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The synthesis of single enantiomers of *trans*-alkene-containing mycolic acids

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ABSTRACT

We report routes to single enantiomers of hydroxy and ketomycolic acids containing an α-methyl-*trans*-alkene unit.

Mycolic acids (MAs) are characteristic components of the cell envelopes of mycobacteria, often covalently bound to an arabinogalactan polysaccharide.[1] They are also seen as sugar esters such as trehalose dimycolate (TDM—one of the most potent signalling agents in the immune system) that are not bound to the cell wall and there is increasing evidence for the presence of free mycolic acids.[2] MAs can be divided into two elements, the hydroxy-acid, for which d is generally 21 or 23, and the meromycolate (Fig. 1). The latter is a long chain which usually contains two groups X and Y. In the most common classes of MA, the two groups are *cis*-cyclopropanes (α-MA), or group X is an α-methyl-β-methoxy and α-methyl-β-keto group (methoxy- and keto-MA, respectively). MAs are generally present in mycobacteria as complex mixtures containing several different chain lengths of each class, and the detailed composition is dependent on the mycobacterium concerned.[3,4] The presence and proportion of individual classes of MA is known to be important for the virulence of diseases such as tuberculosis.[5,6]

In addition, there are a range of generally less abundant MAs which contain other combinations of X and Y; these include molecules containing a β-methyl-*trans*-alkene unit (Type 2 mycolic acids)[3,4] (Fig. 1). The methyl substituent in group Y of this unit is proximal to the hydroxy-acid group of the MA. The methyl group in the X position of keto- and methoxy-MAs and at the Y-position in an α-methyl-*trans*-cyclopropane-containing MA is distal from it.[3,4]

Type 2 ketomycolic acids **1** with a variety of combinations of chain lengths a, b and c have been reported (Table 1).[3,4,7–9]

An analogue, a derivative of a hydroxy-MA with a proximal α-methyl-*trans*-alkene, has also been reported.[10] The fast growing *Mycobacterium smegmatis* synthesizes MAs containing epoxy-groups as well as keto- and hydroxy-MAs. The keto-compounds contain about 33% of a *trans*-alkene. The same percentage is seen in the hydroxy-mycolate.[7] Keto- and hydroxy-MA have been isolated from *Mycobacterium tuberculosis* and *Mycobacterium bovis* BCG; although these predominantly contain a *cis*-cyclopropane at the proximal position, about 15% and 14%, respectively, have an alkene at that position, predominantly an α-methyl-*trans*-alkene.[9] The biosynthesis apparently involves conversion of a *cis*-alkene into an α-methyl-*trans*-alkene caused by MmaS1 and SAM. This

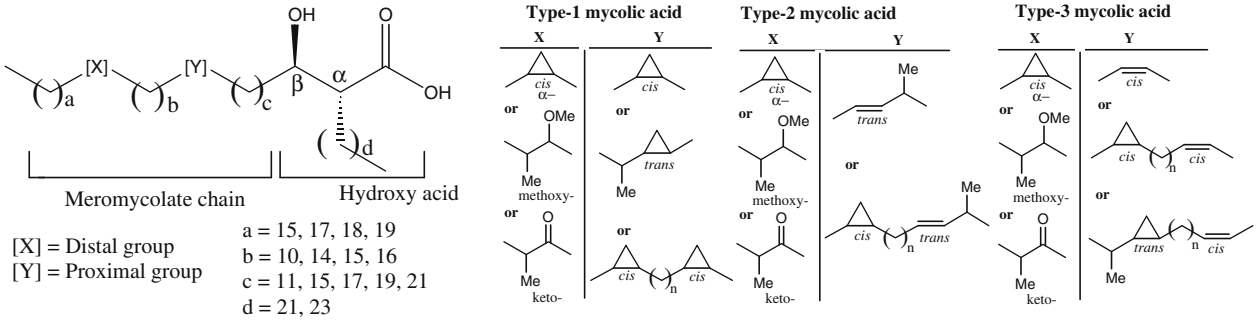

Figure 1. Common types of mycobacterial mycolic acid.

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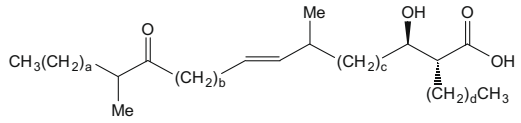
Table 1
Typical chain lengths of major β-methyl-*trans*-alkene-containing mycobacterial ketomycolic acids (**1**)

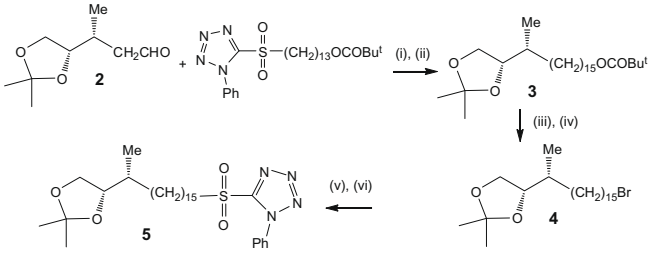


Species	a	b	c	d	Ref.
Mycobacterium tuberculosis, *Mycobacterium bovis*, *M. bovis BCG*, *Mycobacterium microti*	17	19	15	23	3
M. tuberculosis, *M. bovis*, *M. bovis BCG*, *M. microti*	17	19	13	23	3
M. tuberculosis canetti	17	17	17	23	3
Mycobacterium avium, *complex* (MAC)	17	17	17	21	3
Mycobacterium marinum	17	15	17	21	3
Mycobacterium scrofulaceum	15	17	17	21	3
Mycobacterium aurum	15	13	19	19	8
M. aurum	15	13	17	19	8

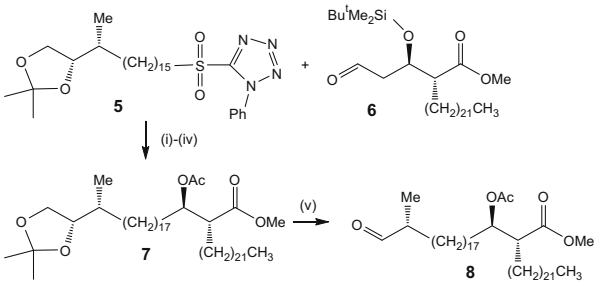
is then cyclopropanated by the CmaA2 gene, again with SAM, to give the α-methyl-*trans*-cyclopropane.[11–15] Inactivation of CmaA2 causes the accumulation of unsaturated derivatives in both methoxy- and keto-MA and the lack of *trans*-cyclopropanes.[16] It should be noted, however, that there are alternative models for the creation of these functional groups.[17] It is also interesting that the labelling pattern of an α-methyl-*trans*-cyclopropane-containing keto-MA of *M. tuberculosis* derived from [1-^{13}C]acetic acid places a labelled carbon at the cyclopropane carbon nearest to the methyl group, while in an unsaturated α2-MA from *M. smegmatis*, the label in the proximal α-methyl-*trans*-alkene is on the carbon adjacent to the methyl group – consistent with biochemical alkylation at opposite ends of a *cis*-alkene.[18] Another type of mycolic acid isolated from *M. smegmatis* and *Mycobacterium aurum*,[8] *Mycobacterium chelonei*,[19] and *Mycobacterium fortuitum*,[20] contains an α-methyl-*trans*-alkene at the proximal position and a *cis*-alkene at the distal position.[21–23] Examples with a *cis*-cyclopropane or an α-methyl-*trans*-oxirane at the distal position have also been reported.[3] In the former case the specific rotation of its methyl ester has been reported to be +1.4 ($CHCl_3$),[24] while that of the acetoxy methyl ester is reported as +3 ($CHCl_3$), and that of a deacetoxy derivative of a mycolate containing only an α-methyl-*trans*-alkene chiral centre corresponds to a molecular rotation (M_D) of −19.5.[24] In addition, the specific rotation of a wax ester containing the α-methyl-*trans*-alkene unit has been reported to be +4.3 ($CHCl_3$).[8,25,26] This allows the contribution to the molecular rotation from the α-methyl-*trans*-alkene unit to be calculated, as the only other chiral centres in these molecules are at the hydroxy-acid position, the contribution of which to the molecular rotation is known (+40), leading to a value of −25 for the α-methyl-*trans*-alkene (for 30% of methyl branched molecules); this in turn suggests that this subunit has an (*R*)-stereochemistry based on model compounds.[9]

As part of a study to determine the biological significance of individual MAs and particularly of their stereochemistry, we have reported routes to α-, methoxy- and keto-MAs as single stereoisomers, as well as to related wax esters.[27–31] We now report the first synthesis of type-2 hydroxy-MA **16** (R = R′ = H) and **18** and the related keto-MA (**21**).

In order to fix the (*R*)-stereochemistry of the β-methyl-*trans*-alkene fragment, aldehyde **2**,[32–34] was chain-extended using a modified Julia–Kocienski reaction,[35] followed by hydrogenation of the derived alkenes to give **3**. The pivalate group was removed and the primary alcohol was converted into sulfone **5** via bromide **4** (Scheme 1).



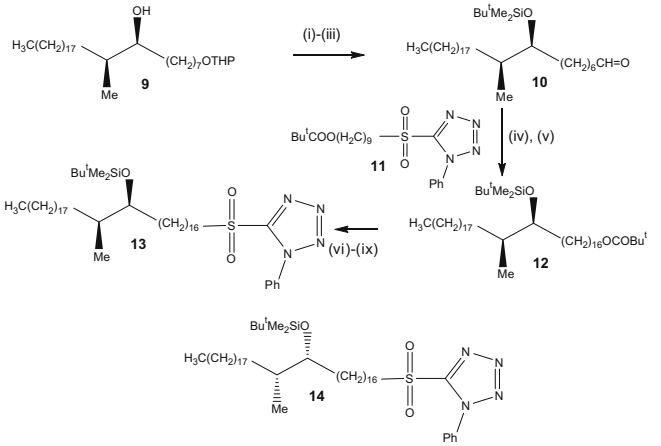
Scheme 1. Reagents: (i) LiHMDS, THF (86%); (ii) H_2, Pd/C, EtOAc/EtOH (96%); (iii) KOH, THF, MeOH, H_2O (97%); (iv) *N*-bromosuccinimide, PPh_3, $NaHCO_3$, CH_2Cl_2 (92%); (v) 1-phenyl-1*H*-tetrazole-5-thiol, K_2CO_3, acetone (95%); (vi) H_2O_2, $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, THF/IMS (81%).



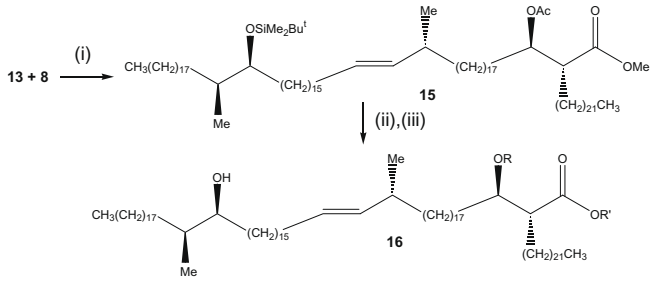
Scheme 2. Reagents: (i) LHMDS (86%); (ii) Pd/C, EtOAc (96%); (iii) HF·pyridine (83%); (iv) Ac_2O, pyridine (94%); (v) periodic acid (80%).

The hydroxy-acid unit was introduced in the form of **6**, prepared as described earlier from l-aspartic acid.[36] A further modified Julia reaction between the aldehyde **6** and sulfone **5** gave a mixture of alkenes which was hydrogenated. The silyl ether was changed to acetyl to avoid the presence of two identical protecting groups at a later stage to yield **7**, and the acetal group was converted into aldehyde **8** with periodic acid (Scheme 2).

The alcohol **9**,[28] was protected as a TBDMS ether, the THP-group was removed and the resulting alcohol was oxidized to the aldehyde **10** (Scheme 3). A Julia–Kocienski reaction between **10** and the sulfone **11**[37] gave an *E*/*Z*-alkene mixture which was hydrogenated to give **12**. This was converted into the sulfone **13**. In the same way the enantiomer of **9** was converted into the enantiomer **14**.



Scheme 3. Reagents: (i) TBDMSCl, imidazole, DMF (91%); (ii) PPTS, THF/ MeOH (80%); (iii) PCC, CH_2Cl_2 (94%); (iv) LiHMDS, **11**, THF (76%); (v) H_2, Pd/C, THF/IMS (70%); (vi) $LiAlH_4$·THF (90%); (vii) *N*-bromosuccinimide, PPh_3, CH_2Cl_2 (81%); (viii) 1-phenyl-1*H*-tetrazole-5-thiol, K_2CO_3, acetone (99%); (ix) H_2O_2, $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, THF/IMS (85%).



Scheme 4. Reagents: (i) KHMDS, 1,2-dimethoxyethane (44%); (ii) HF·pyridine, THF [**16** (R = Ac, R′ = Me), 91%]; (iii) LiOH, MeOH, THF, H_2O [**16** (R = H, R′ = H), 65%)].

The modified Julia–Kocienski reaction using a 1-phenyl-1*H*-tetrazole sulfone and an aldehyde with potassium bis (trimethylsilyl)amide in 1,2-dimethoxyethane is known to lead to an *E*-alkene with good stereoselectivity, especially if the sulfone or aldehyde is α-substituted.[38–40] Reaction of the sulfone **13** with aldehyde **8** gave the *trans*-alkene **15** (Scheme 4). It is also clearly essential that no epimerization occurs adjacent to the aldehyde during this process. There is considerable precedent for the retention of the chirality.[39,41–44] Removal of the silyl group gave **16** (R = Ac, R′ = Me) and hydrolysis of the two esters produced the free hydroxy-acid **16** (R = H, R′ = H).[45] The $[\alpha]_D^{21}$ of this, −2.07 ($CHCl_3$, 0.743 μmol), corresponding to M_D −26, is in agreement with that reported for the hydroxymycolates of *M. smegmatis* (M_D −16, though it must be noted that these only contain 30% *trans*-alkene).[9]

In the same way the enantiomer **14** was converted into **17** and free hydroxy-MA **18** (Fig. 2).[46] The ^1H NMR spectra of each of these in the alkene region were identical to those reported in the literature,[10,14] and to minor signals in fractions of α-mycolates derived from *M. tuberculosis*.[46]

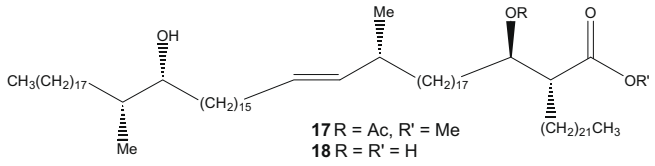
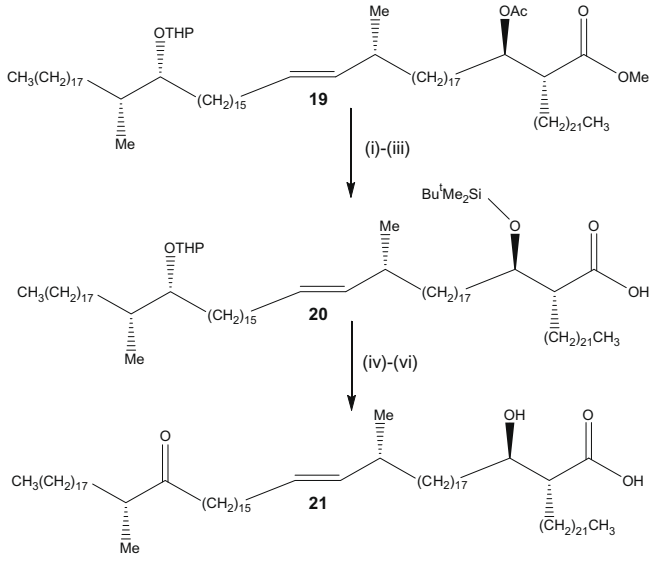


Figure 2.



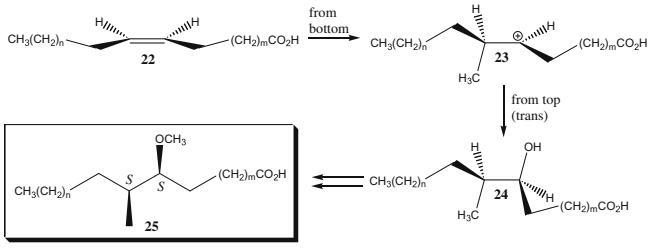
Scheme 5. Reagents: (i) LiOH, MeOH, H_2O, THF (72%); (ii) TBDMSCl, imidazole, DMF; (iii) K_2CO_3, MeOH/H_2O, then $KHSO_4$, (70%); (iv) PPTS, MeOH, H_2O, THF (73%); (v) PCC (89%); (vi) HF·pyridine (44%).

Oxidation of either **16** (R = Ac, R′ = Me) or **17** gave the corresponding protected keto-MAs.[47] However, attempted deprotection of these using LiOH led to epimerization at the position adjacent to the ketone. In order to avoid this, the alcohol **17** was first protected as THP-ether **19**, followed by hydrolysis of the esters and then reprotection at the alcohol group as a silyl ether **20**. Removal of the THP-group, oxidation, and then deprotection now proceeded without epimerization,[48] leading to the free acid **21** (Scheme 5), matching the major *trans*-alkene ketomycolate reported for *M. marinum*.[49]

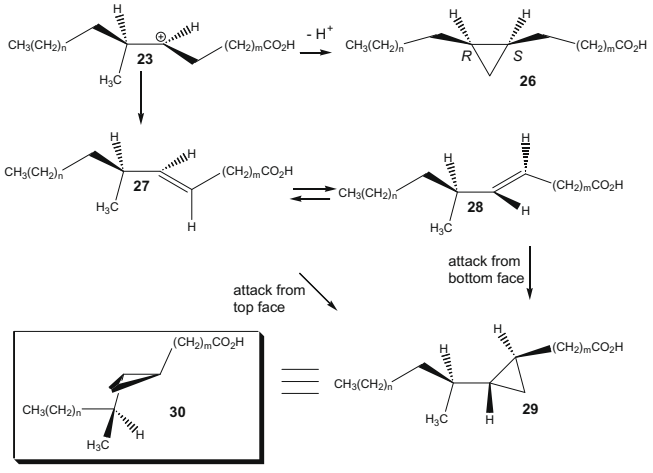
These results provide the first synthetic approaches to both keto and hydroxy-MAs containing a proximal (*R*)-α-methyl-*trans*-alkene unit, and a comparison of molecular rotations with those in the literature confirmed the stereochemistry of this subunit. It has been proposed that alkylation of a *cis*-alkene by SAM (S-adenosylmethionine) provides a common intermediate cation leading to each of the other MA functionalities.[50] Although the actual mechanism may well be different, this does provide a model by which to analyse the stereochemistry of the various classes of MA. It is known that the stereochemistry at the distal groups in methoxy-MA is (*S*,*S*);[7,11] using **22** as a model for a MA precursor, formal addition of a methyl cation to the distal carbon of the alkene from the bottom face would produce cation **23**; trapping could lead to **24** and hence to the (*S*,*S*)-molecule **25** (Scheme 6).

If the same species were involved at the proximal position, this would be consistent with the stereochemistry proposed for the proximal α-methyl-*trans*-cyclopropane unit **30** (Scheme 7).[28]

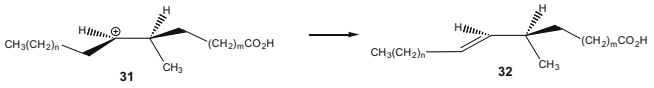
In applying this to the stereochemistry of *trans*-alkene mycolates, one possibility is that alkylation again occurs from the bottom face, this time to the proximal carbon of the alkene, leading



Scheme 6.



Scheme 7.



Scheme 8.

formally to **31**; elimination of a proton would then lead to the (*R*)-α-methylalkene **32** (Scheme 8).

The *trans*-MA content of the cell wall is linked to its permeability and growth in vitro.[18] The keto- and hydroxy-MAs described above are currently being tested to determine whether or not they show specific effects in a range of appropriate biological screens.

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45. Compound **16** (R = R′ = H): δ_H (500 MHz, $CDCl_3$): 5.33 (1H, dt, *J* 6.6, 15.45 Hz), 5.23 (1H, dd, *J* 7.55, 15.45 Hz), 3.70–3.69 (1H, m), 3.52–3.51 (1H, m), 2.45 (1H, br pent, *J* 4.7 Hz), 2.05–2.00 (1H, m), 1.97 (2H, q, *J* 6.9 Hz), 1.79–1.71 (1H, m), 1.66–1.59 (2H, m), 1.64–1.23 (139H, br m, including br s at 1.26), 0.94 (3H, d, *J* 6.6 Hz), 0.89 (6H, t, *J* 6.95 Hz), 0.86 (3H, d, *J* 7.25 Hz); δ_C: 177.3, 136.5, 128.5, 75.4, 72.2, 50.5, 37.3, 36.7, 34.4, 33.4, 32.6, 31.9, 30.0, 29.7, 29.6, 29.52, 29.46, 29.4, 29.1, 27.4, 22.7, 21.0, 16.6, 14.1; ν_{max}: 3534, 2922, 2854, 1751, 1466 cm^{-1} [found M+Na^+: 1234.39; $C_{82}H_{162}NaO_4$ requires: 1234.24]; $[\alpha]_D^{21}$ −2.07 ($CHCl_3$, 0.743 μmol).
46. Compound **18** showed $[\alpha]_D^{20}$ +1.67 ($CHCl_3$, 1.287 μmol); [Found M+Na^+: 1234.18; $C_{82}H_{162}NaO_4$ requires: 1234.24].
47. The protected ketone derived by PCC oxidation of **17** showed δ_H (500 MHz, $CDCl_3$): 5.33 (1H, dt, *J* 6.6, 15.1 Hz), 5.24 (1H, dd, *J* 7.25, 15.1 Hz), 5.09 (1H, dt, *J* 4.1, 8.15 Hz), 3.68 (3H, s), 2.62 (1H, ddd, *J* 4.45, 6.95, 11.05 Hz), 2.50 (1H, sext, *J* 6.95 Hz), 2.41 (2H, dt, *J* 2.25, 7.25 Hz), 2.03 (3H, s), 1.97 (2H, q, *J* 6.6 Hz), 1.63–1.18 (137H, m, including s at 1.26), 1.05 (3H, d, *J* 6.6 Hz), 0.94 (3H, d, *J* 6.6 Hz), 0.89 (6H, t, *J* 6.95 Hz); δ_C: 215.2, 173.7, 170.3, 128.4, 74.1, 51.5, 49.6, 46.3, 41.2, 37.3, 36.7, 33.1, 32.6, 31.9, 31.7, 29.8, 29.7, 29.64, 29.59, 29.54, 29.51, 29.48, 29.43, 29.39, 29.3, 29.2, 28.1, 27.5, 27.4, 27.3, 25.0, 23.7, 22.7, 21.0, 16.4, 14.1; ν_{max}: 2932, 2853, 1748, 1711, 1466 cm^{-1}; $[\alpha]_D^{22}$ +3.52 ($CHCl_3$, 1.094 μmol); [found M+Na^+: 1288.22; $C_{85}H_{164}NaO_5$ requires: 1288.25]. That from **16** (R = Ac, R′ = Me), $[\alpha]_D^{23}$ −2.39 ($CHCl_3$, 0.55 μmol), showed essentially identical NMR spectra.
48. Koza, G.; Al Dulayymi, J. R.; Theunissen, C.; Baird, M. S. *Tetrahedron* **2009**, 10214–10229.
49. The ketone **21** showed δ_H (500 MHz, $CDCl_3$): 5.32 (1H, td, *J* 6.65, 15.45 Hz), 5.24 (1H, dd, *J* 7.55, 15.45 Hz), 2.50 (1H, pent, *J* 6.6 Hz), 2.47 (1H, m), 2.42 (2H, dt, *J* 1.55, 6.95 Hz), 2.36 (1H, t, *J* 7.55 Hz), 1.97 (2H, q, *J* 6.95 Hz), 1.65–1.17 (139H, br m, including br s at 1.26), 1.05 (3H, d, *J* 6.95 Hz), 0.95 (3H, d, *J* 6.95 Hz), 0.89 (6H, t, *J* 6.65 Hz); δ_C: 215.5, 177.9, 136.5, 128.4, 72.2, 50.6, 46.4, 41.2, 37.3, 36.7, 35.6, 33.1, 32.6, 31.9, 29.8, 29.7, 29.64, 29.59, 29.54, 29.51, 29.48, 29.43, 29.39, 29.3, 29.1, 28.9, 27.4, 27.3, 25.7, 23.7, 22.7, 22.6, 21.0, 19.4, 16.4, 14.1; ν_{max}: 3420, 3019, 2926, 2855, 1521, 1420, 1215 cm^{-1}; $[\alpha]_D^{23}$ +2.90 ($CHCl_3$, 0.471 μmol); [found M+Na^+: 1232.36; $C_{82}H_{160}NaO_4$ requires: 1232.22].
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