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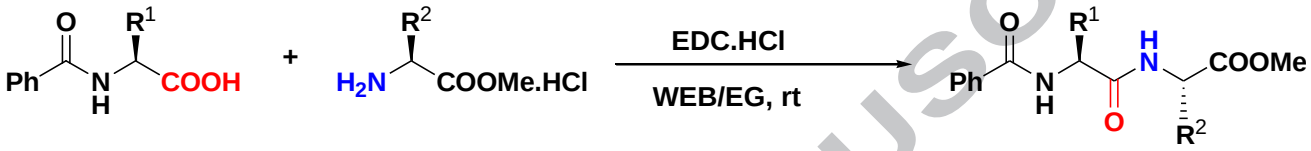

R^1= -H, $-CH_3$, $-CH_2Ph$, $-CH(CH_3)_2$, $-CH_2CH(CH_3)_2$
R^2= -H, $-CH_3$

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ABSTRACT

A simple, efficient and environmentally friendly approach has been developed for the synthesis of peptides in aqueous medium. In this work, peptides are easily synthesized in water extract of banana (WEB)/ethylene glycol and without using external base under mild condition.

Introduction

The peptide formation reaction between two molecules of amino acids is one of the important carbon–nitrogen bond formation reactions in organic chemistry.[1] It has a great importance not only in the field of biology but also in the fields of agriculture, pharmaceuticals and industrial applications.[2-6] However, the choice of peptide synthesis in water is a relatively challenging work in the field of organic chemistry. However, in our body proteins are naturally synthesized in aqueous medium.[7-9]

Usually, peptide synthesis is carried out in presence of an activating agent, coupling agent catalyst and a base.[8] Traditional bases such as K_2CO_3, $NaHCO_3$, triethylamine are generally used in peptide couplings reactions. The use of bases in peptide synthesis is proved to be detrimental when the coupling reaction is carried out in water. Base catalyzes the hydrolysis of amino acid ester, which is used as a coupling partner; thus inhibiting the reaction progress. Therefore, development of a basic green solvent medium will have a great impact on the peptide synthesis so as to avoid the need of an external base.

Due to inflammability and toxicity of organic solvents, water is considered as a green and superior solvent for organic reactions.[10-11] Water is easily available and its low cost leads the reaction more economical towards the green process. Over the past few years, a lot of experiments have been carried out in the field of peptide chemistry trying to perform the reaction in the aqueous media with great efficiency and in a relatively shorter reaction time.[12-14] Literature revels that lot of experiments are carried out in organic solvents using different ligands but there are only few reports presenting a relatively resourceful results of peptide synthesis in aqueous media without use of any kind of ligands.[15-16]

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Results and discussion

In this work, we present a green methodology towards the formation of peptides in aqueous medium. Peptides are easily synthesized in water extract of banana (WEB) in presence of trace amount of ethylene glycol and without using external base under mild condition in a relatively shorter reaction time with good to excellent yields. We recently reported the synthesis of biaryls through Suzuki-Miyaura (SM) cross coupling reaction performed in novel 'WEB' in a highly efficient and green manner.[17] WEB, "water extract of banana" (scientific name: Musa balbisiana Colla; family: Musaceae; species: Musa balbisiana), is prepared by first drying the banana peels followed by burning them to ash (**Fig. 1**). Water is added to the ash, mixed well and then filtered. The filtrate is termed as WEB. It is envisaged that WEB has a very great potential in green chemical processes from the economic and environmental points of view in the near future. Here we have chosen WEB as a reaction medium due to the fact that it has a dual function of green solvent as well as a base. However, our main objective is to develop a green and efficient method for the peptide bond formation in aqueous solvent at room temperature. This solvent system has a superior catalytic activity than aqueous medium since it has basic nature. In this method, the yields are relatively high, reaction proceeds in short time interval, the reaction is highly economical and most importantly it is eco-friendly leading to green chemistry protocols.[18-24]

The reaction of N-benzoyl glycine with glycine methyl ester hydrochloride catalyzed in WEB at room temperature in presence of EDC.HCl afforded 70% yields after 4 h (**Table 1**, entry 1). However, the same reaction while carried out in presence of little amount of ethylene glycol gave almost quantitative yields (95% isolated yields) after 4 h (**Table 1**, entry 2) which acts as a promoter for the reaction. The co-solvent effect on peptide coupling was tested with two other solvents viz. ^{i}PrOH and ^{t}BuOH (**Table 1**, entries 5-6); unlike ethylene glycol the yields obtained with ^{i}PrOH and ^{t}BuOH were relatively low. In absence of the coupling agent EDC.HCl, the reaction did not proceed at

all (**Table 1**, entry 3) indicating the necessity of the coupling agent under our reaction condition. To understand the role of base, the

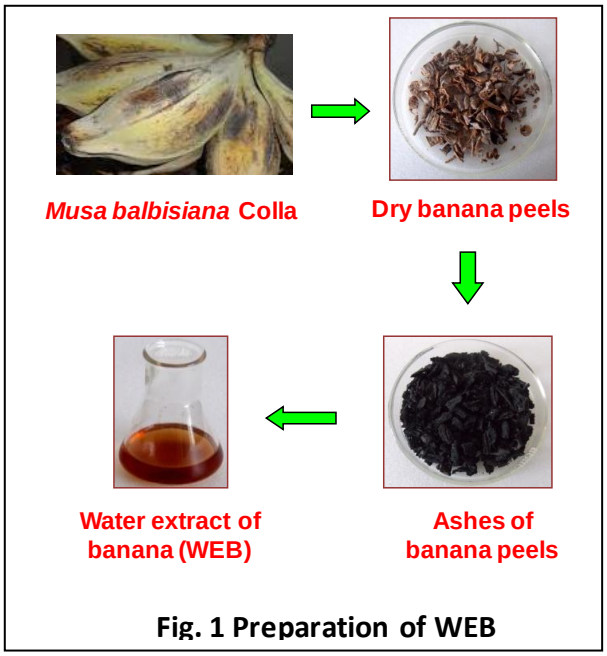


Fig. 1 Preparation of WEB

reaction of N-benzoyl glycine with glycine methyl ester hydrochloride was carried out in water in absence of any base, the reaction did not proceed at all (**Table 1**, entry 4). In short, under our optimized conditions, the peptide coupling reaction precedes in presence of a coupling agent (EDC.HCl), basic catalytic medium (WEB) and a promoter (Ethylene glycol) at room temperature.

Table 1: Optimization of reaction conditions for peptide synthesis.[a]

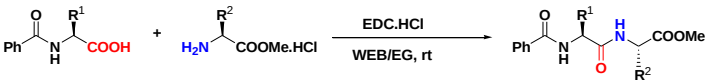
Entry	Solvent	Coupling agent	Time (h)	Yield[b](%)
1	WEB	EDC.HCl	4	70
2	WEB/EG	EDC.HCl	4	95
3	WEB/EG	-	12	-
4	Water	EDC.HCl	12	-
5	WEB/ ^{i}PrOH	EDC.HCl	4	65
6	WEB/ ^{t}BuOH	EDC.HCl	4	60

[a] Reaction conditions: benzoyl protected amino acid (1 mmol), amino acid methyl ester hydrochloride (1.5 mmol), EDC.HCl (1 mmol) in WEB (3 mL), Ethylene Glycol (0.2 mL) at room temperature.

[b] isolated yields

Different benzoyl protected amino acids and various amino acid methyl ester hydrochloride salts were used to construct the peptide moieties in a base free environment (**Table 2**). The coupling of benzoyl glycine with alanine methyl ester hydrochloride afforded 67% yields in 4 h (**Table 2**, entry 3). Identical results were obtained when Bz-alanine, Bz-phenylalanine, Bz-valine and Bz-leucine were coupled with the glycine methyl ester and alanine methyl ester hydrochloride (**Table 2**, entries 3-10).

Table 2: Effect of substituents on peptide synthesis[a]



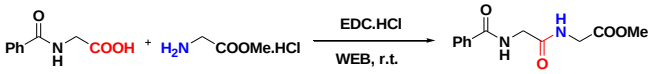
Entry	R^1	R^2	Yield[b](%)
1	-H	-H	95
2	$-CH_3$	$-CH_3$	71
3	-H	$-CH_3$	67
4	$-CH_3$	-H	76
5	$-CH_2Ph$	-H	58
6	$-CH_2Ph$	$-CH_3$	61
7	$-CH(CH_3)_2$	-H	76
8	$-CH(CH_3)_2$	$-CH_3$	72
9	$-CH_2CH(CH_3)_2$	-H	68
10	$-CH_2CH(CH_3)_2$	$-CH_3$	77

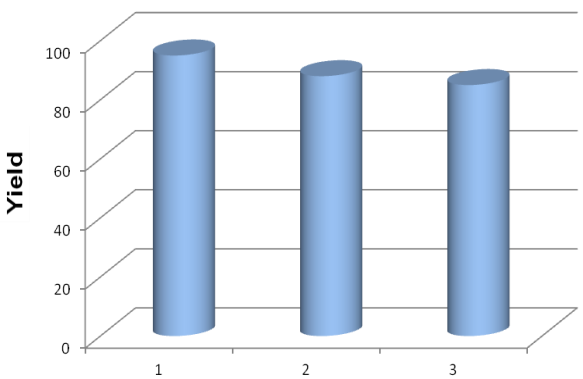
[a] Reaction conditions:- benzoyl protected amino acid (1 mmol), amino acid methyl ester hydrochloride(1.5 mmol), EDC.HCl (1 mmol) in WEB (3 mL) and ethylene glycol (0.2mL) at room temperature for 4 h.

[b] Isolated yields.

In order to demonstrate the practical importance and wide application of the catalytic system consisting of WEB, coupling agent and ethylene glycol, it is essential to show the efficient recycling of the catalyst system.[25-26] The results obtained in our experiment confirmed that it was possible to recycle and reuse the catalytic medium after extraction of products up to at least three times. For this recyclability study, the reaction between N-benzoyl glycine and glycine methyl ester hydrochloride was carried out under our optimized condition, affording the desired dipeptide in almost quantitative yields (95% isolated yields) after 4 h (**Table 3**, entry 1) without adding extra ethylene glycol on successive runs. The resulting mixture was extracted with diethyl ether (3x10 mL). The ether layer was separated and the catalytic medium was reused for next two runs (**Table 3**, entries 2–3). For every repeated run, homogeneous solution was obtained. The yields obtained for successive runs were 88% and 85% respectively.

Table 3: Recyclability test[a]





Run

[a] Reaction conditions: benzoyl protected amino acid (1 mmol), amino acid methyl ester hydrochloride (1.5 mmol), EDC.HCl (1 mmol) in WEB (3 mL) and ethylene glycol (0.2 mL) at room temperature.

[b]isolated yield.

The coupling reaction of two amino acid moieties in WEB has a great importance as the coupling reaction proceeds in aqueous media, at room temperature and in absence of external base. Literature reports reveal that banana peels contain potassium, sodium, carbonate and chloride as major constituents along with a host of other trace elements.[27] Therefore, it is believed that carbonates of sodium and potassium of banana peels act as a base here and chlorides of sodium and potassium act as promoters for peptide bond forming reactions. Burning of the banana peels may also cause the decomposition of the carbonates to oxides but this decomposition takes place at a very high temperature of more than 1200^0 C and the heating of Bunsen burner is in the range of 1000^0 C. Again literature reveals that, potassium carbonate has the thermal stability near its melting point because the vapor pressure over K_2CO_3 suggest a low rate of decomposition near the melting point (13 mbar is achieved only by 1200^0C).[28] The effects of salt additives such as LiCl, LiBr, $LiClO_4$, $ZnCl_2$ etc. on yields, side-product formation, racemisation and reaction rates on peptide coupling are reported earlier in literature.[29]

Conclusion

In summary, we have demonstrated the efficient green method for the peptide bond formation reaction in WEB at room temperature in presence of ethylene glycol as promoter/additive and without using external base.[30] The field of peptide chemistry is a very broad subject which also includes the protein synthesis as the higher branch of peptide coupling reactions. [31-32] By using this coupling protocol of the two amino acid residues we expect that it will give a great utility in the synthesis of polypeptides in near future. [33]

Acknowledgments

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34. ***General experimental procedure for peptide synthesis:*** In a 50 ml round bottom flask, a mixture of benzoyl protected glycine (1 mmol, 0.179g), glycine methyl ester hydrochloride (1.5 mmol, 0.188g), EDC.HCl (1 mmol, 0.192g) in WEB (3 mL) and 0.2 ml ethylene glycol was added and stirred at room temperature for 4 hours. After completion of the reaction (by TLC monitoring); the reaction mixture was extracted with diethyl ether (3x10 ml) and the organic layer was washed with distilled water (20 mL), dried over Na_2SO_4 and organic layer is evaporated in vacuuo. The residue was purified by column chromatography on silica gel using ethyl acetate/hexanes (1:1) as eluent to give the corresponding peptide. The products were characterized by ^{1}HNMR and ESI-MS spectrometry.

HIGHLIGHTS

- Economical and environmentally friendly aqueous system as the medium
- No external base is required for peptide coupling
- Peptides are synthesized in water at room temperature
- Organic solvents are not used