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Efficient approach to acyloxymethyl esters of nalidixic acid and in vitro evaluation as intra-ocular prodrugs

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Abstract—Various alkylcarbonyloxymethyl esters of nalidixic acid ranging from 3 to 15 carbon units in the pro-moiety have been prepared and assessed as potential prodrugs. Their chromatographic retention factors k′, silicone oil solubilities and in vitro conversion to nalidixic acid by a commercial esterase were determined together with their in vitro antimicrobial activity and cytotoxicity. The preliminary results suggest that silicone oil may have potential for the intra-ocular delivery of antibacterial compounds. Moreover, the in vitro release rate can be controlled by the lipophilicity of the prodrug.

1. Introduction

The prognosis for retinal detachments complicated with proliferative vitreoretinopathy (PVR) has improved with close vitrectomy and the use of silicone oil for prolonged retinal tamponade.[1] However, multiple surgical interventions are often necessary to treat PVR, and final visual results are often disappointing.[2] Corticosteroids, antibiotics, and antiproliferative drugs have been found to be effective in controlling the development of PVR.[3] Several authors have investigated the possibility of using silicone oil not only as an extended retinal tamponade agent but also for the delivery of lipophilic antiproliferative agents.[4–6] However, in many cases, these compounds could not be dissolved in silicone oil. In previous studies,[7] we reported a method for the synthesis of lipophilic 5-fluorouracil prodrugs. Formulation of such derivatives in silicone oil and in vitro release of the antiproliferative drug were also examined.[8]

Fluoroquinolones are antibacterial agents, which cover a host of Gram-negative and anaerobic species responsible for ocular infections. The third-generation quinolones, ciprofloxacin and ofloxacin (Fig. 1), are preferred agents as first-line topical therapy for bacterial keratitis and conjunctivitis; they are also routinely used for the prophylaxis of postoperative infections.[9] Topically applied fluoroquinolone (eye drops) penetrates into the anterior chamber but hardly reaches the posterior segment of the eye due to strong barriers involved and high dilution factor arising from the larger volume of vitreous.[10,11] Thus, infections affecting this segment are difficult to treat by topical administration. Systemic administration can deliver fluoroquinolones to the posterior eye, but due to the presence of the blood–retinal barrier, sufficiently high concentrations may not be guaranteed, for example in the treatment of endophthalmitis.[12] Although intravitreal injections of antimicrobial agent provide the most direct approach, they have the inherent potential side effects of retinal detachment, haemorrhage, endophthalmitis and cataract.[13] For these reasons, retinal drug delivery is a challenging area, and a number of approaches have been developed.[14] Despite extensive research in the field of sustained release formulations of fluoroquinolone antibiotics, an ideal system has not yet been found.[15–20]

In the present work, we investigate the possibility of using silicone oil (SiO) for the solubilisation of lipophilic

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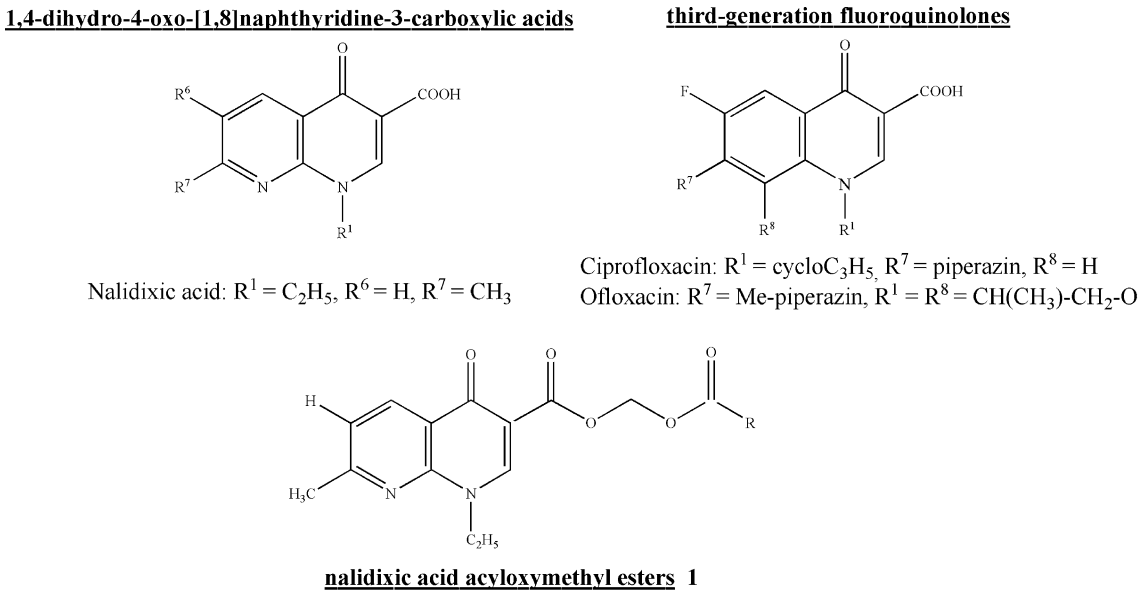


Figure 1. Chemical structures of nalidixic acid (NA), ciprofloxacin and ofloxacin, and acyloxymethylesters of NA.

prodrugs of fluoroquinolone and the delivery of the active compound to the posterior segment of the eye. The prototype quinolone is nalidixic acid (NA; Fig. 1), which exhibits activity, although modest, against a few Gram-negative species. NA has also been suggested to be a potentially useful agent for antiproliferative therapy.[21] Various esters of this naphthyridine-3-carboxylic acid derivative were synthesised by Bundgaard,[22] among them the butyryloxymethyl ester has been shown to function as a prodrug form capable of increasing the dermal delivery of NA.

More recently, in an attempt to reduce the metabolic rate of butyric acid, Nudelman[23,24] synthesised a variety of acyloxyalkylbutyrates as anticancer histone deacetylase inhibitor prodrugs and examined the contribution of the aldehyde (formaldehyde or acetaldehyde) and acids released upon cellular hydrolysis to their anticancer activity. Detailed SAR studies were performed and the results showed that among the aldehydes released, formaldehyde was the dominant factor affecting proliferation and cell death. Among the low molecular weight (C3-5) acids, butyric acid elicited the greatest antiproliferative activity, but the nature of the acid had minor impact on proliferation.

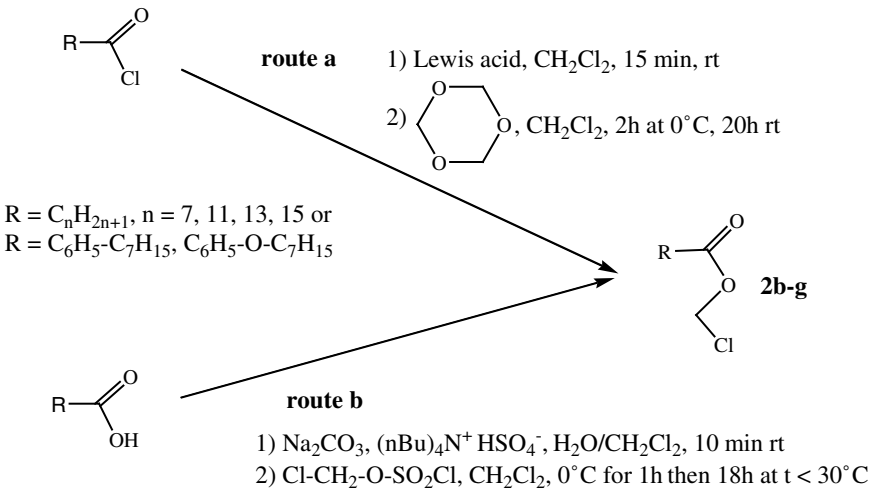
As part of a programme to identify suitable intra-ocular prodrugs of fluoroquinolone, we thus chose to prepare various lipophilic acyloxymethyl esters of NA (**1**, Fig. 1). We wished to investigate modifications of the acyl chain (with diverse steric properties) at the prodrug moieties as a means of modulating their solubility in silicone oil and susceptibility to enzymatic hydrolysis. Therefore, a general and efficient synthesis was required. The present paper also describes characterisation of lipophilicity, silicone oil solubility along with enzymatic hydrolysis by a commercial esterase of the synthesised derivatives. Moreover, in vitro antibacterial activity (MIC values) and cytotoxicity (CC_{50} values) of the investigated compounds were determined.

2. Results and discussion

2.1. Chemistry

The (acyloxy)alkyl ester prodrug moiety is generally introduced by alkylation of the drug carboxylate with a haloalkylester. We first attempted the preparation of the requisite lipophilic alkylating agents **2** as shown in Scheme 1. Examination of the literature on the preparation of the chloromethyl esters identified two main protocols: (route a) condensation of an aldehyde (paraformaldehyde or the easier to handle 1,3,5-trioxane) with acid chlorides in the presence of a Lewis acid,[25] and (route b) alkylation of carboxylates with chloromethylchlorosulfonate in phase transfer conditions.[26,27]

Chloromethylpivalate or chloromethylbutyrate can be obtained in a reasonably good yield[28,29] from the corresponding acid chlorides using the classical route a. However, contradictory results were reported when this method was applied to long-chain acid chlorides (with more than seven carbon atoms).[30,31] To improve this procedure, we explored the effect of several Lewis acids ($SnCl_4$, $ZrCl_4$, $AlCl_3$ and $ZnCl_2$ in the presence or absence of $CaCl_2$) for the preparation of octanoyloxymethylchloride **2b** (Table 1, entries 2–6). We observed that the use of zinc chloride in the presence of calcium chloride improved the yield in **2b** compound. The role of calcium chloride is unknown, but it presumably acts as a drying agent in the reaction. However, even under these conditions, mixtures were formed (**2b** with octanoic acid and octanoyloxymethyloctanoate) resulting in tedious purification.



Scheme 1.

Table 1. Yields of synthesis of acyloxymethylchlorides **2b–g** (according to Scheme 1) and double esters **1a–c** and **1f–g**

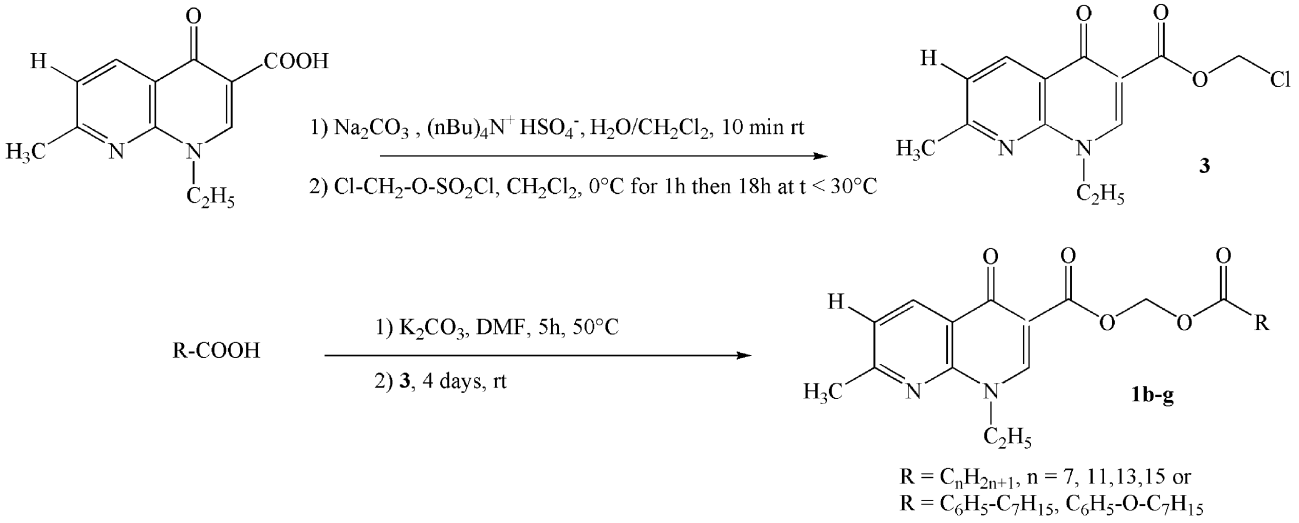
Entry	Product	R	Method (Lewis acid for route a)	% yield of **2**	% yield of **1**
1	**a**	C_3H_7	—	—	39
2	**b**	C_7H_{15}	a ($SnCl_4$)	33[a]	—
3			a ($ZrCl_4$)	9[a]	—
4			a ($AlCl_3$)	16[a]	—
5			a ($ZnCl_2$)	25[a]	—
6			a ($ZnCl_2$ + $CaCl_2$)	57[a]–52[b]	—
7			b	60[b]	35
8	**c**	$C_{11}H_{23}$	b	84[b]	22
9	**d**	$C_{13}H_{27}$	b	82[b]	—
10	**e**	$C_{15}H_{31}$	b	80[b]	—
11	**f**	C_6H_5–C_7H_{15}	b	70[b]	20
12	**g**	C_6H_5–O–C_7H_{15}	b	73[b]	11

[a] Yields calculated from ^{1}H NMR spectra of the mixtures obtained after usual workup. They were estimated by the ratio of the singlet area at δ = 5.70 ppm ($COOCH_2Cl$) to that of the multiplet at δ = 1.6 ppm ($CH_2\beta COOH$ and $CH_2\beta COOCH_2Cl$).
[b] Yields after isolation.

Although route b involved the use of potentially carcinogenic alkylating agent, we next examined this procedure along with various purification conditions (chromatography on florisil, silica gel, or activated aluminium oxide) because of the mild reaction conditions and high yield reported with carboxylic acids containing more than six carbon atoms.[32] We found that higher yields of alkylcarbonyloxymethyl chlorides could be obtained. Compounds **2b–g** were prepared using chloromethylchlorosulfate in a two-phase system of dichloromethane and aqueous sodium carbonate in the presence of tetra-*n*-butylammonium hydrogenosulfate ($(n\text{-Bu})_4NHSO_4$) as phase transfer catalyst. This reaction gave good yields after isolation, ranging from 60% to 84% (Table 1, entries 7–12).

Unfortunately, when attempting the alkylation of NA potassium salt with one equivalent of these reagents in *N*,*N*-dimethylformamide (DMF) at 50 °C for 24 h, we obtained complex mixtures resulting from incomplete reaction (even in the presence of sodium iodide) or decomposition. The corresponding double esters **1** were then isolated in poor yields ranging from 11% to 39% (Table 1).

In order to obtain a more general and efficient method for the preparation of desired compounds **1**, we decided to reverse our strategy. The general synthetic sequence is shown in Scheme 2. Chloromethylation of NA with chloromethylchlorosulfate (1.4 equiv) in phase transfer conditions ($(n\text{-Bu})_4NHSO_4$, 0.16 equiv) cleanly provided



Scheme 2.

the key derivative **3**, isolated in a 55% yield. The use of these reaction conditions was necessary to avoid the degradation of NA observed when the chloromethylation was attempted via the acid chloride intermediate. Subsequent condensation of commercially available carboxylic acids with **3** afforded the corresponding double esters **1b**–**g** in good yields as shown in Table 2.

2.2. Determination of lipophilicity

Since the introduction of the Hansch[33] approach to drug design and the first proposal by Fujita,[34] the octanol–water partition coefficient ($P_{o/w}$) is the most widely used measurement of lipophilicity in modelling biological partition/distribution. It has long been recognised that the retention of a compound in reverse-phase liquid chromatography is governed by its lipophilicity/hydrophilicity ratio and thus shows correlation with $P_{o/w}$. It is therefore a plausible alternative to use reverse-phase HPLC as a substitute for the classical slow and uncomfortable shake-flask method to characterise lipophilic properties of a molecule. A great number of publications on the efforts made to adjust HPLC methods and improve stationary phases to substitute $P_{o/w}$ measurements are well reviewed by Valkó.[35]

The chromatographic retention factor k' of the synthesised derivatives was determined using HPLC retention times as previously described by Thomas et al. and Hallgas et al.[36,37] The method is based on the partition of compounds between a reverse-phase C_8 column and various isocratic acetonitrile (+0.1% trifluoroacetic acid (TFA))/water (+0.1% TFA) phase mobile combinations (90%, 80%, 70%, 60% and 50%). The log of k' in each mobile phase composition is calculated using the equation $\log k' = \log[(t_r - t_0)/t_0]$, where t_r refers to the retention time of the compound and t_0 to the column 'dead time' (elution time of formaldehyde, a non-retained compound). A linear regression analysis of the plot of $\log k'$ versus the percentage of acetonitrile in the mobile phase gives y-intercepts which estimate $\log k'_0$ of each compound (Fig. 2). This value predicts the elution time of the compound if it were partitioned between 100% water and a C_8 reverse-phase column. The correlation coefficients ranged from 0.959 to 0.998. $\log k'_0$ values are presented in Table 3.

Table 2. Yields of synthesis of alkylcarbonyloxymethyl esters **1b**–**g** according to Scheme 2

Product	R	% yield from **3**
1b	C_7H_{15}	70
1c	$C_{11}H_{23}$	50
1d	$C_{13}H_{27}$	60
1e	$C_{15}H_{31}$	56
1f	C_6H_5–C_7H_{15}	58
1g	C_6H_5–O–C_7H_{15}	65

The lipophilicity of compounds **1a**–**g** was also evaluated by in silico calculation based on their chemical structure. Software-predicted lipophilicity was estimated by means of the on-line software CS$\log P$ (Chemsilico, www.chemsilico.com). The CS$\log P$ predictor is based on topological structure descriptors and was developed with artificial neural networks. The calculated CS$\log P$ values are collected in Table 3. In general, there is a good correlation between measured and calculated lipophilicities with the exception of structure isomers and compounds capable of hydrogen bonding.[38] The comparison of the experimentally determined ($\log k'_0$) and computer-estimated (CS$\log P$) lipophilicity parameters revealed a good linear correlation (CS$\log P = A \log k'_0 + B$) for the set of compounds evaluated here ($A = 2.0046$; $B = -0.0606$; $n = 8$; $R^2 = 0.9862$). Moreover, the computer-estimated values (CS$\log P$) for NA and the propyl ester **1a** are consistent with the experimentally determined values by the shake-flask method,[22] the true $P_{octanol/water}$ partition coefficient,[39–41] or the parameter calculated with the C$\log P$ program version 2.0.0b (running under evaluation licence of Biobyte Corporation, Claremont, CA).[42]

As expected, the introduction of an alkyl chain from 3 to 15 carbon atoms in the acyl group increases lipophilicity. Only a slight deviation from expected value was ob-

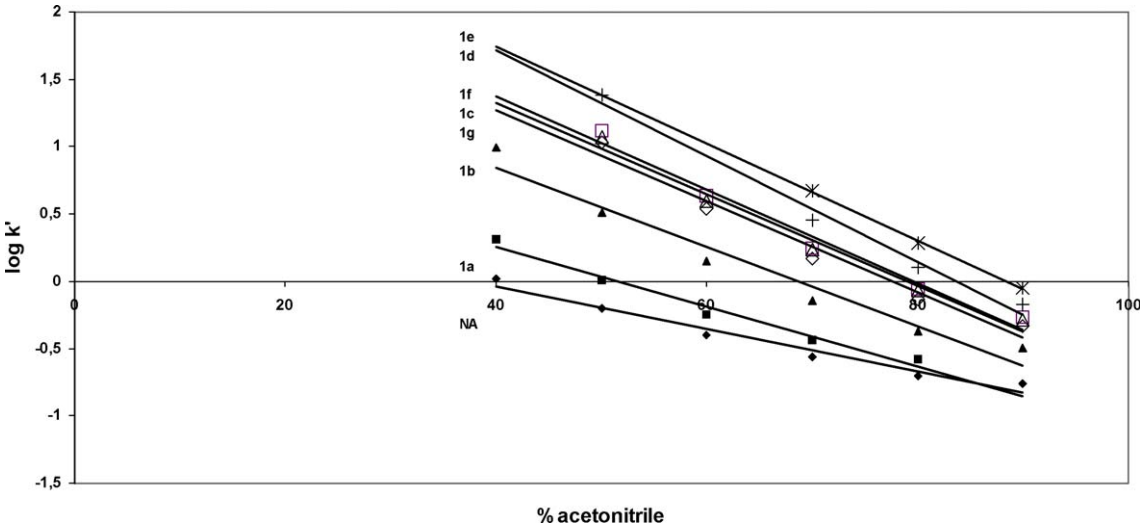


Figure 2. Plots of the chromatographic retention factors ($\log k'$) of esters **1a**–**g** and nalidixic acid (NA) determined in various acetonitrile (+0.1% TFA)/water (+0.1% TFA) phase mobile combinations. A linear regression analysis was used to obtain $\log k'_0$ values; the correlation coefficients were: 0.9719 for NA, 0.9782 for **1a**, 0.9594 for **1b**, 0.9814 for **1c**, 0.9862 for **1d**, 0.9977 for **1e**, 0.9769 for **1f**, 0.9743 for **1g**.

Table 3. Data of lipophilicity (measured by $\log k'_0$ and calculated CS$\log P$), solubility in silicone oil and enzymatic hydrolysis for the esters **1a**–**g** and nalidixic acid

Compound (R)	$\log k'_0$[a]	CS$\log P$ ± SD ($\log P^{lit}$)	Solubility in SiO (μg/g)	% esterase hydrolysis 24 h, 37 °C
1a (C_3H_7)	1.2	2.1 ± 0.7 (2.2[22])	35	100
1b (C_7H_{15})	2.0	4.1 ± 0.7	38	100
1c ($C_{11}H_{23}$)	2.7	5.5 ± 1.0	45	65
1d ($C_{13}H_{27}$)	3.3	6.1 ± 0.9	29	43
1e ($C_{15}H_{31}$)	3.2	6.4 ± 0.7	22	ND[b]
1f (C_6H_5–C_7H_{15})	2.8	5.4 ± 0.8	10	68
1g (C_6H_5–O–C_7H_{15})	2.6	5.5 ± 0.7	7	50
Nalidixic acid	0.6	1.0 ± 0.5 (1.5,[22,40,41] 1.6,[39] 1.4[42])	<2	—

[a] Values are means of three parallel measurements.
[b] Not determined.

served with compounds **1d** and **1e** probably due to the high lipophilicity of these derivatives. Moreover, the introduction of a benzene ring (compounds **1f** and **1b**) resulted in an increase in lipophilicity comparable with that obtained for a *n*-butyl chain, which is in agreement with the results described by Leo et al.[43]

Linear regression analysis of the plots of the lipophilicity parameter ($\log k'_0$ or CS$\log P$) versus the length of the alkyl chain (Fig. 3) provided the incremental methylene contribution (π) to lipophilicity, which is the slope of the straight line. The values obtained from HPLC retention factors and CS$\log P$ values were 0.18 and 0.36, respectively. They are lower than the average of 0.5 per CH_2, which is generally accepted,[44] but similar to those obtained in a homologous series of alkyl derivatives at the N-4 position of the piperazinyl group of ciprofloxacin (π = 0.18[45], 0.34[46]).

2.3. Determination of silicone oil solubility

The solubility of NA and its ester derivatives **1a**–**g** in 1300 centistokes purified SiO was determined at 25 °C by UV spectrophotometry as a function of time. As expected, the prodrugs **1a**–**g**, which are more lipophilic than NA, are more soluble in SiO (Table 3). The solubility of alkylesters increases from R = C_3H_7 to $C_{11}H_{23}$, then decreases for R = $C_{13}H_{27}$ and $C_{15}H_{31}$. These results are probably due to the high steric hindrance of these longer-alkyl chains. The solubilisation profiles are different for alkyl esters (R = C_3H_7–$C_{15}H_{31}$) and for aromatic ones (R = C_6H_5–C_7H_{15}, C_6H_5–O–C_7H_{15}) (Fig. 4). Compound **1c**, which is the most soluble, reached its maximum concentration (45 μg/g SiO) after 6 days. In contrast, although their lipophilicities are comparable to that of **1c** and higher than those of **1a** and **1b**, the aromatic derivatives **1f** and **1g** are less soluble ($\leqslant$10 μg/g SiO), and the saturation concentration was observed after 2 days. Similar results were described with the prodrug N_1-retinoyl-5-fluorouracil. The electronic delocalisation within the bulky retinoyl chain conferring it a certain rigidity hindered the solubilisation of the compound in SiO.[8]

2.4. Enzymatic hydrolysis

In the hypothesis of the use of compounds **1a**–**1g** in SiO during prolonged retinal tamponade after surgery, the rate of their hydrolysis by a commercial esterase was studied in a phosphate buffer at pH 7.4 and 37 °C. Ester-

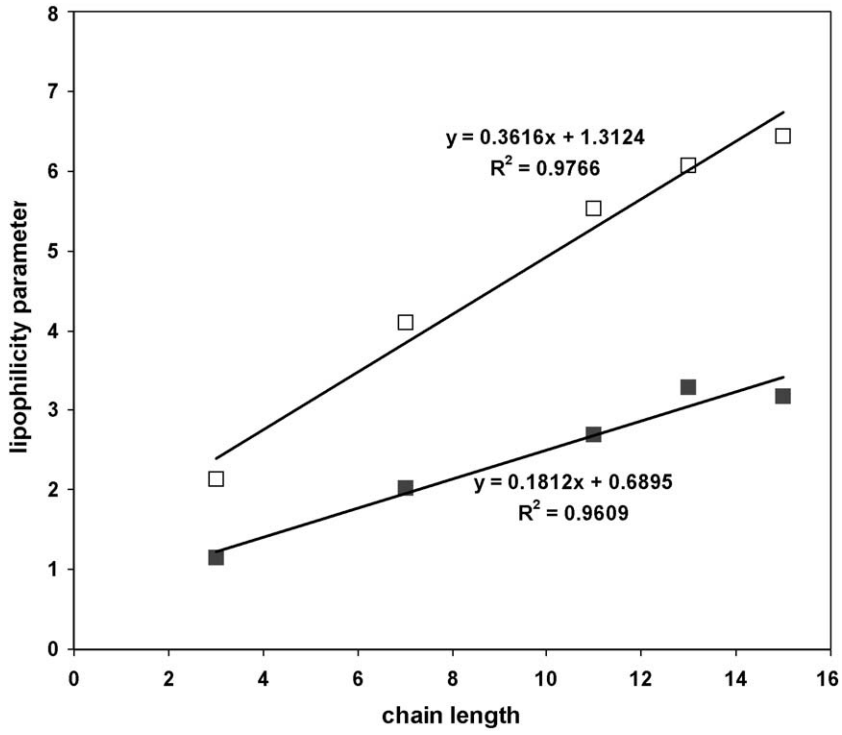


Figure 3. Relationship between (□) experimental ($\log k'_0$) or (■) calculated CS log *P* lipophilicity parameters and the number of methylene groups for derivatives **1a**–**g**.

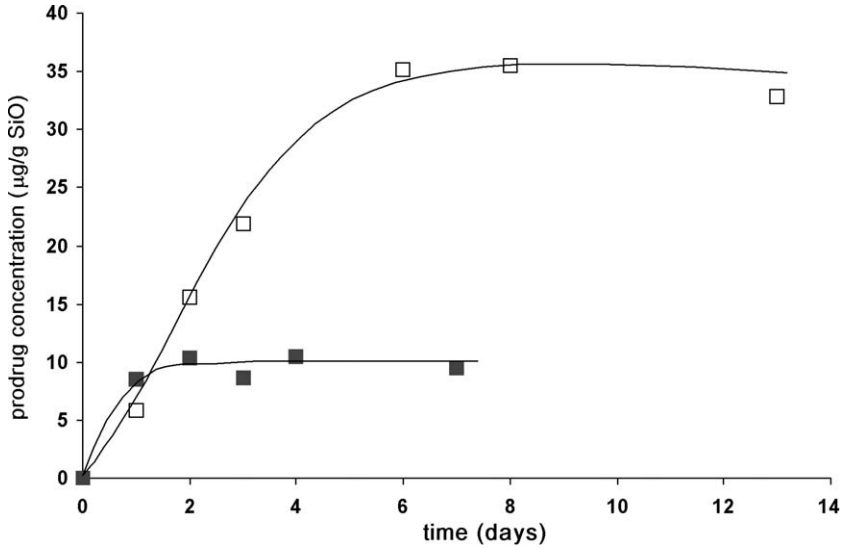


Figure 4. Graphs showing the solubilisation of compounds **1c** (□) and **1f** (■) in silicone oil.

ase from porcine liver was chosen as it is stable, does not require a cofactor and hydrolyses a wide range of esters. Moreover, Foroutan and Watson[47] have compared the hydrolysis of polyethylene glycol esters of hydrocortisone 21-succinate by this commercial esterase and by enzymes extracted from ovine cornea and found similar prodrug half-lives in both media.

Double esters **1** give a ^{1}H NMR singlet for the methylene group 11 in the range 5.9–6.2 ppm, depending on the acyl part R of the molecule (Fig. 5 and experimental part). The absence of this signal confirms the total degradation of the prodrug as formaldehyde that is formed during the hydrolysis remains in the aqueous phase when the medium is extracted with dichloromethane. The percentage of hydrolysis was evaluated by comparing the signal area of the prodrug CH_2-9 quadruplet at 4.4 ppm to that of the NA CH_2-9 quadruplet at 4.6 ppm (Fig. 5).

The percentages of porcine esterase hydrolysis after 24 h at 37 °C are reported in Table 3. For the less lipophilic prodrugs **1a** and **1b**, the release of NA is fast and achieved after 24 h. In contrast, the more lipophilic derivatives are more slowly hydrolysed. Compounds

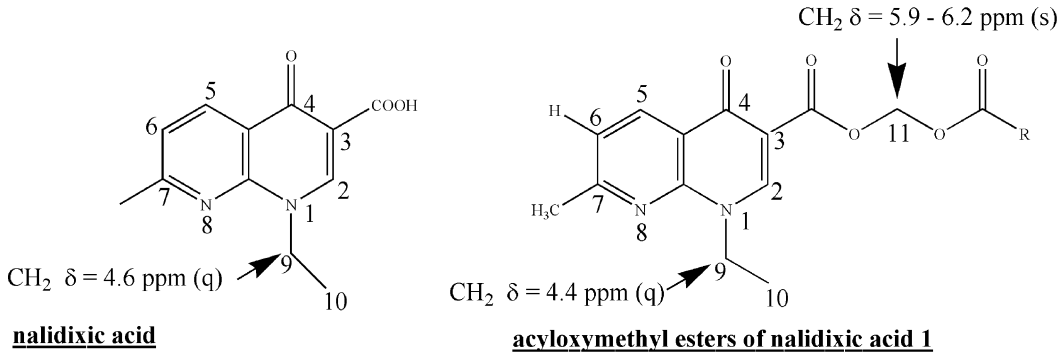


Figure 5. Characteristic ^{1}H NMR chemical shifts δ (ppm) of nalidixic acid (NA) and compounds **1a**–**g** used in the enzymatic hydrolysis study.

1d (R = $C_{13}H_{27}$) and **1g** (R = C_6H_5–O–C_7H_{15}) are the most stable prodrugs. The results obtained here at solid/liquid interface are comparable with previous data described at liquid/liquid interface (SiO/aqueous phase) on N_1-alkylcarbonyl[8] or N_1-alkoxycarbonyl[48] prodrugs of 5-fluorouracil.

2.5. In vitro antibacterial and cytotoxic activities

In the past decade a number of compounds containing a 1,4-dihydro-4-oxo-1,8-naphthyridine carboxylic acid moiety have been developed and structural modifications have altered both the antibacterial spectrum and potency. The newly synthesised acyloxymethyl esters **1a**–**g** were tested for antimicrobial activity by broth dilution micromethod against standard strains of potent pathogenic species including two Gram-positive (*Staphylococcus aureus* CIP 4.83 and *Bacillus subtilis* CIP 52.62) and two Gram-negative (*Escherichia coli* CIP 54127 and *Pseudomonas aeruginosa* CIP 82.118). The minimum inhibitory concentrations (MICs, μg/mL) are reported in Table 4 along with those of NA for comparison.[49–52] Our results show that, in good accordance with the concept of prodrug, the MIC values for acyloxymethyl esters **1** are higher than those of NA on *S. aureus* and *E. coli*. *P. aeruginosa* is resistant to all esters as to NA. However, all the synthesised esters demonstrate MIC values comparable to that of NA against *B. subtilis* which may be explained by the exocellular production of esterase in this microorganism.[53,54]

The cytotoxic activity of compounds **1a**–**g** was also tested on six human cancer cell lines including various histopathological types (glioblastoma Hs683 and U-373MG, colon HCT-15 and LoVo, lung A549 and breast MCF-7) at nine dilutions ranging from 10^{-5} to 10^{-9} M.[55] The colourimetric MTT assay, which indirectly assesses the effect of potentially anticancer compounds on overall growth of adherent cell lines, was used.[56] Compounds **1a**–**g** did not show any cytotoxic activity.

3. Conclusions

In the present study, a general and efficient method for the preparation of alkylcarbonyloxymethyl esters of NA is described. Lipophilicity of the synthesised derivatives was characterised by both chromatographic retention factor k' and computer prediction data (CS log P); a good correlation was found between these parameters. As expected, the lipophilicity of the prodrugs increased with the length of the acyl chain, and lipophilic derivatives could be solubilised in silicone oil. A commercial esterase hydrolysed the alkylcarbonyloxymethyl esters to NA; the release of NA was fast and achieved after one day for the short-chain derivatives, whereas longer-chain compounds were more stable. The newly synthesised derivatives were screened for in vitro antibacterial activity, and the MIC values obtained were comparable to or higher than those of NA. Moreover, they did not show any cytotoxic activity. This study indicates that silicone oil may have potential for the intra-ocular delivery of antibacterial compounds. Moreover, the in vitro release rate can be controlled by the lipophilicity of the prodrug. Further development is in progress on alkylcarbonyloxyalkyl esters of fluoroquinolones.

4. Experimental

4.1. General

4.1.1. Materials. All commercial starting materials were used as supplied without further purification. Dry DMF

Table 4. In vitro antibacterial activity of compounds **1a**–**g** and nalidixic acid (NA)

Microorganism (strain no.)	MIC (μg/mL)							
	1a (C_3H_7)	**1b** (C_7H_{15})	**1c** ($C_{11}H_{23}$)	**1d** ($C_{13}H_{27}$)	**1e** ($C_{15}H_{31}$)	**1f** (ArC_7H_{15})	**1g** ($ArOC_7H_{15}$)	NA[49–52]
Staphylococcus aureus (CIP 4.83)	>128	>128	>128	>128	>128	>128	>128	128
Bacillus subtilis (CIP 52.62)	1	2	2	4	8	4	4	6
Escherichia coli (CIP 54127)	16	>128	>128	120	>128	>128	>128	4
Pseudomonas aeruginosa (CIP 82.118)	>128	>128	>128	>128	>128	>128	>128	>128

and NA (99%) were purchased from Aldrich, butyryloxymethyl chloride (99%), octanoic acid (99%), 4-heptylbenzoic acid (99%) and chloromethylchlorosulfate (98%) from Acros, dodecanoic acid (>99%) from Fluka, tetradecanoic acid (99%) from Alfa Aesar, $(n\text{-Bu})_4NHSO_4$ (97%) from Sigma and 4-heptyloxybenzoic acid (98%) and hexadecanoic acid (95%) from Avocado. Esterase from porcine liver was purchased from Sigma (20 U/mg); one unit hydrolyses 1.0 μmol of ethyl butyrate to butyric acid and ethanol per min at pH 8.0 and 25 °C.

4.1.2. Analytical procedures. ^{1}H NMR spectra were recorded on a Bruker AC-250 spectrometer. Data are reported in the following order: chemical shift δ in ppm, signal multiplicity, value(s) of coupling constant(s), number of protons and assignment. The methylene groups of alkyl chains are reported in Greek alphabetic order starting from CH_3. ^{13}C NMR spectra were recorded on a Bruker Avance-300 spectrometer. Elemental analyses were carried out by the 'Service Commun de Microanalyse élémentaire UPS-INP' in Toulouse. Mass spectra were recorded on a Perkin Elmer SCIEX API 100 operating in chemical ionisation (NH_3) mode. Spectrophotometric determinations were conducted on a HP 8452A diode array spectrophotometer (Hewlett-Packard Co.); ε are expressed in L mol^{-1} cm^{-1}.

Flash chromatography on silica gel Si60 (Merck) finer than 70 μm was used for purifications. HPLC analysis of the samples was performed on a Waters system (Waters Associates Inc., Milford, MA, USA) consisting of a 600 controller pump, a PDA996 diode array detector, a 717 plus autosampler and a Lisa 30 Ecrosas (ICS) oven at 25 °C. The instrument was controlled by the Empower software. The experiments were carried out on a C8 protect (Macherey-Nagel) reverse-phase column (length 50 mm × 3 mm id, 5 μm particle size) eluted with a mobile phase consisting of various mixtures of acetonitrile (+0.1% TFA)/water (+0.1% TFA) at a flow rate of 0.6 mL/min. The eluting solvents were prepared daily and degassed with helium during analyses. The detection was set at 260 nm. Stock solutions of the compounds analysed (1 mg/mL) were prepared in acetonitrile/water (50:50). All injections were made in triplicate.

4.2. General procedure for the synthesis of alkylcarbonyloxymethylchlorides (2b–g)

A mixture of alkanoic acid and Na_2CO_3 in water (15 mL) was stirred at room temperature (rt) (or 100 °C for **2d** and **2e)** until the solubilisation was complete. After cooling to 0 °C, dichloromethane (20 mL), $(n\text{-Bu})_4NHSO_4$ and chloromethylchlorosulfate were added. The reaction mixture was vigorously stirred at 0 °C for 1 h, then at rt for 18 h. The organic layer was separated and the aqueous layer extracted with dichloromethane (3× 20 mL). The organic layers were dried over anhydrous Na_2SO_4 and evaporated. The remaining residue was purified by flash chromatography on silica gel.

4.2.1. Chloromethyl octanoate (2b). Compound **2b** was prepared from octanoic acid (1 g, 6.93 mmol), Na_2CO_3 (2.94 g, 27.7 mmol), $(n\text{-Bu})_4NHSO_4$ (0.52 g, 1.52 mmol) and chloromethylchlorosulfate (0.93 mL, 9.01 mmol). This compound was flash chromatographed on silica gel with cyclohexane/diethyl ether (99:1; v/v) to give **2b** (801 mg, 60% yield) as an oil.

^{1}H NMR ($CDCl_3$, 250 MHz) δ: 0.88 (t, ^3J = 7.0 Hz, 3H, CH_3); 1.29 (m, 8H, $CH_2\alpha$–δ); 1.65 (m, 2H, $CH_2\varepsilon$); 2.38 (t, ^3J = 7.5 Hz, 2H, $CH_2\zeta$); 5.68 (s, 2H, OCH_2Cl). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ: 14.02 (CH_3); 22.55 ($CH_2\alpha$); 24.53 ($CH_2\beta$); 28.83 ($CH_2\gamma$); 28.88 ($CH_2\delta$); 31.58 ($CH_2\varepsilon$); 33.98 ($CH_2\zeta$); 68.55 (CH_2Cl); 171.77 (CO).

4.2.2. Chloromethyl dodecanoate (2c). Compound **2c** was prepared from dodecanoic acid (1 g, 4.99 mmol), Na_2CO_3 (2.1 g, 20 mmol), $(n\text{-Bu})_4NHSO_4$ (0.4 g, 1.1 mmol) and chloromethylchlorosulfate (0.67 mL, 6.49 mmol). This compound was flash chromatographed on silica gel with CH_2Cl_2/triethylamine (Et_3N) (99.5:0.5; v/v) to give **2c** (1.04 g, 84% yield) as an oil.

^{1}H NMR ($CDCl_3$, 250 MHz) δ: 0.87 (t, ^3J = 7.0 Hz, 3H, CH_3); 1.25 (m, 16H, $CH_2\alpha$–θ); 1.63 (m, 2H, $CH_2\iota$); 2.38 (t, ^3J = 6.6 Hz, 2H, $CH_2\kappa$); 5.70 (s, 2H, OCH_2Cl). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ: 14.08 (CH_3); 22.67 ($CH_2\alpha$); 24.53 ($CH_2\beta$); 28.92, 29.17, 29.31, 29.38, 29.55, 29.57 ($CH_2\gamma-\theta$); 31.89 ($CH_2\iota$); 33.97 ($CH_2\kappa$); 68.53 (CH_2Cl); 171.74 (CO).

4.2.3. Chloromethyl tetradecanoate (2d). Compound **2d** was prepared from tetradecanoic acid (1 g, 4.38 mmol), Na_2CO_3 (1.8 g, 17.5 mmol), $(n\text{-Bu})_4NHSO_4$ (0.3 g, 0.964 mmol) and chloromethylchlorosulfate (0.59 mL, 6.69 mmol). This compound was flash chromatographed on silica gel with CH_2Cl_2/petroleum ether (50:50; v/v) to give **2d** (1 g, 82% yield) as a white solid.

^{1}H NMR ($CDCl_3$, 250 MHz) δ: 0.87 (t, ^3J = 6.7 Hz, 3H, CH_3); 1.25 (m, 20H, $CH_2\alpha$–κ); 1.62 (m, 2H, $CH_2\lambda$); 2.38 (t, ^3J = 7.3 Hz, 2H, $CH_2\mu$); 5.70 (s, 2H, OCH_2Cl). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ: 14.10 (CH_3); 22.68 ($CH_2\alpha$); 24.54 ($CH_2\beta$); 28.93, 29.17, 29.35, 29.39, 29.56, 29.63, 29.66 ($CH_2\gamma$–κ); 31.92 ($CH_2\lambda$); 33.99 ($CH_2\mu$); 68.55 (CH_2Cl); 171.78 (CO).

4.2.4. Chloromethyl hexadecanoate (2e). Compound **2e** was prepared from hexadecanoic acid (1 g, 3.90 mmol), Na_2CO_3 (1.6 g, 15.6 mmol), $(n\text{-Bu})_4NHSO_4$ (0.3 g, 0.858 mmol), and chloromethylchlorosulfate (0.52 mL, 5.07 mmol). This compound was flash chromatographed on silica gel with CH_2Cl_2/petroleum ether (50:50; v/v) to give **2e** (0.95 g, 80% yield) as a white solid.

^{1}H NMR ($CDCl_3$, 250 MHz) δ: 0.87 (t, ^3J = 7.0 Hz, 3H, CH_3); 1.24 (m, 24H, $CH_2\alpha$–μ); 1.64 (m, 2H, $CH_2\nu$); 2.37 (t, ^3J = 7.3 Hz, 2H, $CH_2\zeta$); 5.69 (s, 2H, OCH_2Cl). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ: 14.09 (CH_3); 22.68 ($CH_2\alpha$); 24.53 ($CH_2\beta$); 28.93, 29.17, 29.36, 29.39, 29.56, 29.62, 29.66, 29.68 ($CH_2\gamma$–μ); 31.92 ($CH_2\nu$); 33.97 ($CH_2\zeta$); 68.53 (CH_2Cl); 171.73 (CO).

4.2.5. Chloromethyl-4-heptylbenzoate (2f). Compound **2f** was prepared from 4-heptylbenzoic acid (1.01 g, 4.59 mmol), Na_2CO_3 (2.08 g, 19.62 mmol), $(n\text{-}Bu)_4NHSO_4$ (0.17 g, 0.5 mmol) and chloromethylchlorosulfate (0.6 mL, 5.82 mmol). This compound was flash chromatographed on silica gel with hexane/diethyl ether (99:1; v/v) to give **2f** (863 mg, 70% yield) as an oil.

^{1}H NMR ($CDCl_3$, 250 MHz) δ: 0.88 (t, ^3J = 6.7 Hz, 3H, CH_3); 1.30 (m, 8H, $CH_2\alpha$–δ); 1.62 (tt, ^3J = 7.0 Hz and ^3J = 7.4 Hz, 2H, $CH_2\varepsilon$); 2.66 (t, ^3J = 7.4 Hz, 2H, $CH_2\zeta$); 5.94 (s, 2H, CH_2Cl); 7.25 (d, ^3J = 8.2 Hz, 2H, ArH); 7.96 (d, ^3J = 8.2 Hz, 2H, ArH). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ: 14.07 (CH_3); 22.64 ($CH_2\alpha$); 29.11, 29.19 ($CH_2\beta,\gamma$); 31.07 ($CH_2\delta$); 31.76 ($CH_2\varepsilon$); 36.08 ($CH_2\zeta$); 69.22 (CH_2Cl); 126.06 (C_q); 128.68 (CHAr); 130.16 (CHAr); 149.83 (C_q); 164.6 (CO).

4.2.6. Chloromethyl-4-heptyloxybenzoate (2g). Compound **2g** was prepared from 4-heptyloxybenzoic acid (1 g, 4.25 mmol), Na_2CO_3 (2.04 g, 19.24 mmol), $(n\text{-}Bu)_4NHSO_4$ (0.2 g, 0.59 mmol), chloromethylchlorosulfate (0.52 mL, 5.04 mmol). This compound was flash chromatographed on silica gel with hexane/diethyl ether (99:1; v/v) to give **2g** (884 mg, 73% yield) as an oil.

^{1}H NMR ($CDCl_3$, 250 MHz) δ: 0.88 (t, ^3J = 6.7 Hz, 3H, CH_3); 1.35 (m, 8H, $CH_2\alpha$–δ); 1.78 (tt, ^3J = 6.7 Hz and ^3J = 7.6 Hz, 2H, $CH_2\varepsilon$); 3.99 (t, ^3J = 6.7 Hz, 2H, $CH_2\zeta$); 5.92 (s, 2H, CH_2Cl); 6.9 (d, ^3J = 9.1 Hz, 2H, ArH); 7.98 (d, ^3J = 9.0 Hz, 2H, ArH). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ: 14.06 (CH_3); 22.59 ($CH_2\alpha$); 25.92, 26.13 ($CH_2\beta,\gamma$); 29.06 ($CH_2\delta$); 31.75 ($CH_2\varepsilon$); 68.33 ($CH_2\zeta$); 69.20 (CH_2Cl); 114.33 (CHAr); 120.60 (C_q); 132.22 (CHAr); 163.83 (C_q); 164.23 (CO).

4.3. Synthesis of 1-ethyl-7-methyl-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carbonyloxy-methylchloride (3)

To a solution of NA (0.5 g, 2.15 mmol) in water (10 mL), Na_2CO_3 (0.95 g, 8.7 mmol) and $(n\text{-}Bu)_4NHSO_4$ (0.12 g, 0.35 mmol) were added at rt and the reaction mixture was stirred for 90 min at rt. Chloromethylchlorosulfate (0.48 g, 3 mmol) in dichloromethane (20 mL) was added and the mixture was vigorously stirred at 0 °C for 1 h and at rt for 18 h. The organic layer was separated and the aqueous layer extracted with dichloromethane (3× 10 mL). The organic layers were dried over anhydrous Na_2SO_4 and evaporated. The remaining residue was purified by flash chromatography on silica gel with petroleum ether/ethyl acetate/isopropanol/Et_3N) (8:2:0.5:0.1; v/v/v/v) to give **3** (0.33 g, 55% yield) as a white solid.

^{1}H NMR ($CDCl_3$, 250 MHz) δ: 1.49 (t, ^3J = 7.0 Hz, 3H, CH_3); 2.63 (s, 3H, CH_3); 4.5 (q, ^3J = 7.0 Hz, 2H, CH_2); 5.95 (s, 2H, CH_2Cl); 7.26 (d, ^3J = 8.0 Hz, 1H, CH); 8.57 (d, ^3J = 8.0 Hz, 1H, CH); 8.59 (s, 1H, CH). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ: 15.20, 22.03 (CH_3); 46.88 (CH_2); 69.07 (CH_2Cl); 110.12, 148.53, 163.00 (C_q); 121.54, 136.87, 149.53 (CH); 163.10 (COO); 174.37 (CO).

4.4. Synthesis of 3-[(butyryloxy)methyl]1-ethyl-7-methyl-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylate (1a)

To a solution of NA (0.5 g, 2.15 mmol) in dry DMF (10 mL), K_2CO_3 (0.384 g, 2.8 mmol) was added at rt and the reaction mixture was stirred for 5 h at 50 °C. Butyryloxymethyl chloride (0.305 g, 2.2 mmol) was then added. The mixture was stirred at 50 °C for 24 h. DMF was evaporated in vacuo and the residue was diluted with dichloromethane (20 mL). The organic layer was washed with water (60 mL). The aqueous layer was extracted with dichloromethane (8× 20 mL). The organic layers were dried over anhydrous Na_2SO_4 and evaporated. The remaining residue was flash chromatographed on silica gel with petroleum ether/ethyl acetate/isopropanol/Et_3N (8:2:0.5:0.1; v/v/v/v) to give **1a** (0.278 g, 39% yield).

^{1}H NMR ($CDCl_3$, 250 MHz) δ: 0.94 (t, ^3J = 7.5 Hz, 3H, CH_3); 1.49 (t, ^3J = 7.2 Hz, 3H, CH_3 of the NA moiety); 1.66 (tt, ^3J = 7.3 Hz and ^3J = 7.6 Hz, 2H, $CH_2\alpha$); 2.35 (t, ^3J = 7.3 Hz, 2H, $CH_2\beta$); 2.65 (s, 3H, CH_3 of the NA moiety); 4.47 (q, ^3J = 7.2 Hz, 2H, CH_2 of the NA moiety); 5.98 (s, 2H, O–CH_2–O); 7.25 (d, ^3J = 7.9 Hz, 1H, CH); 8.61 (d and s, ^3J = 7.9 Hz, 2H, CH). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ: 13.55 (CH_3); 15.20, 25.07 (CH_3 of the NA moiety); 18.10, 35.87 ($CH_2\alpha$–β); 46.79 (CH_2 of the NA moiety); 79.45 (O–CH_2–O); 110.33, 121.48, 148.55, 162.86 (C_q); 121.41, 136.90, 149.17 (CH); 163.20, 172.47 (COO); 174.61 (CO). UV ($CHCl_3$) λ_{max}: 260 nm (ε = 14010), λ_{max}: 336 nm (ε = 12220). MS (CI/NH_3) *m/z* 333 (MH^+), 233 ($MH^+ - H_2COCOC_3H_7$). Anal. ($C_{17}H_{20}N_2O_5 \cdot 0.7H_2O$) C, H, N: calcd 59.19, 6.25, 8.12; found 59.09, 6.21, 8.15.

4.5. General procedure for the synthesis of 3-[(alkyloxy)methyl]1-ethyl-7-methyl-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylate (1b–g)

To a solution of carboxylic acid in dry DMF, K_2CO_3 was added at rt and the reaction mixture was stirred for 5 h at 50 °C. Compound **3** was then added at rt. The mixture was stirred for 4 days at rt. DMF was evaporated in vacuo and the residue was diluted with dichloromethane (20 mL). The organic layer was washed with water (10 mL) and the aqueous layer extracted with dichloromethane (6× 10 mL). The organic layers were dried over anhydrous Na_2SO_4 and evaporated. The remaining residue was flash chromatographed on silica gel.

4.5.1. 3-[(Octanoyloxy)methyl]1-ethyl-7-methyl-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylate 1b. This compound was synthesised from octanoic acid (0.025 g, 0.18 mmol) and K_2CO_3 (0.024 g, 0.21 mmol) in dry DMF (2.5 mL). Compound **3** (0.05 g, 0.18 mmol) was added. The remaining residue was flash chromatographed on silica gel with petroleum ether/ethyl acetate/isopropanol/Et_3N (8:2:0.5:0.1; v/v/v/v) to give **1b** (0.048 g, 70% yield) as a white solid.

^{1}H NMR ($CDCl_3$, 250 MHz) δ: 0.79 (t, ^3J = 7.0 Hz, 3H, CH_3); 1.23 (m, 8H, $CH_2\alpha$–δ); 1.47 (t, ^3J = 7.0 Hz, 3H,

CH_3 of the NA moiety); 1.61 (tt, ^3J = 7.0 Hz and ^3J = 7.3 Hz, 2H, $CH_2\varepsilon$); 2.36 (t, ^3J = 7.3 Hz, 2H, $CH_2\zeta$); 2.63 (s, 3H, CH_3 of the NA moiety); 4.46 (q, ^3J = 7.0 Hz, 2H, CH_2 of the NA moiety); 5.96 (s, 2H, O–CH_2–O); 7.25 (d, ^3J = 7.9 Hz, 1H, CH); 8.6 (d and s, ^3J = 7.9 Hz, 2H, CH). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ: 14.00 (CH_3); 15.19, 25.05 (CH_3 of the NA moiety); 22.53, 24.56, 28.84, 28.95, 31.59, 34.01 ($CH_2\alpha$–ϕ); 46.78 (CH_2 of the NA moiety); 79.43 (O–CH_2–O); 110.29, 121.43, 148.53, 162.86 (C_q); 121.38, 136.85, 149.17 (CH); 163.16, 172.63 (COO); 174.57 (CO). UV ($CHCl_3$) λ_{max}: 260 nm (ε = 14740), λ_{max}: 336 nm (ε = 12900). MS (CI/NH_3) *m/z* 389 (MH^+), 233 ($MH^+ - H_2COCOC_7H_{15}$). Anal. ($C_{21}H_{28}N_2O_5$·0.15-H_2O) C, H, N: calcd 64.48, 7.29, 7.16; found 64.43, 7.29, 7.58.

4.5.2. 3-[(Dodecanoyloxy)methyl]1-ethyl-7-methyl-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylate (1c). This compound was synthesised from dodecanoic acid (0.081 g, 0.4 mmol) and K_2CO_3 (0.09 g, 0.85 mmol) in dry DMF (2 mL). Compound **3** (0.1 g, 0.35 mmol) was added. The remaining residue was flash chromatographed on silica gel with diethyl ether/ethyl acetate (98:2; v/v) to give **1c** (0.08 g, 50% yield) as a white solid.

^{1}H NMR ($CDCl_3$, 250 MHz) δ: 0.86 (t, ^3J = 6.7 Hz, 3H, CH_3); 1.21 (m, 16H, $CH_2\alpha$–θ); 1.49 (t, ^3J = 7.0 Hz, 3H, CH_3 of the NA moiety); 1.62 (tt, ^3J = 7.0 Hz and ^3J = 7.6 Hz, 2H, $CH_2\iota$); 2.36 (t, ^3J = 7.6 Hz, 2H, $CH_2\kappa$); 2.66 (s, 3H, CH_3 of the NA moiety); 4.47 (q, 2H, ^3J = 7.0 Hz, CH_2 of the NA moiety); 5.98 (s, 2H, O–CH_2–O); 7.26 (d, ^3J = 7.95 Hz, 1H, CH); 8.62 (d and s, ^3J = 7.95 Hz, 2H, CH). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ: 14.09 (CH_3); 15.21, 25.06 (CH_3 of the NA moiety); 22.66, 24.60, 29.04, 29.22, 29.30, 29.44, 29.57, 29.69, 31.89, 34.06 ($CH_2\alpha$–κ); 46.79 (CH_2 of the NA moiety); 79.46 (O–CH_2–O); 110.37, 148.57, 162.86 (C_q); 121.41, 136.94, 149.17 (CH); 163.22, 172.68 (COO); 174.61 (CO). UV ($CHCl_3$) λ_{max}: 260 nm (ε = 14426), λ_{max}: 336 nm (ε = 12527). MS (CI/NH_3) *m/z* 445 (MH^+), 233 ($MH^+ - H_2COCOC_{11}H_{23}$). Anal. ($C_{25}H_{36}N_2O_5$) C, H, N: calcd 67.54, 8.16, 6.30; found 67.78, 8.60, 6.04.

4.5.3. 3-[(Tetradecanoyloxy)methyl]1-ethyl-7-methyl-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylate (1d). This compound was synthesised from tetradecanoic acid (0.08 g, 0.35 mmol) and K_2CO_3 (0.07 g, 0.66 mmol) in dry DMF (2 mL). Compound **3** (0.1 g, 0.35 mmol) was added. The remaining residue was flash chromatographed on silica gel with diethyl ether to give **1d** (0.1 g, 60% yield) as a white solid.

^{1}H NMR ($CDCl_3$, 250 MHz) δ: 0.85 (t, ^3J = 6.5 Hz, 3H, CH_3); 1.20 (m, 20H, $CH_2\alpha$–κ); 1.48 (t, ^3J = 7.0 Hz, 3H, CH_3 of the NA moiety); 1.61 (tt, ^3J = 7.3 Hz and ^3J = 7.6 Hz, 2H, $CH_2\lambda$); 2.35 (t, ^3J = 7.3 Hz, 2H, $CH_2\mu$); 2.64 (s, 3H, CH_3 of the NA moiety); 4.47 (q, ^3J = 7.0 Hz, 2H, CH_2 of the NA moiety); 5.97 (s, 2H, O–CH_2–O); 7.25 (d, ^3J = 8.25 Hz, 1H, CH); 8.62 (d and s, ^3J = 8.25 Hz, 2H, CH). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ: 14.10 (CH_3); 15.20, 25.05 (CH_3 of the NA moiety); 22.66, 24.59, 29.03, 29.14, 29.22, 29.32, 29.43, 29.57, 29.62, 29.64, 31.90, 34.04 ($CH_2\alpha$–μ); 46.78 (CH_2 of the NA moiety); 79.45 (O–CH_2–O); 110.34, 148.55, 162.84 (C_q); 121.39, 136.90, 149.16 (CH); 163.19, 172.64 (COO); 174.57 (CO). UV ($CHCl_3$) λ_{max}: 260 nm (ε = 14124), λ_{max}: 336 nm (ε = 12180). MS (CI/NH_3) *m/z* 473 (MH^+), 233 ($MH^+ - H_2CO$-$COC_{13}H_{27}$). Anal. ($C_{25}H_{36}N_2O_5$·0.2C_8H_{18}) C, H, N: calcd 69.33, 8.87, 5.65; found 69.37, 9.06, 5.72.

4.5.4. 3-[(Hexadecanoyloxy)methyl]1-ethyl-7-methyl-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylate (1e). This compound was synthesised from hexadecanoic acid (0.091 g, 0.35 mmol) and K_2CO_3 (0.07 g, 0.66 mmol) in dry DMF (2 mL). Compound **3** (0.1 g, 0.35 mmol) was added. The remaining residue was flash chromatographed on silica gel with diethyl ether to give **1e** (0.08 g, 56% yield) as a white solid.

^{1}H NMR ($CDCl_3$, 250 MHz) δ: 0.86 (t, ^3J = 6.5 Hz, 3H, CH_3); 1.22 (m, 24H, $CH_2\alpha$–μ); 1.48 (t, ^3J = 7.0 Hz, 3H, CH_3 of the NA moiety); 1.62 (tt, ^3J = 7.0 Hz and ^3J = 7.6 Hz, 2H, $CH_2\nu$); 2.36 (t, ^3J = 7.6 Hz, 2H, $CH_2\zeta$); 2.65 (s, 3H, CH_3 of the NA moiety); 4.47 (q, ^3J = 7.0 Hz, 2H, CH_2 of the NA moiety); 5.98 (s, 2H, O–CH_2–O); 7.25 (d, ^3J = 8.3 Hz, 1H, CH); 8.62 (d and s, ^3J = 8.3 Hz, 2H, CH). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ: 14.11 (CH_3); 15.21, 25.06 (CH_3 of the NA moiety); 22.68, 24.60, 29.04, 29.23, 29.34, 29.45, 29.58, 29.64, 29.67, 31.91, 34.05 ($CH_2\alpha$–ζ); 46.79 (CH_2 of the NA moiety); 79.45 (O–CH_2–O); 110.35, 148.55, 162.85 (C_q); 121.40, 136.92, 149.16 (CH); 163.20, 172.66 (COO); 174.59 (CO). UV ($CHCl_3$) λ_{max}: 260 nm (ε = 15995), λ_{max}: 336 nm (ε = 13938). MS (CI/NH_3) *m/z* 205 (MH^+), 233 ($MH^+ - H_2COCOC_{15}H_{31}$). Anal. ($C_{29}H_{44}N_2O_5$·0.4$H_2O$) C, H, N: calcd 68.58, 8.89, 5.52; found 68.48, 8.88, 5.87.

4.5.5. 3-[(4-Heptylbenzoyloxy)methyl]1-ethyl-7-methyl-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylate (1f). This compound was synthesised from 4-heptylbenzoic acid (0.077 g, 0.35 mmol) and K_2CO_3 (0.06 g, 0.56 mmol) in dry DMF (2 mL). Compound **3** (0.1 g, 0.35 mmol) was added. The remaining residue was flash chromatographed on silica gel with dichloromethane/ethyl acetate/Et_3N (10:2:0.1; v/v/v) to give **1f** (0.094 g, 58% yield) as a white solid.

^{1}H NMR ($CDCl_3$, 250 MHz) δ: 0.85 (t, ^3J = 6.5 Hz, 3H, CH_3); 1.26 (m, 8H, $CH_2\alpha$–δ); 1.48 (t, ^3J = 7.3 Hz, 3H, CH_3 of the NA moiety); 1.6 (tt, ^3J = 6.7 Hz and ^3J = 7.6 Hz, 2H, $CH_2\varepsilon$); 2.65 (s and t, ^3J = 7.6 Hz, 5H, CH_3 of the NA moiety and $CH_2\zeta$); 4.48 (q, ^3J = 7.3 Hz, 2H, CH_2 of the NA moiety); 6.22 (s, 2H, O–CH_2–O); 7.21–7.26 (2d, $^3J_{CH(aro)}$ = $^3J_{CH(NA)}$ = 8.25 Hz, 3H, $CH_{(aro)}$ and CH); 7.99 (d, ^3J = 8.25 Hz, 2H, CH); 8.63 (d and s, ^3J = 8.25 Hz, 2H, CH). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ: 14.05 (CH_3); 15.19, 23.05 (CH_3 of the NA moiety); 22.60, 29.07, 29.15, 31.06, 31.73, 36.02 ($CH_2\alpha$–ζ); 46.81 (CH_2 of the NA moiety); 79.68 (O–CH_2–O); 121.37, 136.84, 149.23 (CH); 110.23, 149.35, 162.84 (C_q of the NA moiety); 126.50, 148.52 ($C_{q(aro)}$); 128.91, 130.16 ($CH_{(aro)}$);

162.94, 165.42 (COO); 174.66 (CO). UV ($CHCl_3$) λ_{max}: 260 nm (ε = 26640), λ_{max}: 336 nm (ε = 12800). MS (CI/NH_3) *m/z* 465 (MH^+), 233 ($MH^+-H_2COCOPhC_7H_{15}$). Anal. ($C_{27}H_{32}N_2O_5{\cdot}0.6H_2O$) C, H, N: calcd 68.22, 7.04, 5.89; found 68.29, 7.08, 6.06.

4.5.6. 3-[(4-Heptyloxybenzoyloxy)methyl]1-ethyl-7-methyl-4-oxo-1,4-dihydro-[1,8]naphthyridine-3-carboxylate (1g). This compound was synthesised from 4-heptyloxybenzoic acid (0.084 g, 0.35 mmol) and K_2CO_3 (0.06 g, 0.56 mmol) in dry DMF (2 mL). Compound **3** (0.1 g, 0.35 mmol) was added. The remaining residue was flash chromatographed on silica gel with petroleum ether/isopropanol (10:1; v/v) to give **1g** (0.11 g, 65% yield) as a white solid.

^{1}H NMR ($CDCl_3$, 250 MHz) δ: 0.85 (t, ^3J = 6.7 Hz, 3H, CH_3); 1.27 (m, 8H, $CH_2\alpha$–δ); 1.45 (t, ^3J = 7.3 Hz, 3H, CH_3 of the NA moiety); 1.75 (tt, ^3J = 6.4 Hz and ^3J = 7.6 Hz, 2H, $CH_2\varepsilon$); 2.62 (s, 3H, CH_3 of the NA moiety); 3.96 (t, ^3J = 6.4 Hz, 2H, $CH_2\zeta$); 4.44 (q, ^3J = 7.3 Hz, 2H, CH_2 of the NA moiety); 6.18 (s, 2H, O–CH_2–O); 6.85 (d, ^3J = 8.8 Hz, 2H, $CH_{(aro)}$); 7.21 (d, ^3J = 8.0 Hz, 1H, CH); 8.00 (d, ^3J = 8.8 Hz, 2H, $CH_{(aro)}$); 8.60 (d and s, ^3J = 8.0 Hz, 2H, CH). ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ: 14.04 (CH_3); 15.18, 25.04 (CH_3 of the NA moiety); 22.56, 25.90, 28.98, 29.05, 31.71, 68.25 ($CH_2\alpha$–ζ); 46.79 (CH_2 of the NA moiety); 79.59 (O–CH_2–O); 121.34, 136.84, 149.19 (CH); 121.34, 162.96 ($C_{q(aro)}$); 110.28, 148.52, 162.81 (C_q of the NA moiety); 163.52, 165.09 (COO); 174.66 (CO). UV ($CHCl_3$) λ_{max}: 260 nm (ε = 30260), λ_{max}: 336 nm (ε = 12100). MS (CI/NH_3) *m/z* 481 (MH^+), 233 ($MH^+-H_2COCOPhOC_7H_{15}$). Anal. ($C_{27}H_{32}N_2O_6{\cdot}1.2H_2O$) C, H, N: calcd 64.57, 6.85, 5.57; found 64.67, 6.66, 5.98.

4.6. Determination of silicone oil solubility

The SiO solutions containing the prodrugs were prepared as follows: an excess amount of each compound (0.015 mmol) was added to SiO (50 g). The suspensions were stirred for several days at 25 °C to reach equilibrium. At appropriate times between 1 and 15 days, an aliquot of the suspension (5 g) was removed, centrifuged for 10 min at 14,000 rpm and then filtered on 0.45 μm Millipore filter. The amount of prodrug solubilised in SiO (SiO + P) was then determined by UV spectrophotometry.

The molar extinction coefficient (ε) was first determined for each prodrug at two λ_{max} (260 and 336 nm). As it was not possible to prepare solutions of known concentration in SiO alone, the calibration was done in a mixture of SiO and chloroform. The SiO/$CHCl_3$ ratio was 25/75. The absorbance of (SiO + P) was then measured in the same conditions at λ_{max} of each prodrug (260 and 336 nm) and the concentration calculated from the Beer–Lambert expression.

4.7. Enzymatic hydrolysis

The synthesised compounds being insoluble in the buffer, suspensions of prodrug (0.015 g) were prepared in phosphate buffer (0.5 mL, 0.1 M, pH 7.4). Separately, the appropriate amount of commercial esterase was dissolved in phosphate buffer (0.5 mL, pH 7.4) to give a number of enzymatic units in a 6-fold excess for the hydrolysis of the two-ester functions of the prodrugs. The reaction was initiated by adding the pre-heated esterase solution to the prodrug suspension in a screw-capped vial placed in a shaking incubator at 37.0 ± 0.1 °C and let for 24 h. In order to stop the enzymatic hydrolysis, dichloromethane (1 mL) was added, the organic phase was separated and the aqueous one extracted with dichloromethane (3× 1 mL). The combined organic phases were dried over anhydrous Na_2SO_4, filtered and evaporated. The resulting residue was dissolved in deuterated chloroform (0.5 mL) and analysed by ^{1}H NMR at 250 MHz.

4.8. Microbiology

MICs were determined by broth dilution micromethod. Briefly, 100 μL of Trypcase soy broth (Biomérieux, France) was placed in each well of a 96-well microtiter plate. Hundred microlitres of the solutions to be tested (prepared in 10% DMSO) was added to the first column. Then 2-fold dilutions were carried out from one column to the next up to column 10. Columns 11 and 12 were used as a sterility control (without product and without bacteria) and a growth control (without product, with inoculum) of the medium. Fresh bacterial suspensions were prepared at 10^8cells/mL using standard strains (*Staphylococcus aureus* CIP 4.83, *Bacillus subtilis* CIP 52.62, *Escherichia coli* CIP 54127 and *Pseudomonas aeruginosa* CIP 82.118) obtained from the Institut Pasteur Collection (France). The microplates were inoculated using a multipoint inoculator (Denley) to obtain a final concentration of 10^6cells/mL. Plates were then incubated at 37 °C for 18–24 h under aerobic conditions. MIC (Minimal Inhibitory Concentration) was determined as the lowest concentration with no visible growth. Assays were performed in duplicate.

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