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Diazo ester insertion in N—H bonds of amino acid derivatives and insulin catalyzed by water-soluble iron and ruthenium porphyrin complexes (FeTSPPCl) as application of carbenoid transfer in aqueous media

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ABSTRACT

The metal complex FeTSPPCl (5,10,15,20-tetrakis)-(4-sulfonato-phenyl)-porphyrin-iron(III) chloride is an active catalyst for carbenoid insertion in N—H bonds of aminoacid derivatives in aqueous media. A variety of diazoacetates and methyl diazophosphonate were used as carbenoid precursors. The commercially available iron porphyrin complex can also selectively catalyze alkylation of the *N*-terminus of insulin (chain B).

1. Introduction

The most common bioconjugations are coupling of a small molecule (such as biotin or a fluorescent dye) to a protein. Usual types of bioconjugation chemistry are amine coupling of lysine amino acid residues (typically through amine-reactive succinimidyl esters) and sulfhydryl coupling of cysteine residues (via a sulfhydryl-reactive maleimide) [1]. Generally, the preparation of bifunctional agents involves multistep syntheses, and their subsequent incorporation into biomolecules is often hampered by cross-reactivity or nonspecific interactions with other functional groups present. Thus, the quest for novel, efficient strategies for the synthesis of functional agents, and their incorporation into biomolecules, has led to a burgeoning interest in "metallocarbenoid" chemistry [2].

The catalytic N—H insertion of α-diazocarbonyl compounds is a powerful organic reaction which has various useful applications [3–5]. One of the most successful intramolecular N—H insertions has been the conversion of a penicillin analogue into the carbapenem nucleus via rhodium-catalyzed insertion of a keto carbenoid into the N—H bond of the β-lactam [6]. More generally, this insertion reaction can lead to the synthesis of α-amino esters, dipeptides, and nitrogen-containing heterocycles. The catalytic preparation of optically active amines, recently reported by Zhou et al. [7] is also a nice example of application in asymmetric synthesis. Protein modification through alkylation of the *N*-terminal α-amino acid is also a potentially useful approach such as recently reported by the group of Che [8]. Among the previously reported metal-catalyzed carbenoid transfer, selective tryptophan modification with rhodium carbenoids [9,10] and terminal amino acid modification of proteins with ruthenium porphyrins have been previously explored [11], although in the latter case, a stoichiometric amount of the metalloporphyrin was necessary.

We reported the first success in the use of ruthenium porphyrin catalysts for intermolecular insertion of diazo derivatives into N—H bonds in 1997 [12]. Since then, we [13,14] and others [11,15–18] have found that ruthenium and iron porphyrins are active catalysts, for carbenoid transfer, not only in organic solvents but also in protic solvents and water. Although, there are examples showing reactions with diazoacetonitrile [19] diazomethyl phosphonate [20] and trifluoro diazomethane [21] in organic solvents, ethyl dia-

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zoacetate has been mainly used as the carbenoid precursors in water.

In this paper, we report NH insertion of amino esters using various diazo esters [5] as reagents and the site selective modification of terminal NH_2 group of insulin using water-soluble 5,10,15,20-tetrakis-(4-sulfonato-phenyl)-porphyrin-iron(III) chloride (FeTSPPCl) **1** as catalyst (Scheme 1). Iron porphyrin-catalyzed NH insertions have been previously studied in organic solvents [15–18]. Aviv and Gross [18] also reported intermolecular N—H insertion of anilines in water/THF catalyzed by myoglobin. Although, there are many examples showing that 5,10,15,20-tetrakis-(4-sulfonato-phenyl)-porphyrin-iron(III) chloride is a versatile water-soluble catalyst for oxidations in organic syntheses [22] and few other reactions [23,24], its use for carbenoid transfer in water seems to be neglected [25]. Comparative evaluation of reactivity for N-H insertion of different diazo derivatives with iron and ruthenium porphyrins will also be described.

2. Experimental

2.1. General

All reactions were performed under argon and were magnetically stirred. Solvents were distilled from appropriate drying agent prior to use: MeOH from turning Mg. Commercially available reagents (Acros) were used without further purification unless otherwise stated. All reactions were monitored by TLC with Merck pre-coated aluminium foil sheets (Silica gel 60 with fluorescent indicator UV254). Compounds were visualized with UV light at 254 nm. Column chromatographies were carried out using silica gel from Merck (0.063–0.200 mm). UV–vis spectra were recorded on a UVIKON XL from Biotech. MALDI-TOF mass spectra were recorded on microflex Bruker. The analysis were performed on a ThermoFisher LC–MS system (a DionexUltiMate 3000 quaternary RSLC Nano-UPHLC system coupled with a Q-Exactive Hybrid Quadrupole-Orbitrap Mass Spectrometer). Ethyl diazoacetate and 5,10,15,20-tetrakis-(4-sulfonato-phenyl)-porphyrin-iron(III) chloride are commercially available. 5,10,15,20-tetrakis-(4-sulfonatophenyl)-porphyrin-ruthenium(II) carbonyl was prepared as previously reported [14]. Diisopropyl diazomethylphosphonate was prepared as previously reported [26,27].

2.2. Syntheses of diazo compounds

The synthesis of substituted benzyl diazoacetates was adapted from the method previously reported by Fukuyama [28]. For the synthesis of the 4-(trifluoromethyl)benzyl 2-diazoacetate, 4-(trifluoromethyl)benzyl bromoacetate was first prepared: 4-(trifluoromethyl)benzyl alcohol (548 μL, 4.0 mmol) and $NaHCO_3$ (1.0 g, 12.0 mmol) were dissolved in acetonitrile (20 ml) and bromoacetylbromide (524 μl, 6.0 mmol) was added slowly at 0 °C in a 100 ml round bottom flask. After stirring for 15 min at room temperature, the reaction was quenched with H_2O. The solution was extracted with CH_2Cl_2 three times. The organic phase was washed with brine and dried over $MgSO_4$. The solvent was evaporated, and the residue was used in the next reaction without purification. The 4-(trifluoromethyl)benzyl bromoacetate thus obtained and *N*,*N*′-ditosylhydrazine (272 mg, 8.0 mmol) were dissolved in THF (20 ml) and cooled to 0 °C. DBU (3 ml, 20 mmol) was added dropwise and stirred at room temperature for 10 min. After quenching the reaction by the addition of saturated $NaHCO_3$ solution, this was extracted three times with diethylether. The organic phase was washed with brine, dried over $MgSO_4$ and evaporated to give the crude diazo acetate. Purification of the diazo acetate was performed with neutral silica gel to give 4-(trifluoromethyl)benzyl 2-diazoacetate as a pale yellow oil (897 mg, 90%, two steps). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 4.83 (s, 1H, CH=N_2), 5.25 (s, 2H, CH_2), 7.47 (d, *J* = 8.02 Hz, 2H), 7.63 (d, *J* = 7.63 Hz, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz, ppm): δ 46.48 (CH=N_2), 65.56 (CH_2), δ 123.05 (CF_3), 125.65 (CHPh), 128.23 (CHPh), 130.57 (Cq-CF_3), 140.06 (Cq-CH_2), 166.57 (C=O). ^{19}F NMR ($CDCl_3$, 400 MHz, ppm): δ −62.67 (s). HR-MS (*m*/*z*): calculated mass for $C_{10}H_7N_2O_2F_3Na$ [M + Na]$^+$: 267.03573, found *m*/*z*: 267.0356 (0 ppm).

For the synthesis of the 4-(methylsulfide)benzyl 2-diazoacetate, the previous experimental procedure was employed. The expected product was obtained as a yellow oil (yield 88%). ^{1}H NMR ($CDCl_3$, 400 MHz, ppm): δ 2.48 (s, 3H, SCH_3), 4.79 (s, 1H, CH=N_2), 5.16 (s, 2H, CH2), 7.24–7.30 (m, 4H, Ph). ^{13}C NMR ($CDCl_3$, 125 MHz, ppm): δ 15.85 (SCH_3), 46.48 (CH=N_2), 66.28 (CH_2), 126.68 (CHPh), 129.07 (CHPh), 132.72 (Cq), 139.08 (Cq), 166.8 (CO_2). HR-MS (*m*/*z*): calculated mass for $C_{10}H_{10}N_2O_2NaS$ [M + Na]$^+$: 245.03607, found *m*/*z*: 245.0361 (0 ppm).

2.3. General procedure for NH insertion in water

In a typical experiment, the α-amino ester (0.18 mmol) and FeTSPPCl catalyst (1.8 μmol) were placed in a schlenck tube under argon and dissolved in 2 ml of degazed bicarbonate buffer solution (pH 10). Ethyl diazoacetate (0.18 mmol) was then added at room temperature. After 15 min of stirring the reaction was stopped and the mixture was then extracted three times with CH_2Cl_2, dried by $MgSO_4$ and purified by column chromatography on silica gel (CH_2Cl_2/Methanol:97/3). A similar procedure was effective for N—H insertion catalyzed by RuTSPPCO, excepted that the reaction was stopped after 4 h.

2.4. General procedure for NH insertion in organic solvent

In a typical experiment the α-amino ester (0.18 mmol) and FeTPPCl (1.3 mg, 1.8 μmol) were placed in a schlenck tube under argon and dissolved in 2 ml of distilled CH_2Cl_2. NEt_3 (0.27 mmol) was added to the solution. (Ethyl diazoacetate) (0.18 mmol) was then added at room temperature. After 2 min of stirring, the insertion product was purified by column chromatography on silica gel (CH_2Cl_2/Methanol: 95/5).

2.5. NH insertion of insulin

In a typical experiment, insulin (5 mg, 0.87 μmol) and 0.2 equiv of TPPSFeCl (0.2 mg, 0.17 μmol) were dissolved in 10 ml of PBS and 5 ml of acetonitrile under nitrogen. 0.32 mg of cobaltocene was first added to the solution and then, the diazo derivative (1.3 mmol) diluted in 10 μl of acetonitrile. The solution was stirred for 1 h. The yield was estimated by MALDI-TOF. After centrifugation, insulin was purified by preparative HPLC and then dried under vacuum to give a white powder.

2.6. Characterization of N—H inserted compounds

2.6.1. Reaction of ethyl diazoacetate with amino esters

Mono-inserted tyrosine methyl ester: ^{1}H NMR (500 MHz, $CDCl_3$, ppm): δ 1.23 (t, *J* = 7.2 Hz, 3H, CH_3), 2.88–2.98 (m, 2H, CH_2), 3.37 (sys AB, *J* = 17 Hz, 2H, CH_2), 3.57 (t, 6.8 Hz, 1H, CH), 3.67 (s, 3H, OCH_3), 4.14 (q, 7 Hz,2H, CH_2), 6.71 (d, *J* = 8.5 Hz, 2H, CH), 7.02 (d, 8.5 Hz, 2H, CH). ^{13}C NMR (125 MHz, $CDCl_3$, ppm): δ 14.13 (CH_3 (CO_2Et)), 38.56 (CH_2Ph), 49.11 (CH_2NH), 51.92 (CH_3 (CO_2Me)), 61.02 (CH_2 (CO_2Et)), 62.23 (*CH), 115.51 (CH, Ph), 128.33 (Cq, CH_2Ph), 130.32 (CH, Ph), 154.9 (Cq (PhOH)), 171.75 (Cq (CO_2Et)), 174.22 (Cq (CO_2Me)). HR-MS (*m*/*z*): calculated mass for $C_{14}H_{19}NO_5$ [M + Na]$^+$: 304.29, found *m*/*z*: 304.11(3 ppm)