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Mucor miehei Lipase Catalyzed Transesterifications on Aromatic and Heteroaromatic Substrates. A General Survey§

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Abstract: An investigation on *Mucor miehei* lipase-catalyzed transesterifications of 16 aromatic and heteroaromatic esters in organic solvents is described. The points studied were the activity and regioselectivity of the enzyme-catalyzed reaction of either one or two ester groups situated in different positions on several heterocyclic systems with an aliphatic alcohol. The reactions took place in moderate to good yields and, in some cases, regioselectively.

INTRODUCTION

There are few references dealing with aromatic esters as acyl donors in the transesterification reactions in organic solvents catalyzed by lipases.[1] Although some of them are referred to activated esters,[2] they are generally presented as poor substrates.[3] This reaction could be a useful method to be applied in our line of podands and macrocycles containing heterocyclic rings, but no works have been published with examples of heteroaromatic esters. We have recently demonstrated that the *Mucor miehei* lipase (MML) regioselectively catalyzes the transesterification of 3,5-diethoxycarbonyl-pyrazole derivatives, producing good yields of new pyrazole esters with aliphatic and polyetheric chains.[4] In the search for the usefulness of the method in our line and as an extension of that work, we have now studied the transesterification of several aromatic and heteroaromatic mono- and diethylesters with n-octanol, catalyzed by MML[5] in an anhydrous organic solvent. The heteroaromatic systems constitute a heterogeneous group of compounds with strong differences among them.[6] The starting esters were selected to remark the possible distinct effects of the three most common heteroatoms, N, O and S, on the activity and regioselectivity of the reaction: all the heterocyclic systems studied were monoheteroatomic and the ethoxycarbonyl group was situated at different positions on the ring, 2-, 3-, and 4-pyridine (**1a-c**), 2- and 3-furan (**1d,e**), and 2-thiophene (**1f**). The influence of a second ethoxycarbonyl group was also investigated in all the diethyl pyridinedicarboxylates (2,3-, 2,4-, 2,5-, 2,6-, 3,4- and 3,5-; **3a-f**). Ethyl benzoate (**1g**) and the three diethyl phthalates (**3g-i**) were also studied. The

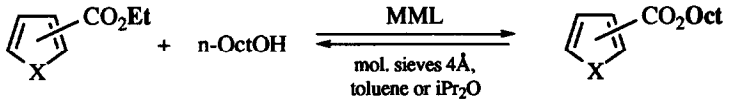
§ *Dedicated to Prof. Carlos Corral on the occasion of his 65th birthday.*

reactions were carried out in two anhydrous organic solvents, toluene and diisopropylether: they both are hydrophobic at an aproximately comparable order and have moderate dielectric constants (log P = 2.5 and 1.9; ε = 2.38 and 3.88 respectively) but diisopropylether can form hydrogen bonds whereas toluene cannot.

RESULTS AND DISCUSSION

MONOESTERS

Initially, the reactions were performed on the monoesters (Scheme 1) at analytical scale (see Experimental Part). Aliquots were withdrawn at 1, 3, 7, and 10 days and analyzed by gas chromatography.[7] In all the examples studied, parallel blank reactions without enzyme were allowed to react under the same experimental conditions; they were analyzed after 7 days.



1a-g **2a-g**

X = HC=N, O, S, HC=CH

a: 2-Pyridyl **b**: 3-Pyridyl **c**: 4-Pyridyl **d**: 2-Furyl **e**: 3-Furyl **f**: 2-Thienyl **g**: Phenyl

Scheme 1

In the blank reactions only one substrate reacted to a detectable degree, ethyl 2-pyridinecarboxylate **1a**, that was transesterified into the octylester in absence of the enzyme. This result is not surprising as the π-deficient pyridine system exerts a remarkable electron-withdrawing effect on 2- and 4-substituents whereas the influence on 3-substituents is significantly smaller.[8] However, this single effect does not seem to be strong enough to promote transesterification (both **1b** and **1c** remained unchanged) under the experimental conditions that we have used, as it only took place on the single substrate **1a** where the electron-withdrawing effect is combined with the chelating properties of the nitrogen pair of electrons which can play an important role on stabilizing a transition state in the nucleophilic substitution.[9] The solvent may also help this stabilization since, although conversions were significant in both solvents, it was slightly higher in diisopropylether (85%) than in toluene (79%). No conversion was detected at all in the rest of the blank reactions, either in toluene or in diisopropylether.

Besides **1a**, which reacted chemically, all the substrates underwent the enzyme-catalyzed reaction to a considerable extent in both solvents. There were some noteworthy features: all the substrates reacted faster in diisopropylether than in toluene (Table 1); there was moderate regioselective preference for 4-pyridyl (**1c**) over 3-pyridyl (**1c**) derivatives in toluene, but slight between 2- and 3-furyl esters (**1d** and **1e**). Both ethyl 2-thienylcarboxylate (**1f**) and ethyl benzoate (**1g**) afforded low conversion.

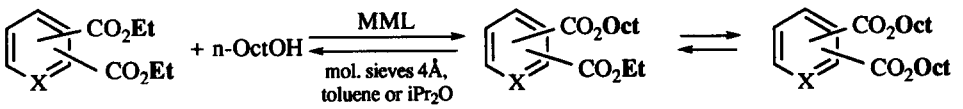
TABLE 1. Transesterification of monoesters **1a-g**. Conversions

Time (days)	**1a**[a]		**1b**		**1c**		**1d**		**1e**		**1f**		**1g**	
	Tol.	iPr_2O	Tol	iPr_2O	Tol.	iPr_2O	Tol.	iPr_2O	Tol.	iPr_2O	Tol.	iPr_2O	Tol.	iPr_2O
1	52	68	7	21	18	48	6	12	6	11	5	4	3	7
3	66	71	23	45	38	60	15	36	12	23	8	16	5	14
7	70	76	46	61	65	65	32	50	24	47	20	35	19	27
10	75	86	50	74	74	76	43	62	41	57	25	46	24	34

[a] The conversion of this product cannot be entirely attributable to enzymatic catalysis

DIESTERS

The diesters (**3a-i**) were subjected to the same experimental conditions as the monoesters (**1a-g**) except that the concentration of n-octanol was doubled. In this case it was a two-steps reaction through a mixed intermediate diester (Scheme 2). Aliquots were also withdrawn after 1, 3, 7, and 10 days, and parallel blank reactions were analyzed after 10 days (Table 2).



3a-i **4a-i** **5a-i**

X = N, CH.

a: 2,3-Pyridinediyl **b**: 2,4-Pyridinediyl **c**: 2,5-Pyridinediyl **d**: 2,6-Pyridinediyl **e**: 3,4-Pyridinediyl **f**: 3,5-Pyridinediyl **g**: *o*-Phenylene **h**: *m*-Phenylene **i**: *p*-Phenylene

Scheme 2

In the blank reactions, only the ethoxycarbonyl group attached on the 2-position of the pyridine ring readily changed into the octylester in absence of the enzyme (Table 2), producing monosubstituted products devoid of disubstituted ones. This is also true for 2,6-diethyl pyridinedicarboxylate (**3d**): though 2- and 6-positions are equivalents, the blank reaction, surprisingly, afforded only monosubstituted 2-ethyl-6-octyl diester (**4d**) while 2,6-dioctylester was not detected. Moreover, whereas 2,3-, 2,4-, and 2,5-diethyl pyridinedicarboxylate (**3a**, **3b**, and **3c**, respectively) displayed similar levels of conversion in the blank reactions, the corresponding 2,6-derivative (**3d**) appeared significantly lower in both solvents: we suspect that in the first step of the chemical reaction, 2- and 6-ethoxycarbonyl groups interfere each other and the substitution is more difficult, and then bulky octylester absolutely prevents the second nucleophilic attack.

However, when the same reaction was carried out with the enzyme, a great amount of 2,6-dioctyl ester (**5d**) was obtained: 65% in toluene and 78% in diisopropylether.

TABLE 2. Transesterifications on diesters **3a-i**. Conversion after 10 days

	Toluene			Diisopropylether		
Initial substrate	Monosust **4a-i**	Disust **5a-i**	Blank[a,b]	Monosust **4a-i**	Disust **5a-i**	Blank[a,b]
3a	13[c]	n.d.	75	19[c]	n.d.	90
3b	10[d]	84	71	15[d]	80	83
3c	45[d]	49	67	20[d]	75	84
3d	16	65	40	15	78	47
3e	4[d]	n.d.	n.d.	22[d]	12	n.d.
3f	25	73	n.d.	18	78	n.d.
3g	6	n.d.	n.d.	12	n.d.	n.d.
3h	45	20	n.d.	16	81	n.d.
3i	62	9	n.d.	30	66	n.d.

n.d.: not detected; [a] measured after 7 days; [b] all the products obtained, including those from **3d**, are monosubstituted; [c] only one regioisomer detected; [d] mixture of regioisomers

There is hardly any difference between the chemical reactivity of 2-ethoxycarbonyl and the enzymatic reactivity of 4-ethoxycarbonyl groups in **3b** (Fig. 1): 2- and 4-monosubstituted products (**4b** and **4b'**) are initially formed at a similar rate from different routes (mostly chemical and enzymatic, respectively) and, as they have been formed, both readily react at nearly identical speeds in interchanged ways (enzymatic and mostly chemical, respectively) to yield the dioctyl ester (**5b**). The combined chemo- and enzymatic regioselectivity could be useful in obtaining mixed 2,5-pyridyl diesters (Fig. 2): whereas the 2-monosubstituted (**4c**) reaches its peak conversion (74 %) in toluene in 1 day and then slowly transforms into the disubstituted (**5c**), the less favoured 5-monosubstituted (**4c'**) always remains below 10 % as the little amount formed is quickly changed into the disubstituted diester (**5c**), keeping roughly a constant regioisomer ratio of 92:8 (**4c**:**4c'**). Good conversions into the monosubstituted mixed diesters can also be found with 2,6-diethyl pyridinedicarboxylate, **3d** (peak conversion into 2-ethyl-6-octyl **4d**, 52 % at 1 day) and the 3,5-diethyl pyridinedicarboxylate, **3f** (peak conversion into 3-ethyl-5-octyl **4f**, 48 % at 3 days), both in toluene.

The proximity of two ethoxycarbonyl groups dramatically influenced the enzymatic activity as can be seen in Table 2, since the reactions of 2,3- and 3,4-diethyl pyridinedicarboxylate (**3a** and **3e**) and diethyl phthalate (**3g**) are strongly inhibited compared with other substrates. Moreover, the 3,4-pyridyl monosubstituted product (**4e**, **4e'**) is a mixture 14:86 (GC) of both regioisomers; regioselective results given above and contrast with ^{1}H NMR spectra of 2,4- and 2,5-pyridyl derivatives point out that the major regioisomer is 3-ethyl-4-octyl pyridinedicarboxylate (**4e'**). However, it could not be separated by any means from minor 3-octyl-4-ethyl analog (**4e**) and were analyzed jointly. The transesterification of 2,3-diethyl pyridinedicarboxylate (**3a**) afforded new and unexpected results when the conversion appeared much lower

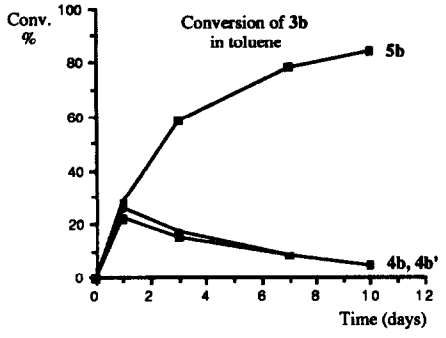


Figure 1

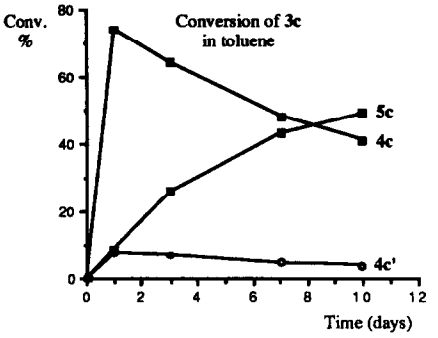


Figure 2

with than without the enzyme (Table 2)[10] as if MML could inhibit the chemical process. We hypothesized that the combined effects of high electron-density and strong chelating properties of contiguous N atom and two ethoxycarbonyl groups could reversibly bind the substrate to the protein molecule, making the approach of nucleophiles troublesome and reducing temporarily the activity of the enzyme by the expected conformational changes. This idea was confirmed when we found that the initial rate of transesterification of 4-ethyl pyridinecarboxylate **1c** (20 mM, see Table 1) decreased up to 60 % when it was carried out in diisopropylether with 2,3-diethyl pyridinedicarboxylate **3a** (20 mM) also present in the solution. Moreover after 10 days of reaction, the recovered enzyme of reaction from 2,3-diethyl pyridinedicarboxylate (**3a**), washed with fresh dry solvent and dried, displayed the same remaining activity (84 %) that the enzyme coming from other reactions.[7]

As above, the reactions of diesters also took place a little slower in toluene than in diisopropylether, specially when referred to phenyl and 3-pyridyl ethoxycarbonyl derivatives (Table 2). This kinetic difference would make toluene the solvent of choice if the reactions were carried out for regioselective synthetic purposes or to get the highest yield of monosubstituted (mixed diesters) *vs.* disubstituted products. The most outstanding example of this difference was diethyl terephthalate (**3i**): the reaction in toluene (Fig. 3) occurred slowly and the monosubstituted ethyloctyldiester (**4i**) transformed even at a slower speed into the dioctylester

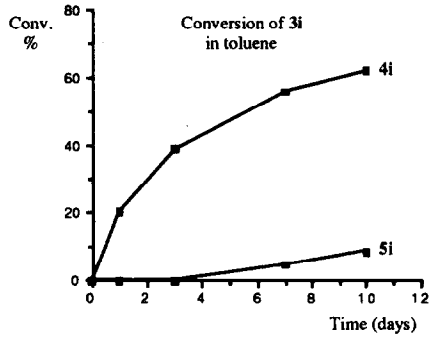


Figure 3

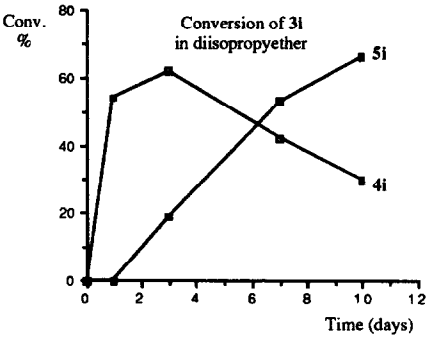


Figure 4

(**5i**), while it happened quicker in diisopropylether (Fig. 4) since the peak conversion of the monosubstituted product (**4i**) was reached in 2-3 days and then decreased on transforming into the dioctylester (**5i**). The most similarly shaped curves in both solvents were obtained with 2,6- and 3,5-diethyl pyridinedicarboxylate (**3d** and **3f**) and diethyl isophthalate (**3h**), although significant differences were observed in all the substrates.

We have carried out all the reactions at preparative scale of 1 g for the purpose of obtaining the products and confirming their structures. As the aim of this work is not synthetic, yields of isolated products were not optimized and are not given. The structural assignements were made comparing ^{1}H NMR spectra of mixed ethyl-octyl esters with those of diethyl and dioctyl esters, and taking into account two features: (i) The triplet from the octyl group ($CO_2CH_2(CH_2)_6CH_3$) appears to a higher field (about 0.10 ppm) than the triplet from the ethyl group ($CO_2CH_2CH_3$). (ii) The pyridinic nitrogen atom exerts an electron-withdrawing effect on the substituents, displacing the signals to lower field, $\delta(\alpha)>\delta(\gamma)>\delta(\beta)$[11] although this effect is weaker than the former. The spectroscopic data confirmed the expected structures and the regioselectivity of the reaction.

CONCLUSIONS

In this paper we have shown that esters of aromatic and heteroaromatic carboxylic acids undergo *Mucor meihei* lipase catalyzed transesterification in moderate to good yields, so the procedure could be synthetically useful. In general, the reactions take place slower in toluene than in diisopropylether. Derivatives of pyridine show the highest reactivity, followed by furan ones and, at the end, by thiophene and benzene esters which react at a slow rate. A remarkable chemical conversion has been detected in all 2-pyridine ethoxycarbonyl groups while not at all in the rest of substrates. In the pyridinic diesters, a chemo-enzymatic regioselectivity appears in 2- and 4- *vs.* 3-position; this could be helpful to obtain mixed esters. Good conversions into the monosubstituted mixed diesters could also be obtained with 2,6- and 3,5-diethyl pyridinedicarboxylate, using toluene as solvent. The reaction is strongly inhibited by a contiguous second group.

EXPERIMENTAL

All the starting monoesters and some diesters (3,4-diethyl pyridinedicarboxylate **3e** and diethyl phthalate **3g**) were commercially available products (Aldrich) and were used without any purification; the rest of diesters were synthesized following a standard procedure.[12] GC Hewlett-Packard apparatus was equipped with a 25 m capillary column of phenylmethyl silicone. Chromatographic separations were performed on silica gel, using flash column chromatography (on Kieselgel 60 Merck of 230-400 mesh) and preparative centrifugal circular thin layer chromatography (cctlc, on a circular plate coated with a 1 mm layer of Kieselgel 60 PF_{254} gipshaltig, purchased from Merck, using a Chromatotron®). Compounds were detected with UV light (λ:254 nm). NMR spectra were recorded with Varian XL-300 or Gemini-200 spectrometers. Diisopropylether and toluene were both refluxed on sodium wire, distilled and stored on molecular sieves 4Å before using. Microanalytical and spectroscopic data of not previously described products are given; data of described compounds have been omitted but they are in accordance with the expected structures.

GENERAL PROCEDURE

Lipozyme (20 mg/ml) and molecular sieves 4Å powder (20 mg/ml) were added to a solution of acyl donor (20 mM) and n-octanol (100 mM when monoesters **1a-g** and 200 mM when diesters **3a-i**). The reaction vessel was sealed and stirred in a rotary evaporator while heated at 60°C in a silicone bath.

Analytical scale.- The reactions were carried out in 2 ml vials containing 1.5 ml of the reaction mixture in the two dry solvents toluene and diisopropylether. Aliquots were withdrawn after 1, 3, 7 and 10 days and analyzed by GC.

Preparative scale.- The reactions were carried out in 1 L round-bottom flasks containing 300 ml of the reaction mixture, using only dry diisopropylether as solvent, and they were periodically analyzed by GC in order to stop them when stabilized or at the maximum of some desired intermediate mixed diester such as **4c**. The reactions were stopped by cooling them at room temperatures and filtering the enzyme and sieve powder off. The clear solution was evaporated to dryness and the excess of n-octanol was azeotropically removed by destillation with water in vacuum (bp of the azeotrope: 99°C) and the final residue chromatographied.

2-Octyl-3-ethyl pyridinedicarboxylate (**4a**).^{1}H NMR ($CDCl_3$): 8.70 (d,1H, $J_{5,6}$=4.7 Hz, H_6-pyridine), 8.14 (d, 1H, $J_{4,5}$=7.1 Hz, H_4-pyridine), 7.43 (dd, 1H, $J_{4,5}$=7.1 Hz, $J_{5,6}$=4.7 Hz, H_5-pyridine), 4.34 (t, 2H, J=6.6 Hz, $CO_2CH_2CH_2$-), 4.33 (q, 2H, J=7.3 Hz, $CO_2CH_2CH_3$), 1.72 (quint, 2H, J=6.6 Hz, $CO_2CH_2CH_2$-), 1.32 (t, 2H, J= 7.3 Hz, $CO_2CH_2CH_3$), 1.21 (m, 10H, rest of CH_2), 0.81 (t, 3H, J=6.6 Hz, $(CH_2)_7CH_3$). ^{13}C NMR ($CDCl_3$): 166.35, 165.06, 151.63, 151.30, 137.54, 126.12, 124.52, 66.34, 61.93, 31.64, 29.24, 29.09, 28.34, 25.75, 22.50, 13.94. Anal. Calcd. for $C_{17}H_{25}NO_4$: C, 66.45; H, 8.14; N, 4.56. Found: C, 66.35; H,8.01; N, 4.70.

2-Octyl-4-ethyl pyridinedicarboxylate (**4b**). ^{1}H NMR ($CDCl_3$): 8.90 (d, 1H, $J_{5,6}$=4.6 Hz, H_6-pyridine), 8.61 (s, 1H, H_3-pyridine), 8.01 (d, 1H, $J_{5,6}$=4.6 Hz, H_5-pyridine), 4.43 (q, 2H, J=7.0 Hz, $CO_2CH_2CH_3$), 4.36 (t, 2H, J=6.9 Hz, $CO_2CH_2CH_2$-), 1.82 (quint, 2H, J=6.9 Hz, $CO_2CH_2CH_2$-), 1.42 (t, 3H, J=7.0 Hz, $CO_2CH_2CH_3$), 1.27 (m, 10H, rest of CH_2), 0.86 (t, 3H, J=6.9 Hz, $(CH_2)_7CH_3$). ^{13}C NMR ($CDCl_3$): 164.56, 164.33, 150.69, 149.28, 138.99, 125.93, 125.80, 66.44, 62.19, 31.74, 29.19, 29.14, 28.61, 25.85, 22.61, 14.17, 14.07. Anal.Calcd. for $C_{17}H_{25}NO_4$: C, 66.45; H, 8.14; N, 4.56. Found: C, 66.58; H, 8.40; N, 4.49.

2-Ethyl-4-octyl pyridinedicarboxylate (**4b'**).^{1}H NMR ($CDCl_3$): 8.90 (d, 1H, $J_{5,6}$=4.9 Hz, H_6-pyridine), 8.62 (s, 1H, H_3-pyridine), 8.02 (d, 1H, $J_{5,6}$=4.9 Hz, H_5-pyridine), 4.50 (q, 2H, J=7.1 Hz, $CO_2CH_2CH_3$), 4.37 (t, 2H, J=6.7 Hz, $CO_2CH_2CH_2$-), 1.78 (quint, 2H, J=6.7 Hz, $CO_2CH_2CH_2$-), 1.45 (t, 3H, J=7.1Hz, $CO_2CH_2CH_3$), 1.24 (m, 10H, rest of CH_2), 0.87 (t, 3H, J=6.7 Hz, $(CH_2)_7CH_3$). ^{13}C NMR ($CDCl_3$): 164.58, 164.37, 150.65, 149.20, 139.20, 125.95, 124.28, 66.35, 62.27, 31.73, 29.12, 28.53, 25.90, 22.60, 14.29, 14.05. Anal. Calcd. for $C_{17}H_{25}NO_4$: C, 66.45; H, 8.14; N, 4.56. Found: C, 66.58; H, 8.40; N, 4.49.

2,4-Dioctyl pyridinedicarboxylate (**5b**). ^{1}H NMR ($CDCl_3$): 8.87 (dd, 1H, $J_{5,6}$=4.9 Hz, $J_{3,6}$=0.8 Hz, H_6-pyridine), 8.59 (dd, 1H, $J_{3,5}$=1.6 Hz, $J_{3,6}$=0.8 Hz, H_3-pyridine), 7.99 (dd, 1H, $J_{5,6}$=4.9 Hz, $J_{3,5}$=1.6 Hz, H_5-pyridine), 4.40 (t, 2H, J=6.8 Hz, $CO_2CH_2CH_2$-), 4.35 (t, 2H, J=6.8 Hz, $CO_2CH_2CH_2$-), 1.77 (quint, 4H, J=6.8 Hz, $CO_2CH_2CH_2$-), 1.25 (m, 20H, rest of CH_2), 0.84 (t, 6H, J=6.8 Hz, CH_3). ^{13}C NMR ($CDCl_3$): 164.54, 164.29, 150.61, 149.29, 138.99, 125.80, 124.17, 66.30, 66.23, 31.66, 29.09, 29.05,

28.56, 28.46, 25.83, 25.78, 22.51, 13.96. Anal. Calcd for $C_{23}H_{37}NO_4$: C, 70.59; H, 9.46; N, 3.58. Found:C, 70.31; H, 9.18; N,3.35.

2-Octyl-5-ethyl pyridinedicarboxylate (**4c**). ^{1}H NMR ($CDCl_3$): 9.28 (s, 1H, H_6-pyridine), 8.39 (d, 1H, $J_{3,4}$=8.2 Hz, H_4-pyridine), 8.15 (d, 1H, $J_{3,4}$=8.2 Hz, H_3-pyridine), 4.41 (q, 2H, J=6.9 Hz, $CO_2CH_2CH_2$), 4.34 (t, 2H, J=6.7 Hz, $CO_2CH_2CH_3$), 1.77 (quint, 2H, J=6.7 Hz, $CO_2CH_2CH_2$), 1.39 (t, 3H, J=6.9 Hz, $CO_2CH_2CH_3$), 1.23 (m, 10H, rest of CH_2), 0.83 (t, 3H, J=6.7 Hz, $(CH_2)_7CH_3$). ^{13}C NMR ($CDCl_3$): 164.97, 164.88, 151.60, 151.27, 138.61, 129.16, 125.01, 66.95, 62.34, 32.20, 29.60, 29.05, 26.32, 23.07, 14.68, 14.53. Anal. Calcd. for $C_{17}H_{25}NO_4$: C, 66.45; H, 8.14; N, 4.56. Found: C, 66.25; H, 7.89; N, 4.64

2-Ethyl-6-octyl pyridinedicarboxylate (**4d**). ^{1}H NMR ($CDCl_3$): 8.21 (d, 1H, J=7.8 Hz, H_3-pyridine), 8.19 (d, 1H, J=7.8 Hz, H_5-pyridine), 7.94 (t, 1H, J=7.8 Hz, H_4-pyridine), 4.38 (q, 2H, J=7.0 Hz, $CO_2CH_2CH_3$), 4.34 (t, 2H, J=7.0 Hz, $CO_2CH_2CH_2$), 1.75 (quint, 2H, J=7.0 Hz, $CO_2CH_2CH_2$), 1.38 (t, 3H, J=7.0 Hz, $CO_2CH_2CH_3$), 1.21 (m, 10H, rest of CH_2), 0.79 (t, 3H, J=7.0 Hz, $(CH_2)_7CH_3$). ^{13}C NMR ($CDCl_3$): 164.51, 164.46, 148.53, 148.49, 138.08, 127.63, 66.27, 62.14, 31.63, 29.10, 29.03, 28.42, 25.77, 22.49, 14.07, 13.94. Anal. Calcd. for $C_{17}H_{25}NO_4$: C, 66.45; H, 8.14; N, 4.56. Found: C, 66.27; H, 8.30; N, 4.60.

2,6-Dioctyl pyridinedicarboxylate (**5d**). ^{1}H NMR ($CDCl_3$): 8.22 (d, 2H, J=7.7 Hz, $H_{3,5}$-pyridine), 7.96 (t, 1H, J=7.7 Hz, H_4-pyridine), 4.37 (t, 4H, J=7.0 Hz, $CO_2CH_2CH_2$), 1.79 (quint, 4H, J=7.0 Hz, $CO_2CH_2CH_2$), 1.30 (m, 20H, rest of CH_2), 0.83 (t, 6H, J=7.0 Hz, CH_3). ^{13}C NMR ($CDCl_3$): 164.58, 148.62, 138.05, 127.62, 66.30, 31.71, 29.17, 29.11, 28.49, 25.83, 22.57, 14.01. Anal. Calcd. for $C_{23}H_{37}NO_4$: C, 70.59; H, 9.46; N, 3.58. Found: C, 70.10; H, 9.65; N, 3.41.

Mixture of 3-octyl-4-ethyl and 3-ethyl-4-octyl pyridinedicarboxylate (**4e**, **4e'**). ^{1}H NMR ($CDCl_3$): 8.99 (s, 1H, H_2-pyridine), 8.74 (d, 1H, J=5.1 Hz, H_6-pyridine), 7.42 (d, 1H, J=5.1 Hz, H_5-pyridine), 4.34 (q, J=7.1 Hz, $CO_2CH_2CH_3$: 3-octyl), 4.33 (q, J=7.1Hz, $CO_2CH_2CH_3$: 4-octyl), 4.27 (t, J=6.7 Hz, $CO_2CH_2CH_2$: 4-octyl), 4.26 (t, J=6.7Hz, $CO_2CH_2CH_2$: 3-octyl), 1.67 (m, 2H, $CO_2CH_2CH_2$-), 1.31 (t, J=7.1 Hz, $CO_2CH_2CH_3$: 4-octyl), 1.30 (t, J=7.1 Hz, $CO_2CH_2CH_3$: 3-octyl), 1.20 (m, 10H, rest of CH_2), 0.80 (t, 3H, J=6.7Hz, $(CH_2)_7CH_3$). ^{13}C NMR ($CDCl_3$): 166.17, 165.02, 152.55, 150.25, 140.39, 125.16, 121.59, 66.30, 66.01, 62.11, 61.83, 31.55, 28.96, 28.30, 28.19, 25.68, 22.42, 13.87, 13.79. Anal. Calcd. for $C_{17}H_{25}NO_4$: C, 66.45; H, 8.14; N, 4.56. Found: C, 66.45; H, 7.98; N, 4.35.

3,4-Dioctyl pyridinedicarboxylate (**5e**). ^{1}H NMR ($CDCl_3$): 8.99 (s, 1H, H_2-pyridine), 8.75 (d, 1H, J=5.0 Hz, H_6-pyridine), 7.42 (d, 1H, J=5.0 Hz, H_5-pyridine), 4.27 (t, 2H, J=6.7 Hz, $CO_2CH_2CH_2$-), 4.26 (t, 2H, J=6.7 Hz, $CO_2CH_2CH_2$-), 1.67 (m, 4H, $CO_2CH_2CH_2$-), 1.29 (m, 20H, rest of CH_2), 0.80 (t, 6H, J=6.7 Hz, CH_3). ^{13}C NMR ($CDCl_3$): 166.37, 165.26, 152.70, 150.48, 140.62, 125.65, 121.80, 66.54, 66.25, 31.75, 29.15, 28.52, 28.42, 25.89, 22.60, 14.03. Anal. Calcd. for $C_{23}H_{37}NO_4$: C, 70.59; H, 9.46; N, 3.58. Found: C, 70.71; H, 9.70; N, 3.28.

3-Ethyl-5-octyl pyridinedicarboxylate (**4f**). ^{1}H NMR ($CDCl_3$): 9.31 (s, 2H, $H_{2,6}$-pyridine), 8.81 (s, 1H, H_4-pyridine), 4.41 (q, 2H, J=7.1 Hz, $CO_2CH_2CH_3$), 4.34 (t, 2H, J=6.6 Hz, $CO_2CH_2CH_2$), 1.75 (quint, 2H, J=6.6 Hz, $CO_2CH_2CH_2$), 1.39 (t, 3H, J=7.1 Hz, $CO_2CH_2CH_3$), 1.24 (m, 10H, rest of CH_2), 0.82 (t, 3H, J=6.6 Hz, $(CH_2)_7CH_3$). ^{13}C NMR ($CDCl_3$): 164.45, 164.38, 154.01, 137.60, 126.22, 65.88,

61.70, 31.66, 29.10, 29.06, 28.54, 25.86, 22.51, 14.15, 13.94. Anal. Calcd. for $C_{17}H_{25}NO_4$: C, 66.45; H, 8.14; N, 4.56. Found: C, 66.70; H, 8.31; N, 4.79.

3,5-Dioctyl pyridinedicarboxylate (**5f**). ^{1}H NMR ($CDCl_3$): 9.32 (s, 2H, $H_{2,6}$-pyridine), 8.81 (s, 1H, H_4-pyridine), 4.34 (t, 4H, J=6.6 Hz, $CO_2CH_2CH_2$), 1.76 (quint, 4H, J=6.6 Hz, $CO_2CH_2CH_2$), 1.32 (m, 20H, rest of CH_2), 0.84 (t, 6H, J=6.6Hz, CH_3). ^{13}C NMR ($CDCl_3$): 164.48, 154.04, 137.85, 126.28, 65.91, 31.70, 29.13, 29.10, 28.57, 25.90, 22.55, 13.98. Anal. Calcd. for $C_{23}H_{37}NO_4$: C, 70.59; H, 9.46; N, 3.58. Found: C, 70.70; H, 9.70; N, 3.70.

1-Octyl-3-ethyl benzenedicarboxylate (**4h**). ^{1}H NMR ($CDCl_3$): 8.67 (t, 1H, $J_{2,4}=J_{2,6}=1.7$ Hz, H_2-benzene), 8.21 (dd, 2H, $J_{2,4}=1.7$ Hz, $J_{4,5}=7.8$ Hz, $H_{4,6}$-benzene), 7.51 (t, 1H, $J_{4,5}=J_{5,6}=7.8$ Hz, H_5-benzene), 4.40 (q, 2H, J=7.1 Hz, $CO_2CH_2CH_3$), 4.33 (t, 2H, J=6.7 Hz, $CO_2CH_2CH_2$), 1.78 (quint, 2H, J=6.7 Hz, $CO_2CH_2CH_2$), 1.40 (t, 3H, J=7.1 Hz, $CO_2CH_2CH_3$), 1.28 (m, 10H, rest of -CH_2-), 0.87 (t, 3H, J=6.7 Hz, $(CH_2)_7CH_3$). ^{13}C NMR ($CDCl_3$): 165.59, 133.43, 130.89, 130.46, 128.29, 65.31, 61.08, 31.61, 29.02, 28.58, 25.86, 22.44, 14.12, 13.83. Anal. Calcd for $C_{18}H_{26}O_4$: C, 70.59; H, 8.49. Found: C, 70.18; H, 8.43.

1-Octyl-4-ethyl benzenedicarboxylate (**4i**). ^{1}H NMR ($CDCl_3$): 8.07 (s, 4H, aromatics), 4.37 (q, 2H, J=7.1 Hz, $CO_2CH_2CH_3$), 4.30 (t, 2H, J=6.6 Hz, $CO_2CH_2CH_2$-), 1.75 (quint, 2H, J=6.6 Hz, $CO_2CH_2CH_2$-), 1.38 (t, 3H, J=7.1 Hz, $CO_2CH_2CH_3$), 1.26 (m, 10H, rest of CH_2), 0.85 (t, 3H, J=6.6 Hz, $(CH_2)_7CH_3$). ^{13}C NMR ($CDCl_3$): 165.87, 165.81, 134.16, 134.11, 129.43, 65.56, 61.35, 31.74, 29.17, 29.15, 28.61, 25.97, 22.59, 14.23, 14.04. Anal. Calcd for $C_{18}H_{26}O_4$: C, 70.59; H, 8.49; Found: C, 70.35; H, 8.31.

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