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Enzyme-catalyzed cascade synthesis of hydroxyiminoacetamides

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ABSTRACT

In order to synthesize *N*-(3-azido-1-phenylpropyl)-2-hydroxyiminoacetamide, a key compound for the preparation of acetylcholinesterase (AChE) reactivators of the *N*-substituted 2-hydroxyiminoacetamide type, it was necessary to develop a method for forming an amide bond between an ethyl glyoxylate oxime and an amine. Using *Candida antarctica* lipase B (CAL-B) in a cascade enzyme-BOP catalyzed reaction, the efficient synthesis of the target hydroxyiminoacetamide was achieved.

Organophosphorus (OP) nerve agents are extremely toxic compounds used as chemical warfare agents in armed conflicts and terrorist attacks[1] and as pest control agents.[2] The acute toxicity of these compounds is due to their irreversible inhibition of acetylcholinesterase (AChE; EC 3.1.1.7).[3] Current therapy in cases of OP nerve agent poisoning includes an antimuscarinic drug (e.g., atropine), an anticonvulsant drug (e.g., diazepam), and an AChE reactivator from the quaternary pyridinium oxime family (2-PAM, trimedoxime, obidoxime, HI-6, Hlö-7).[4] However, current therapy directed at the reactivation of inhibited AChE is limited to peripheral circulation because commonly used quaternary pyridinium oximes do not cross the blood–brain barrier due to their permanent positive charge.[5] In order to achieve an efficient reactivation of central nervous system AChE, attempts have been made to develop efficient uncharged reactivators.[6] Recently, a new series of AChE reactivators, including *N*-substituted 2-hydroxyiminoacetamides, were reported.[7] A few *N*-substituted 2-hydroxyiminoacetamides have shown high reactivation potential toward sarin, cyclosarin, and VX inhibited AChE.[8] Because of the significant role of non-bonding interactions between the quaternary pyridinium ring of 2-PAM or HI-6 and the surrounding aromatic amino acids in the AChE active site in the overall stabilization of these compounds, it is reasonable to assume that the introduction of a phenyl ring into the structure of *N*-substituted 2-hydroxyiminoacetamides would help their stabilization and possibly improve the geometry of the oxime group access to phosphylated serine. Thus, the aim of our work was to synthesize several structurally diverse aromatic *N*-substituted 2-hydroxyiminoacetamides **2a–c** (Fig. 1).

The synthesis of key compound **1**, [*N*-(3-azido-1-phenylpropyl)-2-hydroxyiminoacetamide], from which the target molecules **2a–c** could be prepared by the well-known copper catalyzed azide–alkyne cycloaddition,[9] started from readily available cinnamyl alcohol (**3**) (Scheme 1).

1-Phenyl-allylamine (**4**) was prepared from alcohol **3** according to a procedure described in the literature[10] and an azide group was then introduced via a three-step process. The final reaction was the formation of an amide bond between 3-azido-1-phenylpropyl-amine (**5**) and ethyl glyoxylate oxime, which was previously

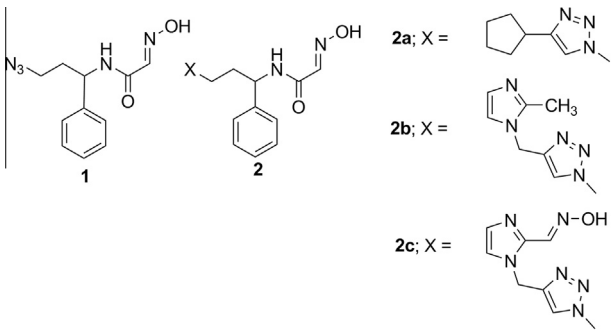

Figure 1. Targeted aromatic *N*-substituted 2-hydroxyiminoacetamides.

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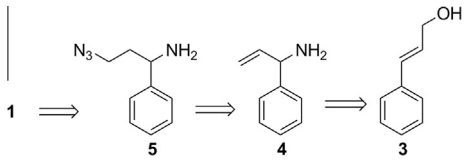


Scheme 1. Retrosynthetic pathway toward **1**.

described for similar compounds.[7] However, as in numerous other cases, amide bond formation turned out to represent a significant challenge.[11]

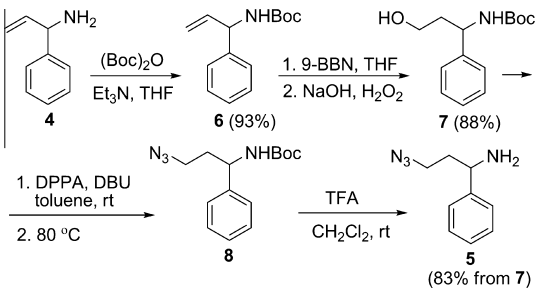
The preparation of 1-phenyl-allylamine (**4**) from cinnamyl alcohol (**3**) includes an Overman reaction, which has been well-described in the literature.[12] The hydroxyl group was introduced to Boc protected 1-phenyl-allylamine (**6**) via hydroboration–oxidation using 9-BBN as the hydroboration reagent (Scheme 2).

The hydroxyl group in **7** was replaced with an azide using diphenylphosphoryl azide (DPPA) as the azide source.[13] The reaction was performed in dry toluene with 1,8-diazabicycloundec-7-ene (DBU) as the base. The first step of the reaction, which was performed at room temperature, was the formation of the diphenylphosphate, which can easily be isolated, and the azide salt of DBU. By raising the temperature, nucleophilic substitution of the diphenylphosphate group with azide occurs. The resulting Boc-protected 3-azido-1-phenyl-propylamine (**8**) was difficult to purify from excess DPPA, so the crude residue was deprotected with trifluoroacetic acid (TFA) in dichloromethane to afford pure 3-azido-1-phenylpropylamine (**5**).

The remaining step for the synthesis of **1** was assumed to follow the same path as the synthesis of structurally similar reported compounds RS41A and RS194B.[7,14] The previously described condensation of ethyl glyoxylate with hydroxylamine provided ethyl glyoxylate oxime (**9**).[15] The formation of an amide between ethyl glyoxylate oxime and an amine was reported in EtOH at 50 °C. However, in our case, reaction at 50 °C, as well as in boiling EtOH, did not produce the desired product. Addition of Et_3N as a base provided a somewhat poor conversion. The search for a solvent and base that would result in a satisfactory conversion of the reactants into the desired product was unsuccessful.

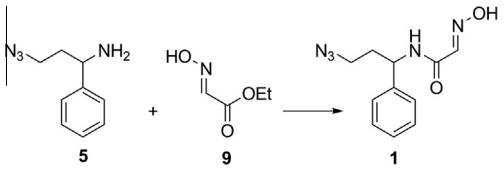
Microwave-assisted reactions, which are known to be very fast and straightforward, also proved unsuccessful.[16,17] MW irradiation of **5** and **9**, with or without an additional base, for 30 min under solvent-free conditions did not demonstrate enhanced conversion compared to reactions in boiling EtOH. Longer reaction times did not increase the conversion, however new peaks appeared in the HPLC chromatogram indicating the formation of by-products. The results of several attempts to carry out this reaction are presented in Table 1.

Bearing in mind our previous experience with *Candida antarctica* lipase B (CAL-B),[10] we decided to test this enzyme for



Scheme 2. Synthesis of 3-azido-1-phenylpropylamine (**5**).

Table 1
Testing different conditions for the synthesis of compound **1**



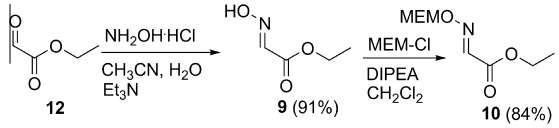
Solvent	Base	*T* (°C)	*t* (h)	Conversion[a] (%)
EtOH	—	Reflux	24	0
EtOH	Et_3N	Reflux	72	26
1,4-Dioxane	Et_3N	100	24	0
1,4-Dioxane	DIPEA	80	24	0
1,4-Dioxane	1,2,4-Triazole/DBU	80	24	0
1,4-Dioxane	DMAP	80	24	0
Diglyme	DIPEA	100	48	0
Diglyme	DIPEA	110	48	8
DMSO	DIPEA	100	48	0
THF	KO*t*-Bu	rt	2	0
—	—	MW-110	0.5	17
—	DIPEA	MW-95	0.5	18

[a] Conversion of the product was determined using HPLC with UV detection at 220 nm.

our reaction. CAL-B is a very selective biocatalyst in enzymatic resolution of primary amines, as well as an excellent tool for amide bond formation.[18] However, the test enzymatic reaction of amine **5** and ester **9** using CAL-B did not proceed as desired. The target product was obtained, but in very low yield. We first assumed that these poor results were the consequence of the somewhat ambiguous placement of ester **9** in the active site of the enzyme due to its oxime group. In fact, oximes are used as nucleophiles in enzyme-catalyzed oximolysis for the preparation of oxime esters.[19] Although these activated oxime esters are known to react with amines to give amides,[20] the success of amide formation depends on the structures of the amines and oxime esters.[21]

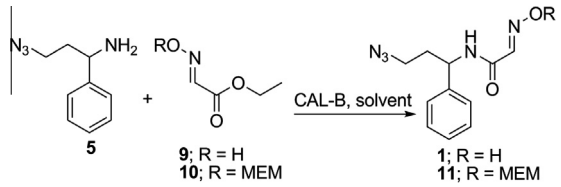
Therefore, to exclude the possibility of other side reactions, the oxime group was protected using a 2-methoxyethoxymethyl ether (MEM) protecting group (Scheme 3), starting from ethyl glyoxylate (**12**). Next, the reaction of the obtained ester **10** with amine **5** was examined. As was the case with unprotected oxime **9**, the desired product was obtained in low yield using the classic [EtOH, *N*,*N*-diisopropylethylamine (DIPEA), reflux for 48 h, 16% yield], as well as the enzymatic approach (Table 2). However, some solid was isolated which was insoluble in methyl *tert*-butyl ether (MTBE) and diisopropyl ether (DIPE), but was very soluble in dichloromethane. NMR and MS analysis of the solid showed that this substance (**13**, see Scheme 4) was actually the salt of amine **5** and hydrolyzed ester **10**.

Considering the results listed in Table 2, it was clear that hydrolysis of ester **10** catalyzed by the enzyme was faster than the expected amide bond formation reaction. It is generally known that during enzyme-catalyzed aminolysis, even traces of water cause hydrolysis of the acyl donor, which is why such reactions must be performed in dry solvents.[18,23] DIPE is a solvent which probably contains a sufficient amount of water to prevent amide formation. Hence, salt **13** was isolated in almost quantitative yield (96%), indicating that under these reaction conditions complete



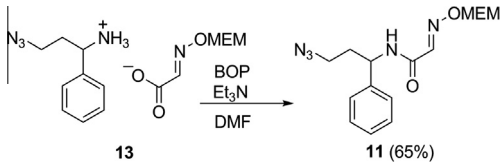
Scheme 3. Synthesis of the MEM-protected oxime **10**.

Table 2
Attempts at preparing compound **1** using CAL-B



Ester	Solvent	T (°C)	t (h)	Yield of **1** or **11** (%)
9	DIPE	40	48	0
9	MTBE	30	24	9
9	MTBE	40	24	2
10	MTBE	40	72	31
10	MTBE[a]	40	48	23
10	DIPE	45	48	0[b]

[a] Et_3N was added to the reaction mixure.[22]
[b] Salt **13** (see Scheme 4) was isolated from the reaction mixture (96%).



Scheme 4. Formation of amide **11** from salt **13**.

hydrolysis of the MEM-protected ester **10** occurred. Furthermore, ammoniolysis catalyzed by Novozym 435 (CAL-B immobilized on macroporous acrylic resin) has been reported to sometimes be accompanied by unexpected hydrolysis of the ester in spite of the rigorous exclusion of moisture.[23] It is assumed that these results are the consequence of acrylic carrier adsorption of traces of water flushed out by ammonia. Since we also used Novozym 435, it is reasonable to assume that this problem is also present.

The reaction yield of amide **11** could be enhanced by using a very large excess of ester that would ensure an ample amount of acyl donor for amide formation. However, ester **10** is not commercially available and has to be synthesized, so this approach was not suitable. Considering the above-mentioned facts and since we needed amide **1** in racemic form and higher yield if possible, we decided to try an alternative approach.

A large number of coupling reagents for amide bond formation between amines and carboxylic acids have been developed, principally for their use in peptide synthesis.[11] Benzotriazolyloxy-tris(dimethylamino)hexafluorophosphate (BOP) was selected due to its ability to work in situ. Typically, the activating agent is added to a 1:1 mixture of the amine and the carboxylic acid.[24] Accordingly, the isolated salt **13** was converted into the desired amide **11** in a BOP-catalyzed reaction (Scheme 4) in satisfactory yield.

Considering this reaction proved its potential, we decided to investigate a one-pot cascade enzyme-BOP catalyzed reaction of ester **10** and amine **5** (Scheme 5).[25] For reasons mentioned previously, DIPE was selected as the solvent. In this reaction, CAL-B first catalyzed the hydrolysis of ester **10** which produced salt **13**, and then the resulting salt was converted into amide **11** using the BOP/Et_3N catalyzed reaction. The reaction yield was similar to that obtained when the salt was first isolated from the reaction mixture and then subjected to BOP coupling in DMF. The desired amide **1** was now easily obtained from amide **11** by removing the MEM protecting group under mild reaction conditions using $ZnBr_2$.[26,27]

In conclusion, we have described the synthesis of hydroxyiminoacetamide **1** via a cascade enzyme-BOP catalyzed reaction. After several unsuccessful attempts in forming an amide bond between ethyl glyoxylate oxime and 3-azido-1-phenylpropylamine, the problem was solved in three steps. The oxime group was protected using the 2-methoxyethoxymethyl ether (MEM) protecting group. The obtained MEM-protected oxime ester was hydrolyzed using *Candida antarctica* lipase B (CAL-B) in an organic solvent and then coupled with an amine using BOP as the coupling reagent in a one-pot cascade reaction. After removing the MEM protecting group under mild conditions, the target hydroxyiminoacetamide **1** was obtained.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2014.06.027. These data include MOL files and InChiKeys of the most important compounds described in this article.

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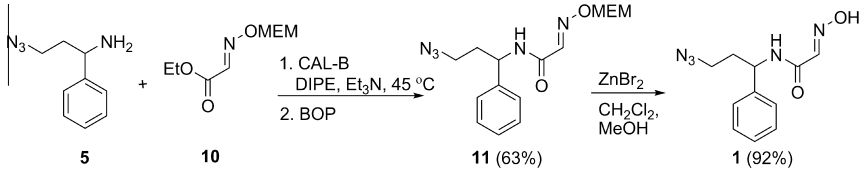

Scheme 5. Synthesis of hydroxyiminoacetamide **1** from ester **10** and amine **5**.

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25. *Enzyme-BOP catalyzed reaction:* MEM-protected ester **10** (132 mg, 0.64 mmol) was added to a solution of amine **5** (105 mg, 0.60 mmol) in DIPE (4 mL) followed by Et_3N (86 μL, 0.61 mmol) and immobilized lipase B from *C. antarctica* (200 mg). The mixture was stirred at 45 °C for 6 h. Next, BOP (267 mg, 0.60 mmol) was added and the mixture was stirred at the same temperature for another 2 h. The enzyme was filtered off and washed with DIPE and CH_2Cl_2. The filtrate was extracted with saturated aqueous NH_4Cl solution, dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude residue was purified by silica gel column chromatography (hexane–EtOAc, 1:1, R_f = 0.21) to give a clear oil (125 mg, 63%).
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27. *MEM-amide deprotection:* MEM-protected amide **11** (68 mg, 0.20 mmol) was dissolved in a mixture of CH_2Cl_2 (2 mL) and MeOH (0.4 mL). $ZnBr_2$ (oven-dried at 200 °C overnight) was added (400 mg, 1.8 mmol) to the solution. The mixture was stirred at 35 °C until completion of the reaction (monitored by HPLC). The mixture was diluted with CH_2Cl_2 (5 mL) and extracted with saturated aqueous $NaHCO_3$ solution, brine and water, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. Amide **1** was obtained as a white solid (46 mg, 92%).