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POLYMER BOUND 4-DIALKYLAMINO PYRIDINES: SYNTHESIS, CHARACTERIZATION AND CATALYTIC EFFICIENCY.

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Abstract: This work describes the synthesis of 4-carboxy-N-(4'-pyridino) piperidine (CPP), a functionalized analogue of the 4-dialkylamino pyridines, and its anchorage to various polymers by means of an amide bond. Some methods of titration of these supported CPP are pointed out. The efficiency of various supported CPP in the acetylation reaction of 1-methyl cyclohexanol are compared with DMAP as standard. The influence of various factors (polymer type, spacer, loading and temperature) are interpreted in relation to the nature of the microenvironment. An important decrease of the apparent pK of supported CPP as compared to DMAP (about 2 pK units) is assessed.

Compared to their soluble analogues, supported catalysts present a number of advantages: easy separation at the end of the reaction, possibility of using the reagent in repeated cycles, ability to work with continuous flow methods, expected favourable polymer effects , etc

A number of catalysts anchored to polymers are described in the literature[1,2], but generally their degree of reactivity is distinctly lower by comparison with their soluble analogues. This is due to the fact that in most cases authors have not tried any optimization of the catalytic properties.

4-Dialkylamino pyridines, and more particularly 4-dimethylamino pyridine (DMAP), are extremely powerful acylation catalysts which are now commonly used[3-5]. Besides papers concerning 4-dialkylamino pyridines fixed onto soluble polymers[6-8], some publications describe 4-dialkylamino pyridines anchored to crosslinked polystyrene[9-15] and to polyacrylamide[16]. In a preliminary note[17], we described several 4-carboxy N-(4'-pyridino) piperidines (CPP 1) bound to polystyrene, some of which attained 90% of the DMAP efficiency.

In this work, we studied the influence of a number of factors not only in order to improve catalytic properties, but also to bring to light some general features which could be applied to other types of supported reagents. Furthemore, we have illustrated some specific properties caused by the presence of the polymer, such as the variation of pK, with a view to apply these compounds to peptide synthesis.

In order to synthesize these reagents, we chose to anchor a soluble analogue of DMAP, possessing an acid function, to amine-bound polymers, in order to create an amide bond which would remain stable towards the classical conditions of acylation reactions. This procedure is preferable to the synthesis by copolymerisation, as it allows the nature and loading of the polymer to be easily varied, as well as the introduction of several types of spacers. We

were effectively able to synthesize a large number of supported catalysts (Table 1) and to estimate the influence of different factors.

Table 1

Polymer*	Spacer	N°
a	-	3a
b	-	3b
c	-	3c
d	-	3d
a	$-CO-(CH_2)_5-NH-$	4a
b	" "	4b
c	" "	4c
d	" "	4d
c	$-CO-(CH_2)_2-NH-$	5c
d	" "	5d
c	$-CO-(CH_2)_2-NH-CO-(CH_2)_5-NH-$	6c
c	$-CO-(CH_2)_5-NH-CO-(CH_2)_5-NH-$	7c
c	$-CO-(CH_2)_5-NH-CO-(CH_2)_2-NH-$	8c
c	$-CO-(CH_2)_5-NMe-CO-(CH_2)_2-NH-$	9c

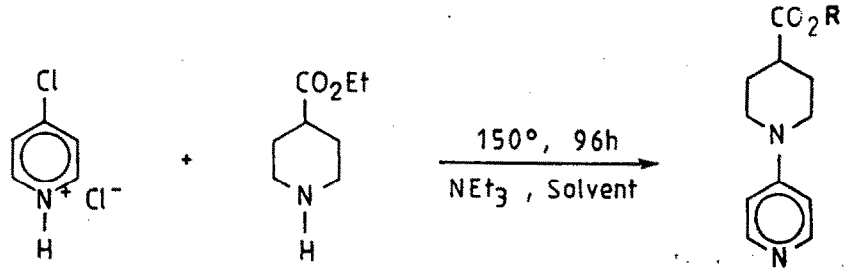
*) a = cross-linked polyacrylamide functionalized with amine function[23]
b = benzhydrylamine resin
c = aminomethyl cross-linked polystyrene
d = methylaminomethyl cross-linked polystyrene

Choice of a soluble analogue of DMAP

The choice of a functionalized 4-dialkylamino pyridine with a catalytic efficiency at least equal to that of DMAP appeared essential. For example, the supported N-benzyl N-methyl 4-amino pyridine synthesized by TOMOI[9] was a bad choice, because N-benzyl N-methyl 4-amino pyridine is clearly less efficient than DMAP. On the other hand, 4-cycloalkylamino pyridines, such as 4-pyrrolidino pyridine (PPY)[3-5] and the corresponding analogs supported on soluble polymers[18-21] are known for a catalytic activity superior to DMAP.

Taking into account the difficulties encountered in the synthesis of PPY analogues containing functional groups, we chose 4-carboxy N-(4'-pyridino) piperidine CPP 1, because it is easily obtained in one single step, following the technique of PENTIMALLI[22] (Scheme 1).

Scheme 1



1 R=H (CPP) Solvent EtOH/H2O
2 R=Et (CPPOEt) Solvent EtOH

The efficiency of CPPOEt 2 was tested in several difficult acetylation reactions and compared with other 4-dialkylamino pyridines under the conditions specified in the experimental part. The results are summarized in Table 2.

Table 2

Yield (%) in tertiary alcohol acetates

catalyst	t- butanol	1-methylcyclohexanol	1,1-diphenylethanol
DMAP	43	92	59
PPY	-	100	69
CPPOEt	48	94	62.5

The yields observed for DMAP and PPY were in accordance with those of the literature[4,24].

These results showed that CPPOEt had an excellent catalytic activity, superior to DMAP, but nevertheless without attaining the capacity of PPY.

Synthesis of polymer supported CPP

CPP was bound to several amine functionalized polymers by forming an amide bond, either directly, or with the intermediary of a spacer composed of one or two linear aminoacids, according to Scheme 2.

These amide bonds were generally generated by using diisopropylcarbodiimide (DIC). However, the low solubility of CPP required the use of hot DMF in the coupling reactions, conditions which favour the formation of undesired N-acylureas. Moreover, the anchoring of CPP often required repetitive couplings in order to obtain a complete reaction. When coupling by DIC appeared particularly difficult, as was the case with compound 3d, DIC was replaced by N-dimethyl chloroiminium chloride obtained from oxalyl chloride and DMF[25]. Under these conditions, a single coupling generally led to a complete reaction.

Titration of supported CPP

The synthesis of a supported reagent must always be followed with an accurate titration method allowing the reagent loading to be known.

In the case of supported CPP, we looked for quick methods that would enable us to differentiate the unreacted amines from CPP itself.

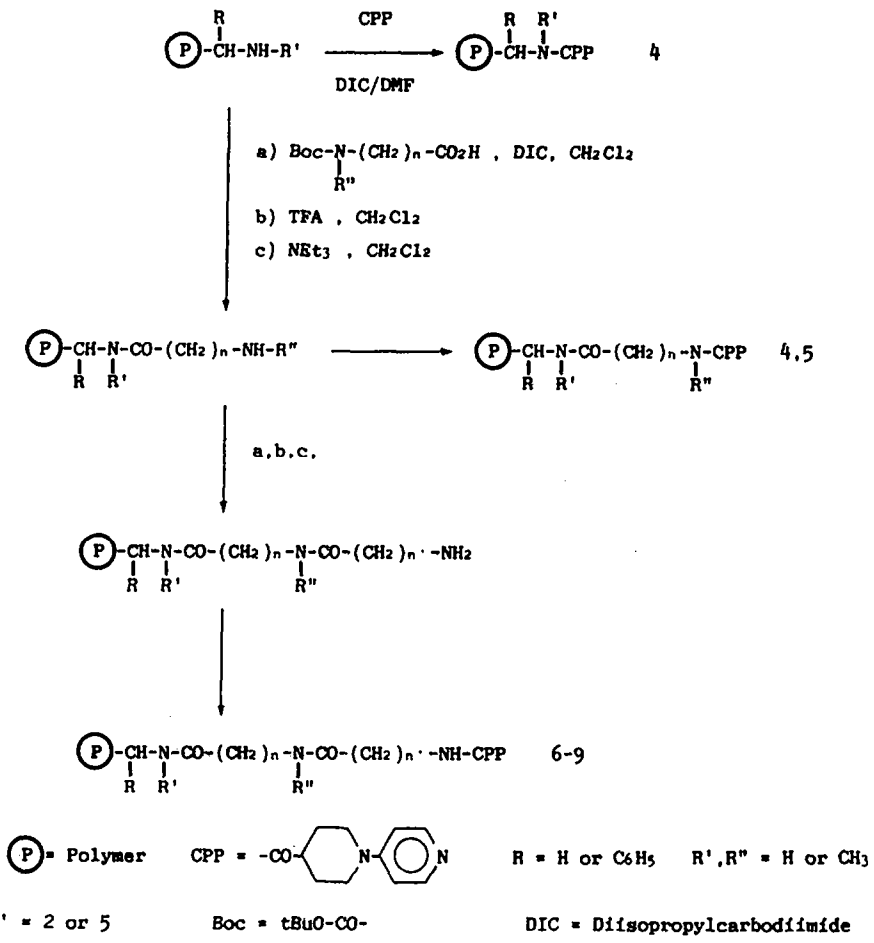
1- Titration of the supported reagent by acid hydrolysis

Hydrolysis of all the amide functions of polyacrylamide resins, such as 3a, was achieved by heating 24 hrs at 110°C in 6N hydrochloric acid. The amount of CPP hydrochloride was then obtained by measuring the UV absorption as compared with a solution of CPP in 6N hydrochloric acid (λ_{max} = 280 nm, ε = 17330).

In this way, the catalyst 3a, which afforded a negative ninhydrine test[26], and was obtained from a 0,70 meq/g NH_2 functionalized polyacrylamide resin, titrated 0,70 meq/g of CPP.

On the other hand, acid hydrolysis could not cleave the amide function of polystyrenic resins even under forcing conditions, which is probably due to the strong hydrophobic character of polystyrene.

Scheme 2



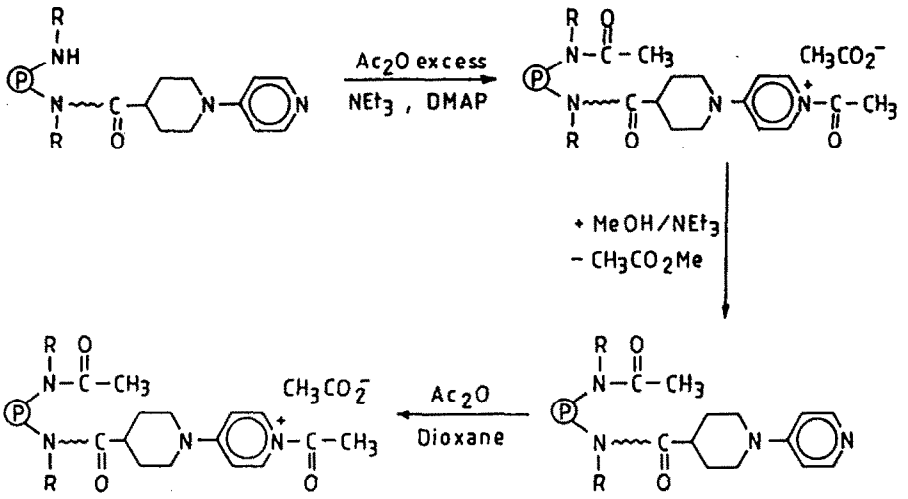
2- Titration by acetylation

The acetylation reaction (Scheme 3) was chosen with polystyrene reagents.

The titration was carried out in two steps. Peracetylation was first performed with excess acetic anhydride in the presence of DMAP. This enabled the unreacted amine functions to be blocked, which is particularly useful in the case of secondary amine functionalized polystyrenic resins. The completion of the coupling reaction of CCP with these secondary amines could not be verified because the Kaiser test[26] became inoperative. Treatment with methanol and triethylamine then led to the free CPP which was acetylated again with a known quantity of acetic anhydride. After elimination of the acetylated polymer by filtration and hydrolysis of the acetic anhydride in excess, the back titration of the consumed acetic acid enabled the quantity of CPP bound to the polymer to be determined.

The validity of this method was verified in the case of CPP anchored to aminomethyl polystyrenes loaded with less than 1.5 meq/g. In this case, the titration of supported CPP and the initial NH_2 loading (determined by quantitative titration with ninhydrine[27]) gave the same results. In the case of secondary amine functionalized resins, the CPP loading determined by this method was generally equal to the initial chlorine loading (taking into account the weight modifications), demonstrating that titration by acetylation was also valid in this case.

Scheme 3



A problem was encountered in the case of highly charged resins (obtained from Merrifield resins with 3-5 meq/g of chlorine). Titration by acetylation gave loadings superior to those theoretically possible (e.g. 5 meq/g of CPP from the resin at 5 meq/ of chlorine). It is possible that the high increase of the polymer polarity favoured a partial diacetylation of CPP. Another type of titration was therefore developed for these strongly charged compounds.

3-Titration with acetic acid

Supported CPP were transformed by reaction with acetic acid in dioxane into the corresponding supported pyridinium acetates. It was therefore possible to determine the CPP loading by titration of the remaining acetic acid. However, this method could only be applied if no unreacted amine functions remained, since they were partially titrated with acetic acid.

This titration was applied to several resins loaded at about 1 meq/g and identical results to those obtained with the acetylation method were found. The same titration was also applied to two highly loaded resins (obtained from Merrifield resins at 3 and 5 meq/g of Cl respectively) and the results (1.80 and 3.30 meq/g) were compatible with the maximum theoritical loading.

Catalytic efficiency

The 4-dialkylamino pyridines (Table 1) were meant to allow an optimization of the catalytic properties, by modifiying several factors bearing on the microenvironment of the reaction site. This approach was comparable to the work recently published by FRECHET et al.[14-15].

In order to measure the catalytic activity of these supported CPP, we chose the acetylation reaction of 1-methylcyclohexanol, which can be easily followed by gas chromatography. The quantities of 1-methylcyclohexanol acetate obtained after 24 hrs in the presence of supported CPP and DMAP respectively enabled us to determine the relative efficiency of these catalysts (with DMAP as standard) and discuss the influence of several factors.

2- Influence of the type of polymer.

The relative reactivities are given in Table 3 which showed that reagents bound to polyacrylic resins have no or little activity, although the polystyrene analogues are very efficient. These results can be explained by the high polarity of polyacrylamides in accordance with the decrease of the catalytic activity of 4-dialkylamino pyridines with increased solvent polarity[3]. Similar results were obtained by FRECHET[14,15] for 4-dialkylamino pyridines anchored to co-polyvinyl pyridines.

Table 3

Efficiency depending on the polymer type

Polymer*	Spacer	N°	Efficiency (%)*
a	-	3a	0
b	-	3b	85
c	-	3c	75
d	-	3d	95
a	-CO-$(CH_2)_5$-NH	4a	14
b	" "	4b	85
c	" "	4c	89
d	" "	4d	93

*) Relative efficiency by comparison with DMAP after 24hrs reaction = 100 X conversion % in presence of supported catalyst / conversion % in presence of DMAP(accuracy 1%).

In the case of our polyacrylamide supported CPP, the high deactivation can also be explained by formation of hydrogen bonds between the pyridine nitrogen and the matrix.

As a consequence of these results, only polystyrene supported reagents were subsequently studied.

2- Influence of the nature and length of the spacer.

It is generally taken for granted that the introduction of a spacer between the polymer and the catalytic site improves accessibility and therefore reactivity. The results of Table 4 showed that in our case, with the specific acetylation reaction chosen as a test, this factor generally played an insignificant role.

Indeed, the comparison between benzhydrylamine- bound catalysts 3b and 4b showed that the efficiency was not modified by introducing an additional aminocaproic spacer. In the same way, a second aminocaproic group did not improve 7c, as compared to 4c. With compounds 3d, 4d, 5d and 9c, the presence or the lack of a spacer did not appreciably alter the reactivity. These results, apparently in contradiction with the literature, can be explained by the presence of a piperidine ring which, when added to the amide bond, afforded a chain long enough to keep the catalytic site away from the polymer.

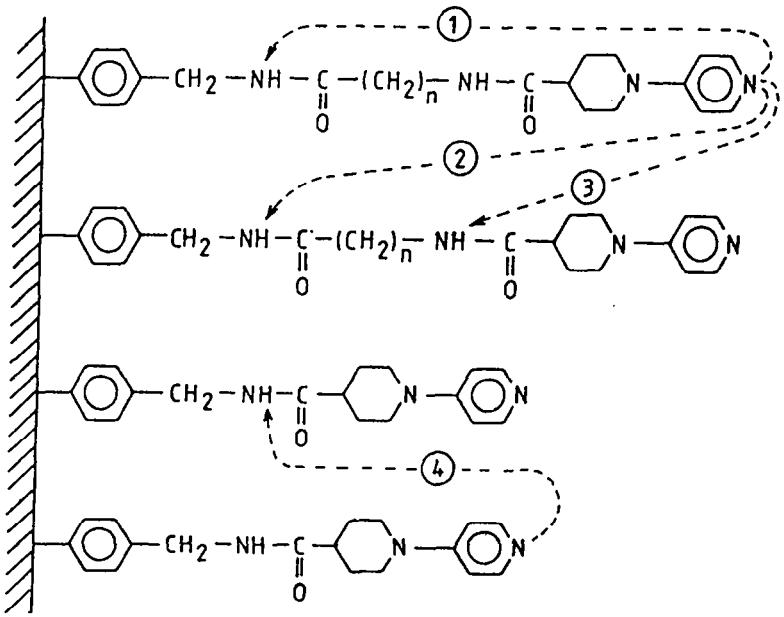
On the contrary, the spacer type played an essential part with several compounds of the c series obtained from aminomethyl benzyl functionalized polystyrene. This can be explained by the presence of amide nitrogen atoms, but not as proposed by FRECHET et al.[14,15] or TOMOI et al.[13] by involving a concentration of charged species or of final product in the vicinity of the catalytic sites. In our case, this phenomenon was overshadowed by the interference of intra- or inter-site hydrogen bonds, resulting in the deactivation of the pyridine nitrogen atom (Scheme 4).

Table 4

Efficiency depending on the spacer

Polymer	Spacer	N°	Efficacity
b	-	3b	85
b	-CO-(CH2)5-NH-	4b	85
c	-	3c	75
c	-CO-(CH2)5-NH-	4c	89
c	-CO-(CH2)2-NH-	5c	64
c	-CO-(CH2)2-NH-CO-(CH2)5-NH-	6c	88
c	-CO-(CH2)5-NH-CO-(CH2)5-NH-	7c	89
c	-CO-(CH2)5-NH-CO-(CH2)2-NH-	8c	36
d	-	3d	95
d	-CO-(CH2)5-NH-	4d	93
d	-CO-(CH2)2-NH-	5d	95
c	-CO-(CH2)5-NMe-CO-(CH2)2-NH-	9c	94

Scheme 4

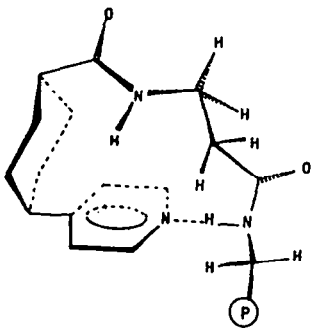


The interactions of type 1 can explain the important deactivation of compounds 5c and 8c in which the CPP moiety is preceded with the β-alanyl group.

Molecular models showed the easy formation of an hydrogen bond between the NH preceding the β-alanyl arm and the pyridine nitrogen (Scheme 5).

This interpretation is supported by the excellent catalytic activity of the N-methyl analogues 5d and 9c (95% and 94%

Scheme 5



respectively), as well as the good reactivity of 6c (88%), where the respective order of the ß-alanine and ε-aminocaproïc groups were reversed.

The same type of interaction can also take place, but to a lesser degree, when the spacer is a simple aminocaproïc group (comparison between 5c and 5d); however, a type 2 interaction can also explain the observed results.

Considering all our supported catalysts, it was not possible to characterize a type 3 hydrogen bond, at least with a loading of 1 meq/g. On the contrary, the comparison of 3c and 3d gave evidence of a type 4 interaction, on account of the deactivation of 3c. With benzhydrylamine resins, compound 3b was not deactivated (as compared with 4b for example); this was probably the result of a steric hindrance by one of the phenyl groups, preventing the formation of a type 4 hydrogen bond.

All these results showed that, with respect to supported CPP loaded to 1 meq/g, the essential factor bearing on the reactivity was not the spacer length, but the intra or intersite interactions between the spacer and the active site.

3- Influence of the cross-linking

TOMOI[9] showed that with polystyrene, an increase of the cross-linking percentage from 2% to 10% induced a decrease amounting to half of the reactivity. All our studies were carried out with 1% cross-linked styrene-divinylbenzene copolymer. With compound 3d, a comparison between 1% and 2% cross-linked copolymers showed a non significative variation of reactivity (94% to 93%)(Table 5).

4- Influence of the loading

An increase in loading resulted in bringing the catalytic sites nearer together,which induced an increase in the previously described interactions, and a decrease in reactivity. But other factors are likely to occur: this decrease could be produced by the "catalyst's self-inhibition"[14] due to the high local concentration in CPP. On the other hand, an increase in the number of polar pyridine reactive sites led to a decrease in the polystyrene apolar character, which influenced the reactivity.

In order to estimate these factors, catalyst 3d was chosen (being unable to form hydrogen bond interactions) and obtained with different loadings, starting with commercial Merrifield resins (Table 5)

Table 5

Compound 3d

Cross-linking (% DVB)	Loading (meq/g)	Efficiency
1	0.67	93
1	1.00	94
1	1.34	90
2	0.70	94
2	1.00	93
2	1.80	82
2	3.30	61

Results from Table 5 showed that an increase in loading above 1 meq/g induced a reactivity decrease, which rose to one third of the initial value with a loading of 3.3

meq/g (all styrene residues are substituted).

In practice, catalysts loaded with about 1 meq/g appeared to be preferred

5- Influence of the temperature and of the solvent

Measurements were carried out in dichloromethane at 35°C and in benzene at 35°C and 70°C (Table 6).

Table 6

Solvent effect

Catalyst	Benzene 70°C Yield	Efficiency	Benzene 35°C Yield	Efficiency	Dichloromethane 35°C Yield	Efficiency
DMAP	92	100	89	100	69	100
3c	69	75	51	57	15	21
4c	82	89	56	63	36	52

The efficiency of the supported catalysts in benzene solution decreased significantly with a lowering of the temperature, although the reactivity of DMAP under the same conditions was not much influenced. This was probably the result of a limitation brought about by the diffusion rates in the polymer, which are strongly dependent on the temperature.

Replacing benzene with dichloromethane led to an important decrease in the reactivity of DMAP, in accordance with the literature[3].

Compound 4c acted in the same way. On the contrary, compound 3c was deactived more than expected. This could have been the result of a specific solvatation effect: an aromatic solvent will preferentially solvate polystyrene chains; the hindrance arising at the proximity of aminobenzyl groups will interfere with the formation of NH type 4 bonds and with the accumulation of ionic species responsable for the decrease in reactivity. With dichloromethane, this specific solvatation was no longer involved and the decrease in efficiency was ever greater.

Thus, solvent effects had a complex influence on our supported catalysts by interfering with the intrinsic reactivity of the active site, and at the same time by modifying the microenvironment through a more or less important polymer solvatation.

5- Apparent pK of the supported CPP

Our initial working hypothesis was that bonding 4-dialkylaminopyridines to an hydrophobic polymer could induce a decrease of the basicity of these reagents, and therefore a decrease in racemization when applied to peptide synthesis. We based this hypothesis on the results of HOOPER et al[28] who showed that the basicity of the terminal amine function of peptides bound to polystyrene supports increased in relation with the length of the peptide chain. We explain this result on the grounds that the shorter the peptide chain, the greater the amine function protection against the hydrophobic environment, and hence the lower the pK.

The decrease of apparent pK of some supported CPP compared to DMAP was measured in the following way (Table 7): An equilibrium was established between equimolecular quantities of a supported CPP hydrochloride and DMAP (or between a supported CPP and DMAP hydrochloride) in dichloromethane solution by stirring 1 hr at room temperature. After filtration, titration of the chloride ions bound to the support and remaining in solution respectively gave the difference of the apparent pK:

$$\Delta pK = -\log \frac{(\%\ Cl^- \text{ bound to the support})^2}{(\%\ Cl^- \text{ in solution})^2}$$

Table 7

Compound	Loading (meq/g)	Spacer	ΔpK
3d	1	-	2.2
4d	1	$-CO-(CH_2)_5-NH-$	1.7
3d	1.8	-	0.8
3d	3.3	-	0.55

An important decrease of pK was observed with 1 meq/g loaded catalysts. This ΔpK was higher when the catalytic site was near to the polymer matrix. On the other hand, with highly loaded 3d catalysts, the ΔpK decreased as the loading increased. This result was consistent with the increase of polymer polarity and with the decrease of efficiency as the loading increased.

Use of the reagents in repeated cycles

One of the advantages of supported reagents consists in the possibility of using these reagents in repeated cycles. Taking compound 3c as an example, and the same acetylation reaction of 1-methylcyclohexanol, there was only a 30% loss of efficiency after 10 cycles, each of them of 24 hrs at 70°C, in spite of these drastic conditions.

Conclusion: Several easily accessible supported acylation catalysts have been synthesized. They present catalytic activities nearly equal to DMAP and excellent stabilities allowing them to be reused.

The important role played by a number of factors has been pointed out. The hydrophilic or hydrophobic nature of the polymer has a essential bearing on the catalytic efficiency.

In opposition to commonly accepted ideas, the spacer length is not an essential factor if it exceeds a minimal value. On the contrary, intra and intersite interactions between the spacer polar group and the catalytic site notably altered its reactivity. Finally, the influence of the loading value was clearly illustrated; it was necessary not to exceed a loading of 1 to 1.2 meq/g in order to avoid an increase in the intersite interactions as well as in the environment polarity, as both of these factors bring about a deactivation of the reaction site.

This approach about factors related to the reactivity of supported catalysts is not exhaustive. The solvent influence, the relation between the reactivity and the diffusion rates into the polymer according to catalytic site density would deserve further studies.

Finally, the occurrence of a pK decrease when a base is anchored to a hydrophobic polymer was clearly shown for the first time; the shorter the spacer length, the greater the decrease of pK. In a following paper, we intend to apply this important property of supported CPP to peptide synthesis.

Experimental part

The uncorrected melting points were determined with a Büchi apparatus. NMR spectra were recorded on Varian T60 or HA 100 apparatus. A Jeol JMS DX 300 apparatus was used for mass spectroscopy. UV spectrum were recorded on a Cary 118 apparatus.

Synthesis of CPP 1

4-Chloropyridine hydrochloride (9,55g, 64 mmol) was dissolved in a mixture of water and ethanol (30 ml/10 ml) with a large excess of triethylamine (26 ml, 190 mmol). Ethyl isonipecotate was added (10g, 64 mmol) and the mixture was heated in a sealed tube 96 hrs at 150°C. The residue was tritured in ethanol and the solvent removed. This operation was repeated twice. A sufficient quantity of chloroform was added to the residual solid. After filtration, the precipitate was recristallized in hot DMF to give CPP 1 in 90% yield. F= 275°; MS m/z 206; ^{1}H NMR (D_2O), δ ppm: 2.00 (m, 4H β from N), 3.40 (m, 1H β from N), 4.00 (m, 4H α from N), 6.60-8.15 (m, 4H of the pyridine). Anal. calc. for $C_{11}H_{14}O_2N_2$: C, 64.08; H, 6.80; N, 13.59; found: C, 64.22; H, 6.53; N, 13.32.

Synthesis of CPPOEt 2

CPPOEt 2 can be obtained by the same procedure as 1, except that no water should be used.However, purification was difficult. The best method to get a pure product was by esterification of 1. Thionyl chloride (8 ml) was slowly added to cooled ethanol (0°C). This solution was then added to CPP (2g, 10 mmol) and the mixture was stirred 20 mn at 0°C, then 2 hrs at room temperature, and finally heated 2 hrs to reflux. After removal of the solvent under reduced pressure, ethanol was added to the residue and the mixture treated with an ion exchange resin (Dowex 1-X8; OH^-). The solvent was removed and distillation gave CPPOEt 2 ($Eb_{0.4}$ = 155-160°) in 78% yield. MS m/z 234; ^{1}H NMR ($CDCl_3$) δ ppm: 1.28 (3H, t, J=7Hz, Et), 1.93-3.97 (m, 5H of the piperidine cycle),4.15 (q,J=7Hz, 2H, Et), 6.55-8.15 (m, 4H of the pyridine); Anal. calc. for $C_{13}H_{18}O_2N_2$: C, 66.67; H, 7.69; N, 11,97; found: C, 66.54; H, 7.49; N, 11,83.

Measurement of the efficiency of soluble 4-dialkylamino pyridines.

Acetylation of t-butanol

Acetic anhydride (10 ml, 105 mmol) was added to a mixture of t- butanol (3.7 g, 50 mmol), triethylamine (7.57 g, 50 mmol) and catalyst (2.5 mmol). The reaction mixture was stirred 10 hrs at room temperature, then 100 ml of ether were added. This solution was washed with 1N hydrochloric acid, with a satured solution of sodium bicarbonate and finally with water. The organic phase was dried over sodium sulfate, and evaporated under vacuum at room temperature. The t- butyl acetate was isolated by distillation (Eb = 90°C) and weighted.

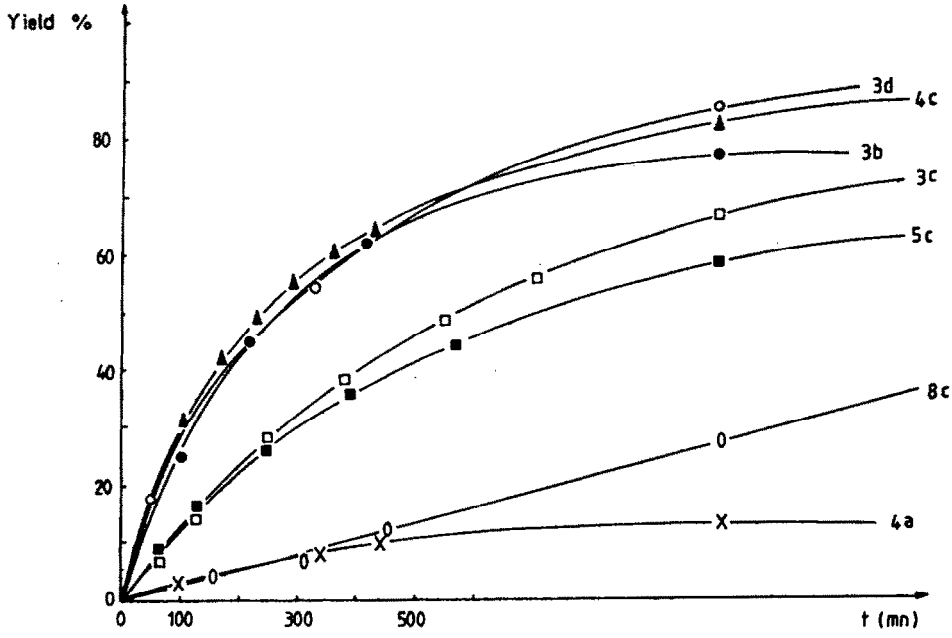
Acetylation of 1-methylcyclohexanol.

The reaction was followed by gas chromatography (PERKIN-ELMER SIGMA 3 connected to an integrator DELSI ENICA 10) under the following conditions:

Column CAW 20%-PEG 20M, length 3m
Temperature 140°C
Injector and detector at 220°

The 1-methylcyclohexanol was distilled twice prior to use. The reaction was carried out in a double wall reactor joined to a thermostated bath (70°). Benzene (5 ml), 1-methylcyclohexanol (2.5 mmol), triethylamine (3.75 mmol), ethylbenzene (0.125 mmol) (used as standard), catalyst (0.5 mmol) and acetic anhydride (3.75 mmol) were successively added. The progress of the reaction was followed as a function of time; Scheme 6 shows several examples of the obtained plots. In each case , the value measured after 24 hrs was considered as an end point and used for assessement of the efficiency (accuracy 2%).

SCHEME 6



Acetylation of 1,1-diphenylethanol.

Triethylamine (0.28 ml, 2 mmol), acetic anhydride (0.19 ml, 2 mmol) and catalyst (0.2 mmol) were added to 1,1-diphenylethanol (0.2 g, 1 mmol) in 25 ml of carbon tetrachloride. After 15 hrs stirring at room temperature, the yield was determined by integration of the methyl peaks of diphenylethanol (δ 1.87) and of the corresponding acetate (δ 2.05) respectively.

Synthesis of supported CPP.

Polyacrylic resin with 0.8 meq NH_2/g was prepared according to the method used in our laboratory[23]. Commercial polystyrene resins were used (from UCB for the benzhydrylamine and from Fluka or Janssen for Merrifield resins). All these resins were washed with DMF, dichloromethane and dried before use.

The chlorine loading of Merrifield resins was measured along the following method[29]: 200 mg of dried resin were heated 2 hrs at 100°C in 3ml of pyridine. After cooling, 30 ml of a 50% aqueous solution of acetic acid, 5 ml of concentrated nitric acid and 3 drops of a saturated ferric alum solution were slowly added and the mixture was stirred 5 mn; 5 ml of a 0.1 N silver nitrate solution were slowly added and the mixture was stirred during 5 mn; 5 ml of water and 2 ml of toluene were then added and the titration made with a 0.1 N ammonium thiocyanate solution until color change.

Aminomethylated resins.

Chloromethylated resins were transformed into aminomethylated resins in two steps, by reaction with potassium phtalimide and then with hydrazine, as shown by WEINSHENKER and SHEN[30]. The substitution was quantitative as shown by the negative Volhard titration of chloride and by the SARRIN-MERRIFIELD[27] titration of amino groups.

N- Methylaminomethylated resins.

Merrifield chloromethylated resin was treated with methylamine according to SHINKAI and al.[10]. The substitution was quantitative as checked with a negative Charpentier-Volhard titration.

Introduction of the spacer.

- Synthesis of N-methyl ε-aminocaproïc acid:

A solution of 55 mmol (7 g) of N-methyl ε-caprolactame[31] in 100 ml of concentrated hydrochloric acid was heated 12 hrs to reflux. After evaporation under vacuum, the residue was cristallized in ethanol-ether. N-Methyl ε-aminocaproïc acid hydrochloride (F.67-69°C) was isolated with a 85% yield.

NMR (D_2O). δ ppm: 1.45 (m, 6H β,γ,δ from N); 2.35 (m, 2H ε from N); 2.65 (s, 3H, N-CH_3); 3.00 (m, 2H α from N); 10.40 (s, 1H, CO_2H).

- Synthesis of Boc-aminoacids.

Boc alanine (F.75°), Boc-ε-aminocaproïc acid and the corresponding N-methyl derivative (oil) were prepared with di-t-butyldicarbonate according to MORODER's technique[32].

- Coupling of the spacer.

A suspension of the functionalized resin (1 meq NH_2 or NH-CH_3) in the minimal quantity of dichloromethane was stirred during 15 mn; 3 mmol of Boc-aminoacid dissolved in 5 ml of dichloromethane, 0.02 mmol (25 mg) of DMAP and a solution of 3 mmol (0.38 g) of DIC in 5 ml of dichloromethane were successively added. After 4 hrs stirring at room temperature, the resin was isolated by filtration and washed three times with dichloromethane.

With NH_2 functionalized resins, substitution is quantitative as verified with Kaiser's test. With NH-CH_3 functionalized resins, a second identical coupling was performed in order to get a quantitative substitution.

In both cases, to deprotect the terminal amine function, the resin was stirred 30mn in 20 ml of 30% trifluoroacetic acid in dichloromethane; after washing with dichloromethane, treatment during 15 mn with a 10% solution of triethylamine in dichloromethane and washing, the resin was dried under vacuum at room temperature.

- Coupling of CPP.

A DMF suspension of 1 meq of amine functionalized resin was stirred 15 mn; a solution of 3 mmol (0.62 g) of CPP in hot DMF was then added, followed by a DMF solution of 3 mmol (0.38 g) of DIC. The reaction mixture was heated at 60°C with stirring, the reaction time being specified below. The resin was then filtered and washed many times successively with DMF and dichloromethane.

The coupling reaction was repeated as many times as necessary in order to ensure a quantitative substitution. This was verified with Kaiser's test when the resins were functionalized with primary amino groups. In the case of secondary amino groups, the CPP loading was measured with an acetylation titration method.

Resin 3a required three successive couplings of 5 hrs each. Compounds 6c,7c,8c and 9c were obtained with a single coupling of 5 hrs, whereas compounds 3b, 3c,4a,4b,4c,4d,5c and 5d required 15 hrs of one coupling step.

Particular case of resin 3d : 3 mmol (0.26 ml) of oxalyl chloride were added drop by drop into 30 ml of DMF in a flask protected from moisture. After stirring 20 mn at room

temperature, 3 mmol (0.62 g) of CPP were added. When the solution became clear, 1 meq of N-methylaminomethylated polystyrenic resin and 9 mmol (1.25 ml) of triethylamine were added. The mixture was heated 12 hrs at 100°C with stirring. After cooling, the resin was filtered, washed two times with DMF, three times with dichloromethane and dried under vacuum at room temperature.

Titration of the supported CPP

Titration by acid hydrolysis

200 mg of polyacrylic supported CPP and 3 ml of 6N hydrochloric acid were introduced into a thick pyrex tube. The tube was sealed and heated 24 hrs at 110° for. After evaporation under reduced pressure, the residue was taken into 50 ml of water. Absorption at 280 nm gave directly the concentration of CPP hydrochloride (ε = 1730).

Titation by acetylation

1 mmol (102 mg) of acetic anhydride, 1 mmol (101 mg) of triethylamine and 0.1 mmol (12 mg) of DMAP were added to a suspension of 500 mg of supported CPP onto 10 ml of anhydrous dioxane. After 3 hrs stirring at room temperature, the resin was filtered, washed three times with dioxane and one with methanol; a suspension of this resin was stirred 3 hrs at room temperature in methanol containing 4 mmol of triethylamine. The resin was filtered and washed several times with dioxane, dried under vacuum and weighted; a suspension of this resin was then stirred 3 hrs at room temperature in 10 ml of a 0.1 N dioxane solution of acetic anhydride. An aliquot of 5 ml of the liquid part was hydrolysed with 20 ml of water; titration of this solution with 0.1 N sodium hydroxide gave the quantity of consumed acetic acid, and consequently the CPP loading (one equivalent of CPP for two equivalents of acetic acid consumed).

Titration with acetic acid

A quantity of dried resin corresponding approximately to 1 meq was weighted and suspended in 20 ml of a 0.1 M dioxane solution of acetic acid. After 1 h stirring at room temperature, an aliquot of 5 ml of solution was titrated with a 0.1 N solution of sodium hydroxide. This allowed the quantity of acetic acid consumed by the resin to be known, and consequently the initial loading of the sample.

Determination of the pK

The supported CPP (1meq) was suspended and stirred in 25 ml of dioxane saturated with gaseous hydrogen chloride. The resin was then filtered, washed four times with dioxane and dried under reduced pressure. One meq of DMAP was added to this resin suspended in 20 ml of dichloromethane, and the mixture stirred 1 hr at room temperature. The resin was then filtered and washed many times with dichloromethane. The chloride ions remaining in the organic phase and bound to the polymer were respectively titrated with the Volhard method. The sum of the two results came to one meq.

We checked with two examples that identical results were obtained from the equilibration of supported CPP with DMAP hydrochloride.

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