Synthesis of ferrocenyl- and pyrenyl-thioimidates of terminal acetylenes. "Click" reaction with 3′-azido-3′-deoxythymidine affording redox-active and fluorescent thymidine conjugates

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Abstract

Thioamides Ar(C=S)NHCOOEt (Ar = ferrocenyl and 1-pyrenyl) obtained from ferrocene and pyrene and ethoxycarbonyl isothiocyanate react under Mitsunobu conditions with propargyl and homopropargyl alcohols, yielding the corresponding *N*-(ethoxycarbonyl)thioimidates containing a terminal acetylene functionality. These compounds undergo Cu(I)-catalyzed "click reaction" with 3′-azido-3′-deoxythymidine (AZT) affording novel redox-active ferrocenyl and fluorescent, environmentally sensitive pyrenyl thymidine conjugates.

Keywords: Ferrocene, pyrene, AZT, thioamide, click reactions, fluorescence

Introduction

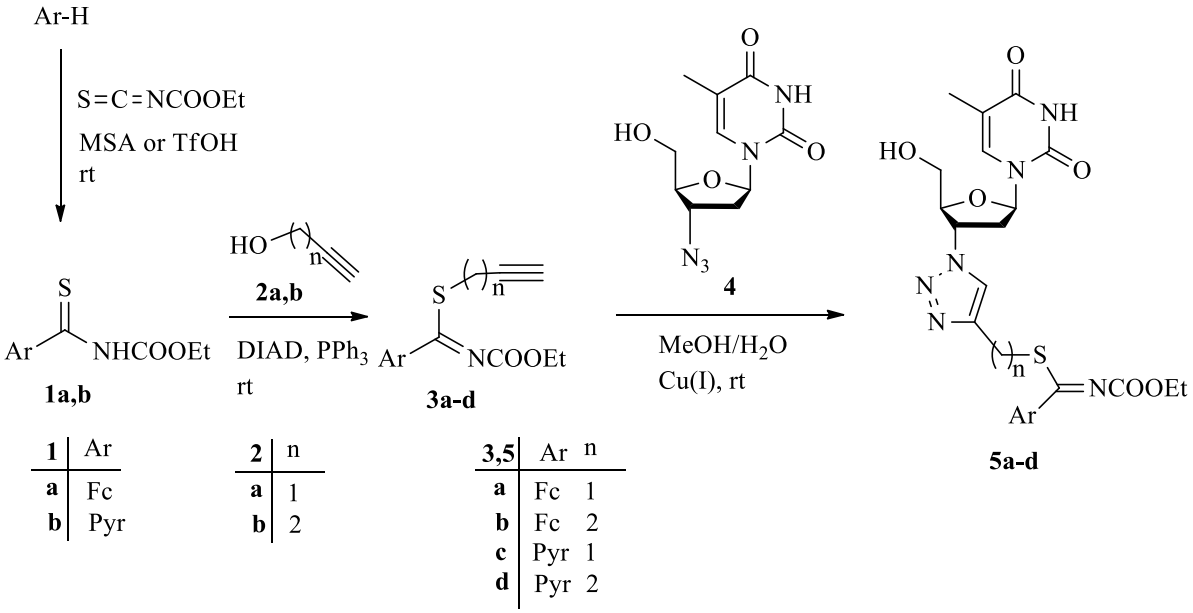
Labeling of biomolecules with redox active or fluorescent reporter groups constitutes a powerful research tool for biological and medical sciences.[1] Among various redox-active markers ferrocenyl compounds are of increased interest due to the accessibility of a large variety of compounds, reversibility of the redox process and the easy tuning by changing substituents at the cyclopentadienyl ligands.[2] On the other hand, pyrene derivatives are attractive fluorescent markers due to long lifetimes of their excited states, ability to π-stacking resulting in exciplex and excimer formation and sensitivity to the polarity of the surrounding medium.[3]

We have recently reported that ferrocenyl thioamide Fc(C=S)NHCOOEt (**1a**) reacts as an *S*-pronucleophile in the Mitsunobu reaction with alcohols to afford the corresponding ferrocenyl (*N*-ethoxycarbonyl)thioimidates.[4] This reaction has been applied for redox labeling of adenosine, 2′-deoxyadenosine and some steroidal alcohols.

Herein we report that **1a** can be readily transformed by Mitsunobu reaction into thioimidates **3a–b** (Scheme 1) with terminal acetylene groups. These compounds can be used as "clickable" markers for azide-functionalized biomolecules as exemplified by 3′-azido-3′-deoxythymidine (AZT, **4**), applying the Cu(I)-catalyzed Huisgen-Meldal azide-alkyne cycloaddition (CuAAC),[5] thus affording redox-active conjugates **5a,b**. Furthermore, we have extended this approach to pyrenyl compounds, synthesizing the *N*-ethoxycarbonyl thioamide **1b**, acetylenic thioimidates **3c,d**, and AZT conjugates **5c,d**. We have found that these conjugates show intense solvent-dependent fluorescence.

Results and Discussion

It has been reported that the Friedel-Crafts reaction of ferrocene with *N*-ethoxycarbonyl isothio–cyanate carried out in methanesufonic acid at rt affords thioamide **1a** in 48% yield.[6] We have found that with pyrene this reaction proceeds better in the presence of trifluoromethanesulfonic acid in dichloromethane affording thioamide **1b** in 95% yield. The ^{13}C NMR signals of the thiocarbonyl and carbonyl groups of this compound appear at δ 201.5 and 149.5, respectively. The Mitsunobu reaction of **1a,b** with propargyl alcohol **2a** and homopropargyl alcohol **2b** was carried out in the presence of diisopropyl azodicarboxylate (DIAD) and triphenylphosphine and afforded acetylenic thioimidates **3a–d** in 78–92% yield (Scheme 1).



1	Ar
a	Fc
b	Pyr

2	n
a	1
b	2

3,5	Ar	n
a	Fc	1
b	Fc	2
c	Pyr	1
d	Pyr	2

Scheme 1. Synthesis of acetylenic thioimidates **3a–d** and their "click" reaction with AZT (**4**). (MSA = methanesulfonic acid, TfOH = trifluoromethanesulfonic acid, DIAD = diisopropyl azodicarboxylate; Fc = ferrocenyl; Pyr = 1-pyrenyl).

The ^{13}C NMR spectra of **3a–d** displaying characteristic signals at δ 171–175 and δ ~161 confirm the *S*-propargylation and homopropargylation.[4] The IR spectra of **3a–d** show strong absorption bands at 1700–1725 and 1600–1620 cm^{-1}, indicative of *N*-ethoxycarbonylthioimidates.

To test the "clickability" of **3a–d**, we chose 3′-azido-3′-deoxythymidine (AZT, **4**) as a model azide-modified biomolecule. Modification of nucleosides using the Cu(I)-catalyzed Huisgen-Meldal azide-alkyne cycloaddition (CuAAC) reaction has attracted considerable interest; numerous compounds obtained in this way exhibit interesting biological or pharmaceutical properties.[7] Furthermore, the 1,2,3-triazole formed in CuAAC reactions is now considered a promising pharmacophore.[8] AZT (**4**) is a commercially available and cheap azide-containing nucleoside, which has been used in "click" reactions with various terminal acetylenes affording conjugates displaying interesting biological properties.[9] However, to the best of our knowledge, up to now there is no report on the reaction of **4** with ferrocenyl or pyrenyl acetylenes.

We have found that AZT (**4**) reacted with **3a–d** under the usual CuAAC reaction conditions[5] to afford **5a–d** in 40–70% yield. The formation of the 1,4-disubstituted 1,2,3-triazole ring was confirmed by the ^{1}H NMR signals of the H-5 triazolyl protons at δ 7.5–8.3, and by the absence of acetylenic proton signals at δ 2–2.5.

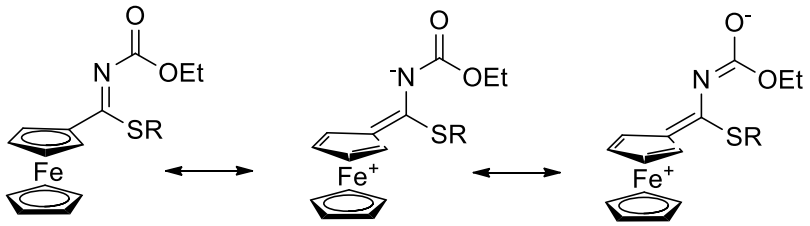
Redox properties of ferrocenyl-AZT conjugates 5a–b

Since ferrocenyl groups are widely used as redox-active reporters in biochemistry,[2] it seemed interesting to study the redox properties of **5a,b**. Cyclic voltammetry showed that the Fe(II)/Fe(III) redox process in these compounds is electrochemically quasi-reversible (ΔE ~80–100 mV) and chemically reversible (i_{pa}/i_{pc} ~ 1) occurring at substantially higher potential than that of the parent ferrocene (Table 1).

Table 1. Cyclic voltammetry data for **5a,b** and acetylferrocene (MeCN, Pt, $Bu_4N^+PF_6^-$, 50 mV/s)

Compound	$E_{1/2}$ (mV)[a]	ΔE (mV)
5a	282	80
5b	265	84
FcAc	248	102

Compounds **5a,b** undergo oxidation at slightly higher potential than acetylferrocene, indicating that the (*N*-ethoxycarbonyl)thioimidato groups in these compounds behave as relatively strong electron-withdrawing groups (stronger than the acetyl group). This may be explained by delocalization of the negative charge on the carbonyl group and the cation stabilizing ability of the ferrocenyl group (Scheme 2).[10]



Scheme 2. Resonance structures of a ferrocenyl (*N*-ethoxycarbonyl)thioimidate (*Z*-isomer shown).

Electronic absorption and emission spectra of 5c,d

Electronic absorption and emission spectra of **5c** and **5d** are closely similar. Those of **5c** are shown in Fig.1 and the data are collected in Table 2.

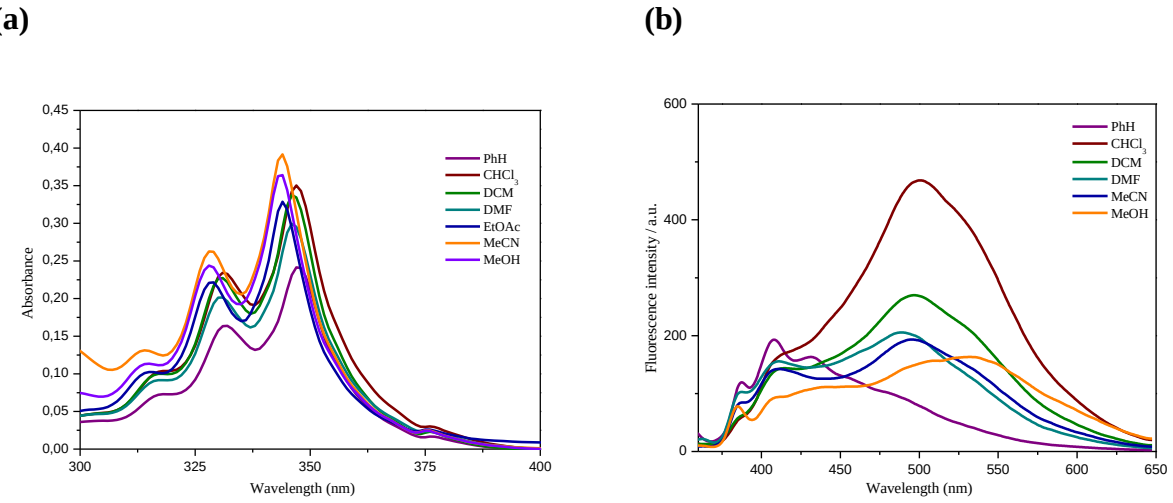


Figure 1. (a) Electronic absorption and (b) fluorescence emission spectra (λ_{excit} = 345 nm) of **5c** in various solvents.

The electronic absorption spectra of **5c,d** exhibit typical vibronic features of the pyrenyl chromophore[3c] attributable to the S_0–S_1 transitions.

The fluorescence displayed by these compounds is strongly solvent-dependent (Table 2). In a nonpolar solvent like benzene, a relatively weak, structured fluorescence spectrum is observed, whereas in other solvents stronger and red-shifted unstructured signals were also present. These signals were observed even at concentrations as low as 10^{-7} M, suggesting that they correspond to electronic transitions in monomers **5c,d**. The strongest fluorescence was observed in chloroform and in dichloromethane (DCM). For **5c**, the fluorescence quantum yield of 0.04 was determined (in DCM, excitation wavelength 376 nm, quinine sulfate in 0.1 M H_2SO_4 used as a reference[11]). The solvent-dependence of fluorescent properties of **5c,d** suggests that pyrenyl

thioimidato compounds may be used, similarly to pyrenyl carbonyl compounds,[12] as environment-sensitive probes in biological systems and in materials science.

Table 2. Electronic absorption spectra of **5c,d** in various solvents

5	Solvent	Absorption λ_{max} [nm] (ε_{max} [$M^{-1}\cdot cm^{-1}$])	Fluorescence λ_{max} [nm]
5c	Benzene	377 (890), 348 (19280), 332 (12790), 316 (6600)	387, 408, 432
	Chloroform	376 (3200), 348 (33390), 332 (22370), 317 (11070)	501
	Dichloromethane	376 (2890), 346 (40590), 331 (27610), 316 (13490)	416, 496
	DMF	376 (1470), 347 (28090), 331 (18610), 317 (7610)	386, 411, 489
	Acetonitrile	375 (1940), 344 (37690), 329 (25150), 314 (12650)	386, 410, 494
	Methanol	375 (2030), 344 (33700), 329 (22200), 316 (9630)	385, 542
5d	Benzene	376 (1660), 347 (24150), 332 (16370), 316 (7300)	386, 450
	Chloroform	376 (3010), 347 (35030), 331 (23440), 318 (10370)	498
	Dichloromethane	376 (2380), 346 (33760), 331 (22730), 317(10000)	414, 435, 496
	DMF	376 (2310), 346 (29930), 331 (20150), 317(9160)	386, 411, 487
	Acetonitrile	375 (2500), 344 (39160), 329 (26270), 314 (13130)	387, 412, 494
	Methanol	375 (2460), 344 (36400), 329 (24380), 316 (11340)	384, 528

Ferrocenyl compounds **5a,b** are not fluorescent (it is known that ferrocene is an efficient quencher of excited states).[13]

Conclusions

Ferrocenylthioamide **1a** is an efficient labeling reagent for hydroxyl-containing biomolecules via Mitsunobu reaction; it can be transformed into ferrocenylthioimidato acetylenes **3a,b** that are "clickable" with biomolecules bearing an azido function (exemplified by AZT, **4**) affording redox-active **5a,b**. The same approach applied to 1-pyrenylthioamide **1b** opens an entry to bioconjugates **5c,d** with a new environmentally sensitive pyrenylthioimidato fluorophore.

Experimental Section

General. Compound **1a** was prepared according to a published procedure.[6] All other reagents were purchased from Sigma-Aldrich and were used as such. Before use, solvents were thoroughly purified using standard methods. Reactions with ferrocenyl compounds were carried out under argon. Chromatographic separations were performed on silica gel Merck 60 (230–400 mesh ASTM). NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Bruker Avance III 600

spectrometer (600 MHz for ^{1}H) and referenced to internal TMS. IR spectra were recorded on a FT-IR NEXUS (Thermo Nicolet) spectrometer. Electronic absorption and fluorescence spectra were run on a Perkin Elmer LS 55 spectrofluorimeter. Cyclic voltammetry experiments were carried out on an AUTOLAB (Eco Chimie BV) apparatus in a three electrode system, the working electrode was a Pt disk (1.5 mm diameter), the reference electrode was a ferrocene electrode, and the counter electrode was a cylindrical Pt gauze. These experiments were carried out using argon-saturated $5x10^{-4}$ M solutions of **5a,b** in acetonitrile containing 0.1 M [(*n*-Bu)$_4$N][PF$_6$] as supporting electrolyte. Elemental analyses were performed by Analytical Services of the Center of Molecular and Macromolecular Studies of the Polish Academy of the Sciences (Łódź).

Ethyl pyrene-1-carbonothioylcarbamate (1b). To a solution of pyrene (202 mg, 1 mmol) in DCM (5 mL) were added at rt ethoxycarbonylisothiocyanate (262 mg, 2 mmol) and TfOH (348 μL, 4 mmol). After stirring for 2 h, the reaction mixture was poured onto ice-water (30 mL) and extracted with DCM. Flash chromatography (DCM) afforded yellow crystals **1b** (316 mg, 95%); mp 159–160 °C. ^{1}H NMR (600 MHz, CDCl$_3$): δ 9.48 (s, 1H, NH), 8.24 (d, *J* = 9 Hz, 1H), 8.21 (dd, J_1 = 3.6 Hz, J_2 = 11.4 Hz, 2H), 8.16 (d, *J* = 7.8 Hz, 1H), 8.17 (dd, J_1 = 1.8 Hz, J_2 = 10.8 Hz, 2H), 8.05 (d, *J* = 9 Hz, 1H), 8.03 (t, *J* = 7.8 Hz, 1H), 8.01 (d, *J* = 7.8 Hz, 1H), 4.04 (q, *J* = 6.6 Hz, 2H), 1.08 (t, *J* = 7.2 Hz, 3H). ^{13}C NMR (150 MHz, CDCl$_3$): δ 201.5, 149.5, 137.2, 132.3, 131.4, 130.7, 128.8, 128.5, 127.3, 126.4, 126.3, 126.0, 125.7, 124.8, 124.6, 124.3, 123.3, 62.9, 13.9. IR (KBr): 3137, 2961, 1762, 1509, 1247, 1235, 1168, 1043, 847 cm^{-1}. Anal. calcd for $C_{20}H_{15}NO_2S$: C, 72.05; H, 4.53; N, 4.20. Found: C, 72.01; H, 4.63; N, 4.37%.

Synthesis of 3a–d. General procedure

To a stirred solution of **1a** or **1b** (1.0 mmol) in anhydrous THF (10 mL) under argon at 0 °C were added triphenylphosphine (393 mg, 1.5 mmol), propargyl alcohol **2a** or homopropargyl alcohol **2b** (1.0 mmol) in THF (5 mL), and DIAD (295 μL, 1.5 mmol). The reaction mixture was allowed to warm to rt and was stirred for 2 h. Flash chromatography (DCM) afforded products **3**.

Prop-2-yn-1-yl *N*-ethoxycarbonylpyrene-1-carbimidothioate (3a). Red oil (277 mg, 78%). ^{1}H NMR (600 MHz, CDCl$_3$): δ 4.65 (t, *J* 1.8 Hz, 2H), 4.31 (t, *J* 1.8 Hz, 2H), 4.27 (s, 5H), 4.23 (q, *J* 7.2 Hz, 2H), 3.79 (d, *J* 2.4 Hz, 2H), 2.23 (t, *J* 2.4 Hz, 1H), 1.30 (t, *J* 7.2 Hz, 3H). ^{13}C NMR (150 MHz, CDCl$_3$): δ 171.1, 161.4, 78.5, 77.1, 71.4, 71.2, 70.9, 68.9, 62.5, 19.6, 14.3. IR (KBr): 3287, 2964, 2926, 1699, 1618, 1254 cm^{-1}. Anal. calcd for $C_{17}H_{17}FeNO_2S$: C, 57.48; H, 4.82; N, 3.94. Found: C, 57.58; H, 4.91; N, 3.92.

But-3-yn-1-yl *N*-ethoxycarbonylpyrene-1-carbimidothioate (3b). Red oil (321 mg, 87%). ^{1}H NMR (600 MHz, CDCl$_3$): δ 4.65 (t, *J* 1.8 Hz, 2H), 4.13 (t, *J* 1.8 Hz, 2H), 4.25 (s, 5H), 4.23 (q, *J* 7.2 Hz, 2H), 3.16 (t, *J* 7.2 Hz, 2H), 2.61 (td, J_1 2.4 Hz, J_2 7.8 Hz, 2H), 2.04 (t, *J* 2.4 Hz, 1H), 1.30 (t, *J* 7.2 Hz, 3H). ^{13}C NMR (150 MHz, CDCl$_3$): δ 171.8, 161.6, 82.4, 77.7, 71.0, 70.9, 69.6, 69.0, 62.4, 29.9, 18.8, 14.3. IR (KBr): 3292,3097, 2980, 2936, 1705, 1616, 1253 cm^{-1}. Anal. calcd for $C_{18}H_{19}FeNO_2S$: C, 58.55; H, 5.19; N, 3.79. Found: C, 58.72; H, 5.22; N, 3.89

Prop-2-yn-1-yl *N*-ethoxycarbonylferrocenecarbimidothioate (3c). Colorless crystals (342 mg, 92%); mp 105–106 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.52 (d, *J* 9 Hz, 1H), 8.22 (dd, J_1 4.8 Hz, J_2 10.8 Hz, 2H), 8.16 (d, *J* 9 Hz, 1H), 8.15 (d, *J* 7,8 Hz, 1H), 8.11 (d, *J* 9 Hz, 1H), 8.04 (d, *J* 9 Hz, 1H), 8.03 (d, *J* 7.8 Hz, 1H), 7.99 (d, *J* 7.8 Hz, 1H), 4.05 (d, *J* 2.4 Hz, 2H), 3.80 (q, *J* 7.2 Hz, 2H), 2.33 (t, *J* 2.4 Hz, 1H), 0.73 (t, *J* 7.2 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 174.8, 160.6, 132.6, 131.1, 130.7, 129.5, 128.8, 128.7, 127.6, 127.0, 126.4, 126.0, 125.9, 125.0, 124.4, 124.2, 124.1, 124.0, 77.9, 71.9, 62.4, 20.5, 13.7. IR (KBr): 3277, 2981, 1720, 1617, 1220, 846 cm^{-1}. Anal. calcd for $C_{23}H_{17}NO_2S$: C, 74.37; H, 4.61; N, 3.77. Found: C, 74.45; H, 4.71; N, 3.87.

But-3-yn-1-yl *N*-ethoxycarbonylferrocenecarbimidothioate (3d). Light yellow crystals (343 mg, 89%); mp 92–94 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.23 (d, *J* 9 Hz, 3H), 8.15 (dd, J_1 4.2 Hz, J_2 12.6 Hz, 3H), 8.06 (d, *J* 9 Hz, 1H), 8.05 (t, *J* 7,8 Hz, 1H), 7.97 (d, *J* 7.8 Hz, 1H), 3.76 (q, *J* 7.2 Hz, 2H), 2.42 (t, *J* 7.2 Hz, 2H), 2.61 (td, J_1 2.4 Hz, J_2 7.2 Hz, 2H), 2.11 (t, *J* 2.4 Hz, 1H), 0.70 (t, *J* 7.2 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 175.9, 160.9, 132.5, 131.1, 130.8, 130.5, 128.8, 128.6, 127.4, 127.1, 126.4, 125.9, 125.8, 125.0, 124.4, 124.3, 124.1, 82.0, 70.0, 62.3, 30.8, 18.6, 13.7. IR (KBr): 3291, 3048, 2977, 1723, 1610, 1236, 846 cm^{-1}. Anal. calcd for $C_{24}H_{19}NO_2S$: C, 74.78; H, 4.97; N, 3.63. Found: C, 74.82; H, 4.90; N, 3.67%.

Synthesis of 5a–d via "click" reaction. General procedure

To a solution of **3a–d** (0.29 mmol) and AZT (**4**) (90 mg, 0.34 mmol, 1.16 equiv.) in methanol/water (20:2 mL) were added aqueous solutions of TTTA (0.01 M, 250 μL), $CuSO_4$ (0.1 M, 50 μL) and sodium ascorbate (0.1 M, 50 μL). The resulting solution was stirred at rt for 72 h. Then, water (50 mL) was added and products were extracted with ethyl acetate. Pure products were obtained by chromatography using DCM / ethyl acetate / methanol 25:25:1 (v/v/v) as eluent.

5a. Orange crystals (80 mg, 42%); mp 130–131 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.34 (br. s., 1H), 8.22 (s, 1H), 7.82 (br. s., 1H), 6.42 (t, *J* 6.4 Hz, 1H), 5.37 (d, *J* 7.5 Hz, 1H), 5.28 (br s, 1H), 4.63 (br. s., 2H), 4.58 (br s, 2H), 4.29 (s, 2H), 4.25 (s, 5H), 4.23–4.16 (m, 3H), 3.76–3.67 (m, 1H), 3.63 (d, *J* 11.7 Hz, 1H), 2.73 (d, *J* 6.4 Hz, 1H), 2.69–2.60 (m, 1H), 1.82 (s, 3H), 1.26 (t, *J* 7.0 Hz, 3H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 170.78, 164.17, 161.10, 150.90, 143.08, 136.68, 123.77, 110.08, 84.93, 84.41, 71.98, 71.15, 69.10, 62.53, 61.18, 59.68, 37.59, 25.77, 21.22, 14.62, 12.69, IR IR (KBr): 3432, 1697, 1236 cm^{-1}. Anal. calcd for $C_{27}H_{30}FeN_6O_6S{\cdot}CH_3OH$: C, 51.38; H, 5.24; N, 12.84. Found: C, 51.58; H, 5.54; N, 13.12%.

5b. Orange crystals (76 mg, 41%); mp 163–166 °C. ^{1}H NMR (600 MHz, $CDCl_3$): δ 9.20 (br. s., 1 H), 7.58 (s, 1H), 7.50 (s, 1H), 6.24 (t, *J* 6.2 Hz, 1H), 5.42–5.33 (m, 1H), 4.60 (br s, 2H), 4.41 (br s, 3H), 4.29–4.18 (m, 7H), 3.96 (d, *J* 11.7 Hz, 1H), 3.79–3.65 (m, 2H), 3.36 (qd, J_1 6.9 Hz, J_2 17.4 Hz, 2H), 3.14 (t, *J* 7.0 Hz, 2H), 3.01–2.84 (m, 2H), 1.32 (t, *J* 7.2 Hz, 3H), 1.26 (br s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 172.63, 163.77, 161.96, 150.42, 146.20, 137.65, 121.67, 111.13, 88.21, 85.22, 77.69, 71.18, 70.93, 68.95, 62.57, 61.41, 58.97, 37.56, 29.96, 25.44, 14.33, 12.41. IR IR (KBr): 3437, 1697, 1670, 1236 cm^{-1}. Anal. calcd for $C_{28}H_{32}FeN_6O_6S$: C, 52.10; H, 5.15; N,13.02. Found: C, 52.47; H, 5.19 ; N, 13.11%.

5c. Pale yellow crystals (80 mg, 41%); mp 147–149 °C. ^{1}H NMR (600 MHz, $CDCl_3$): 9.52 (s, 1H), 8.08 (dd, J_1 4.3 Hz, J_2 7.3 Hz, 2H), 8.02–7.94 (m, 4H), 7.92–7.87 (m, 2H), 7.84 (s, 1H), 7.80 (d, *J* 7.9 Hz, 1H), 7.41 (s, 1H), 6.19 (t, *J* 6.2 Hz, 1H), 5.44–5.33 (m, 2H), 4.46 (d, *J* 2.6 Hz, 2H), 4.35 (br s, 2H), 3.93 (d, *J* 11.3 Hz, 1H), 3.76 (d, *J* 11.3 Hz, 1H), 3.67 (q, *J* 6.9 Hz, 2H), 2.96–2.87 (m, 2H), 2.84 (d, *J* 6.0 Hz, 1H), 1.76 (s, 3H), 0.57 (t, *J* 7.0 Hz, 3H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 175.6, 164.0, 160.9, 150.5, 143.7, 137.6, 132.5, 131.0, 130.5, 129.6, 128.8, 128.6, 127.3, 126.9, 126.4, 126.0, 125.9, 124.9, 124.2, 124.1, 124.0, 123.9, 123.1, 111.0, 88.0, 85.2, 62.5, 61.4, 59.3, 37.4, 26.7, 13.6, 12.3. IR (KBr): 3432, 1705, 1236 cm^{-1}. Anal. calcd for $C_{33}H_{30}N_6O_6S{\cdot}CH_3OH$: C, 60.88; H,5.11; N, 12.53. Found: C,60.43; H, 5.73; N, 12.93%.

5d. Pale yellow crystals (152 mg, 71%); mp 182–183 °C. ^{1}H NMR (600 MHz, DMSO-d_6): δ 11.27 (s, 1H), 8.34–8.30 (m, 2H), 8.27 (d, *J* 7.9 Hz, 1H), 8.24 (t, *J* 9.2 Hz, 2H), 8.19–8.14 (m, 2H), 8.08 (t, *J* 7.7 Hz, 1H), 8.01 (d, *J* 9.4 Hz, 1H), 7.89 (d, *J* 7.9 Hz, 1H), 7.75 (s, 1H), 6.38 (t, *J* 6.8 Hz, 1H), 5.30 (td, *J* 5.4, 8.5 Hz, 1H), 5.19 (br. s., 1H), 4.19–4.14 (m, 1H), 3.68–3.60 (m, 2H), 3.59–3.53 (m, 1H), 3.47 (t, *J* 6.8 Hz, 2H), 3.24 (br. s., 1H), 3.15 (t, *J* 7.2 Hz, 2H), 2.75–2.65 (m, 1 H), 2.63–2.50 (m, 1H),1.78 (s, 3H), 0.49 (t, *J* 7.2 Hz, 3H). ^{13}C NMR (150 MHz, DMSO-d_6): δ 175.7, 164.2, 160.6, 150.9, 145.5, 136.7, 132.4, 131.1, 130.7, 130.6, 129.3, 129.1, 127.6, 127.4, 127.1, 126.8, 126.6, 125.4, 124.9, 124.3, 123.9, 123.8, 122.7, 110.1, 85.0, 84.4, 62.2, 61.3, 59.6, 37.6, 31.3, 25.1, 14.0, 12.7. IR (KBr): 3432, 1693, 1667, 1236 cm^{-1}. Anal. calcd for $C_{34}H_{32}N_6O_6S{\cdot}CH_3COOC_2H_5$: C, 62.97; H, 5.56; N, 11.59 Found: C, 63.12 ; H, 5.50; N, 11.98%.

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