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The synthesis of paleic acid, an antimicrobial agent effective against *Mannheimia* and *Pasteurella*, and its structurally related derivatives

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ABSTRACT

A synthetic route to paleic acid **1**, antimicrobial agent effective against *Mannheimia haemolytica* and *Pasteurella multocida*, has been established. The absolute configuration of the secondary hydroxyl group was controlled by a catalytic asymmetric alkylation of an aldehyde using a chiral titanium sulfonamide complex and the cis double bond was installed using a Wittig reaction. This synthetic route was also applied to the preparation of structurally related analogs, which were used in structure–activity relationship studies for antibacterial activity.

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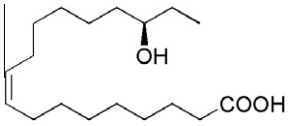
Mannheimia haemolytica[1] and *Pasteurella multocida*[2] are pathogenic and cause a wide range of diseases in food-producing animals such as cattle, poultry, pigs and rabbits. Antimicrobial agents are powerful tools for controlling infection. In fact, tilmicosin,[3,4] a derivative of desmycosin, was found to be effective against bovine respiratory disease (BRD) and was introduced into the US market in 1990. Since that time, a variety of antibiotics including β-lactams, macrolides, tetracyclines and sulfonamides have been revealed to be effective against the infectious diseases.[5,6] One of the most successful veterinary drugs available is the triamilide macrolide, tulathromycin[7] (the active ingredient in Draxxin Injectable Solution launched by Pfizer Animal Health), which has been approved for use in the treatment and prevention of BRD and the treatment of swine respiratory disease in the EU and the USA. The prevalence of antimicrobial resistance among pathogens[8] urges us to search for new classes of antibiotics with unique structure and/or mode of action.

In the previous research,[9] the isolation and structural elucidation of paleic acid **1** (Fig. 1) is described. Paleic acid is the first reported fatty acid derivative to display antibacterial activity against diverse strains of *Mannheimia haemolytica* and *Pasteurella multocida*. Since paleic acid has a structure completely different from those of other known antibiotics and may have a unique mode of action, the compound is expected to be a new lead in veterinary drugs.



Paleic acid (**1**)

Figure 1. Structure of paleic acid.

Herein we describe the synthesis of paleic acid **1** in detail. The synthesis was easily applied to the preparation of a variety of paleic acid analogs with structural modification at C16 and the carboxylate moiety. Once the synthesis of paleic acid analogs was achieved, a study on the structure–activity relationship (SAR) was conducted.

The structure of paleic acid is characterized as a (1) hydroxylated, (2) *cis*-unsaturated C18 fatty acid. We envisioned installing the *Z* double bond through a Wittig reaction. An asymmetric alkylation of an aldehyde using diethylzinc and a titanium sulfonamide ligand complex reported by Ohno and co-workers[10–12] was chosen to provide the chiral secondary alcohol at C16. The synthetic route is displayed in Scheme 1.

The 7-hydroxyheptylaldehyde, protected as a *tert*-butyldiphenylsilyl (TBDPS) ether **2**,[13] was uneventfully alkylated in an enantioselective manner (62% yield, >99% ee; vide infra) according to the reported procedure employing (1*S*,2*S*)-bis(trifluoromethanesulfo-

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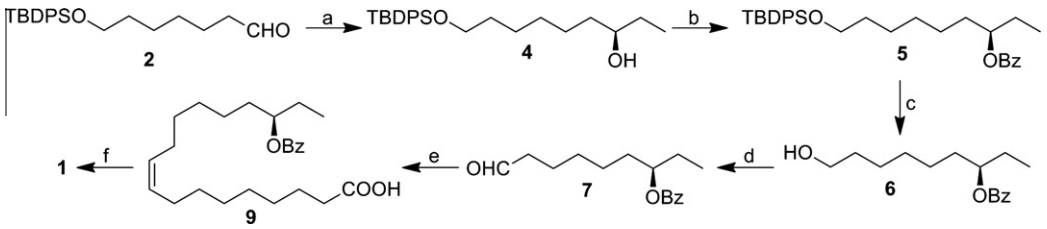


Scheme 1. Reagents and conditions: (a) (1*S*,2*S*)-1,2-*N*,*N*′-bis (trifuluoromethylsulfonylamino)cyclohexane **3**, Et_2Zn, Ti(O*i*-Pr)$_4$, toluene, −78 °C to −50 °C, 5 h, 62%, >99% ee; (b) BzCl, pyridine, DCM, rt, 16 h, 67%, (c) TBAF, THF, rt, 16 h, quant.; (d) TPAP, NMO, MS4A, DCM, rt, 1 h, 53%; (e) $Br^-Ph_3P^+(CH_2)_8COOH$ (**8**), KHMDS, THF, rt, 2 h, 72%; (f) NaOH, aq MeOH, rt, 3 d, 82%.

nylamino)cyclohexane **3** as a chiral ligand. Although the enantioselectivity of the reaction could not be determined by HPLC at this stage, the absolute configuration of the newly formed stereocenter was confirmed to be *R* by modified Mosher's method. Based on the analysis of the NMR spectra, the Mosher esters were not contaminated by ester derived from the *S*-isomer. The resulting alcohol **4** was protected as the benzoate **5** (67%), and the subsequent desilylation gave the primary alcohol **6** in quantitative yield, with which the enantioselectivity of the first reaction was determined to be >99% ee by chiral HPLC. Oxidation of the alcohol **6** with TPAP afforded the desired aldehyde **7** in 53% yield, which is the substrate for the subsequent Wittig reaction.

A phosphonium salt **8**[14] was treated with potassium bis(trimethylsilyl)amide (KHMDS) in THF to give the corresponding ylide, to which aldehyde **7** was added to afford the desired *cis*-olefin **9** (72% yield, based on the aldehyde). Last, saponification afforded paleic acid **1** in 82% yield. All the physicochemical properties and antimicrobial activity of the synthetic sample were indistinguishable from those of the natural product.

In order to obtain insight into the structural features responsible for the biological activity of paleic acid, a number of analogs were synthesized utilizing a synthetic route similar to **1** (Scheme 2).

Epimeric paleic acid **12** was prepared to evaluate the effect of the stereochemistry at C16 on antibacterial activity. The carboxylate of paleic acid **1** was esterified with trimethylsilyldiazomethane to give compound **10** in quantitative yield. The subsequent Mitsunobu reaction using benzoic acid as a nucleophile resulted in benzoate **11** (67%), with the stereochemistry at C16 position inverted. Finally, hydrolysis of the both protecting groups in a basic condition afforded the requisite epimeric paleic acid **12** in 49% yield.

The oxime-derivative **16** could be obtained synthetically as shown in Scheme 3. A C9 aldehyde **13**,[15] with the C16 carbonyl protected as an acetal, was subjected to a Wittig reaction under the same reaction conditions to that in the paleic acid synthesis, where the whole 18-carbon framework containing a *cis* double bond was constructed (**14**: 59%). After hydrolysis of the acetal (**15**: 93%), the liberated carbonyl group was reacted with hydroxylamine hydrochloride to give the oxime derivative of paleic acid **16**. The compound was produced as an inseparable equilibrating mixture of *E* and *Z*-isomers (in approximately a 1:1 ratio in $CDCl_3$).

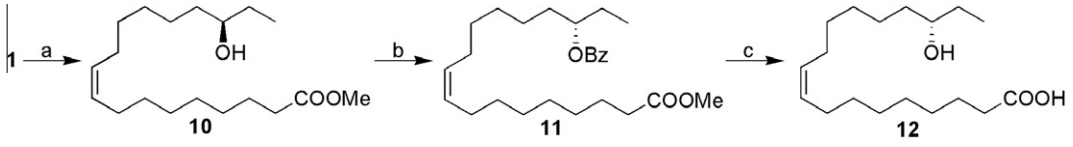
To examine the significance of the carboxyl group of paleic acid on antibacterial activity, a primary amide derivative **18** was prepared (Scheme 4). Benzoyl paleic acid (**9**) was treated with trimethylsilyldiazomethane to give methyl ester **17** (99%), which was converted to **18** with methanolic ammonia and a catalytic amount of sodium cyanide (NaCN)[16] in 67% yield.

A preliminary experiment showed that the antibacterial activity of paleic acid and the related compounds was ruined in the presence of serum albumin (unpublished results). Since the deleterious effect may come from the high affinity of acidic carboxylate groups albumin, paleic acid derivatives in which the carboxyl groups are replaced with its bioisosters were synthesized,[17] namely, hydroxamate **19**, sulfonimide **24**, and tetrazole **27** (Scheme 5).

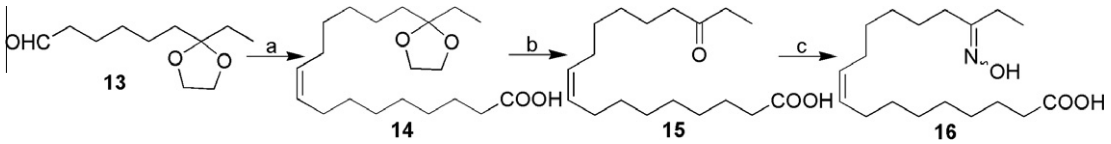
The hydroxamate analog **19** was obtained in a single step from compound **17** (treated with hydroxylamine in methanol) in 34% yield. Hydrolysis of the product by water gave rise to a reduction in the yield of the reaction.

The sulfonamide **24** could be synthesized by changing the phosphonate **8** to **22** that was prepared from 9-bromononanoic acid **20** in three steps (formation of the sulfonamide **21**, 46% over two steps and substitution by triphenylphosphine, quantitative yield). The Wittig reaction with phosphonium salt **22** produced the *cis*-olefin **23** in low yield (23% isolated yield). The poor yield was likely due to the instability of the phosphonium ylide generated from **22**. The final deprotection was performed to afford the sulfonimide **24** in 86% yield.

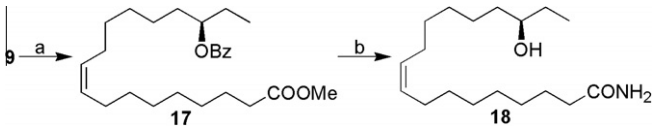
The tetrazole derivative **27** was prepared from benzoyl paleic acid **9** in three steps according to Duncia's procedure.[18] The compound **9** and 3-aminopropionitrile were condensed by a standard coupling method employing *N*-ethyl-*N*′-(3-dimethylaminopro-



Scheme 2. Reagents and conditions: (a) $TMSCHN_2$, MeOH, 0 °C, 20 min, quant.; (b) PhCOOH, DIAD, PPh_3, Et_2O, rt, 16 h, 67%; (c) NaOH, aq MeOH, rt, 5 h, 49%.



Scheme 3. Reagents and conditions: (a) $Br^-Ph_3P^+(CH_2)_8COOH$ (**8**), KHMDS, THF, rt, 16 h, 59%; (b) AcOH, aq THF, 50 °C, 5 h, 93%; (c) $NH_2OH{\cdot}HCl$, AcONa, MeOH, rt, 2 h, 96%.



Scheme 4. Reagents and conditions: (a) $TMSCHN_2$, MeOH, 0 °C, 20 min, 99%; (b) NH_3, NaCN, MeOH, 45 °C, 16 h, 67%.

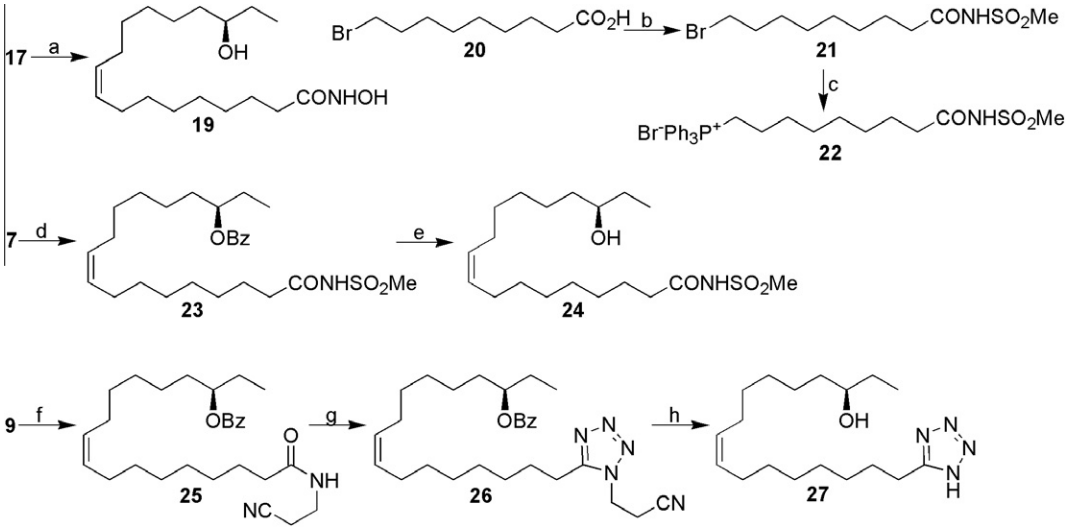
pyl)carbodiimide hydrochloride (EDCI) and 1-hydroxybenzotriazole (HOBt) to afford **25** in 70% yield. The amide was directly converted to a tetrazole ring under Mitsunobu conditions in the presence of trimethylsilylazide as the azide anion source to provide **26** in 86% yield. The final deprotection step completed the synthesis of the tetrazole analog **27** in 76% yield.

Antibacterial activities of paleic acid and its derivatives synthesized above were examined by subjection to a panel of bacteria covering various strains of *M. haemolytica* and *P. multocida*, and representative pathogens of human disease for comparison. To evaluate the significance of oxygen functionality in the biological activity of paleic acid, two intermediates in the oxime analog synthesis, acetal **14** and ketone **15**, were also tested.

Table 1 summarizes the results of the antibacterial assays. The antibacterial activities of the synthetic and natural sample of paleic acid **1** are identical. Interestingly, paleic acid epimeric at C16 **12** has essentially the same antibacterial spectra as the parent compound **1**. This result shows that the stereochemistry of the hydroxyl group of paleic acid has almost no influence on its biological activity. The C16 position allows structural modification with limited diversity. While the oxime **16** and ketone **15** analogs showed almost the same antibacterial activity than that of paleic acid toward many of the strains of *M. haemolytica* and *P. multocida* tested here, the acetal derivative **14** was far less active. In addition, **1**, **15**, and **16** have comparable antibacterial activity to that of the positive control, tilmicosin,[3,4] against several strains examined in this study.

The carboxyl group of paleic acid was revealed to be indispensable to its antibacterial activity, as the amide derivative **18** and the bioisosteric analogs **19**, **24**, and **27** showed high MIC values compared to the parent compound **1**. Finally, no paleic acid-related compounds displayed antibacterial activity against *Histophilus somni*, another important pathogen that can cause BRD.

This study established a synthetic route toward paleic acid **1**, a novel naturally occurring anti-*Mannheimia* and anti-*Pasteurella* substance. The synthesis could be applied to the preparation of a variety of analogs, which demonstrated the structural requirements to display biological activity. Modifications of the oxygen functionality at the C16 position were tolerated in limited degree and the carboxylate group was found to be indispensable. This study is expected to pave the way for further SAR studies to yield



Scheme 5. Reagents and conditions: (a) NH_2OH, MeOH, rt, 16 h, 34%; (b) (i) $(COCl)_2$, cat. DMF, 1 h, 70 °C; (ii) $MeSO_2NH_2$, pyridine, DCM, rt, 46% for two steps; (c) PPh_3, MeCN, 95 °C, 4 d, quant.; (d) **22**, KHMDS, THF, rt, 16 h, 23%; (e) NaOH, aq MeOH, rt, 5 h, 86%; (f) $H_2N(CH_2)_2CN$, EDCI, HOBt, TEA, DCM, rt, 16 h, 70%; (g) $TMSN_3$, DIAD, PPh_3, THF, rt, 16 h, 86%; (h) NaOH, aq MeOH, rt, 4 d, 76%.

Table 1
Antibacterial activity of paleic acid and its derivatives

Test Organisms	MIC (μg/mL)										
	1[a]	1[b]	12	14	15	16	18	19	24	27	Timicosin
Mannheimia haemolytica N791	0.78	0.78	0.78	25	3.13	1.56	25	25	50	50	1.56
M. haemolytica N811	1.56	0.78	0.78	25	6.25	1.56	12.5	25	50	50	0.39
M. haemolytica S801	1.56	0.78	0.39	25	6.25	1.56	50	50	25	50	3.13
Pasteurella multocida No.6	6.25	12.5	50	>50	50	3.13	>50	12.5	50	50	1.56
P. multocida Kobe	>50	>50	>50	50	>50	>50	>50	>50	>50	50	1.56
P. multocida TS-8	3.13	6.25	6.25	25	50	25	50	50	25	50	1.56
P. multocida M-17	>50	>50	>50	>50	>50	>50	>50	>50	>50	50	1.56
Acrinobacillus pleuroneumoniae NB001	6.25	6.25	12.5	>100	6.25	6.25	>50	50	>50	50	12.5
Histophilus somni 23N2359	>50	>50	>50	>50	>50	>50	>50	>50	>50	50	3.13

[a] Natural.
[b] Synthetic.

more potent paleic acid derivatives, especially in vivo. Synthetic studies along these lines are currently underway.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.07.115.

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