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[c013]

Synthesis and structural characterization of metallated bioconjugates: C-terminal labeling of amino acids with ferroceneamine

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Abstract

Ferroceneamine, H_2N-Fc, has been substituted to the C-terminus of 6 amino acids using the HBTU / HOBt coupling protocol. The synthesized bioconjugates Boc-Aaa-Fc, Aaa = Gly (**1**), Leu (**2**), Phe (3), Val (4), Cys(Acm) (5), Tyr(^{t}Bu) (6) (Acm = acetamidomethyl, ^{t}Bu = t-buthyl), have been characterized by ^{1}H NMR, ^{13}C NMR EI-MS, EI-HRMS, UV and CD spectroscopies. In addition, a VT NMR study on **4** and the X-ray structure of **1** are presented.

Key words: Amino acids, bioorganometallic chemistry, ferrocene, peptides, X-ray

Introduction

Organometallic compounds are often considered as instable and sensitive to air and water, and therefore not suitable for the use in biology and medicine. However, in addition to vitamin B12 and the active sites of the metal-containing hydrogenazes found in nature, the rapidly growing field of bioorganometallic chemistry [1-3] has created a number of applications including efficient organometallic derivatives of the anticancer drug tamoxifen [4], the antimalaria drug chloroquin [5] and the kinase inhibitor staurosphorine [6].

An important aspect of bioorganometallic chemistry is the synthesis and characterization of bioconjugates of organometallics with biological molecules like carbohydrates, nucleic acids and peptides [7]. In particular, recent research has focused on ferrocenoyl labeled amino acids and peptides [7-8]. While extensive studies have been reported for the other four members of the ferrocene-derived peptide family shown in Figure 1 [9-12], only initial studies have been performed on amino acids labeled with ferrocenamine [12b]. In this paper we describe the synthesis and characterization of amino acids substituted with ferroceneamine at the C-terminus.

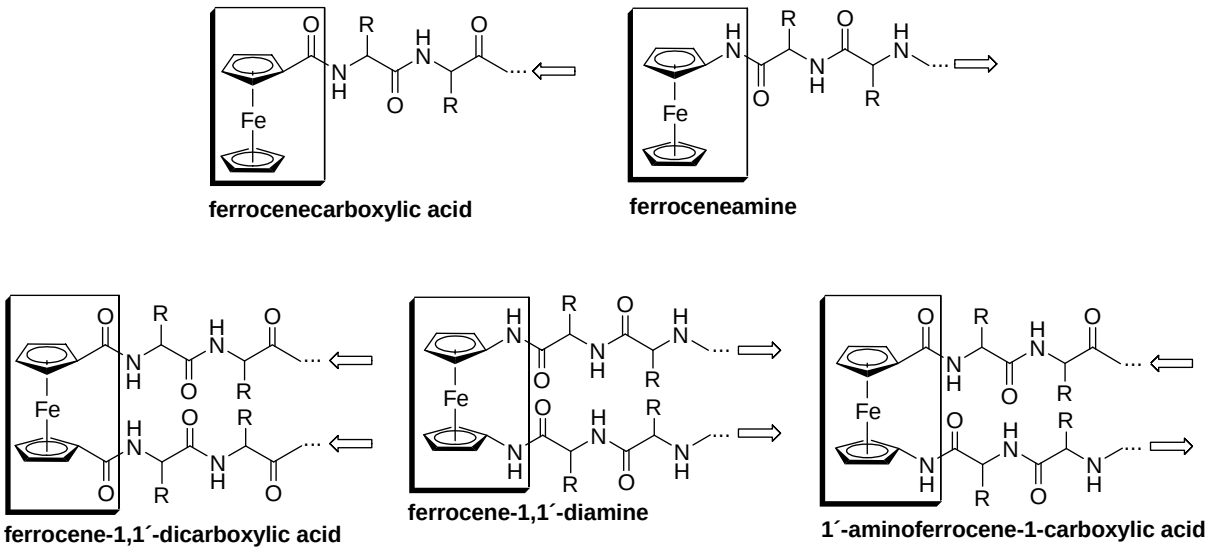
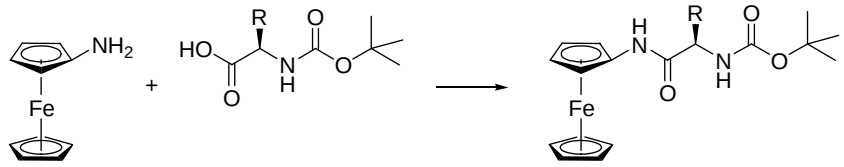


Figure 1. The ferrocene-derived peptide family. Arrows point from the C to the N termini of the peptides.

Results and Discussion

Synthesis. The unstable ferrocenylamine constitute the simplest amine of ferrocene and was the compound of choice for the coupling at the C-terminus of the amino acid. From the scarce methods found in the literature for their preparation, we followed the procedure of Leusen and Hessen as the most convenient synthesis route [13]. The procedure involves lithiation of ferrocene with t-BuLi followed by reaction with α-azidostyrene at -70°C and subsequent acidification. The required α-azidostyrene was prepared following a reported procedure from 1,2-dibromo-1-phenylethane [14]. Ferrocenylamine was coupled to the C-terminal end of six amino acids using the HBTU / HOBt protocol, Scheme 1. The bioconjugates **1** - **6** could be obtained in good yields (58 - 70 %) as orange solids.



1, R = H
2, R = $CH_2CH(CH_3)_2$
3, R = $CH_2C_6H_5$
4, R = $CH(CH_3)_2$
5, R = $CH_2SCH_2NHCOCH_3$
6, R = $CH_2C_6H_4OC(CH_3)_3$

Scheme 1. Synthesis of ferrocene peptides **1** - **6**. Reaction conditions: HBTU / HOBt / DIPEA / DCM.

Spectroscopy. The molecular structure of **1** - **6** was confirmed by EI-MS spectrometry. As known from previous studies [10j, 12b], the M^+ of ferrocene peptides are very stable and are found to be the base peak in all compounds presented in this study. The UV/VIS spectra in CH_2Cl_2 show the characteristic ferrocene peak at about 440 nm with ε ~ 180 M^{-1} cm^{-1}. The CD spectra (CH_2Cl_2) display only weak signals with M_θ = 5 mM^{-1} cm^{-1} or below, as expected for monosubstituted ferrocene peptides [8, 10b, 10j]. An interesting feature is provided by the ^1H NMR spectra ($CDCl_3$) were the NH protons are found above 7 ppm indicating intramolecular H-bond [8b, 10h]. Variable temperature ^1H NMR spectroscopy shows a weakening of the both N-H hydrogen bonding when the temperature is increased (see Figure 2). For the amide proton of the amino acid the temperature dependence is higher than the amide hydrogen attached to the Cp ring of ferrocene. The NMR spectra are also concentration dependent and both acidic protons are shifted to lower field when the concentration is increased (see Figure 2b).

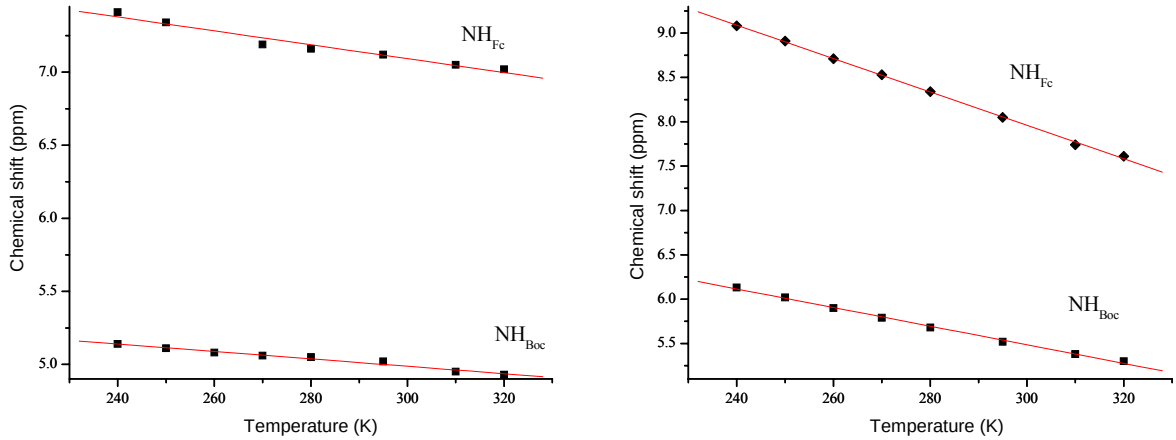


Figure 2a. Plot of chemical shift *vs.* temperature for compound **4** in 4 mM (left) and 200 mM (right) in $CDCl_3$. NH_{Fc} (slopes = -18.8 and -4.76 ppb/K for concentrated and diluted dissolutions respectively) refers to the amide hydrogen attached to the Cp ring of ferrocene and NH_{Boc} (slopes = -10.49 and -2.54 ppb/K for concentrated and diluted dissolutions respectively) is the amide proton of valine.

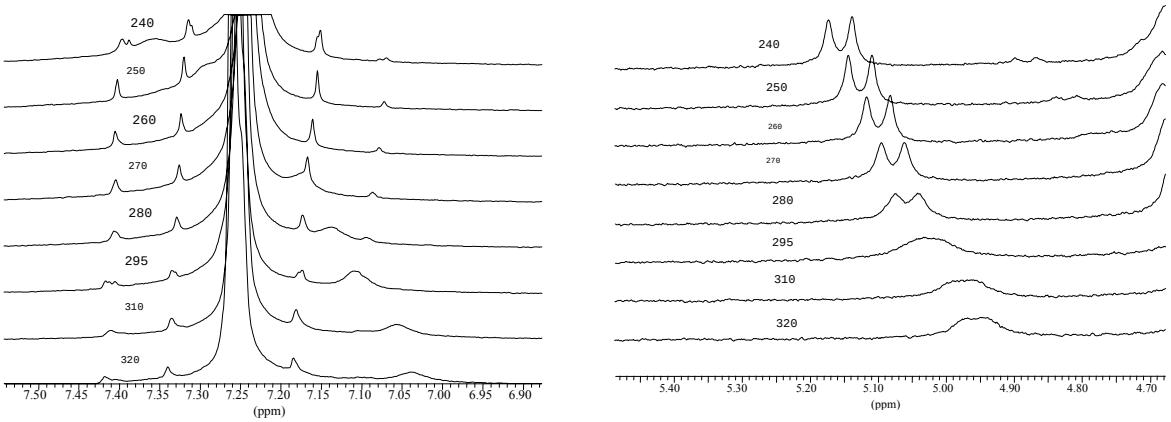
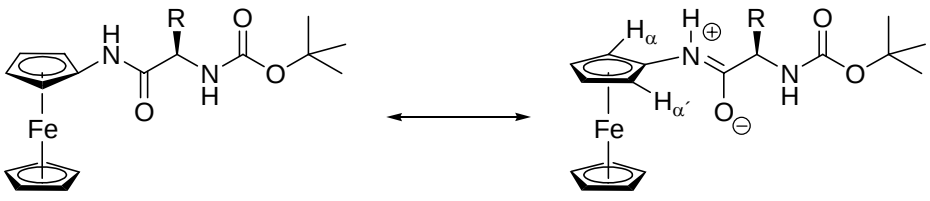


Figure 2b. ^1H NMR spectra of compound **4** at different temperatures in 4 mM solution in $CDCl_3$. The NH_{Fc} proton (left) is superimposed for the solvent residual peak at 260 K. The NH_{Boc} proton (right) is resolved in a doublet below 280 K

The conformation with an intramolecular six-membered hydrogen bond that involves the proton H_B of the cyclopentadienyl ring and the –N-C(O)- moiety is favored for the mesomeric canonical form of the amide group (see Scheme 2). In compounds **2**, **3-5** this preferred conformation places the hydrogens H_α and H_α, in different magnetic environments. In our assignment the nucleus H_α and C_α refers to the ones involved in the intramolecular hydrogen bond (see Scheme 2).



Scheme 2. Mesomeric canonical forms of Boc-Aaa-NH-Fc bioconjugates.

X-ray crystallography. The solid state structure of Boc-Gly-Fc **1** was studied by x-ray crystallography. The molecular geometry and atom labeling of **1** are shown in Fig. 3, while the hydrogen bonding pattern is displayed in Fig. 4. The crystal structure confirms the proposed composition of the compound. Bond lengths and angles are within the expected range. The ferrocene moiety is found in the eclipsed conformation, ω = 5°. The two Cp rings as well as the amide group are coplanar, the angles between the corresponding planes are 1.5°. Two hydrogen bonds, O1 ... N1A and N2 ... O3A, dominate the crystal packing and induce the formation of chains along the crystallographic *c* axis, Figure 4.

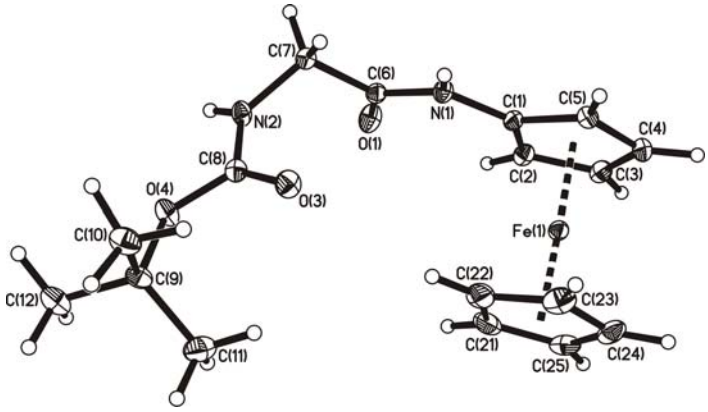


Figure 3. ORTEP plot of the asymmetric unit of **1**. Thermal ellipsoids are depicted at 50% probability.

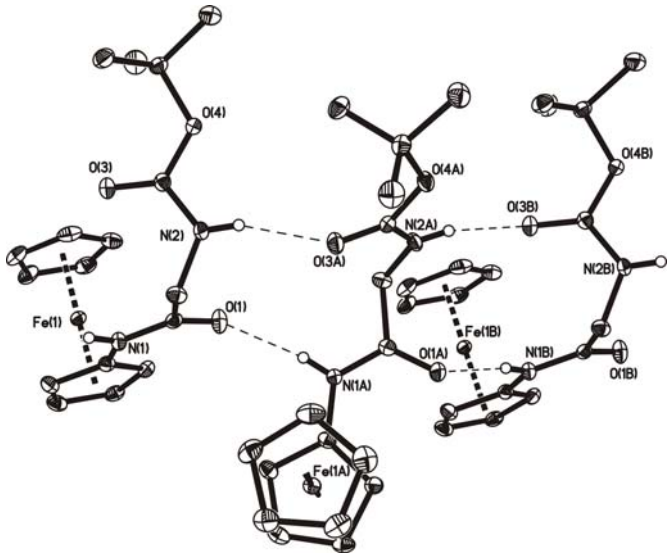


Figure 4. ORTEP plot of the hydrogen bond pattern in the solid state structure of **1**.

Experimental

General remarks. Reactions were carried out in ordinary glassware and chemicals used without further purification. All chemicals were obtained from Aldrich, Iris, Fluka or Novabiochem. Only pure N-Boc-protected L-amino acids were used. Variable temperature (320 – 240 K), ^{1}H (250.13 MHz) and ^{13}C (62.98 MHz) NMR measurements were performed on a Bruker AC 250 spectrometer. The chemical shift standard was internal tetramethylsilane for ^{1}H and ^{13}C (δ 0 ppm). Digital resolutions in the 1D NMR spectra were 0.17 Hz/point for 1H, 0.94 Hz/point for ^{13}C, pulse angles were *ca*. 30°, and sample concentrations were 2 mM. Signal assignments were assisted by gs-COSY, gs-HMQC, and gs-HMBC experiments performed on a Bruker Avance DPX-400 spectrometer using standard Bruker software. Mass spectra were measured on a Mat 8200 instrument, only characteristic fragments with intensities and possible composition are given in brackets. UV/VIS spectra were measured on a Varian CARY 100 instrument in 1 cm quartz Suprasil cells thermostated at 20 °C. Absorption maxima, λ_{max}, and molar absorption coefficients, ε_{max}, are given in nm and M^{-1} cm^{-1}, respectively. CD spectra were recorded on a JASCO J-810 spectropolarimeter in 1 cm quartz Suprasil cells under nitrogen thermostated at 20 °C, at 2 ± 0.2 mM concentrations. Ellipticity maxima, λ_{max}, and molar elliptisity coefficients, M_θ, are given in nm and mM^{-1} cm^{-1}, respectively.

General procedure of preparation of the title compounds 1-6. The corresponding Boc-protected α-amino acid (1 mmol) was dissolved in 35 ml of CH_2Cl_2. Solid HOBt (1 mmol, 0.153 g) was added under cooling (0 °C) and stirred for 10 minutes. HBTU (1 mmol, 0.380 g) and DIPEA (2 mmol, 0.646 g) were added sequentially and the reaction mixture was allowed to warm up and kept at room temperature for 30 minutes under stirring. To the reaction solution ferrocenylamine (1.1 mmol, 0.220 g) [13] was added. After completion (TLC analysis), the reaction was diluted to 100 ml with CH_2Cl_2 and washed successively with saturated $NaHCO_3$, 10% citric acid, and water. Then it was dried over Na_2SO_4 and evaporated under reduced pressure. The crude product was purified by flash column chromatography using hexane - ethyl acetate as eluent to get the pure product after crystallization (hexane or hexane - ethyl acetate mixtures).

Boc-Gly-Fc (1). Orange solid, yield 61%. M_r ($C_{17}H_{22}FeN_2O_3$) = 358.21; MS (EI): 358 (84) $[M]^+$, 302 (100) [M - *t*Bu]$^+$, 284 (20) [M - *t*BuOH]$^+$, 258 (91) [M - Boc]$^+$, 227 (24) [Fc-NHCO]$^+$, 201 (45) [M - Gly-OMe]$^+$. HRMS (EI): *m/z* exp. 358.0980 and calc. 358.0980. ^{1}H NMR (250.13 MHz, $CDCl_3$, 294 K), see Table 1. ^{13}C NMR (62.98 MHz, $CDCl_3$, 294 K), see Table 2. UV / VIS (CH_2Cl_2): λ_{max}. (ε) 441 (168).

Boc-Leu-Fc (2). Orange solid, yield 59%. M_r ($C_{21}H_{30}FeN_2O_3$) = 414.32; MS (EI): 414 (85) $[M]^+$, 358 (100) [M - *t*Bu]$^+$, 340 (54) [M - *t*BuOH]$^+$, 314 (79) [M - Boc]$^+$, 227 (67) [Fc-NHCO]$^+$, 201 (99) [M - Leu-OMe]$^+$. HRMS (EI): *m/z* exp. 414.1606 and calc. 414.1606. ^{1}H NMR (250.13 MHz, $CDCl_3$, 294 K), see Table 1. ^{13}C NMR (62.98 MHz, $CDCl_3$, 294 K), see Table 2. UV / VIS (CH_2Cl_2): λ_{max}. (ε) 441 (174). CD (CH_2Cl_2): λ_{max} (M_θ) 466 (+ 5.3), 350 (-0.3), 327 (+0.4).

Boc-Phe-Fc (3). Orange solid, yield 65%. M_r ($C_{24}H_{28}FeN_2O_3$) = 448.34; MS (EI): 448 (100) $[M]^+$, 392 (88) $[M - t\text{Bu}]^+$, 374 (60) $[M - t\text{BuOH}]^+$, 348 (76) $[M - \text{Boc}]^+$, 227 (64) $[\text{Fc-NHCO}]^+$, 201 (85) $[M - \text{Phe-OMe}]^+$. HRMS (EI): *m/z* exp. 448.1450 and calc. 448.1449. ^{1}H NMR (250.13 MHz, $CDCl_3$, 294 K), see Table 1. ^{13}C NMR (62.98 MHz, $CDCl_3$, 294 K), see Table 2. UV / VIS (CH_2Cl_2): λ_{max}. (ε) 442 (183). CD (CH_2Cl_2): λ_{max} (M_θ) 470 (+1.6), 324 (+0.4).

Boc-Val-Fc (4). Orange solid, yield 65%. M_r ($C_{20}H_{28}FeN_2O_3$) = 400.29; MS (EI): 400 (100) $[M]^+$, 344 (96) $[M - t\text{Bu}]^+$, 326 (19) $[M - t\text{BuOH}]^+$, 300 (78) $[M - \text{Boc}]^+$, 227 (39) $[\text{Fc-NHCO}]^+$, 201 (83) $[M - \text{Val-OMe}]^+$. HRMS (EI): *m/z* exp. 400.1448 and calc. 400.1449. ^{1}H NMR (250.13 MHz, $CDCl_3$, 294 K), see Table 1. ^{13}C NMR (62.98 MHz, $CDCl_3$, 294 K), see Table 2. UV / VIS (CH_2Cl_2): λ_{max}. (ε) 441 (196). CD (CH_2Cl_2): λ_{max} (M_θ) 478 (-1.1), 333 (+0.8).

Boc-Cys(Acm)-Fc (5). Orange solid, yield 58%. M_r ($C_{21}H_{29}FeN_3O_4S$) = 475.38; MS (EI): 475 (100) $[M]^+$, 419 (15) $[M - t\text{Bu}]^+$, 404 (18) $[M - \text{Acm}]^+$, 375 (43) $[M - \text{Boc}]^+$, 348 (29) $[M - \text{Acm} - t\text{Bu}]^+$; 304 (46) $[M - \text{Acm} - \text{Boc}]^+$; 227 (41) $[\text{Fc-NHCO}]^+$, 201 (37) $[M - \text{Cys(Acm)-OMe}]^+$. HRMS (EI): *m/z* exp. 475.1230 and calc. 475.1228. ^{1}H NMR (250.13 MHz, $CDCl_3$, 294 K), see Table 1. ^{13}C NMR (62.98 MHz, $CDCl_3$, 294 K), see Table 2. UV / VIS (CH_2Cl_2): λ_{max}. (ε) 441 (184). CD (CH_2Cl_2): λ_{max} (M_θ) 471 (-4.4), 304 (+4.4).

Boc-Tyr(^{t}Bu)-Fc (6). Orange solid, yield 70%. M_r ($C_{28}H_{36}FeN_2O_4$) = 520.44; MS (EI): 520 (100) $[M]^+$, 464 (100) $[M - t\text{Bu}]^+$, 446 (39) $[M - t\text{BuOH}]^+$, 420 (42) $[M - \text{Boc}]^+$, 390 (23) $[M - t\text{Bu} - t\text{BuOH}]^+$, 227 (50) $[\text{Fc-NHCO}]^+$, 201 (61) $[M - \text{Tyr}(t\text{Bu})\text{-OMe}]^+$. HRMS (EI): *m/z* exp. 520.2023 and calc. 520.2024. ^{1}H NMR (250.13 MHz, $CDCl_3$, 294 K), see Table 1. ^{13}C NMR (62.98 MHz, $CDCl_3$, 294 K), see Table 2. UV / VIS (CH_2Cl_2): λ_{max}. (ε) 441 (175). CD (CH_2Cl_2): θ_{max} (M_θ) 466 (+3.2), 346 (-0.5), 320 (+0.3).

X-ray Crystallographic. Yellowish needle-shaped crystals of **1** were obtained by slow evaporation from a hexane - ethyl acetate solution mixture (9 : 1). Crystal data for **1**: Bruker SMART-CCD diffractometer, SHELX-97 programs. Mo-K_α radiation (λ = 0.71073 Å). $C_{17}H_{22}N_2O_3Fe$, *M* = 358.22, monoclinic, space group $P2_1/c$, a = 11.6361(5) Å, b = 16.1791(7) Å, g = 9.4167(4) Å, β = 107.303(5)º, V = 1692.57(13) Å^3, Z = 4, T = 100(2) K, m(Mo-K_α) = 0.907 mm^{-1}, 19764 reflections measured, 5168 unique (Rint = 0.0460) which were used in all calculations. R1 = 0.0427 (all data) and wR2(F2) = 0.0784 (all data).

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Table 1. ^{1}H chemical shifts of compounds **1-6**, ^{1}H,^{1}H coupling constants (in Hz) in parentheses[a,b].

	H_5C_5	H_α	$H_{\alpha'}$	$H_{\beta,\beta'}$	NH	α-CH	NH_{Boc}	$(CH_3)_{3Boc}$	R		
									CH	β-CH_2	CH_3
1	4.16 s	4.60 t (2.0)	4.60 t (2.0)	3.99 t (1.8)	7.64 s	-	5.38 s	1.49 s		3.82 d (5.8)	
2	4.13 s	4.62 s	4.60 s	3.97 s	7.73 s	3.97 s	5.08 s	1.47 s	1.26 s	1.71 m	0.95 s
3	4.07 s	4.51 m	4.51 m	3.96 t (1.8)	7.36-7.19 [c]	4.35 ddd (7.2, 7.2, 7.2)	5.14 d (7.2)	1.44 s		3.10 d (7.0)	
4	4.13 s	4.67 s	4.56 s	3.92 m	7.77 s	3.92 m	5.35 d (8.4)	1.46 s	2.14 d (5.6)	-	1.01 t (6.7)
5	4.16 s	4.57-4.67 m	4.57-4.67 m	3.98 br s	8.53 s	4.57-4.67 m	5.75 d (8.1)	1.48 s		2.78 dd (14.2, 7.6)	2.05 s
6	4.11 s	4.49-4.54 m	4.49-4.54 m	3.96 t (1.9)	7.31 br s	4.33 ddd (7.2, 7.2, 7.2)	5.21 d (6.8)	1.18 s		3.04 dd (7.0, 2.5)	1.23 s

a) δ(^{1}H) in ppm relative to TMS; $CDCl_3$ as solvent; letters denote signal multiplicities: s = singlet, d = doublet, m = multiplet, t = triplet, br = broad.

b) ^{1}H chemical shifts of the substituents not included in the table: **3:** δ = 7.36-7.19 m (C_6H_5); **5:** δ = 7.34 br s, (NH), 2.96 dd, *J* = 15.0, 14.0 Hz (S-CH_2-); **6**: δ = 7.18-7.12 m (*meta*-CH of -C_6H_4-O*t*Bu), 6.96-6.90 m, (*ortho*-CH of -C_6H_4-O*t*Bu).

c) Superimposed with the aromatic protons.

Table 2. ^{13}C chemical shifts of compounds **1-6**[a,b].

	C_5H_5	C_{ipso}	C_α	$C_{\alpha'}$	$C_{\beta,\beta'}$	CO	α-CH	CO_{Boc}	CH_{3Boc}	C_{Boc}	R		
											β-CH_2	CH	CH_3
1	69.2	80.6	61.6	61.6	64.7	156.4	45.3	167.8	28.3	93.8		-	-
2	69.1	94.1	61.3	61.3	64.4	156.0	53.5	170.0	28.3	80.4	22.8	40.5	22.6
3	69.1	93.5	61.8	61.6	64.6	155.7	56.4	169.8	28.3	80.5	38.1	-	-
4	69.0	94.1	61.5	61.2	64.4	156.0	61.2	170.0	28.3	82.8	-	30.4	19.3
5	69.2	93.6	61.8	61.6	64.7	156.1	53.9	171.2	28.3	80.4	34.8	-	21.0
6	69.1	93.5	61.5	61.5	64.5	156.6	56.3	169.5	28.2	80.3	37.3	-	28.7

a) δ(^{13}C) in ppm relative to TMS; $CDCl_3$ as solvent.

b) ^{13}C chemical shifts of the substituents not included in the table; **3:** δ = 136.6 (*ipso*-C_6H_5), 129.3 (*ortho*- C_6H_5), 128.7 (*meta*-C_6H_5), 127.1 (*para*- C_6H_5); **5:** δ = 169.0 (CO), 41.3 (S-CH_2); **6:** δ = 154.3 (*ipso*-CH of -C_6H_4-O*t*Bu), 131.3 (*para*-CH of -C_6H_4-O*t*Bu), 129.6 (*meta*-CH of -C_6H_4-O*t*Bu), 124.0 (*ortho*-CH of -C_6H_4-O*t*Bu), 78.4 (quaternary C of O*t*Bu).