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OPTIMIZATION OF THE REACTION CONDITIONS USING A NADH MODEL GRAFTED ON A MERRIFIELD TYPE RESIN

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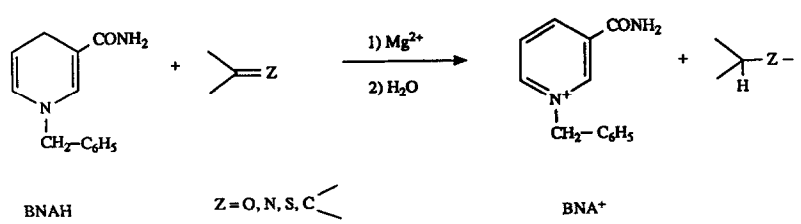
Abstract : The synthesis of reducing agent 3, 1,4-dihydronicotinamide grafted on a Merrifield resin, is described. The reaction conditions of reagent 3 are optimized, with respect to the solvent and the catalyst amount. The reagent 3 is more sensitive to traces of water than free NADH models such as N-benzyl 1,4-dihydronicotinamide. However in hyper-dry conditions reagent 3 is very much more stable and more efficient.It is shown that insertion of a spacer between the dihydronicotinamide moiety and the matrix enhances the rate of reductions but lowers the yields.

Nicotinamide adenine dinucleotide and its phosphate derivative are widely distributed as the coenzymes for biological redox reactions. It has been established that in the reduced form of the coenzyme the active part is the 1,4-dihydronicotinamide moiety. Thus, a 1,4-dihydropyridine derivative where the ring nitrogen is substituted by a simple substituent, such as 1-benzyl 1,4-dihydronicotinamide (BNAH), can be considered as a model compound for NAD(P)H. Simulation of the reactions of dehydrogenases using 1,4-dihydropyridine derivatives has been widely investigated (1)(2). These reagents allow the chemoselective reduction of C=O, C=N, C=S and C=C double bonds under mild conditions. Moreover with chiral models high enantiomeric excesses have been obtained in asymmetric syntheses.

Scheme 1



However the work-up of resulting mixtures of reactions performed with NADH models is rather difficult. In some cases, special techniques such as preparative gas phase chromatography or high performance liquid chromatography are required for the isolation of the products. Moreover it is very important to work in hyper-dry conditions so as to have good yields in reductions (3)(4). It must be mentioned that in biological systems, the reaction generally occurs in a hydrophobic site of the apoenzyme cavity.

An alternative which permits a modification of the treatment of the reaction mixture is the use of grafted reagents on a polymeric matrix. Generally, a simple filtration allows the product formed in the reaction to be isolated. Moreover,if the polymeric matrix is derived from polystyrene, its hydrophobic character can favor the enzymatic type reaction.

This approach has already been described in the literature (5 to 12). In some cases, only kinetic studies were performed. In most cases, only very reactive substrates were successfully reduced, or reduction of classical carbonyl compounds gave average yields.

In our laboratory, we described the first truly reactive polymer bound NADH model (13). The dihydronicotinamide moiety was grafted on to a Merrifield type resin. Since our initial publication we have obtained new, interesting results in the reduction of various substrates with this reagent (14). The purpose of this article is to describe the grafting of the dihydropyridine derivative on the matrix, to show the optimization of the use of the reagent and the comparison of the results obtained with those from free NADH models and to describe the modification of the initial grafted reagent by the introduction of a spacer.

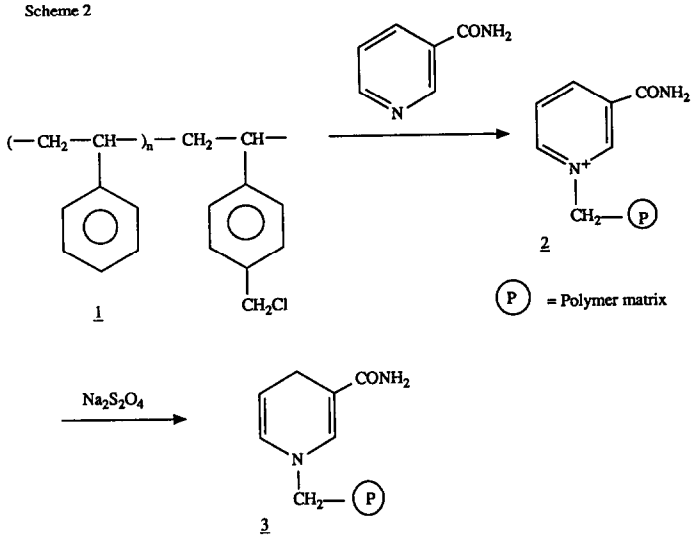
SYNTHESIS OF THE GRAFTED REAGENT

The initial Merrifield resin was purchased from the Merck Co (The chlorine content was 4-5%; cross-linking was insured by 2% of divinylbenzene). This product was referred to chloromethylated polystyrene art. N°818178. The results described in the present paper were obtained with reagent grafted on to this matrix. Some years later we needed new samples of the same polymer. The specifications seemed to be the same. However the results were irreproducible. Quantitative yields in the reduction of p-nitro benzaldehyde could be obtained only by the use of a two fold excess of grafted reagent.

It must be noticed that the inconstancy in reproduction of results with polymer supported reagents has already been reported in the literature (15).

Quaternization was carried out by treatment of nicotinamide with the Merrifield resin in refluxing acetonitrile as solvent. After cooling, the resin was filtered, and without drying reduced regioselectively with sodium dithionite (scheme 2). It must be stressed that if the pyridinium salt 2 is completely free of solvent, swelling cannot occur and the reduction leading to the dihydropyridine derivative 3 is unsuccessful.

Scheme 2



The changes in the elemental analysis from 1 to 2 and 3 show that the relevant reactions proceed quantitatively. For example the nitrogen contents in 2 and 3 are consistent with that predicted from the degree of chloromethylation of the commercial Merrifield resin. Moreover, the absence of chlorine in 3 constitutes supplementary evidence that the reduction with $Na_2S_2O_4$ is quantitative. From these results the amount of 1,4-dihydronicotinamide grafted per gram of resin is about 1.2 milliequivalent.

OPTIMAL CONDITIONS FOR REDUCTIONS.

The substrate used for these studies is p. nitro-benzaldehyde (p.NBA). The reactivity of this compound towards NADH models is good and the NMR spectra of the aldehyde

and those of the corresponding alcohol are easy to interpret : this situation allows a facile monitoring of the course of the reductions.

1) Choice of the solvent.

With free models such as BNAH, the most widely used solvent is acetonitrile, since it is a polar solvent which favours interactions between the model and the substrate and an excellent solvent for magnesium perchlorate necessary in most of the reductions performed with NADH models. Unfortunately it could not be used with reagent 3 grafted as it did not swell the polymer, so that access to 1,4-dihydronicotinamide was impossible. We studied the influence of benzene when added as swelling cosolvent with acetonitrile. We performed the reductions of p.NBA in various mixtures of these two solvents : the amount of benzene was increased until $Mg(ClO_4)_2$ no longer dissolved. At room temperature the reductions were too slow, so the experiments were performed at 65°C. At highest temperatures the rates were faster but the matrix was notably degraded. The reductions were conducted in sealed tubes and stopped after 21 hours, before completion. However reductions were also performed for 112 hours in order to confirm the efficiency of the reagent. (table 1).

V benzene/V solvent	Yield in alcohol %
0.05	42 [a] (92 [b])
0.2	48 [a] (98 [b])
0.35	56 [a] (99 [b])
0.5	65 [a] (99 [b])
0.6	60 [a] (99 [b])

Table 1: reduction of p.NBA in various mixtures of CH_3CN/C_6H_6
a: 1 mmole p.NBA, 1 mmole 3, time 21 h at 65°C.
b: same conditions but time 112h.

It appears that the optimum composition of the benzene/acetonitrile solvent mixture is 1/1. This is the best compromise between the necessary polarity of the solvent and the necessary swelling which allows access to the active sites. We must point out that, as might be expected, the reduction of p.NBA with the grafted reagent is slower that the similar

reaction performed with BNAH. With the latter the reduction is complete after 4 hours at 65°C. This phenomenon is generally observed with reagents grafted on a cross-linked matrix where the rate determining factor is the diffusion through the matrix. This factor is generally governed by the swelling. However, with the grafted NADH model, the rate of reduction was very similar to that observed with a model grafted on a non-cross-linked acrylic resin type (16).

2) Role of traces of water.

In preliminary studies with BNAH we showed that small amounts of water dramatically decrease the efficiency of the reagent (4) : the reduction yields of p.NBA in the presence of one or two equivalents of water fell respectively to 84% and 76%. We think that water complexes a part of the magnesium ions and lowers their ability to insure the interaction between the substrate and the model.

Molar ratio water/p.NBA [a]	Yield in alcohol (%) [b]
0.035	74
0.120	62
0.255	52
0.470	42
0.970	28
2.160	18

Table 2: Effect of water added in the reduction of p.NBA with 3
a: water titrated by the Karl Fisher method
b: reductions were performed with 1 mmole of p.NBA
1 g of model 3 in a 1/1 mixture of C_6H_6/CH_3CN at 65°C.

With two equivalents of water the yield is very low : only 18% of alcohol is obtained. As can be seen the influence of water is much greater than in the case of a free model such as BNAH (4). We think that with the polymeric reagent, the microenvironment has a high hydrophobic non-polar character. So, magnesium perchlorate could have difficulty in penetrating the resin and thus in reaching the active sites. As a consequence it becomes more sensitive to the presence of water which

is also repulsed by the matrix : the complexation of magnesium ions is important and as a result, the catalytic efficiency for a reduction is lowered significantly (17).

3) Influence of magnesium concentration.

With free models the reduction rate is generally enhanced by using increasing amounts of magnesium ions.

Mmoles Mg^{2+} [a]	Yield in alcohol (%) [b]
0.25	40
0.75	70
1.00	80
1.25	70
1.75	40

Table 3: Influence of magnesium perchlorate concentration [a].
a: reductions were performed with 1 mmole of p.NBA, 1 g of model 3 in a 1/1 mixture of C_6H_6/CH_3CN at 65°C.
b: time was 70h.

It appears clearly that the optimal amount of catalyst is about one equivalent of substrate to one of model. With an excess of magnesium ions the yields decreased. This feature occurs in some rare cases with free models where the structure of the substrate can inhibit interactions with Mg^{2+} (18). With p.NBA this does not occur. We have here another important difference between free models and our grafted model. Moreover it must be mentioned that with models grafted on to acrylic resins the behaviour is very similar to that observed with BNAH (16).

It can be assumed that with the cross-linked matrix of 3, the access to the active sites is hindered by large amounts of magnesium ions.

The standard conditions to perform the reduction of 1 millimole of a substrate with the grafted reagent can be summarized as follows :

- 1 mmole of the substrate.
- 1.0 g. of reagent 3, that is a slight excess with respect to the substrate.
- 1 mmole of anhydrous magnesium perchlorate.
- 12 ml. of an equivolumic amount of hyper-dry-acetonitrile and benzene.
- Reaction performed in a sealed tube (to avoid moisture) for 4 to 5 days at 65°C.

It must be pointed out that the grafted reagent is remarkably stable for a long time at a rather high temperature (several days at 65°C). In similar conditions, free models such as BNAH are completely destroyed after a few hours by autodecomposition in the presence of magnesium perchlorate. This behaviour is mentioned particularly in kinetic studies (19). In the case of BNAH, with one equivalent of magnesium perchlorate the reagent has totally disapeared after 8 hours at 65°C.

With the grafted reagent this pernicious reaction does not occur probably because the concentration of magnesium ions near the active site is very low or zero : the magnesium is especially complexed by the substrate before slowly diffusing, through the matrix, until it reaches the active site where the dihydropyridine is located.

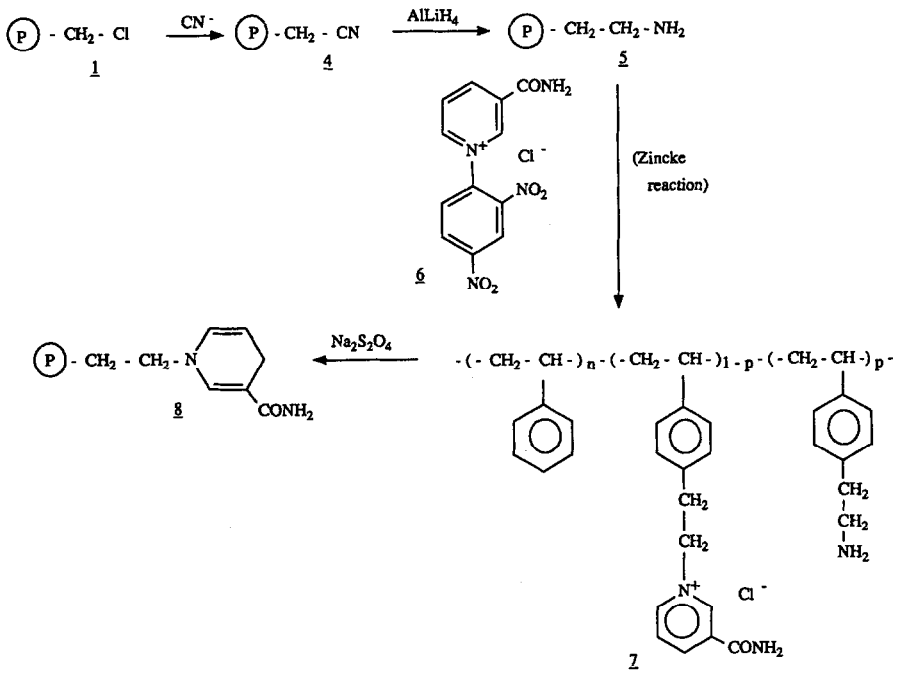
INFLUENCE OF A SPACER.

In standard conditions the immobilized NADH model is efficient. However the maximum yield for the reduction product of p.nitrobenzaldehyde is obtained after several days at 65°C. This feature is generally observed when a comparison is made between free and grafted reagents.

One method for enhancing the reactivity of a grafted reagent is to insert a spacer between the reagent and the matrix. (20)(21). The reagent is then less closely related to the polymeric matrix and the polymer effect is diminished. This approach has been developed in some cases with NADH models. The improvements were not important (22).

We have synthezised a new reagent in which the 1,4-dihydronicotinamide moiety is put away from the matrix by a supplementary carbon atom. The synthesis of this reagent is summarized in the scheme 3.

Scheme 3



The key step of the method is the Zincke reaction (23) applied to the aminoethylated polymer 5 (24). In fact, the exchange between 5 and the pyridinium Zincke's salt 6 is not complete. The course of the reaction can be estimated through the change in nitrogen content of the different polymers implicated in the scheme 3 (table 4).

Compound	% N Exp	% N Calc
4	2.15	2.00
5	2.0	1.99
7	2.8	3.31
8	3.05	3.45

Table 4 : Nitrogen contents in compounds 4, 5, 7, 8.

The chlorine content in the starting commercial product (5%) allows virtual molecular masses to be estimated. For compound 7 this mass can be deduced from the formula : $M=104n + 288.5 (1-p) + 147 p = 850.1 - 141.5 p$.

An approached calculation gave p=0.4 which means that only 60% of the amino groups of 5 were submitted to the Zincke's reaction leading to polymer 7. We think that the access to these functions is difficult owing to the large size of the pyridinium Zincke's salt 6. However, we have studied with this reagent the rate of the reduction of p.NBA. The reaction was stopped a long time before completion (after 17 hours) and the yield in alcohol compared with the result obtained with 3 in the same conditions.

Reagent	Alcohol	Recovered aldehyde	Total
3	24	76	100
8	35	28	63

Table 5: Reduction of p.NBA with reagents 3 and 8.

It can be seen that the reagent with a spacer enhances notably the rate of the reduction. However with 8, the global balance of the reaction is bad. The reduction can be limited by : -1) partial reaction of the remaining amine functions with the aldehyde through iminic derivatives. -2) The instability of the reagent in presence of traces of water retained by the hydrophilic character of the remaining amino group. As a consequence, the efficiency of the grafted reagent 8 is diminished, and in further experiments we shall use only reagent 3. The reactive polymer was recycled one time and we observed a small loss of activity (95 % reduction yield instead of 100 %) in the reduction of p-NBA.

EXPERIMENTAL

Melting points are uncorrected. IR spectra were recorded on a BECKMAN IR 4250 spectrophotometer in KBr pellets unless otherwise stated. ^1H NMR spectra were recorded on a VARIAN EM 360L (60 MHz) spectrometer in $CDCl_3$ solution unless otherwise noticed : chemical shifts are reported in ppm downfield from internal TMS. Elemental analysis were performed on a CARLO ERBA 1106 device. MERRIFIELD resin was purchased from MERCK Co. Acetonitrile was first distilled on phosphoric anhydride then redistilled on calcium hydride and storred on molecular sieves (3 Å) for two days prior to use. The water amounts in solvents were determined with a METROHM KF 652 coulometer. Para nitrobenzaldehyde was purchased from ALDRICH Co and used without further purification. Anhydrous magnesium perchlorate was purchased from MERCK Co (Art. 5879).

Nicotinamide grafted on MERRIFIELD resin :
This compound was obtained as described earlier (25).

Cyanomethylated resin : 4

This reaction had to be carried out in a vessel connected to an hydrogen cyanide trap. To a stirred mixture of MERRIFIELD resin (25g of 4-5 % Cl) and freshly distilled dimethyl formamide (200 ml) was added sodium cyanide (4.9 g,0.1 mole). The reaction mixture was stirred for 4h at 75-80°C. The resin was collected by filtration and then thoroughly washed successively with water, methanol, water, a mixture of dioxane-water 4/1, dimethylformamide, dioxane, acetone, chloroform and methanol. The white resin was dried in a vacuum oven at 40 °C. The yield is 4.5 g: IR 2240 cm^{-1}. Typical Analysis: C, 90.6; H, 7.6; N, 2.0.

Aminomethylated resin : 5

An ice cooled mixture of cyano methylated resin 4, anhydrous aluminium chloride (13.4g, 0.1 mole) in dry tetrahydrofuran (200ml) was treated with lithium aluminium hydride (4 g, 0.105 mole). The resulting mixture was refluxed for 18h. After cooling, the excess of reducing agent was hydrolysed with crushed ice. The aluminium salts were dissolved with diluted aqueous hydrogen chloride. The resin was filtered and washed successively with aqueous sodium hydroxyde, water, tetrahydrofuran, dichloromethane and methanol. The yield is 10 g of a white resin: IR 3450 cm^{-1}. Typical Analysis: C, 87.5; H, 8.0; N, 2.0.

ZINCKE salt : 6

A mixture of nicotinamide (20g, 0.16 mole) and 2,4-dinitro chlorobenzene (100g, 0.49 mole) was heated at 90°C for 4 hours. After cooling, the resulting mixture was dissolved in methanol (120ml) and quenched in ether (1l). The resulting solid was filtered. This procedure was repeated twice. The product was then dissolved in water and boiled with charcoal. The hot solution was filtered and the filtrate concentrated in vacuo. The crude product was cristallized several times from methanol to provide 6 in white needles (48g,82 %) : IR 3300, 3100, 1690, 1350cm^{-1}. ^{1}H NMR (DMSO D_6, ppm/HMDS) 8.16 (s,2H); 8.5-10 (m,7H). Typical Analysis: C, 42.25; H, 3.4; N, 15.65; $C_{12}H_9N_4O_5Cl$, CH_3OH requires C, 43.77; H, 3.67; N, 15.70.

Pyridinium resin : 7

A mixture of amino ethylated resin (9g) and ZINCKE salt (10 g, 0.28 mole) in methanol (250 ml) was heated at 65 °C for 24 hours, without stirring. The resin was filtered, washed successively with methanol and acetone. The product was then extracted with methanol in a SOXHLET device for 24h. The resin was dried in a vacuum oven at 40°C. The yield is 10.5 g of a red product: IR 1695 cm^{-1}. Typical Analysis: C, 80.3; H, 7.2; N, 2.8.

Reducing agent : 8

The reduction had to be carried out under an argon atmosphere. The mechanical stirring had to be slow. A mixture of 10 g of resin 7, hydrated sodium carbonate (10 g, 0.035 mole) and sodium dithionite (20g,0.115 mole) in water (200ml) was stirred cautiously at room temperature for 6 hours. The yellow beads of polymer were then filtered and dried at 40°C in a vacuum oven. The grafted reagent thus obtained must be used immediately or storred in a refrigerator under an argon atmosphere. The yield is 10g of yellow beads: IR 1690 cm^{-1}. Typical Analysis: C, 82.5; H,7.0; N, 3.05.

General procedure for reductions with the grafted NADH models:

In a dry glass tube, flushed with argon were introduced 1.2 meq of the grafted reagent, 1,0 millimole of the compound to be reduced, and magnesium perchlorate (amounts as mentionned in the theoretical section), 7ml of hyper dry acetonitrile and 7 ml of benzene (these solvents were introduced with syringues through a septum). In all cases, the amounts of water in solvents were determined by using the KARL FISCHER method. The tube was sealed and heated in an oil bath at 65°C (reaction times are mentionned in theoretical part). After cooling, the tube was broken, the resin filtered and washed with acetonitrile. The solvents were removed in vacuo and water (5ml) was added. The resulting mixture was extracted twice

with dichloromethane. The combined organic layers were dried over anhydrous magnesium sulfate and concentrated. The residue was submitted to NMR analysis.

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