Thiamine an unexplored, ecologically hormonizable, nontoxic catalyst for benzoylation

Deepak Chowrasia* and Nisha Sharma

University Institute of Pharmacy, Chhatrapati Shahu ji Maharaj University, Kalyanpur, Kanpur 208 024, India

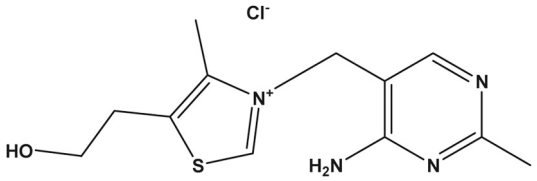
Numerous catalysts have been evaluated to assist benzoylation since its origin (1883), each having their own pros and cons with respect to synthetic environment. However, the one (thiamine) we report here is superior as well as multidimensionally advantageous with reference to non-hygroscopic characteristic, stability at room temperature and reaction conditions, uninflammable, non-volatile, easy to handle, non-corrosive, environmentally harmonizable, biodegradable, recyclable and non-toxic. Our result denies the traditional concept of 'Inorganic alkaline assistance' for benzoylation. The percentage yield of benzoylated product obtained by thiamine assisted method was found to be comparable with traditional methods. Furthermore non-catalyst assisted benzoylation in neat water was also studied and the results coincide with traditional as well as thiamine-assisted method reported here with some limitations.

Keywords: Benzoylation, catalyst, clean water, thiamine.

BENZOYLATION (Scheme 1) is an important synthetic manifestation involving the introduction of ArCO-functionality either to protect/identify –NH or –OH bearing organic compounds, or to yield their subsequent superior (over acetylated)[1,2] synthetically modified derivatives such as amide (Ar'CONHAr/R) or ester (Ar′CO-OAr/R) in the presence of benzoylating agent[3–7] and alkaline catalyst. Till date, numerous catalysts[8–14] have been explored that assist in benzoylation, each having its own merits and demerits. However, most (except few) are unsafe from the environment and human prospective although they are utilized owing to their low cost and ease of availability. Here we report a commercially advantageous and chemically distinct procedure of benzoylation utilizing 'thiamine hydrochloride' as an unexplored, non-corrosive, ecologically safe, non-toxic, non-volatile, handy, non-inflammable and stable catalyst, thus establishing our hypothesis 'Iceberg dancing of molecules'.

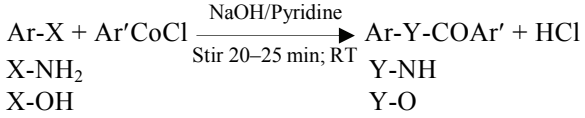
Thiamine (vitamin B_1) is a water-soluble important sub-member of the vitamin-B family. It is responsible for catalysing vital physiological phenomena inside the living system; its deficiency causes morbidity and mortality[15–18]. Chemically thiamine (thi = sulphur, amine = nitrogen) is a hetero-vitamin, consisting of sulphur and nitrogen along with other elements such as carbon, hydrogen and oxygen (Figure 1). Structurally, the vitamin comprises dual heteroaromatic rings, pyrimidine (six-member nitrogen-containing nucleus; substituted) and thiazole (five-member sulphur and nitrogen-containing nucleus; unsubstituted) coupled together via methylene bridge[19]. As enveloping two heterocyclic moieties, it is the thiazole nucleus which is responsible for catalysing power for a reaction[20–23]. On the other hand, the pyrimidine ring serves an ancillary function of assisting the molecule to fit inside the protein basket. Furthermore, when compared with other biologically active heterocyclic moieties, thiamine possesses distinct chemical behaviour owing to its unique chemoskeletal configuration and arrangement of heteroatom inside the rings, especially in the thiazole nucleus. Generally the thiazole ring is an aromatic, π-excessive system containing sulphur (thiophene-type) and nitrogen (pyridine-type) atoms consecutively at the first and third position. When existing as an isolated system and under general circumstances, this extraordinary arrangement of ring heteroatom creates an electron-deficient region at the second position which is prone to attack by nucleophile. However, when combined with other functionality as in thiamine, the thiazole ring exists as thiazolium ion; proton from second position could readily be removed generating stable zwitterion ion (ylide) chemically endowed with unique characteristics, such as balanced nucleophilicity and excellent leaving group property, ideal for catalysing numerous biochemical phenomena.

Aniline, benzoyl chloride, thiamine hydrochloride, methanol, ethanol (Merck, Mumbai) and distilled water (prepared in the lab by double distillation) were used in this study without further modification unless otherwise specified. Qualitative analytical reagents included sodium

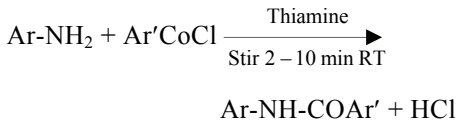


Thiamine

Figure 1. Structure of thiamine hydrochloride.



Scheme 1.



Scheme 2.

*For correspondence. (e-mail: chowrasia.deepak@gmail.com)

Table 1. Comparative study between various reaction parameters of classical and thiamine-assisted benzoylation

Reaction parameters	Classical alkali-catalysed benzoylation	Thiamine-catalysed benzoylation
Host reactant	Aniline	Aniline
Host reactant quantity	0.01 M	0.01 M
Benzoylating agent	Benzoyl chloride	Benzoyl chloride
Benzoylating agent quantity	0.01 M	0.01 M
Matrix system (a)	NaOH/pyridine	Thiamine
Matrix system quantity	1 g	1 g
Preparing medium (b) for matrix system	Water	Water
Preparing medium quantity	10 ml	10 ml
Ease of preparing matrix system (a) in medium (b)	Time-consuming	Instant
Special precautions for preparing (a)	Required	Not required
Reaction temperature	RT	RT
Device to carry out the reaction	Fume hood	Fuming hood
Reaction time	20 min	30 s to 1.5 min
Persistence of benzoyl chloride odour	Remains up to the end of the reaction	Terminated immediately; however, slight odour was perceived even after the reaction
Requirement of product washing	Required	Required
Product washing solvent	Water initially, followed by 1 : 1 water : methanol mixture	Water initially followed by 1 : 1 water : methanol mixture
Number of washings given	Sufficient	Sufficient
Recrystallization needed	Yes	Yes
Reaction handling	Tedious	Easy
Name of product (c) yielded	Benzanilide	Benzanilide
Product (c) yield	86%	82%
Product (c) quality	Good	Good

nitroprusside, ferric chloride, silver nitrate, lead acetate, sulphuric acid, nitric acid, sodium nitrite, sodium hydroxide (Merck); hydrochloric acid (Himedia); ferrous sulphate, sodium metal, hydrogen peroxide (SDFCL). The FTIR spectra of the synthesized compound were recorded on a Shimadzu IR affinity IS-CE spectrophotometer. The melting point of the synthesized compound was recorded using open capillary method and was not corrected. As the yielded product is well reported with surplus physio-chemical data available in the literature it is thus feasible to compare the physio-chemical characteristics (physical appearance, solubility, melting point, etc.) of the synthesized compound with those reported, which is economical, time-saving, and greener in approach.

The benzoylated derivative (benzanilide) of aniline was synthesized (Scheme 2) by suspending equimolar quantity (0.01 M) of host reactant (aniline) and benzoyl chloride (slightly in excess) in aqueous thiamine medium (10%; 10 ml). The reaction mixture was shaken vigorously to obtain crude product, which was further filtered-off, washed thoroughly with cold water initially followed by methanol (50%) and finally recrystallized from ethanol. The progression of the reaction was monitored on pre-coated TLC plates (Merck) using PET ether : ethylacetate (8 : 2) as binary solvent system. The purity of host reactant, thiamine, benzoyl chloride and the synthesized product was evaluated in a UV chamber at 200–400 nm by the procedure as given above.

The aqueous thiamine-based medium for this study was prepared by dissolving appropriate quantity of desiccated thiamine hydrochloride in clean distilled water with quantity of solvent added to obtain as 10% solution. Qualitatively pH of thiamine hydrochloride aqueous medium was measured physically (Supplementary Material), and was found to be acidic, although it catalysed benzoylation quickly (even faster than conventional catalyst (Table 1) and smoothly, thus eliminating the traditional concept of requiring alkaline medium for benzoylation. At equivalent quantity and under the same reaction condition, a detailed comparative study was done to enumerate the synthetic portrait of thiamine in benzoylation (Table 1), revealing that thiamine could be a better in-future catalyst comparatively among the available known analogues. Furthermore, slight compromise in the yield of benzanilide by this method could be neglected after considering some of the superior aspects of thiamine over hazardous traditional catalysts (Table 2).

Since thiamine also contains free –NH as well as –OH groups, principal functionalities prone to benzoylation, micro-scale synthetic procedure were separately performed subsequently in different test tubes (Supplementary Material). The procedure ascertain, whether thiamine itself get benzoylated under similar test conditions, revealing its incapability to do so – the test tube containing the water, thiamine and benzoyl chloride mixture did not give any product; benzoyl chloride initially settled down (as viscous liquid) immediately after its addition and was further converted into a white lump (day after; thiamine still in water) indicating an analogues reaction as was observed in the test tube containing only water and

Table 2. Comparative study between classical and novel benzoylating catalyst

Parameters	Classical benzoylating catalysts		Novel benzoylating catalysts
Catalyst	NaOH	Pyridine	Thiamine
Water solubility	Soluble	Miscible	Very soluble
Ease of solubility	Time consuming	Time consuming	Instant
Solubility-related reaction	Exothermic	None	None
Ease of handling	Difficult	Difficult	Easy
Hygroscopic	Yes	No	No
Stability	Unstable at normal temperature	Unstable at normal temperature	Stable
pH	Highly alkaline	Highly alkaline	Less basic
Corrosive to metal	Yes	Yes	No
Corrosive to skin	Yes	Yes	No
Corrosive to eye	Yes	Yes	No
Corrosive to respiratory tract	Yes	Yes	No
Corrosive to GIT	Yes	Yes	No
Visceral organ damage	Yes	Yes	No
Teratogenic	No	Yes	No
Reproductive effect	None reported so far	Yes	No
Reactive to water	Yes	Yes	No
In atmosphere	May accumulate	May accumulate	No
Carcinogenicity	Possibly	Yes	No
Mutagenic	Yes	Yes	No
Inflammable	No	Yes	No
Volatile	No	Yes	No
Explosive	No	Yes	No
Eco-toxicity	Yes	Yes	No
Disposal consideration	Required	Required	Not required
Recyclable	No	No	Yes

benzoyl chloride (Supplementary Material). This further confirmed that the product obtained in the main reaction (Scheme 2) was benzanilide. While performing micro-scale synthetic procedure in the laboratory (benzoylating aniline under different test conditions), we came across an interesting phenomenon, viz. 'benzoylation of aniline can also be done in clean water'. To confirm and establish the same, sequential benzoylation between aniline and benzoyl chloride was done in neat aqueous medium; the same results were obtained each time (Supplementary Material). To confirm the nature of product, it was subjected to physio-chemical tests (Supplementary Material) including IR spectroscopy and finally established as benzanilide.

Thus thiamine can be used as a safe, ecologically compatible and non-toxic catalyst for industrial as well as small-scale benzoylation, especially for chemical demonstrations in the laboratory, preventing direct exposure of students to hazardous alkaline chemicals. The overall percentage yield of benzanilide found by this method is comparable with traditional methods. According to our prediction, thiazolium is the key intermediate responsible for drifting reaction to forward direction. We thus plan to design a radio-labeled study to explore possible mechanism. This technique may also be used for synthesizing benzoylated derivatives of mono and polynuclear aromatic ring systems containing –OH as well as –NH functionality; traditionally benzoylated in alkaline hazardous matrix system. Furthermore, benzoylation of aniline in water has been successfully established and the same could be implemented as a catalyst-free, cost effective, eco-handy, convenient method with certain limitations, especially removing benzoyl chloride from the final product.

1. Vogel, A. I., *Practical Organic Chemistry*, Longman Group Ltd, London, UK, 1956, pp. 582–583.
2. Mann, F. G. and Saunders, B. S., *Practical Organic Chemistry*, Longman Group Ltd, London, UK, 1978, pp. 243–244.
3. Carey, F. A. and Hodgson, K. O., Efficient syntheses of methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranoside and methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-ribo-hexopyranosid-3-ulose. *Carbohydrate Res.*, 1970, **12**(3), 463–465.
4. Clarke, H. T. and Rahrs, E. J., In *Organic Syntheses,* Wiley, New York, USA, 1941, vol. I, 2nd edn, p. 91.
5. Stawinski, J., Hozumi, T. and Narang, S. A., Benzoyltetrazole: a mild benzoylating reagent for nucleosides. *J. Chem. Soc.*, *Chem. Commun.*, 1976, **7**, 243–244.
6. Bhat, B. and Sanghvi, Y. S., A mild and highly selective *N*-benzoylation of cytosine and adenine bases in nucleosides with *N*-benzoyltetrazole. *Tetrahedron Lett.*, 1997, **38**(51), 8811–8814.
7. Yamada, M., Watabe, Y., Sakakibara, T. and Sudoh, R., Preparation of a water-soluble acylating agent: benzoylation of acids, amines, and phenols with 2-benzoylthio-1-methylpyridinium chloride in aqueous phase. *J. Chem. Soc., Chem. Commun.*, 1979, no. 4, 179–180.
8. Greene, T. W., *Protective Groups in Organic Synthesis*, Wiley, NY, USA, 1981, pp. 261–263.
9. Reese, C. B., *Protective Groups in Organic Chemistry*, Plenum Press, London, UK, 1973, pp. 52–53.

10. Ando, W. and Tsumaki, H., *Synth. Commun.*, 1983, **13**, 1053–1056.
11. Taylor, E. C. Mclay, G. W. and McKillop, A., *J. Am. Chem. Soc.*, 1968, **90**, 2422–2423.
12. Illi, V. O., *Tetrahedron Lett*., 1979, **20**, 2431–2432.
13. Chowrasia, D. and Sharma, N., Solvent substitution evaluation of limestone water as a medium for benzoylation. *Arch. Chem. Res*., 2016, **1**, 1.
14. Paul, S., Nanda, P. and Gupta, R., PhCOCl–Py/basic alumina as a versatile reagent for benzoylation in solvent-free conditions. *Molecules*, 2003, **8**, 374–380.
15. Murray, R. K., Granner, D. K., Mayer, P. A. and Rodwell, V. W., Structure and function of water-soluble vitamins. In *Harper's Biochemistry*, Appleton & Lange, Stamford, 1996, vol. 24, pp. 599–600.
16. Bernhard, S., *The Structure and Function of Enzymes*, W. A. Benjamin, New York, USA, 1968, Chap. 7.
17. Bruice, T. C. and Benkovic, S., *Bioorganic Mechanism*, W. A. Benjamin, New York, USA, 1966, vol. 2.
18. Lowe, J. N. and Ingraham, L. L., *An Introduction to Biochemical Reaction Mechanisms*, Englewood Cliffs, Prentice-Hall, NJ, USA, 1974, Chap. 5.
19. Eicher, T., Hauptmann, S. and Speicher, A., Five membered heterocycles. In *The Chemistry of Heterocycles-Structure, Reaction, Synthesis, and Applications*, Wiley-VCH GmbH & Co. KGaA, Weinheim, 2003, 2nd edn, pp. 154–155.
20. Lobell, M. and Grout, D. H. G., *J. Am. Chem. Soc.*, 1996, **118**, 1867.
21. Stetter, H. and Dämbkes, G., *Synthesis*, 1977, **6**, 403.
22. http://www.umsl.edu/~{}orglab/pdffiles/multistp.pdf
23. http://www.stpaulsschool.org.uk/resource.aspx?id=136714

Received 18 January 2018; accepted 21 May 2018

doi: 10.18520/cs/v115/i11/2130-2133

Degraded products of stem bromelain destabilize aggregates of β-amyloid peptides involved in Alzheimer's disease

Debratna Mukherjee[1], Payel Bhattacharjee[1], Reema Bhattacharya[1], Alok K. Dutta[1] and Debasish Bhattacharyya[1,2,*]

[1]Division of Structural Biology and Bioinformatics, CSIR-Indian Institute of Chemical Biology 4, Raja S.C. Mallick Road, Jadavpur, Kolkata 700 032, India
[2]Department of Zoology, Tripura University, Surjyamani Nagar, Bikramnagar 799 022, India

Deposition of fibrils originating from monomeric β-amyloid (Aβ) peptide in brain cells is responsible for progressive neuronal damages in Alzheimer's disease. Peptides from bromelain, a cysteine protease from *Ananas comosus* (pineapple), were generated after digestion with proteases under conditions similar to human gastrointestinal tract. These peptides not only inhibit the growth of Aβ-amyloid aggregates, but also irreversibly destabilize the preformed aggregates. Gel filtration followed by mass spectrometric analysis identified a pool of peptides of <700 Da in the digest. Probable composition of the peptides interacting with Aβ-peptide was predicted from homology alignment between Aβ-peptide and bromelain using bioinformatics tools. Corresponding synthetic peptides can also destabilize the preformed aggregates as observed from thioflavin T assay, transmission electron microscopy and atomic force microscopy. Aβ aggregates that were preincubated with the bromelain-derived peptides did not exert appreciable toxicity on human neuroblastoma cells (SH-SY5Y) cultured *in vitro*.

Keywords: Alzheimer's disease, Aβ peptide, disaggregation, stem bromelain.

PROTEIN aggregation is one of the consequences of cellular events. Their accumulation is related to neuronal degeneration and organ failure[1]. Alzheimer's disease (AD) is an irreversible degeneration of the brain that causes dementia followed by cognition impairment and loss of memory. The impairment of cognition in human brain is caused by deposition of the aggregates of β-amyloid (Aβ) peptide in a progressive manner since the initiation of the process[2]. Even the soluble oligomers of Aβ peptide are cytotoxic to neuronal cells. It is unclear how the equilibration between the free, nonpathogenic monomeric peptides and the oligomers that are forerunners of the aggregate is disturbed[3]. As of today, no natural product or synthetic compound has been discovered as a drug to prevent or cure AD.

Pineapple (*Ananas comosus*) is a medicinal plant. All parts of the plant contain high level of protease activity. The plant extracts are collectively known as bromelain. Medicinal properties of bromelain are brought about synergistically by an array of enzymes present in it[4]. Stem bromelain, the major cysteine protease of the extract from pineapple stem, is commercially available[5]. Bromelain has broad specificity towards hydrolysis of peptide bonds and offers a wide range of therapeutic activities[6–9]. Due to its efficiency after oral administration, safety and lack of undesired side effects, bromelain is being increasingly considered as a phyto-therapeutical drug[7,10].

In recent years, the role of proteases in the pathogenesis, diagnosis and treatment of amyloid peptide-related diseases has been extensively studied[11,12]. Generally, amyloid fibrils are stable, rich in β-sheet content and are resistant to proteases[13]. However, this notion is not universally valid. Cathepsin B, a lysosomal cysteine protease, plays a crucial role in intracellular proteolysis. Upregulation of this enzyme is observed in a number of clinical conditions. Anti-amiloidogenic and related neuroprotective function of cathepsin B against Aβ peptides has been reported[14]. Subsequently, cystatin C, a cysteine protease inhibitor that inactivates cathepsin B, has further confirmed

*For correspondence. (e-mail: dbhattacharyya1957@gmail.com)