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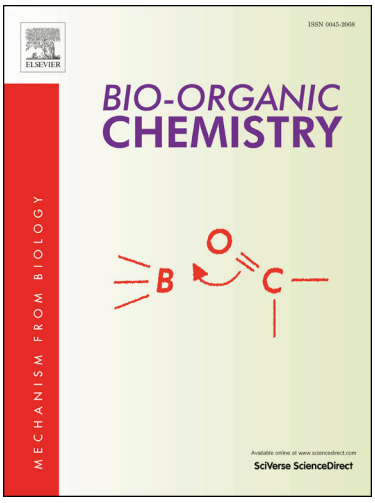

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THE FIRST ENZYME-PROMOTED ADDITION OF NITROMETHANE TO IMINES (AZA-HENRY REACTION)

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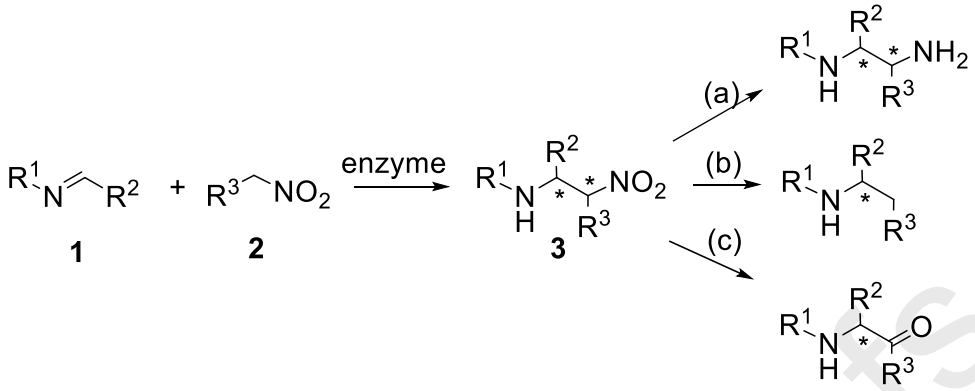
ABSTRACT

Enzyme catalytic promiscuity is the ability of a single enzyme active site to catalyze several chemical transformations, among them those which are different from natural. We have attempted to use this feature of enzymes in the nucleophilic addition of nitromethane to aldimines (the aza-Henry reaction) whose chemically catalyzed version leads to synthetically useful β-nitroamines. We succeded in obtaining for the first time the desired products in the yields up to 81 %. The most efficient proved lipase TL (from *Pseudomonas stutzeri*) and oxynitrilase from *Arabidopsis thaliana*. However, all the reactions investigated were non-stereoselective.

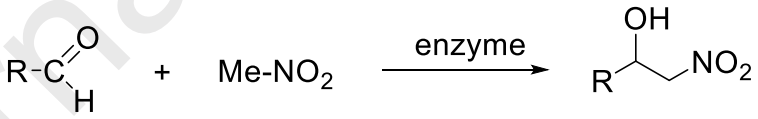
1. Introduction

Enzyme catalytic promiscuity, i.e. the ability of a single active site of the enzyme to catalyze more than one reaction, particularly those which are different from that designed by Nature, has been a subject of increasing research interest in the recent few years [1-6]. As enzyme catalytic promiscuity is highly advantageous to chemists, since it broadens the applicability of enzymes in chemical synthesis and particularly in the stereoselective transformations, it is of great importance to search for new applications of this feature of enzymes. Therefore, and in connection with our previous work on this subject [7], we have decided to make an attempt to discover new, unknown thus far, enzyme-promoted transformations which will be based on this phenomenon. The transformation of choice has been the nucleophilic addition of nitroalkanes **2** to imines **1**, called the "aza-Henry" or "nitro-Mannich" reaction (Scheme 1) which is known to lead (in its chemical version [8,9]) to the formation of synthetically useful substituted β-nitroamines **3** with potentially possible two stereogenic centres. These compounds may be used as interesting building blocks (routes a, b and c). The biocatalytic version of the aza-Henry reaction has not been reported in the literature.



Scheme 1. The aza-Henry reaction

A chance of finding new examples of enzyme catalytic promiscuity is based on numerous literature data. So far, several interesting examples of this phenomenon have been reported. Among them, a very important case seems the ability of hydrolytic enzymes to catalyze a carbon-carbon [10] and carbon-heteroatom bond [11] formation , i.e. to exhibit a lyase activity. Lipases are those hydrolytic enzymes for which numerous examples of such a promiscuous activity have been described. In addition to their original activity comprising hydrolysis of lipids and, generally, catalysis of the hydrolysis or formation of carboxylic esters [12], lipases are able to catalyze, among others, also Michael additions of *C*-acids to nitroalkenes, aldol condensation [13,14] and Knoevenagel condensation [15]. From the point of view of the present account, the most inspiring example is the ability of various hydrolytic enzymes to catalyze the analogous reaction, namely the nucleophilic addition of nitroalkanes **2** to carbonyl compounds (the Henry reaction, Scheme 2).



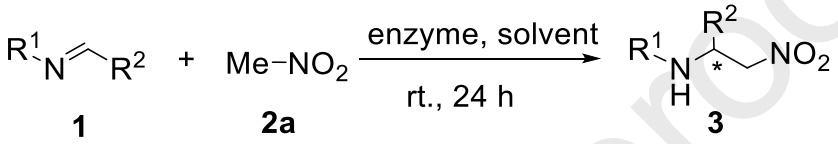
Scheme 2. The Henry reaction

This reaction was found to be efficiently (i.e. giving products in good yield and with good to high enantiomeric excess, ee) catalyzed by esterases (esterase from *Thermotoga naphthophila* and from *Aeropyrum pernix*), lipases (lipase from *Candida antarctica,* CAL-B, and from porcine pancreas) [16] and proteases (aminopeptidase from *Sulfolobus tokodaii* [16], but also by selected lyases, e.g. hydroxynitrilases (hydroxynitrilase from *Hevea* brasiliensis [17] and from *Arabidopsis* thaliana [18]). Moreover, application of protease from *Streptomyces* griseus [19] or acylase I from *Aspergillus* melleus [20] as catalysts in the Mannich reaction gave the products with up to 92/2 diastereomer ratio and up to 89% ee. However, in certain cases the enzymatic reactions were non-

stereoselective, although the yields of the products were up to 95 %. This concerned the use of lipases from *Aspergillus niger*, *Rhizopus niveus*, *Mucor javanicus*, *Pseudomonas fluorescens*, *Candida rugosa*, *Burkholderia* cepacia [21], protease from *Bacillus licheniformis* [22], papain from papaya latex [21], and transglutaminase from *Streptorerticillium griseoverticillatum* [23].

2. Results and discussion

In our preliminary work, we screened the reaction of *N*-arenesulfonylaldimines **1**, synthesized according to known literaturę procedures, with nitromethane **2a** (Scheme 3).



Scheme 3. The enzyme-promoted aza-Henry reaction

In this case, only one stereogenic centre could be created in products **3** (R^3 = H). We applied various reaction conditions and a broad variety of enzymes, including lipases, proteases and hydroxynitrilases. The results are collected in Table 1.

Table 1: Enzyme-promoted aza-Henry reaction

Entry	N-arenesulfonylaldimine **1**		Enzyme	Solvent	Conversion [%]	Product **3**	
	R^1	R^2				Symbol	Isolated yield [%]
1	*p*-TolSO_2	2-thienyl	TL	$MeNO_2$	No reaction	-	-
2	*p*-TolSO_2	2-thienyl	AtHNL	$MeNO_2$	No reaction	-	-
3	*p*-TolSO_2	2-thienyl	AtHNL	H_2O	Hydrolysis of imine	-	
4	*p*-TolSO_2	2-thienyl	AtHNL	EtOH	100	**3a**	74
5	*p*-TolSO_2	2-thienyl	TL	EtOH	60	**3a**	43
6	*p*-TolSO_2	2-thienyl	TL	MeCN	100	**3a**	81
7	*p*-TolSO_2	2-thienyl	AtHNL	BF	ND	**3a**	45

8	*p*-TolSO$_2$	2-thienyl	TL	BF	ND	**3a**	40
9	*p*-TolSO$_2$	1-naphthyl	AtHNL	EtOH	100	**3b**	24
10	*p*-TolSO$_2$	1-naphthyl	TL	EtOH	70	**3b**	15
11	*p*-TolSO$_2$	1-naphthyl	TL	MeCN	70	**3b**	38
12	*p*-TolSO$_2$	phenyl	AtNHL	EtOH	50	**3c**	26
13	*p*-TolSO$_2$	phenyl	AtNHL	MeCN	70	**3c**	32
14	*p*-TolSO$_2$	phenyl	TL	EtOH	70	**3c**	54
15	*p*-TolSO$_2$	phenyl	TL	MeCN	40	**3c**	16
16	PhSO$_2$	phenyl	AtHNL	EtOH (94%)	ND	**3d**	32
17	PhSO$_2$	phenyl	AtHNL	MeCN	ND	**3d**	20
18	PhSO$_2$	phenyl	TL	MeCN	60	**3d**	41
19	PhSO$_2$	phenyl	TL	EtOH	ND	**3d**	30
20	PhSO$_2$	phenyl	PPL	EtOH	ND	**3d**	5

Enzyme: TL– lipase from *Pseudomonas stutzeri*; AtHNL – oxynitrilase from *Arabidopsis thaliana*; PPL – porcine pancrease lipase; BF – ionic liquid: 1-butyl-3 methylimidazolium tetrafluoroborate; Conversion calculated on the basis of the consumption of the starting imine **1**

The results obtained need certain comments. First of all, from among a high number of enzymes examined, only three enzymes proved suitable and gave the desired products **3** in the yield up to 81%. These were: lipase TL from *Pseudomonas stutzeri* (entries 5, 6, 8, 10, 11, 14, 15, 18 and 19); oxynitrilase from *Arabidopsis thaliana*, AtHNL, (entries 4, 7, 9, 12, 13, 16 and 17) and, to a certain extent, porcine pancrease lipase, PPL (entry 20). The products obtained were isolated, purified, fully analyzed and characterized. Interestingly, in many cases the conversion degree was quite high,. This was determined by NMR of the crude reaction mixtures, in which the relative amount of the unreacted starting imine was measured. The lower yield of the isolated products was due to the presence of some by-products, particularly the corresponding aldehydes resulting form the hydrolysis of the imines. No reaction was observed in the absence of enzymes and in the presence of the following enzymes: papain, lipases from: *Candida antarctica B, Mucor javanicus*, *Candida rugosa*, *Pseudomonas species* and *Pseudomonas fluorescens.* No products were formed in the control reactions in which those denatured enzymes, which earlier proved efficient in the reaction investigated, were applied (see Experimental Section). This can be taken

as proof of the real participation of enzymes in the successful reactions. In search for the most favourable reaction conditions, a variety of solvents were checked. No reaction was observed in the case of common organic solvents such as dichloromethane, ethers, toluene or nitromethane (used here both as reagent and solvent and for this reason visualized in Table 1). Water proved useless as it caused hydrolysis of aldimines. Acetonitrile and ethanol proved to be the best solvents although it is difficult at this point to explain unusual differences in the yield of particular products when the same enzymes were used (e.g entries 5 and 6 versus 14 and 15). The use of an ionic liquid 1-butyl-3 methylimidazolium tetrafluoroborate, gave relatively good results (entries 7 and 8). It is worth mentioning that ionic liquids are known as useful solvents in biotransformations [24] and were successfully used also by us in hydrolase-catalyzed reactions [25]).

To our deep disappointment, all the reactions, performed thus far, were non-stereoselective. Although precedents for such outcomes were cited in the literature (*vide supra* and in our paper [7]), it seems necessary to seek an explanation for the lack of the stereoselectivity, as stereoselectivity should be a common feature of enzyme-promoted transformations. In our opinion, the substrates, which possess large electron-withdrawing substituents at the nitrogen atom, may not be properly accommodated by the enzyme active site. On the other side, such substituents enhance the addition of nitromethane to aldimines. Indeed, our experiments using the substrates which are deprived of them, were thus far unsuccessful. Although these preliminary attempts were in certain aspects disappointing, the investigations will be continued in the hope of finding proper substrates, enzymes and reaction conditions.

3. Conclusions

In this preliminary study, the first successful enzyme-promoted addition of nitromethane to *N*-arenesulfonylaldimines was accomplished, which was a result of the application of enzyme catalytic promiscuity. Only three enzymes: lipase TL from *Pseudomonas stutzeri*; oxynitrilase from *Arabidopsis thaliana* (AtHNL) and porcine pancrease lipase (PPL) proved suitable. The best results were obtained using ethanol as solvent. However, all the reactions were non-stereoselective, which is considered to be due to the large electron withdrawing substituents at the nitrogen atom in the substrates. In case of the substrates deprived of such substituents, no reaction was observed. Further investigations are in progress.

4. Experimental Section

All the *N*-sulfonyl imines **1** are known [26,27,28] and for our purposes were obtained according to the procedure described by Garcia Ruano in the reaction of the corresponding aryl aldehydes and *p*-toluenesulfonamide or benzenesulfonamide in the presence of catalytic amounts of pyrrolidine [26].

Enzymes: Lipase TL – Lipase from *Pseudomonas stutzeri;* a generous gift by Meito Sangyo Co., Ltd. AtHNL – oxynitrilase from *Arabidopsis thaliana*; PPL – porcine pancrease lipase, both purchased from Sigma.

4.1.General procedure for enzymatic Aza-Henry reaction of imines 1 with nitromethane.

To the solution of an *N*-sulfonyl imine **1** (0.4 mmol) in the appropriate solvent (6mL), enzyme [AtHNL (63μL), or TL (50 mg), or PPL (50 mg)] was added and the mixture was stirred for 0.5 h. Nitromethane (1mL) was dropped and the reaction mixture was stirred for 24 h at ambient temperature. Than the solvent was evaporated and CH_2Cl_2 (10 mL) was added and (in the case of AtNHL) washed with water (2x5 mL) or (in the case of Lipase TL and PPL) filtered through Celite. The organic fraction was dried over $MgSO_4$ and evaporated. The crude reaction mixture was purified using column chromatography to give pure products. All the products are known form the literature [29,30,31]. Analytical and spectral data of the products obtained were compared with those reported in the literature and proved correct.

Experiments with denatured enzymes

Two enzymes, namely AtHNL and lipase TL, were denatured by heating overnight in boiling water at 100 ºC. The water was evaporated and the recovered denatured enzymes were used in selected reactions according to the procedure described above. In no case was the formation of the appropriate products **3** observed.

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Notes

The authors declare no competing financial interest.

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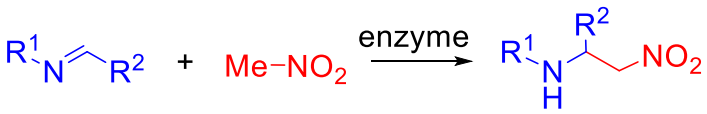

Enzyme catalytic promiscuity

Highlights

- The first successful enzyme-promoted addition of nitromethane to sulfonylimines (aza-Henry reaction) using enzyme catalytic promiscuity is presented.
- A series of various enzymes was screened to identify their promiscuous activity.
- Three enzymes proved efficient and led to the formation of the desired adduct in up to 81% yield
- From among many solvents only three proved suitable for the reaction.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: