaz, S.; Gonzalez, A.; Bradshaw, B.; Cuesta, J.; Bonjoch, J. *J. Org. Chem.* **2005**, *70*, 3749–3752.

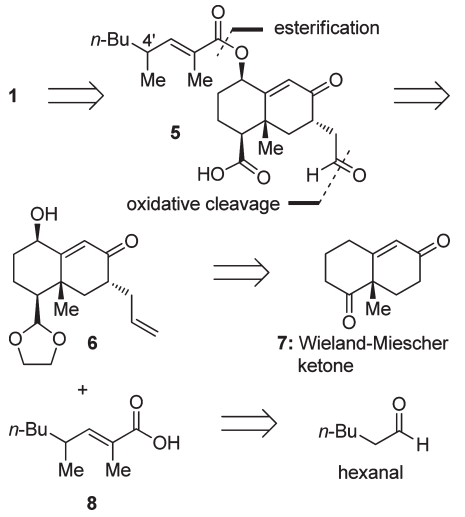
(8) (*S*)-(**8**), $[\alpha]_D$ +37.6 (*c* 1.0, $CHCl_3$); see: Alcaraz, L.; Macdonald, G.; Ragot, J.; Lewis, N. J.; Taylor, R. J. K. *Tetrahedron* **1999**, *55*, 3707–3716.

(9) Singh, S. B.; Felock, P.; Hazuda, D. J. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 235–238.

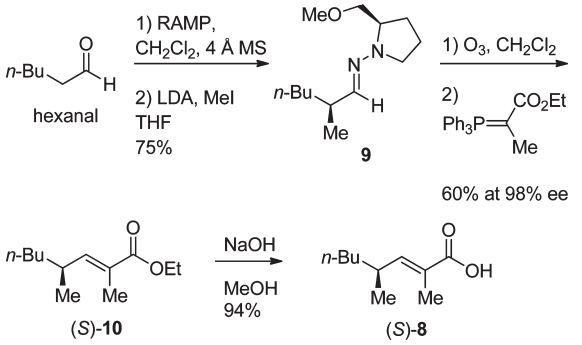
(10) For an enantioselective synthesis of **8** with Evans' chiral oxazolidinone methodology, see ref 8.

Published on Web 10/27/2009 DOI: 10.1021/jo901845r

SCHEME 1. Retrosynthetic Approach to Integric Acid (1)



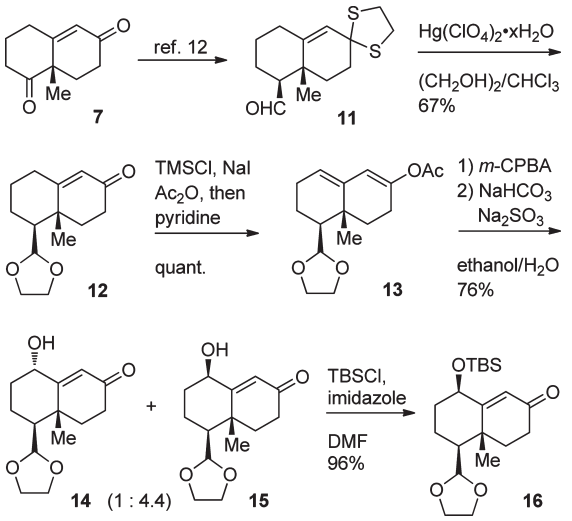
SCHEME 2. Synthesis of (*S*,*E*)-2,4-Dimethyloct-2-enoic Acid ((*S*)-8)



cleavage with ozone.[11] The crude product was immediately reacted in a Wittig reaction to provide ethyl ester **10** (ee 98%). Basic hydrolysis with NaOH then afforded (*S*)-**8** in 42% yield over five steps. In the same manner, (*R*)-**8** was synthesized starting from the corresponding SAMP-hydrazone in similar yields and equal selectivity.

The synthesis of **6** commenced with the known transformation of the Wieland−Miescher ketone (**7**) into aldehyde **11** (Scheme 3).[12] In the next step, deprotection of the dithioacetal with $Hg(ClO_4)_2$ was initially carried out in a mixture of methanol and chloroform. It soon turned out that, although the deprotection proceeded smoothly, the aldehyde partially formed the corresponding dimethyl acetal. Fortunately, we could use this side reaction to our advantage by replacing methanol for ethylene glycol. In this way, the dithioacetal was deprotected with simultaneous dioxolane protection of the aldehyde to give **12** in a single step in 67% yield. Next, formation of dienyl acetate **13** was achieved via reaction of **12** with TMSCl and NaI in acetic anhydride, which appeared superior to other established methods such as treatment with pyridine or PTSA in acetic

SCHEME 3. Synthesis of TBS Ether 16



anhydride at high temperatures.[13] Addition of pyridine at the end of the reaction was essential to prevent dioxolane hydrolysis upon workup. Treatment of **13** with *m*-CPBA then afforded the alcohols **14** and **15** in 76% yield in a 1:4.4 ratio.[14] In the next step, alcohol **15** was protected as the TBS-ether to arrive at intermediate **16** in 96% yield.

One of the more challenging steps in the synthesis was the selective oxidative cleavage of the allyl group in the presence of two other unsaturated bonds (Scheme 1). Unlike xylarenal A, integric acid (**1**) incorporates an exocyclic unsaturated ester that might well interfere with the oxidative cleavage of the allyl group. Although this problem could be circumvented by introducing the side chain after the oxidation, this would require additional steps after installing the labile unsaturated aldehyde moiety, which we wished to avoid. To investigate the selectivity of the oxidation we decided to probe the feasibility of the next part of the synthesis with racemic **16** and use (*E*)-2-methyloct-2-enoic acid (**21**) as a substitute for the side chain to avoid the formation of diastereoisomers (Scheme 4). Thus, TBS ether **16** was diastereoselectively alkylated with allyl bromide in 82% yield and subsequently deprotected with TBAF. Coupling of (*E*)-2-methyloct-2-enoic acid with DIC then gave ester **18** in 51% over two steps. Acidic hydrolysis followed by a Pinnick oxidation delivered key intermediate **19**, which was set to study the oxidative cleavage. Unfortunately, reaction of **19** with ozone or $OsO_4/NaIO_4$ gave a complex mixture and delivered no significant amounts of the desired product. Monitoring of the reaction by mass analysis showed the formation of multiple oxidation products in an early stage of the reaction. Challenged by the lack of selectivity we explored the possibility of using a more nucleophilic alkene to enhance the selectivity. In particular, the known selective oxidative cleavage of the trisubstituted over the monosubstituted double bond in (*S*)-citronellene encouraged us to replace the allyl for the prenyl group.[15] Thus, enantiopure **16** was

(11) For a review on the RAMP/SAMP methodology, see e.g.: Job, A.; Janeck, C. F.; Bettray, W.; Peters, R.; Enders, D. *Tetrahedron* **2002**, *58*, 2253–2329.

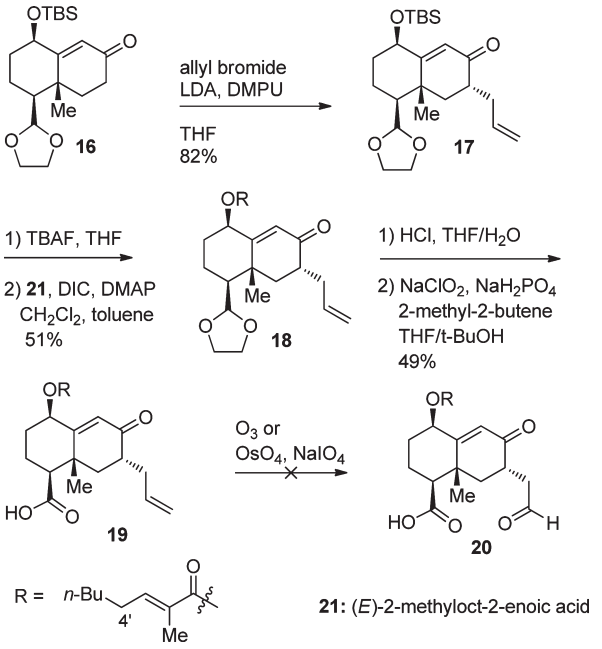
(12) Paquette, L. A.; Wang, T.-Z.; Philippo, C. M. G.; Wang, S. *J. Am. Chem. Soc.* **1994**, *116*, 3367–3374.

(13) Chowdhury, P. K.; Sharma, R. P.; Barua, J. N. *Tetrahedron Lett.* **1983**, *24*, 3383–3384.

(14) This selectivity is in accordance with oxidations of similar dienyl acetates: (a) Suryawanshi, S. N.; Fuchs, P. L. *J. Org. Chem.* **1986**, *51*, 902–921. (b) Kirk, D. N.; Wiles, J. M. *J. Chem. Soc., Chem. Commun.* **1970**, 1015–1016. (c) Kirk, D. N.; Wiles, J. M. *J. Chem. Soc., Chem. Commun.* **1970**, 518.

(15) Barrett, A. G. M.; Carr, R. A. E.; Attwood, S. V.; Richardson, G.; Walshe, N. D. A. *J. Org. Chem.* **1986**, *51*, 4840–4856.

SCHEME 4. Synthesis of a Model System



diastereoselectively alkylated with prenyl bromide to yield **22** in 83% yield (Scheme 5). After deprotection with TBAF, both enantiomers of (*E*)-2,4-dimethyloct-2-enoic acid ((*R*)- and (*S*)-**8**) were coupled with DIC and DMAP to give diastereoisomers **23** and **24** in good yields. The carboxylic acid was then generated by hydrolysis of the dioxolane followed by a Pinnick oxidation yielding **25** and **26** in satisfactory overall yields.

Again we attempted the selective oxidative cleavage and to our delight we found that ozonolysis in the presence of 4 equiv of pyridine in a 1:1 mixture of $MeOH/CH_2Cl_2$, followed by reduction with Ph_3P, gave **5a** and **5b** in acceptable yields of 72% and 50% based on recovered starting material, respectively. In the final step, the unsaturated double bond was introduced by reaction with Eschenmoser's salt in the presence of Et_3N affording both C4′ diastereoisomers **1a** and **1b** of integric acid. On the basis of the optical rotations of **1a** and **1b**,[16] and in particular by comparison of the ^1H and ^{13}C NMR spectral data with an authentic sample of **1**, the stereochemistry at C4′ could be unambiguously assigned the (*S*)-configuration.[17]

In conclusion, we have developed an efficient total synthesis of integric acid and its C4′ diastereoisomer, which has led to the unambiguous assignment of (*S*)-stereochemistry at the C4′ position. The newly developed pathway in principle also allows for the synthesis of related compounds by variation of the ester substituent, such as the eremoxylarins, 07H239-A, and other derivatives.

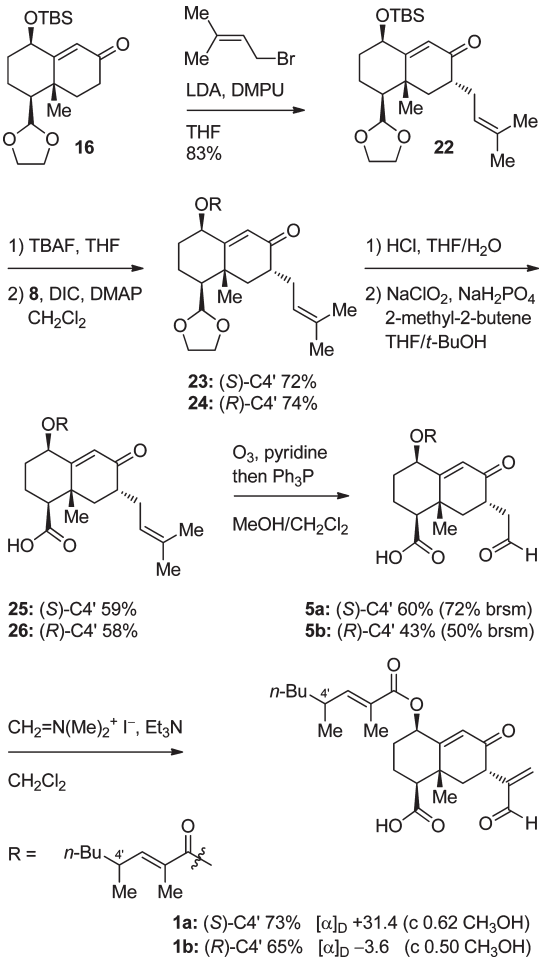
Experimental Section

Dioxolane 12. To a stirred solution of mercury(II) perchlorate hydrate (34.78 g, 87.1 mmol) in ethylene glycol (200 mL) was added a solution of **11** (9.35 g, 34.8 mmol) in $CHCl_3$ (115 mL).

(16) Optical rotation: **1a**, $[\alpha]_D^{20}$ +31.4 (*c* 0.62, MeOH); **1b**, $[\alpha]_D^{20}$ −3.6 (*c* 0.50, MeOH); **1**, $[\alpha]^{25}{}_D$ + 38.3 (*c* 0.63, MeOH) (ref 1).

(17) The ^1H NMR (400 MHz, $CDCl_3$) chemical shift for the proton at C1 should read as δ 5.52 instead of the erroneously reported δ 5.25 as determined by comparison with an authentic sample (ref 1)

SCHEME 5. Synthesis of Integric Acid



After 45 min diethyl ether (100 mL) was added and the reaction mixture was stirred vigorously. The organic layer was subsequently collected by decantation over a plug of cotton wool. This process was repeated until all product was collected according to TLC (approximately ten times). The combined Et_2O layers were washed with water (500 mL) and brine (100 mL) and dried with $MgSO_4$. After filtration, the solvent was evaporated in vacuo and the crude product was purified by flash column chromatography (silica gel, EtOAc/heptane 1:10 → 1:2) to afford **12** (5.51 g, 67%) as a white solid. $[\alpha]_D^{20}$ +194.0 (*c* 1.10, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$) δ 5.72−5.73 (m, 1H), 4.94 (d, J = 2.6 Hz, 1H), 3.89−3.96 (m, 2H), 3.78−3.87 (m, 2H), 2.48 (ddd, 1H, J = 5.0, 14.2, 17.0 Hz, 1H), 2.31−2.39 (m, 2H), 2.20−2.27 (m, 2H), 1.85−1.96 (m, 2H), 1.77−1.83 (m, 1H), 1.55−1.68 (m, 2H), 1.33−1.44 (m, 1H), 1.26 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 199.2, 170.3, 124.3, 103.4, 65.2, 64.5, 51.4, 38.1, 36.0, 33.7, 33.5, 25.7, 21.2, 18.3; IR (film) ν 2943, 2879, 1668, 1614, 1126, 1039 cm^{-1}; HRMS (ESI) calcd for $C_{14}H_{21}O_3$ $[M + H]^+$ 237.1491, found 237.1474.

TBS Ether 16. To a stirred solution of β-alcohol **15** (172 mg, 0.68 mmol) and imidazole (232 mg, 3.41 mmol) in DMF (1 mL) was added *tert*-butyldimethylsilyl chloride (232 mg, 3.41 mmol). The solution was stirred at 40 °C for 7h, and subsequently at rt for 16 h. Next, the reaction mixture was quenched with H_2O and extracted with ether (3 × 5 mL). The combined organic layers were washed with saturated aqueous NH_4Cl (5 mL) and brine (5 mL) and dried with Na_2SO_4. After evaporation of the solvent in vacuo, the crude product was purified with flash column chromatography (silica gel, EtOAc/heptane 1:40 → 1:10) to give **16** (240 mg, 96%)

as a colorless oil. $[\alpha]_D^{20}$ +58.7 (*c* 1.00, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.74 (s, 1H), 4.93 (d, J = 3.6, 1H), 4.23 (t, J = 2.5 Hz, 1H), 3.92−3.98 (m, 2H), 3.80−3.88 (m, 2H), 2.56 (ddd, J = 4.9, 14.9, 17.1 Hz, 1H), 2.33−2.39 (m, 1H), 2.29 (ddd, J = 3.0, 4.8, 13.4 Hz, 1H), 2.04 (dq, J = 3.3, 13.5 Hz, 1H), 1.90 (dq, J = 2.9, 13.5 Hz, 1H), 1.85 (dt, J = 4.1, 14.3 Hz, 1H), 1.65 (dq, J = 3.2, 13.5 Hz, 1H), 1.55−1.60 (m, 2H), 1.41 (s, 3H), 0.87 (s, 9H), 0.06 (s, 3H), 0.00 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 200.4, 167.6, 126.0, 103.8, 73.6, 65.0, 64.5, 51.6, 37.9, 37.8, 34.0, 33.7, 25.7, 20.1, 18.0, 16.1, −4.7, −5.0; IR (film) ν 2937, 2881, 1679, 1045 cm^{-1}; HRMS (ESI) calcd for $C_{20}H_{35}O_4Si$ $[M + H]^+$ 367.2305, found 367.2330.

Integric Acid (1a). Eschenmoser's salt (33.1 mg, 0.179 mmol) was added to a solution of **5a** (15.0 mg, 0.036 mmol) and triethylamine (181 mg, 1.79 mmol) in CH_2Cl_2 (3 mL) at rt. After 22 h, the reaction was quenched with water (15 mL) and acidified to pH 1 with 2 M HCl. The reaction mixture was extracted with CH_2Cl_2 (3 × 15 mL) and the combined organic layers were washed with brine (15 mL) and dried over Na_2SO_4, then the solvent was evaporated. The crude product was purified by flash column chromatography (silica gel, EtOAc/heptane 1:10 + 0.5% AcOH → 1:3 + 0.5% AcOH) to give **1a** (11.3 mg, 73%) as a white powder. $[\alpha]_D^{20}$+31.4 (*c* 0.62, MeOH); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.53 (s, 1H), 6.55 (dd, J = 1.4, 10.1 Hz, 1H), 6.36 (s, 1H), 6.24 (s, 1H), 6.09 (s, 1H), 5.52 (t, J = 2.7 Hz, 1H), 3.73 (dd, J = 4.3, 14.4 Hz, 1H), 2.47−2.54 (m, 1H), 2.44 (dd, J = 3.0, 12.8 Hz, 1H), 2.24−2.35 (m, 1H), 2.27 (t, J = 13.7 Hz, 1H), 2.14−2.18 (m, 1H), 2.13 (dd, J = 4.1, 13.1 Hz, 1H), 1.82−1.86 (m, 1H), 1.84 (d, J = 1.3 Hz, 3H), 1.73 (tt, J = 3.5, 14.1 Hz, 1H), 1.51, (s, 3H), 1.34−1.46 (m, 1H), 1.13−1.34 (m, 5H), 1.00 (d, J = 6.7 Hz, 3H), 0.87 (t, J = 7.1 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 196.9, 193.2, 177.8, 166.8, 159.0, 149.7, 147.7, 136.4, 129.6, 125.8, 72.6, 53.4, 43.2, 43.0, 38.2, 36.5, 33.3, 29.8, 29.6, 22.7, 20.1, 19.9, 19.5, 14.0, 12.6; IR (neat) 2700−3300, 2954, 2926, 2867, 1719, 1687, 1642 cm^{-1}; HRMS (ESI) calcd for $C_{25}H_{34}O_6Na$ $[M + Na]^+$ 453.2253, found 453.2250.

Acknowledgment. We thank Merck & Co., Inc. for generously providing an authentic sample of integric acid. This research has been financially supported (in part) by the Council for Chemical Sciences of The Netherlands Organization for Scientific Research (NWO-CW).

Supporting Information Available: Experimental procedures, spectral data, and copies of ^{1}H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.