ELSEVIER

www.elsevier.nl/locate/jorganchem

Journal of Organometallic Chemistry 617–618 (2001) 376–382

Journal of Organometallic Chemistry

Transition metal ('Fischer-type') carbene complexes as protein labelling reagents

Michèle Salmain [a], Emanuela Licandro [b], Clara Baldoli [b], Stefano Maiorana [b], Hoa Tran-Huy [c], Gérard Jaouen [a,*]

[a] *Ecole Nationale Supérieure de Chimie de Paris, UMR CNRS 7576, 11, rue Pierre et Marie Curie 75231 Paris Cedex 05, France*
[b] *Università di Milano, Dipartimento di Chimica Organica e Industriale and CNR Centro Studio Sintesi Stereochimica Speciali Sistemi Organici, Via Golgi 19, 20133 Milan, Italy*
[c] *Ecole Polytechnique, DCPH, Route de Saclay 91128 Palaiseau Cedex, France*

Received 21 July 2000; accepted 5 September 2000

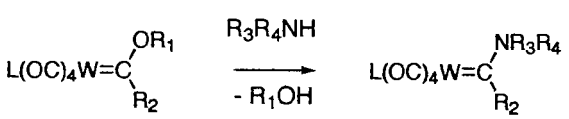
Abstract

The electrophilic property of metallocarbenes of Fischer-type was used to label amino acid derivatives and a model protein in a regio-specific manner with a transition heavy metal complex. Hence, the reaction of $(CO)_5W{=}C(OMe)Me$ (**1**) was shown to involve exclusively the amino group of a series of α-amino esters whether this function was carried by the α-carbon or by the side chain, leading to stable aminocarbenes. With a model protein, namely bovine serum albumin, labelling also occurred involving solely the amine function of some of its lysine residues. Coupling yield was shown to depend both on the pH where the reaction was carried out, and on the quantity of carbene. This work could find an application in the field of protein X-ray crystallography.

Keywords: Fischer-type carbenes; Bioorganometallics; Metal-labelled proteins

1. Introduction

Alkoxy tungsten carbene complexes of Fischer-type of the general formula depicted in Scheme 1 possess a marked electrophilic character at the carbenic carbon. They undergo a facile reaction of aminolysis with primary and secondary amines, leading to stable aminocarbenes, as exemplified in Scheme 1 for complexes **1**–**4** [1,2]. In earlier works, this reaction has been exploited in peptide synthesis and the α-amino function of amino acids has been protected by reaction with alkoxycarbenes to give aminocarbene labelled peptides [3,4].



1 L = CO, $R_1 = R_2 = Me$
2 L = CO, $R_1 = Et$, $R_2 = Ph$
3 L = PBu_3, $R_1 = Et$, $R_2 = Ph$
4 L = CO, $R_1 = Li$, $R_2 = Ph$

Scheme 1. Structure and reactivity of Fischer carbene complexes.

Various ways of derivatization of proteins with transition organometallic complexes have been previously explored. To this purpose, bifunctional complexes combining a stable organometallic moiety and a function targeted towards nucleophilic protein residues were designed from classical protein chemistry reagents [5–8]. The design of such reagents should take into account a certain number of constraints, including sufficient stability in aqueous medium, solubility in water-miscible solvents or better in water, high reactivity and most importantly high selectivity.

One of our targets is to provide protein crystallographers with new heavy metal (electron-dense) reagents to be used in the course of the resolution of protein structures by X-ray diffraction, when phase determination is performed by the classical multiple isomorphous replacement method.

* Corresponding author. Fax: +33-1-43260061.
E-mail address: jaouen@ext.jussieu.fr (G. Jaouen).

0022-328X/01/$ - see front matter
PII: S0022-328X(00)00634-3

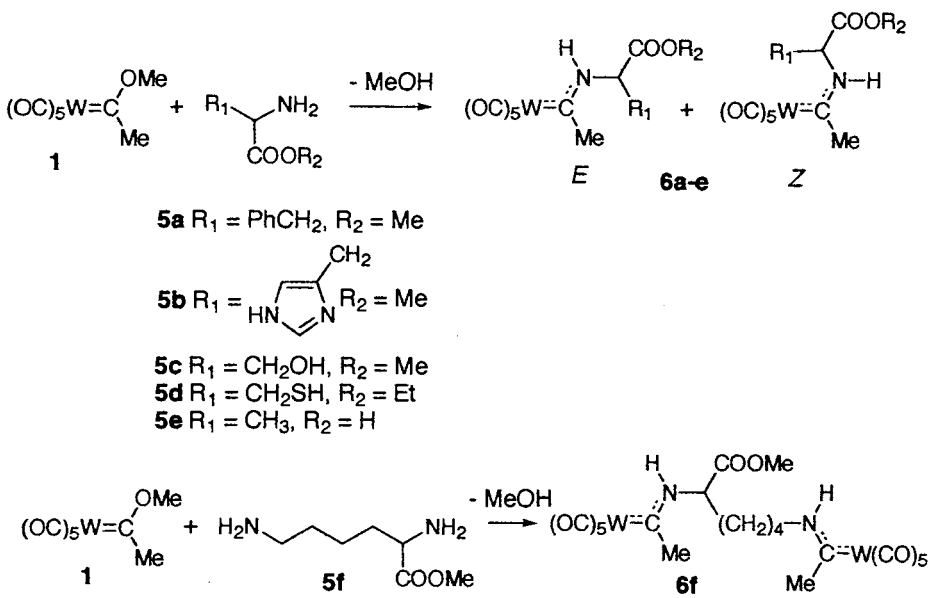
We thought to use the electrophilic property of Fischer-type carbene complexes to label proteins with organotungsten entities through reaction of these complexes with some of the protein residues. However, proteins possess several potentially reactive nucleophilic functions borne by the amino acid residues. Hence, the issue of selectivity towards one particular class of residues had first to be addressed. To do that, the reaction of alkoxycarbenes with well-chosen α-amino esters and one α-amino acid was first studied. In all cases, the expected aminocarbenes were obtained and fully characterized by spectroscopic methods. With the model protein bovine serum albumin (BSA), side-chain specific labelling was observed, involving the α-amino group borne by some of the protein lysine residues. Moreover, the extent of labelling (derivatization) was shown to markedly depend on the pH of the reaction medium and less significantly on the initial amount of carbene.

2. Results and discussion

2.1. Reaction of *1* with amino esters

The reaction of the carbene **1** with a series of α-amino ester hydrochlorides **5a–e** was performed in THF in the presence of one or two molar equivalents of triethylamine (Scheme 2). Some of the amino esters were chosen according to the presence of nucleophilic functions at their side chain. The reactions were monitored by analytical thin layer chromatography (TLC). The formation of products was assessed by the appearance of one or two yellow spots having $R_f < R_f$ of **1**. Qualitatively, a significant difference in the reaction rates was observed along the amino esters series, with reaction times ranging from 2 h (**5b**) to 16 h (**5f**) for the complete disappearance of carbene **1**. Purification of the adducts **6** was achieved by preparative TLC and yellow oils or solids were obtained after work-up.

These products were submitted to spectroscopic analysis (^{1}H-NMR, IR and occasionally ^{13}C-NMR). In the case of amino esters **5a** (PHE–OMe), **5b** (HIS–OMe) and **5c** (SER–OMe), the expected aminocarbenes **6a**, **6b** and **6c** were readily identified by ^{1}H-NMR as reaction products. Their proton chemical shifts are collected in Table 1. For **6a** and **6b**, a set of two triplets of doublets characteristic of the $(CH)_\alpha$ proton were detected between 4.7 and 5.4 ppm, whereas only one set was observed for **6c**. Similarly, the methyl group borne by the carbenic carbon gave a set of two doublets between 2.7 and 2.9 ppm, as a consequence of an allylic coupling between the methyl and the NH protons ($^4J =$ 1 Hz). All these data indicated that as expected aminocarbenes **6a** and **6b** were a mixture of two geometrical isomers *Z* and *E* because of the double-bond character of the C–N bond [9]. Already published NMR data showed that both N–CH and the CH_3–$C_{(carben)}$ protons were more deshielded in the case of the *Z* isomer [10]. This allowed us to identify both isomers and to evaluate their proportion in the mixtures (Table 1). A prevalence of the *E* isomers was obtained for carbenes **6a** and **6e** whereas the *Z* isomer was predominant in the case of **6b**,**d** and resulted to be the only one detectable for **6c**. This behaviour is quite interesting, because in general the *E* isomer is thermodynamically favoured and prevails in *E*/*Z* mixtures of aminocarbenes. A possible explanation could lie in the presence



Scheme 2. Reaction of Fischer carbene complexes with amino esters and an amino acid.

Table 1
^{1}H-NMR spectra of aminocarbenes (solvent: $CDCl_3$) [a]

	Amino acid/ amino ester	Z/E	$C_{carbene}$–Me	NH	$(CH)_\alpha$	$(CH_2)_\beta$	Other peaks
6a	PHE–OMe	1/5	2.86 *Z* (3, d, 0.7), 2.31 *E* (3, d, 1.0)	9.06 *E* (1, br), 8.71 *Z* (1, br)	5.29 *E* (1, dt, 7,1, 4.9), 4.91 *Z* (1, dt, 7.6, 5.9)	3.27 (1, dd, 13.7, 4.5), 3.06 (1, dd, 13.8, 8.3)	7.1–7.7 (5, m), phenyl, 3.87 *E* (3, s), 3.74 *Z* (3, s), OMe
6b	HIS–OMe	10/1	2.92 *Z* (3, d, 1.0), 2.57 *E* (3, d, 1.0)	10.6 *Z* (1, br), 10.3 *E* (1, br)	5.34 *Z* (1, dt, 8.8, 4.4), 4.78 *E* (1, dt, 8.1, 5.3)	3.48 (1, dd, 15.9, 4.3), 3.20 (1, dd, 15.3, 4.5)	7.59 *Z* (1, d, 1.2), 7.60 *E* (1, d, 1.2), 6.91 (1, s), imidazole, 3.79 *E* (3, s), 3.61 Z (3, s), OMe
6c	SER–OMe	$Z \gg E$	2.80 (1, d, 1.0)	10.09 (1, br)	5.00 (1, q, 3.9)	4.77 (1, t), 4.12 (1, br m)	3.77 (3, s), OMe
6d	CYS–Oet	10/1	2.95 *Z* (3, d, 1.0), 2.78 *E* (3, d, 1.0)	9.12 *Z* (1, br)	5.31 *Z* (dt, 8.6, 4.7), 4.70 *E* (dt, 4.7, 8.6)	3.38 (1, ddd, 3.9, 7.9, 14.5), 2.99 (1, m)	4.30 (2, q, 7.1), 1.35 (3, t, 7.1), OEt
6e	ALA [b]	1/5	2.84 *Z* (3, d, 1.1), 2.78 *E* (3, d, 1.1)	10.44 (1, br)	4.52 *Z* (td, 7.1, 9.3), 4.19 *E* (q, 7.1)	–	1.51 *Z* (d, 7.1), .42 *E* (d, 7.1), $(CH_3)_\beta$
6f	LYS–OMe	[c]	2.76 (3, d, 1.0), 2.72 (3, d, 1.0)	9.14 (1, br), 8.65 (1, br)	4.52 (1, dt, 8.4, 6.1)	Between 1.2 and 2.0	3.88 (3, s), OMe, 3.43 (2, q, 7.0), $(CH_2)_\beta$

[a] δ in ppm/TMS isomer (number of protons, multiplicity, coupling constant in Hz).
[b] In acetone-d_6.
[c] One isomer.

of a heteroatom present on the side chain of the amino ester (nitrogen for complex **6b**, oxygen for complex **6c**, and sulfur for complex **6d**) which could give an interaction with the metal atom thus stabilizing the *Z* isomer.

In the case of amino ester **5d** (CYS–OEt), more surprisingly, only the yellow–brown adduct resulting from the reaction of the α-NH_2 group was isolated as shown by ^{1}H-NMR (Table 1) and IR spectroscopy (Table 2). One could have expected that competitive thiolysis of **1** would occur by reaction of the highly nucleophilic sulfhydryl function of cysteine [11]. This behaviour could be explained by a higher rate of aminolysis of **1** in the reaction conditions used (THF in the presence of one equivalent of TEA) [12,13].

In the case of amino ester **5f** (LYS–OMe) which possesses two primary amino groups in α and ε positions, only the diaminocarbene complex was isolated as previously mentioned [4], indicating that both functions had a similar reactivity towards **1** in THF. Although four geometrical isomers would be expected, only one compound was observed by ^{1}H-NMR, probably the *EE* isomer.

The ^{13}C-NMR analysis of compounds **6b–d** and **6f** was also performed (Table 2). Two peaks around 200 ppm were observed corresponding to the two types of carbonyl ligands in the $(CO)_5W–R$ moiety. The structure of **6f** as a diaminocarbene was confirmed by the presence of two peaks for the methyl carbon borne by the carbenic carbon. A weak signal assigned to the carbenic carbon was detected only for compound **6c**.

The IR data of the aminocarbenes **6a–d** and **6f** are reported in Table 3. Four ν_{CO} vibration modes of symmetry A_1, B and E are expected from the C_{4v} local symmetry of the $(CO)_5W–R$ moiety, two of them (the A_1^2 and E modes), giving bands at very close frequencies around 1920 cm^{-1}. The aminocarbenes **6a-d** and **6f** usually displayed three ν_{CO} bands similarly to compound **1** and their position was slightly shifted to the low wavenumbers in agreement with the higher electron donating ability of aminocarbene ligands relative to alkoxycarbene ligands.[1] In addition to the ν_{CO} bands, characteristic $\nu_{C–N}$ bands were observed around 1550 cm^{-1}, $\nu_{N–H}$ around 3400 cm^{-1} and $\nu_{C=O}$ around 1735 cm^{-1} (ester) in agreement with the proposed structures. The presence of a weak $\nu_{S–H}$ band of **6d** confirmed that reaction of **5d** with **1** solely involved the α-amino function. Compound **6c** displayed a narrow ν_{OH} band around 3250 cm^{-1} that could sign the presence of an intramolecular hydrogen bond with the carbonyl group of the ester.

Table 2
^{13}C-NMR spectra of aminocarbenes (δ in ppm/TMS)

	Amino ester	$C_{carbene}$	Me(–$C_{carbene}$)	CO	$(CH)_\alpha$	$(CH_2)_\beta$	COOR	Other peaks
6b	HIS-OMe [b]	nd [a]	46.9	198.1	67.4	29.4	170.1	52.7, OMe, 135.2, 135.0, 113, imidazole ring
6c	SER-OMe [c]	259.4	38.4	199.7, 204.5	62.4	62.3	169.1	53.1, OMe
6d	CYS-OEt [b]	nd	47.6	197.7, 202.4	67.3	27.1	168.3	62.8, 14.0, OEt
6f	LYS–OMe [c]	nd	38.4, 37.5	199.7, 200.0, 204.5	60.4	–	170.5	23.8, 31.5, 48.2, 49.7, $(CH_2)_\gamma$, $(CH_2)_\delta$, $(CH_2)_\varepsilon$, $(CH_2)_\beta$

[a] nd: not detected.
[b] In $CDCl_3$.
[c] In acetone-d_6.

Table 3
IR spectra of aminocarbenes (ν in cm^{-1})

Compound	$\nu_{C=O}$	$\nu_{C=O}$	Other vibrations
5a [a]	2063, 1963, 1923	1744	1515 ($\nu_{C–N}$)
5b [b]	2063, 1978, 1909	1735	1560 ($\nu_{C–N}$), 3416 ($\nu_{N–H}$)
5c [b]	2062, 1963, 1915, 1890	1733	3452 ($\nu_{N–H}$), 3260 ($\nu_{O–H}$), 1544 ($\nu_{C–N}$)
5d [c]	2063, 1974, 1920	1733	3434 ($\nu_{N–H}$), 1545 ($\nu_{C–N}$), 2583 ($\nu_{S–H}$)
5e [b]	2063, 1974, 1904	1963	3393 ($\nu_{N–H}$), 1526 ($\nu_{C–N}$)
5f [c]	2061, 1975, 1915	1740	3354 ($\nu_{N–H}$), 1532 ($\nu_{C–N}$)
Adduct of **1** and BSA [d]	2063, 1972, 1923		
Adduct of **2** with BSA [d]	2064, 1978, 1928		
Adduct of **3** with BSA [d]	2015, 1892		

[a] $CHCl_3$.
[b] KBr pellet.
[c] Neat.
[d] Aqueous solution deposited on nylon membrane.

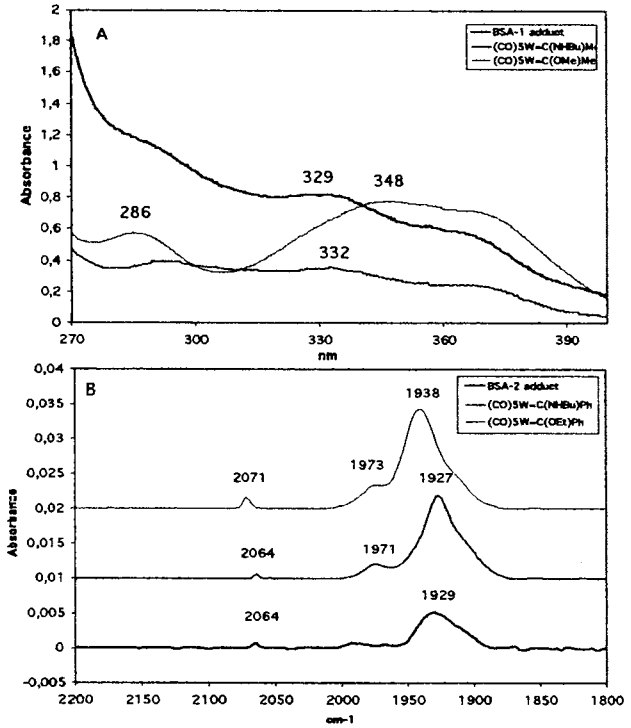


Fig. 1. (A) Superimposition of the UV–vis spectra of complex **1**, $(CO)_5W{=}C(NHBu)Me$ and the BSA-**1** adduct. (B) Superimposition of the IR spectra of **2**, $(CO)_5W{=}C(NHBu)Ph$ and the BSA-**2** adduct. Conditions of reaction and purification described in Section 3.

Because of the insolubility of amino acid **5e** in THF, the reaction with **1** was performed in a mixture of water and THF in the proportion of 1:1 and in the presence of one molar equivalent of TEA. TLC monitoring indicated that **1** disappeared quickly. After work-up, the yellow product was analysed by ^{1}H-NMR. The presence of a set of two characteristic multiplets at 4.2 and 4.5 ppm was good evidence of the formation of the aminocarbene of the expected formula **6e** in the form of two geometrical isomers *E* and *Z* (Table 1). Thus, aminocarbenes can be formed in good yield in aqueous solutions because aminolysis of alkoxy metallocarbenes is much faster than hydrolysis [12,14].

This body of results leave us confident with the ability to use Fischer carbene complexes for the selective labelling of protein amino groups in aqueous medium. We should not expect any side reaction with other nucleophilic functions as long as no interfering of the solvent.

2.2. Reaction of alkoxycarbenes *1–4* with a model protein

In order to investigate the ability of Fischer carbene complexes to label proteins with heavy metals, we chose to work with BSA which is a convenient model for these studies because of its high number of amino groups (59 lysines + the N-terminal function). Four different alkoxycarbene complexes **1–4** were tested to investigate the influence of the different substituents on their reactivity. Labelling tests were performed as follows. Solutions of BSA in an aqueous buffer were reacted overnight with a controlled amount of carbenes **1–3** dissolved in MeCN or **4** dissolved in water. The protein conjugates were separated for low molecular weight molecules by gel filtration chromatography and the resulting samples were submitted to spectroscopic analyses in order to determine whether aminocarbene adducts were formed and their number bound per protein molecule. The influence of the pH of the reaction medium and of the initial carbene to protein molar ratio was studied.

The superimposition of the UV–vis spectra of **1**, $(CO)_5W{=}C(NHBu)Me$ and the BSA-**1** adduct and the superimposition of the IR spectra of **2**, $(CO)_5W{=}C(NHBu)Ph$ and the BSA-**2** adduct are displayed in Fig. 1. In the UV–vis range, the BSA-**1** conjugate displays one characteristic maximum of absorption at 332 nm and a shoulder around 360 nm which are also displayed by $(CO)_5W{=}C(NHBu)Me$, prepared in situ by reaction of **1** with an excess of $BuNH_2$ in MeCN. Similarly, in the mid-IR spectral range, the BSA-**2** adduct displays three ν_{CO} bands at 2064, 1978 and 1928 cm^{-1} (on nylon membranes) which were also found for $(CO)_5W{=}C(NHBu)Ph$ (in MeCN). The three observed ν_{CO} bands were shifted to low wavenumbers as compared to compound **2**, in agreement with the higher electron-donating ability of aminocarbenes mentioned above. Such spectral features were good evidence of the formation of aminocarbene adducts by reaction of some of the amine functions of BSA with alkoxycarbenes **1** and **2**. Similarly, coupling of **3** where one of the carbonyl ligands has been replaced by a phosphine ligand also was evidenced by the presence of a maximum of absorption of the conjugate at 413 nm in the visible wavelength range, while two ν_{CO} bands were detected at 2015 and 1982 cm^{-1}.

Quantitation of the number of aminocarbene adducts per protein molecule (called coupling ratio CR) was done in two steps. First, the concentration of protein was measured by a standard colorimetric assay. Second, the concentration of aminocarbene was measured spectroscopically by taking the adducts resulting from the reaction of carbenes **1**, **2** or **3** with $BuNH_2$ as standards. The coupling ratios measured as function of the initial [carbene]/[protein] ratio of pH of the reaction medium are plotted in Fig. 2. Carbenes **1** and **2** which differ by the substituents on the carbenic carbon behave quite similarly towards BSA.

When the reaction was carried out at pH 9.0, the increase of the quantity of alkoxycarbene led to an increase of the coupling ratio until a plateau at CR $\approx$ 17 was reached for an initial [carbene]/[protein] ratio of 36. When the reaction was carried out with 60 molar

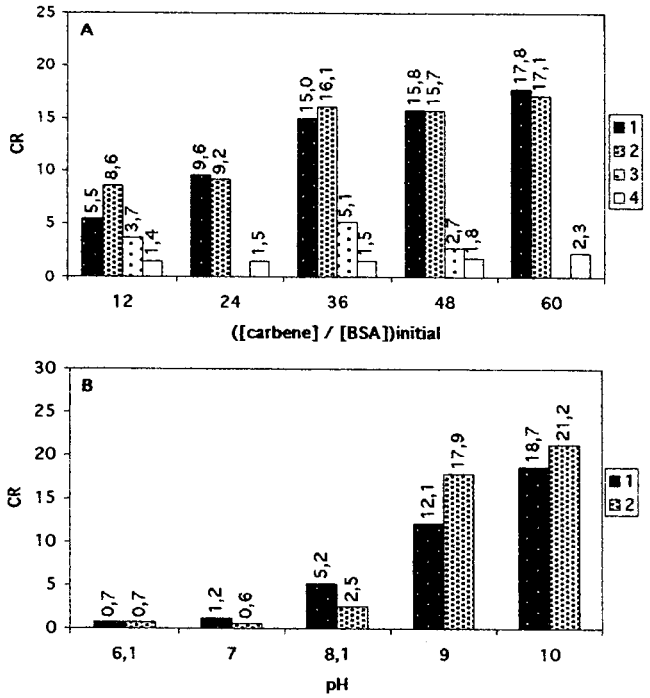


Fig. 2. (A) Coupling ratio of BSA with complexes **1**–**4** as a function of the [carbene]/[BSA] initial molar ratio. (B) Coupling ratio of BSA with complexes **1** and **2** as a function of the pH of the reaction medium.

equivalents of **1** or **2**, coupling ratios were weak at acidic and neutral pH and steadily increased with the pH above 7, in accordance with the necessity for the amine functions to be partially to fully deprotonated to be able to react with alkoxycarbenes.

With carbene **3**, weak and erratic coupling yields were measured. This behaviour was attributed to the fact that complex **3** is completely insoluble in the reaction conditions used (borate buffer pH 9.0/MeCN, 9:1) leading to its precipitation. Compound **4**, which is advantageous as regards its solubility in water, reacted well with simple amines such as *n*-butylamine [15]. It also led to the formation of aminocarbene adducts by reaction with BSA at pH 9.0 as shown by the characteristic UV–vis features of the conjugates ($\lambda_{max} = 338$ nm, shoulder at 360 nm). However, very low coupling ratios were measured and they were almost independent of the quantity of reagent put into reaction. This probably reflects a lower electrophilic character of the carbenic carbon atom.

3. Experimental

3.1. General experimental conditions

Tungsten–carbene complexes **1**, **2** and acylmetallate **4** were prepared according to literature methods [16]. Carbene **3** was prepared according to a published method [17]. Syntheses were carried out under argon atmosphere, using a vacuum line and Schlenck technique. Solvents were purified by classical techniques and deoxygenated. L-Amino esters **5a–d,f**, L-alanine **5e**, and bovine serum albumin (lipid-free, IgG-free grade) were purchased from Sigma and used without further purification. Preparative TLC was performed with Kieselgel 0.02–0.04 mm silica gel (Merck). Gel filtration chromatography was done with a prepacked dextran desalting column (bed volume 5 ml, Pierce chemicals). IR spectra were recorded on a MB 100 FT-spectrometer (Bomem) equipped with a liquid-nitrogen cooled MCT detector. UV–vis spectra were recorded on a uv-mc^2 spectrometer (Safas). ^{1}H- and ^{13}C-NMR spectra were recorded on an AM200 FT spectrometer (Bruker) operating at 200 MHz (^{1}H).

3.2. Reaction of *1* with amino esters *5a–d* and *5f*

To a solution of 5 mmol of amino ester in THF (15 ml) 5 mmol of **1** and 5 mmol or 10 mmol of triethylamine, (depending on the initial protonation state of the α-amino ester) were added. The mixture was stirred from 3 to 19 h at room temperature (r.t.). The reactions were monitored by TLC until complete disappearance of the starting complex. The solvent was evaporated under reduced pressure and products were purified by preparative TLC (**6b**, **6c**: cyclohexane/ethyl acetate/methanol, 4:2:1; **6f**: cyclohexane/ethyl acetate, 2:1) or by simple filtration of the crude mixture over a celite pad in the case of complex **6a** and **6d**, and obtained as yellow oils: **6a** (84%), **6d** (77%), **6f**, (50%) or powders: **6b** (60%), **6c** (51%).

3.3. Reaction of *1* with L-alanine *5e*

To 0.1 mmol of **5e** in 2.5 ml of water was added 0.1 mmol of **1** in 2.5 ml of THF and 0.1 mmol of TEA. The reaction was monitored by TLC. The organic solvent was evaporated under reduced pressure, water was added and the aqueous phase was extracted with dichloromethane. After a standard work-up, **6e** was obtained as a yellow oil (53%).

3.4. Reaction of alkoxycarbenes *1–3* and the acylmetallate *4* with BSA. Effect of the quantity of carbene

Solutions of BSA (50 μM) and complex **1**, **2**, **3** or **4** (600, 1200, 1800, 2400 or 3000 μM) in borate buffer at pH 9.0 containing 10% of MeCN (except for **4** in water) were incubated overnight at r.t. The resulting proteins were purified by gel filtration chromatography with 10 mM NH_4HCO_3 as eluent. Fifteen 0.5 ml fractions were collected. Their protein content was measured by the Coomassie blue assay [18] and the concentration of aminocarbene entities was measured spectroscopically,

taking as standards the adducts generated in situ by reaction of carbenes **1**–**3** with an excess of *n*-butylamine [ε (328 nm) = 5700 l mol^{-1} cm^{-1} for **1**, ε (338 nm) = 11500 l mol^{-1} cm^{-1} for **2** and **4**; ε (413 nm) = 7700 l mol^{-1} cm^{-1} for **3**].

*3.5. Reaction of alkoxycarbenes **1** and **2** with BSA. Effect of the pH*

Solutions of BSA (50 μM) and complex **1** or **2** (3000 μM) in citrate-phosphate pH 6.1, phosphate pH 7.0, borate pH 8.1 or 9.0 or carbonate pH 10.0 containing 10% of MeCN were incubated overnight at r.t. Protein conjugates were purified and analysed as above.

4. Conclusions

In conclusion, several Fischer carbene complexes of the general formula $L(CO)_4W{=}C(OR_1)R_2$ were shown to react in aqueous medium with the protein BSA by reaction of the amino group borne by some of the lysine residues to lead to stable aminocarbene adducts which were characterized by IR and UV–vis spectroscopy. It was shown that the yield of coupling markedly depended on the pH of the reaction medium with the highest yield reached at pH 10 whereas only a maximum of 17 out of the 60 amine functions of BSA reacted with carbenes **1** and **2** at pH 9. The behaviour of carbene **4** towards BSA was completely different from the other carbenes with coupling ratios of only 1.5 reached at pH 9. The ability of carbene **3** to label BSA opens the way to the preparation of a water-soluble Fischer carbene complex by replacing the PBu_3 ligand with a water soluble phosphine like TPPTS.

Acknowledgements

The Centre National de la Recherche Scientifique, CNR-Italy and the European Community (COST action D8, Metals in Medicine) are gratefully acknowledged for their financial support.

References

[1] E.O. Fischer, B. Heckl, H. Werner, J. Organomet. Chem. 28 (1971) 359.
[2] J.A. Connor, E.O. Fischer, J. Chem. Soc. (A) (1969).
[3] K. Weiss, E.O. Fischer, Chem. Ber. 106 (1973) 1277.
[4] K. Weiss, E.O. Fischer, Chem. Ber. 109 (1976) 1868.
[5] S. Blanalt, M. Salmain, B. Malézieux, G. Jaouen, Tetrahedron Lett. 37 (1996) 6561.
[6] D. Osella, P. Pollone, M. Ravera, M. Salmain, G. Jaouen, Bioconjugate Chem. 10 (1999) 607.
[7] B. Rudolf, J. Zakrzewski, M. Salmain, G. Jaouen, New J. Chem. (1998) 813.
[8] M. Salmain, K. Malisza, S. Top, G. Jaouen, M.-C. Sénéchal-Tocquer, D. Sénéchal, B. Caro, Bioconjugate Chem. 5 (1994) 655.
[9] B. Moser, E.O. Fischer, J. Organomet. Chem. 15 (1968) 147.
[10] E.O. Fischer, M. Leupold, Chem. Ber. 105 (1972) 599.
[11] E.O. Fischer, M. Leupold, C.G. Kreiter, J. Müller, Chem. Ber. 105 (1972) 150.
[12] C.F. Bernasconi, M.W. Stronach, J. Am. Chem. Soc. 115 (1993) 1341.
[13] C.F. Bernasconi, K.W. Kittredge, F.X. Flores, J. Am. Chem. Soc. 121 (1999) 6630.
[14] C.F. Bernasconi, F.X. Flores, K.W. Kittredge, J. Am. Chem. Soc. 119 (1997) 2103.
[15] H. Tran-Huy, Unpublished results.
[16] E.O. Fischer, A. Massböl, Chem. Ber. 100 (1967) 2445.
[17] B. Moser, E.O. Fischer, J. Organomet. Chem. 16 (1969) 275.
[18] M.M. Bradford, Anal. Biochem. 72 (1976) 248.