Cent. Eur. J. Chem. • 9(1) • 2011 • 143-148
DOI: 10.2478/s11532-010-0132-x

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Central European Journal of **Chemistry**

Use of pyridinium ionic liquids as catalysts for the synthesis of 3,5-bis(dodecyloxycarbonyl)-1,4-dihydropyridine derivative

Research Article

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Received 29 July 2010; Accepted 19 October 2010

Abstract: The synthesis of cationic amphiphilic 1,4-dihydropyridine derivative, potential gene delivery agent is achieved *via* an efficient multi-step sequence. The key step of this approach is a two-component Hantzsch type cyclisation of 3-oxo-2-[1-phenylmethylidene]-butyric acid dodecyl ester and 3-amino-but-2-enoic acid dodecyl ester utilising bis(2-hydroxyethyl)ether as a solvent and 1-butyl-4-methylpyridinium chloride as a catalyst. The 1,4-dihydropyridine derivative with long alkyl ester chains at positions 3 and 5 of the 1,4-DHP ring - 3,5-bis(dodecyloxycarbonyl)-2,6-dimethyl-4-phenyl-1,4-dihydropyridine was obtained in substantially higher yield with respect to classical Hantzsch synthesis. Bromination of this compound followed by nucleophilic substitution of bromine with pyridine gave the desired cationic amphiphilic 1,4-dihydropyridine.

Keywords: *Hantzsch synthesis • 1,4-dihydropyridine • Ionic liquid • Catalysis • 1-butyl-4-methylpyridinium chloride*

1. Introduction

Ionic liquids (ILs) today have been applied in different ways, for example in catalysis, *i.e.*, as the catalyst itself, as a co-catalyst or catalyst activator, as the source of a new ligand for a catalytic metal centre, or just as the solvent for the reaction [1-3]. Usually melting points of ILs are below 100°C, which can be achieved by incorporating a bulky asymmetric cation into the structure together with a weakly coordinating anion [4]. Immiscibility of ILs with a number of common organic solvents and substances is used to create environmentally friendly two-phase systems for many synthetic purposes, allowing easy separation of products from a reaction mixture and recovery of the catalyst [5].

In recent years, a number of strategies for the synthesis of 1,4-dihydropyridine (1,4-DHP) derivatives have been developed due to the fact that the 1,4-DHP scaffold is a common component of pharmacologically active molecules, which possess a variety of biological activities [6]. Several new efficient methods of 1,4-DHP synthesis in ILs have been also reported [7]. In recent years, catalytic effects of imidazolium-based ILs on 1,4-DHP synthesis [8,9] were investigated. As phase transfer catalyst tetrabutylammonium sulfate was used for the synthesis of glycosyl 1,4-DHPs in diethylene glycol in almost quantitative yields [10]. Currently, much attention is paid to organic reactions catalysed by ILs [11-14]. When pyridinium derived ILs are applied many problems related with use of imidazolium ILs, such as side product formation due to the relatively acidic nature of the C2 hydrogen of the imidazolium ring can be avoided or minimised [12]. Several pyridinium ILs were successfully applied as catalysts in different reactions, for example, in the synthesis of 1,4-dibromonaphthalene [13], Fischer esterifications of long chain aliphatic acids [11], for the *tert*-butylation of phenol and esterifications of cyclic olefins with acetic acid [14], enantioselective Michael addition of cyclic ketones to nitroalkenes [12]. 1-Butyl-4-methylpyridinium chloride (4-MBPy) has found applications in enzymatic hydrolysis of cycloalkyl acetates as co-solvents in an aqueous system or acetonitrile [15]. 2-Chloromethylpyridinium iodide (2-ClMPy) has been used as activating Mukaiyama reagent for esterification and amide formation [16].

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Our research group has reported that cationic amphiphiles comprising 1,4-dihydropyridine cycle are good candidates for the development of new gene delivery systems [17]. One of the most potent and promising compounds with high transfection efficiency *in vitro* is a synthetic cationic lipid – 1,1′-{[3,5-bis(dodecyloxycarbonyl)-4-phenyl-1,4-dihydropyridine-2,6-diyl]dimethylene} bispyridinium dibromide (**6**) [17,18]. In this paper, we report an effective synthetic approach to amphiphilic 1,4-DHP **6**, the key step of which comprises a two-component Hantzsch-type cyclisation using pyridinium ionic liquid as a catalyst, proceeding in a substantially higher yields with respect to classical Hantzsch synthesis.

2. Experimental Procedure

2.1. General procedures

All reagents were purchased from Acros and used without further purification. TLC was performed on Silica gel 60 F254 Aluminium sheets 20×20 cm (Merck). ^{1}H NMR spectra were recorded with a Varian Mercury (200 MHz or 400 MHz) spectrometer. ^{13}C NMR spectra were recorded with a Varian Mercury (100 MHz) spectrometer. Chemical shifts are reported in ppm relative to hexamethyldisiloxane (δ 0.055). Multiplicities are abbreviated as: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. The coupling constants are expressed in Hertz. Alliance Waters 2695 HPLC system connected to a Waters 3100 mass detector operating in the ESI positive or negative ion mode on a Waters Xbridge C18 column (5 µm, 2.1×50 mm) using a gradient elution with methanol/formic acid (0.1% in water). Infrared spectra were recorded with a FTIR spectrometer Prestige-21 (Shimadzu). Melting points were determined on an OptiMelt (SRS Stanford Research Systems). Elemental analyses were determined on an EA 1106 (Carlo Erba Instruments).

2.2. Synthesis of compounds 4-6

2.2.1. 3,5-Bis(dodecyloxycarbonyl)-2,6-dimethyl-4-phenyl-1,4-dihydropyridine (4)

Method A. To a solution of 3-oxo-2-[1-phenylmethylidene]-butyric acid dodecyl ester **3** (360 mg, 1.00 mmol) and 3-amino-but-2-enoic acid dodecyl ester **2** (270 mg, 1.00 mmol) in bis(2-hydroxyethyl)ether (5.0 mL) 1-butyl-4-methylpyridinium chloride (18 mg, 0.10 mmol) was added and the reaction mixture was heated at 80°C for 8 h. Then the reaction mixture was poured into ice water and extracted with ethylacetate (3×20 mL). The combined organic layers were dried with anhydrous $MgSO_4$ and the solvent was removed *in vacuo*. The residue was crystallised from ethanol giving compound **4** as a white powder (440 mg, 72%), mp 55-56°C. ^{1}H NMR spectrum ($CDCl_3$, 200 MHz): δ 0.88 (t, 6H, *J*=6.6), 1.21-1.30 (m, 36H), 1.51-1.64 (m, 4H), 2.33 (s, 6H), 4.02 (t, 4H, *J*=6.6), 4.99 (s, 1H), 5.59 (br s, 1H), 7.10-7.30 (m, 5H); ^{13}C NMR spectrum ($CDCl_3$, 100 MHz): δ 14.09, 19.61, 22.67, 26.08, 28.69, 29.30, 29.34, 29.55, 29.61, 29.63, 29.66, 31.90, 39.49, 63.93, 104.26, 126.08, 127.83, 127.87, 143.76, 147.64, 167.62; IR (film) 3339, 1700, 1653 cm^{-1}; MS(-ESI) *m/z* (relative intensity) 608 ([M-H]$^+$, 100). Anal. Calcd. for $C_{39}H_{63}NO_4$: C, 76.80; H, 10.41; N, 2.30. Found: C, 76.78; H, 10.50; N, 2.27.

Method B. A solution of 3-amino-but-2-enoic acid dodecyl ester **2** (10.80 g, 40.10 mmol) and benzaldehyde **1** (2.16 g, 20.40 mmol) in methanol/acetic acid (40 mL/10 mL) was refluxed for 8 h, after cooling the precipitate was filtered off and crystallised from ethanol giving compound **4** as a light yellow powder (7.80 g, 64%), mp 54-56°C. ^{1}H NMR spectrum ($CDCl_3$, 200 MHz) was identical to that described above for **4** in *Method A*.

2.2.2. 2,6-Di(bromomethyl)-3,5-bis(dodecyloxycarbonyl)-4-phenyl-1,4-dihydropyridine (5)

To a solution of compound **4** (3.03 g, 5.00 mmol) in methanol NBS (1.85 g, 10.40 mmol) was added portion-wise. The reaction mixture was stirred at rt for 20 h. The yellow precipitate was filtered off, and washed with water. The precipitate was crystallised from ethanol giving compound **5** as a yellow powder (2.76 g, 72%), mp 87-90°C. ^{1}H NMR spectrum ($CDCl_3$, 200 MHz): δ 0.88 (t, 6H, *J*=6.6), 1.21-1.33 (m, 36H), 1.61-1.67 (m, 4H), 4.00 (t, 4H, *J*=6.6), 4.62 and 4.91 (AB-system, 4H, *J*=11.4), 5.02 (s, 1H), 6.57 (br s, 1H), 7.17-7.30 (m, 5H); ^{13}C NMR spectrum ($CDCl_3$, 100 MHz): δ 14.09; 22.66; 26.02; 27.32; 28.55; 29.26; 29.34; 29.53; 29.59; 29.62; 29.65; 31.90; 40.07; 64.77; 106.07; 126.84; 127.91; 128.21; 141.60; 145.60; 166.20; IR (film) 3320, 1696, 1676 cm^{-1}. Anal. Calcd. for $C_{39}H_{61}Br_2NO_4$: C, 61.02; H, 8.01; N, 1.82. Found: C, 61.23; H, 8.04; N, 1.73.

2.2.3. 1,1′-{[3,5-Bis(dodecyloxycarbonyl)-4-phenyl-1,4-dihydropyridine-2,6-diyl]dimethylene} bispyridinium dibromide (6)

To a solution of compound **5** (1.00 g, 1.30 mmol) in dry acetone (30 mL), pyridine (200 µL, 2.50 mmol) was added and the reaction mixture was stirred at rt for 48 h. After cooling, the precipitate was filtered off, washed with dry acetone and crystallised from ethanol giving compound **6** as a light yellow powder (0.98 g, 82%), mp 156-158°C (mp 140-145°C [18]). ^{1}H NMR spectrum ($CDCl_3$, 200 MHz): δ 0.88 (t, 6H, *J*=6.6), 1.22-1.30 (m, 36H), 1.61-1.63 (m, 4H), 4.04 (t, 4H, *J*=6.6), 5.10 (s, 1H), 5.89 and 6.40 (AB-system, 4H, *J*=13.5),

7.17-7.40 (m, 5H), 8.27 (d.d, 4H, *J*=6.4 and *J*=6.4), 8.60 (t, 2H, *J*=6.4), 9.34 (d, 4H, *J*=6.4), 10.95 (br s, 1H). The ^{1}H NMR spectrum was in accordance with reported previously [18] spectral data ($CDCl_3$, 90 MHz). ^{13}C NMR spectrum ($CDCl_3$, 100 MHz): δ 14.09, 22.66, 25.96, 28.38, 29.27, 29.34, 29.56, 29.62, 29.63, 29.67, 31.89, 39.74, 57.22, 65.56, 110.46, 127.52, 128.06, 128.59, 128.87, 137.97, 144.83, 145.45, 146.62, 166.41; IR (film) 3385, 1691, 1627 cm^{-1}; MS (+ESI) *m/z* (relative intensity) 766 ([M-2Br]$^{+}$, 15). Anal. Calcd. for $C_{49}H_{71}N_3O_4Br_2{\cdot}2H_2O$: C, 61.18; H, 7.86; N, 4.37. Found: C, 61.42; H, 7.85; N, 4.25.

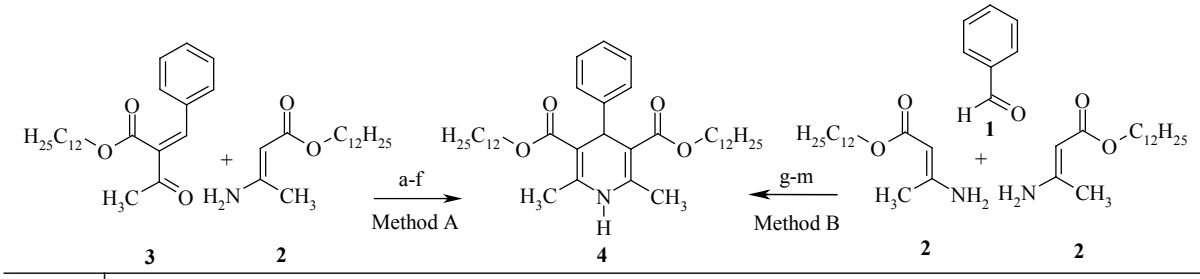
3. Results and Discussion

The synthesis of cationic amphiphilic 1,4-DHP **6** [17,18] was previously reported without giving experimental details for the first step. This synthetic approach involved three sequential steps, where the first step was a three component classical Hantzsch synthesis followed by bromination and nucleophilic substitution of bromine giving the target compound **6** [17,18]. In our hands, the Hantzsch cyclisation of 2 equivalents of β-ketoester – dodecyl acetoacetate, and one equivalent benzaldehyde (**1**) and ammonia in ethanol under reflux for 6 h gave 3,5-bis(dodecyloxycarbonyl)-2,6-dimethyl-4-phenyl-1,4-dihydropyridine (**4**) [17,18] only in 21% yield. The reported yield of the structurally related 1,4-DHPs with long alkyl ester chains at positions 3 and 5 obtained also in the three-component Hantzsch cyclisation did not exceed 25% [19]. Meanwhile, the yield of 3,5-bis(ethoxycarbonyl) analogue of **4** obtained *via* three-component Hantzsch cyclisation was as high as 75% [20]. The low yield of compound **4** might be attributed to poor crystallisation tendency and easier oxidation of 1,4-dihydropyridines with long alkyl ester chains at positions 3 and 5 comparing to respective derivatives with shorter alkyl ester moieties [21].

Consequently, this step could be improved by substituting the three component cyclisation with a modified two component reaction, which could give a more efficient synthesis of 1,4-DHP **4**. The elaborated synthesis of the cationic derivative **6** involves three sequential steps (Table 1; Scheme 1). The first step is a synthesis of the starting 1,4-DHP derivative **4** (Table 1), the second involves the bromination of the methyl groups with N-bromosuccinimide (NBS) of 2,6-dimethyl-1,4-DHP derivative **4,** and the third step consists of the nucleophilic substitution of bromine of compound **5** with pyridine yielding the target compound **6** (Scheme 1) [17,18].

Two methods of a two-component Hantzsch type cyclisation were elaborated. The yields of both synthetic approaches to the 3,5-bis(dodecyloxycarbonyl)-2,6-dimethyl-4-phenyl-1,4-dihydropyridine (**4**) were

Table 1. Two synthetic approaches for the preparation of 1,4-DHP **4**.



Entry	Reagents	IL	Solvent	Conditions	Yield[a],%
a[b]	**3** (1 equiv), **2** (1 equiv)	4-MBPy (10 mol%)	Diethylene glycol	8 h, 80°C	72
b[b]	**3** (1 equiv), **2** (1 equiv)	-	Diethylene glycol	8 h, 80°C	46
c[c]	**3** (1 equiv), **2** (1 equiv)	2-ClMPy (10 mol%)	Diethylene glycol	8 h, 80°C	49
d[b]	**3** (1 equiv), **2** (1 equiv)	4-MBPy (10 mol%)	-	8 h, 80°C	47
e[b]	**3** (1 equiv), **2** (1 equiv)	4-MBPy (6 equiv)	-	2 h, 150°C	38
f[b]	**3** (1 equiv), **2** (1 equiv)	2-ClMPy (6 equiv)	-	2 h, 220°C	decomp.
g[c]	**1** (1 equiv), **2** (2 equiv)	-	Methanol/acetic acid (4:1)	8 h, reflux	64
h[b]	**1** (1 equiv), **2** (2 equiv)	-	Diethylene glycol	8 h, 80°C	29
i[b]	**1** (1 equiv), **2** (2 equiv)	4-MBPy (10 mol%)	Diethylene glycol	8 h, 80°C	31
j[b]	**1** (1 equiv), **2** (2 equiv)	2-ClMPy (10 mol%)	Diethylene glycol	8 h, 80°C	33
k[b]	**1** (1 equiv), **2** (2 equiv)	4-MBPy (10 mol%)	-	8 h, 80°C	16
l[b]	**1** (1 equiv), **2** (2 equiv)	4-MBPy (6 equiv)	-	2 h, 150°C	8
m[b]	**1** (1 equiv), **2** (2 equiv)	2-ClMPy (6 equiv)	-	2 h, 220°C	decomp.

[a] *isolated yields.*
[b] *the isolation procedure was performed as described in experimental part (Method A).*
[c] *for the details see experimental part.*

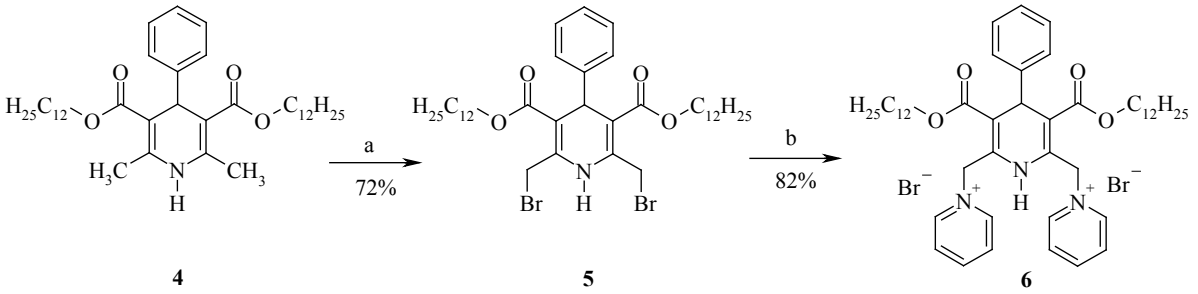
higher (Table 1) compared to the classical Hantzsch cyclisation.

Method A is a reaction between 3-oxo-2-[1-phenylmethylidene]-butyric acid dodecyl ester (2-benzylidene dodecyl acetoacetate) (**3**) and 3-amino-but-2-enoic acid dodecyl ester (β-aminocrotonic acid dodecyl ester) (**2**) (Method A; entries a-f; Table 1). Investigation of the reaction course has demonstrated that the yield of compound **4** was highly dependent on the reaction media and the catalyst employed. Experimental studies using a variety of reaction media led us to the conclusion that the highest yield of 1,4-DHP **4** as a white powder (entry a; Table 1) can be achieved using bis(2-hydroxyethyl)ether (diethylene glycol) as a solvent and 4-MBPy as a catalyst; without a catalyst the target product **4** was obtained as a light yellow solid, which turned oily upon standing and the yield was much lower (46%; entry b; Table 1). Using Mukaiyama reagent as a catalyst instead of 4-MBPy the compound **4** was obtained as a light yellow precipitate with 49% yield (entry c; Table 1). The reaction in solvent free conditions [8] in the presence of 4-MBPy as a catalyst gave the compound **4** as a white powder with 47% yield (entry d; Table 1). When this cyclisation was performed in 4-MBPy as IL at 150°C for 2 h the compound **4** was obtained as a yellow solid, which turned oily upon standing in only 38% yield (entry e; Table 1). Mukaiyama reagent can be applied as IL only at the temperatures above its melting point (200°C) however, under such drastic reaction conditions, a dark coloured mixture of thermal decomposition products containing also 1,4-DHP **4** was formed in 2 h (entry f; Table 1).

In the literature, the synthesis of starting 2-benzylidene dodecyl acetoacetate (**3**) (E/Z forms) was performed from the corresponding acetoacetate [22] and benzaldehyde (**1**) in solvent free conditions in the presence of piperidine [23]. In this paper, Knoevenagel condensation of dodecyl acetoacetate and benzaldehyde (**1**) was performed in 2-propanol using 10% mol. equivalents of piperidine acetate as a catalyst with yield of product **3** ~60%. The starting β-aminocrotonic acid dodecyl ester (**2**) was synthesised from the corresponding β-ketoester by treatment with 25% aqueous ammonia, in analogy with the reported method, in 58% yield [24].

Method B is a reaction of 2 equivalents of β-aminocrotonic acid dodecyl ester (**2**) with 1 equivalent of benzaldehyde (**1**) (Method B; entries g-m; Table 1). In this method dodecyl acetoacetate was replaced by β-aminocrotonic acid dodecyl ester (**2**), which also served as a source of ammonia. Altering the reaction media led to yields of 1,4-DHP **4** varying from 8% to 64%. The best result of this method was achieved in methanol/acetic acid (entry g; Table 1) where 1,4-DHP **4** was obtained as a white powder in 64% of yield. Replacing of methanol/acetic acid mixture by diethylene glycol resulted in decreased yield of compound **4** (as a white powder; entry h; Table 1) to 29%, this reaction in diethylene glycol, but with addition of 4-MBPy gave compound **4** as a light yellow solid, which turned oily upon standing in 31% yield (entry i; Table 1), using Mukaiyama reagent instead of 4-MBPy gave compound **4** as a light yellow powder in 33% yield (entry j; Table 1). Obviously, addition of ILs to the reaction of β-aminocrotonic acid dodecyl ester (**2**) with benzaldehyde (**1**) in diethylene glycol had almost no influence on its course. Performing this reaction in solvent free conditions [7] (entry k; Table 1) in the presence of 4-MBPy as a catalyst gave compound **4** as a light yellow solid, which turned oily upon standing in only 16% of yield. This reaction was also performed in IL 4-MBPy at 150°C for 2 h where the compound **4** (entry l; Table 1) was obtained as a light yellow solid, which turned oily upon standing in only 8% of yield. Use of Mukaiyama reagent instead of 4-MBPy as IL led only to a dark coloured mixture of thermal decomposition products containing also 1,4-DHP **4** (entry m; Table 1).

Promoting effect of ionic catalysts (4-MBPy or Mukaiyama reagent) on Hantzsch cyclisation was proved by performing this reaction in diethylene glycol. At the same reaction conditions without a catalyst the yield and purity of **4** were lower than those in presence of the catalysts. However, both methods performed in



Scheme 1. Synthesis of cationic amphiphilic 1,4-DHP derivative **6**. Reagents and conditions: a) NBS, MeOH, rt, 20 h; b) Pyridine, Acetone, rt, 48 h.

the presence of 4-MBPy under solvent free conditions afforded only very low yields of compound **4**. The best result was obtained using 4-MBPy as a catalyst where 72% yield was achieved (entry a; Table 1). Obviously, 4-MBPy as a catalyst and diethylene glycol as solvent have improved the yield of Hantzsch cyclisation.

The next steps of the synthesis of target compound **6** were studied in detail in order to develop new preparative approach or to improve existing method by fine tuning of reaction conditions (Scheme 1). Previously, bromination of compound **4** with NBS was performed at 0°C for 40 min giving 2,6-di(bromomethyl) substituted 1,4-DHP **5** in 34% yield [18]. The main drawbacks of this step are low yield and complicated work-up procedure. In this paper, bromination of compound **4** with NBS was carried out in methanol at rt for 20 h giving 1,4-DHP **5**, which was precipitated during the reaction. Precipitation of the reaction product has simplified work-up procedure. Longer reaction time and higher temperature for this step improved the yield from 34% of crude substance [17,18] to 72% of a crystalline compound with a satisfactory spectroscopic data. 1,3-Dibromo-5,5-dimethylhydantoin (DBDMH) was also tested as bromination agent [25] for compound **4** under the same reaction conditions, however we found no advantages of this approach, as the yield was moderate. The final step of synthesis of the target compound **6** is nucleophilic substitution of bromine of 2,6-di(bromomethyl) 1,4-DHP **5** with pyridine. Previously [18], nucleophilic substitution of bromine was carried out with one equivalent of dibromoderivative **5** and 2 equivalents of pyridine in dry acetone at rt for 4 h, giving cationic amphiphilic 1,4-DHP **6** in 40% yield. Now we have found, that at the same reaction conditions the prolongation of the reaction time to 48 h improves the yield of target 1,4-DHP **6** from 40 to 82%. Other variations of the reaction conditions, such as addition of excess of pyridine makes only more complicated isolation and purification of compound **6**. The range of variation of the temperature is also limited due to the fact that at the temperatures above 50°C occurs lactonisation of 2,6-di(bromomethyl) substituted 1,4-DHP **5** to 8-phenyl-5,8-dihydro-1*H*,3*H*-difuro[3,4-*b*:3′,4′-*e*]pyridine-1,7(4*H*)-dione [26].

Compared to the previously reported synthetic approach to cationic amphiphilic 1,4-DHP **6** where only 3% overall yield was achieved, the present methodology allowed the synthesis of compound **6** in 23% overall yield by *Method A* or in 21% by *Method B* starting from 3-amino-but-2-enoic acid dodecyl ester (**2**) and benzaldehyde (**1**). On the other hand, the main achievement of this work was elaboration of the preparative method of synthesis of 1,4-dihydropyridine derivative having long alkyl ester moieties at the positions 3 and 5.

4. Conclusions

In conclusion, we have developed an efficient synthesis of 3,5-bis(dodecyloxycarbonyl)-2,6-dimethyl-4-phenyl-1,4-dihydropyridine (**4**), which is a key intermediate for the synthesis of cationic amphiphilic 1,4-dihydropyridines (*e.g.* 1,1′-{[3,5-bis(dodecyloxycarbonyl)-4-phenyl-1,4-dihydropyridine-2,6-diyl]dimethylene} bispyridinium dibromide (**6**)). The present synthetic procedure allowed the synthesis of 1,4-DHP **4** *via* two-component Hantzsch type cyclisation between 3-oxo-2-[1-phenylmethylidene]-butyric acid dodecyl ester (**3**) and 3-amino-but-2-enoic acid dodecyl ester (**2**) utilising 1-butyl-4-methylpyridinium chloride as the catalyst in 72% yield, showing the improvement over the previous synthetic methods due to the simple work-up procedure, which can be easily scaled-up.

Use of pyridinium ionic liquids as catalysts of Hantzsch type cyclisations could become a perspective tool for the synthesis of 1,4-dihydropyridine derivatives with long alkyl ester chains at positions 3 and 5 of the 1,4-DHP ring, where classical Hantzsch synthesis gives only low yields.

Acknowledgement

This research work was supported by European Social Foundation (ESF) project Nr. 2009/0197/1DP/1.1.1.2.0/09/APIA/VIAA/014 and ESF Nr. 2009/0138/1DP/1.1.2.1.2/09/IPIA/VIAA/004 (for K. Pajuste).

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