Tetrahedron Letters 49 (2008) 6311–6313

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Synthesis of ferrocenyl conjugates of thio analogs of hydroxyl-containing biomolecules via the Mitsunobu reaction with *N*-(ethoxycarbonyl)ferrocenecarbothioamide as the pronucleophile

Anna Wrona, Janusz Zakrzewski *

Department of Organic Chemistry, University of Łódź, 90-136 Łódź, Narutowicza 68, Poland

ARTICLE INFO

Article history:
Received 3 July 2008
Revised 8 August 2008
Accepted 19 August 2008
Available online 23 August 2008

Keywords:
Ferrocene
Mitsunobu reaction
Cholesterol
Stigmasterol
Adenosine
Bioorganometallic chemistry

ABSTRACT

A new method for attachment of a ferrocenyl moiety to hydroxyl-containing biomolecules is reported, based on the Mitsunobu reaction with *N*-(ethoxycarbonyl)ferrocenecarbothioamide. The reaction results in the replacement of the OH group with a (ferrocenyl)thioimidoyl moiety. Using this method, ferrocenyl conjugates of cholesterol, stigmasterol, as well as protected and nonprotected adenosine and 2′-deoxy adenosine were obtained in high yield and with high chemo- and stereoselectivity.

Bioorganometallic chemistry of ferrocene has attracted considerable interest in recent years,[1] which has stimulated the search for synthetic methods enabling attachment of ferrocenyl groups to biomolecules. The most frequently used method relies on simple acylation of amino- or hydroxyl groups of biomolecules with derivatives of carboxylic acids containing ferrocenyl moieties.[1a,d] Another method consists of the reaction of ferrocenecarbaldehyde with amino groups of biomolecules with subsequent reduction of the imino function initially formed.[1a] Finally, there are other methods based, for example, on addition of thiol groups to ferrocenyl maleimides or iodoacetamides,[1g] C–C bond forming cross-coupling reactions,[1b,2] 'click' chemistry,[3] and Staudinger ligation.[4]

The Mitsunobu reaction is one of the most versatile methods for the replacement of the hydroxyl groups of alcohols by various nucleophiles[5] and therefore could be of interest for the synthesis of ferrocene conjugates of hydroxyl-containing biomolecules. In fact, the Mitsunobu reaction of ferrocenyl alcohols with thymine/uracil, a protected thymidine and a protein nucleic acid has been used for the synthesis of ferrocenyl conjugates of these biomolecules.[6] However, to our knowledge, there has been no report on the application of this reaction for the replacement of the hydroxyl group by a *ferrocene-containing nucleophile*.

We have recently described the synthesis of *N*-(ethoxycarbonyl)ferrocenecarbothioamide **1**.[7] Herein, we report that this compound can be used as a pronucleophile in Mitsunobu reactions, allowing the synthesis of ferrocene conjugates of thio analogs of

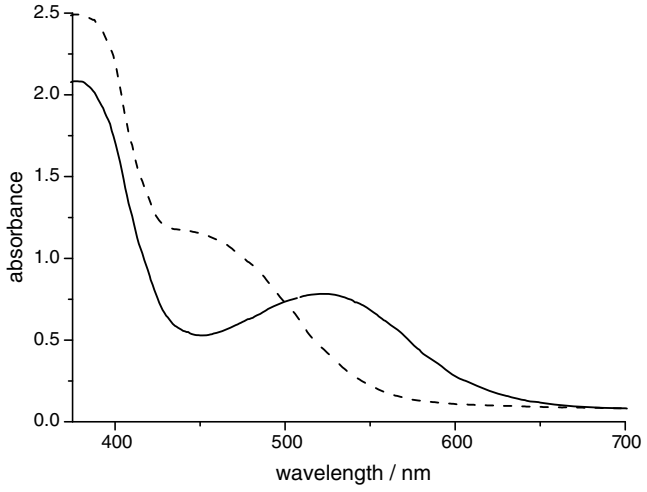


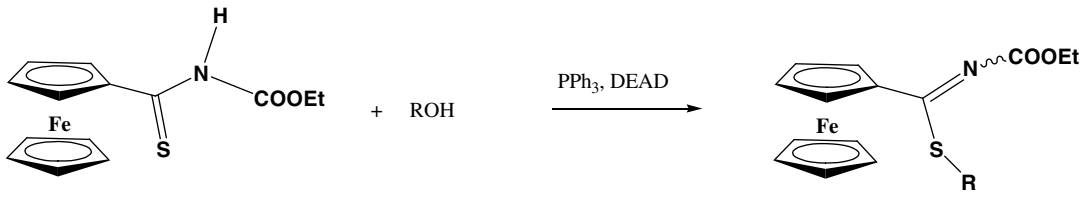
Figure 1. Electronic absorption spectra of **1** in H_2O–MeOH (9:1) at pH 7 (solid line) and pH 12 (dashed line).

* Corresponding author. Tel.: +48 42 6655333; fax: +48 42 6655258.
E-mail address: janzak@uni.lodz.pl (J. Zakrzewski).

0040-4039/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2008.08.070

some hydroxyl-containing biomolecules (the hydroxyl group is replaced by a (ferrocenyl)thioimidoyl unit).

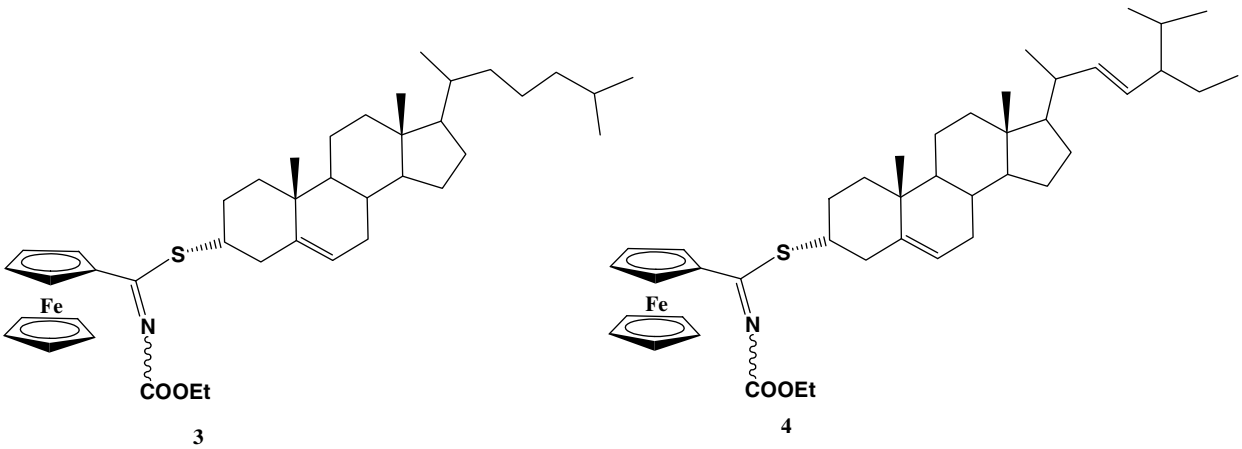
Figure 1 shows the electronic absorption spectra of **1** in MeOH-water solutions at pH 7 and pH 12. In alkaline solution a significant hypsochromic effect is observed, as expected for (at least partial) deprotonation of **1**. This means that the pK_a of this compound is <13, that is, in the range required for classical Mitsunobu conditions (PPh_3, DEAD).[5]



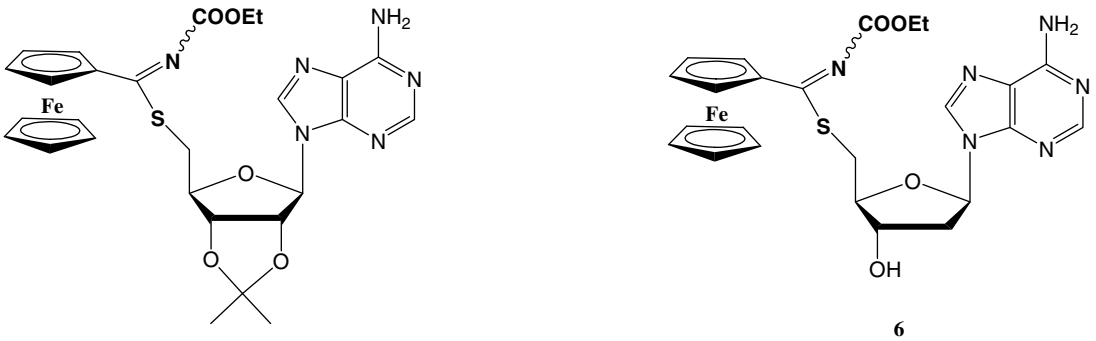
1 **2a-b**

a) R = CH_2Ph
b) R = $CH_2C_6H_4$-NO_2(*p*)

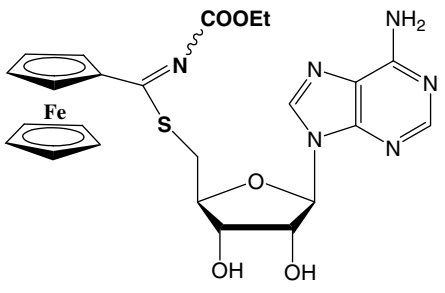
Scheme 1. Mitsunobu reaction of **1** with benzylic alcohols.



3 **4**



5 **6**



7

Chart 1. Structures of the synthesised bioconjugates.

In our first attempts we found that **1** reacts in the presence of PPh_3 and DEAD with benzyl and *p*-nitrobenzyl alcohol to afford (*N*-ethoxycarbonyl)thioimidates **2a–b**, in 56% and 87% isolated yields, respectively (Scheme 1).[8]

The reaction was carried out in THF at room temperature for 4 h. Identification of the products was simple as we had obtained them earlier in the reaction of **1** with the corresponding halides in DMF/K_2CO_3 and we had confirmed the structure of **2b** by X-ray diffraction.[9]

The reaction of **1** with cholesterol, PPh_3 and DEAD afforded **3** (Chart 1). The reaction was relatively slow and the isolated yields of **3** after 2 h, 4 h, 24 h and 3 days were 16%, 21%, 51% and 83%, respectively. Analysis of the coupling constants in the ^{1}H NMR spectrum of **3** (measured using selective decoupling experiments) revealed inversion of the configuration at C-3 typical for the Mitsunobu reaction (coupling constants of H-3 with H-2, 5.5 Hz and 3.5 Hz, reveal the equatorial orientation of H-3). The ^{13}C NMR spectrum of **3** in $CDCl_3$ showed signals characteristic for the –S–*C*═N–*C*O–OEt moiety at 172.6 and 161.9 ppm. Although the stereochemistry of this moiety has not been determined, we presume that it is the same as in **2b**, that is (*E*). Similarly, the reaction of **1**, PPh_3 and DEAD with stigmasterol afforded compound **4** (87% yield after 3 days).

It is worth noting here that Mitsunobu reactions of cholesterol lead in some cases to mixtures of products, resulting from assistance from the ethylenic bond,[10] although examples of the selective C-3 substitution with inversion of the configuration are also known.[11]

A cyclic voltammetry study of **3** in dichloromethane solution showed that this compound undergoes reversible one-electron oxidation at 254 mV (vs Fc^+/Fc) with $\Delta E = \sim 70$ mV. This significant anodic shift reflects the electron-withdrawing character of the thioimidato substituent.

We were also interested in the Mitsunobu reaction of adenine nucleosides, which should lead to ferrocenyl conjugates of 5′-thio-analogs of these compounds. Reaction of **1**, PPh_3 and DEAD with the protected nucleoside, 2′,3′-*O*-isopropylideneadenosine, proceeded cleanly with formation of **5** (91% yield after 24 h). More interestingly, non-protected 2′-deoxyadenosine and adenosine afforded selectively **6** and **7**. The isolated yields of **6** were 15% and 72% after 4 h and 24 h, respectively. The conjugate **7** was isolated in 93% yield after 3 days.

The structures of compounds **6** and **7** were confirmed by their ^{1}H NMR spectra, exhibiting one and two secondary OH groups (doublets, exchangeable with D_2O), respectively.

In conclusion, we have reported the first example of a Mitsunobu reaction using a ferrocenyl pronucleophile and demonstrated the potential of this reaction for the synthesis of ferrocenyl conjugates of hydroxyl-containing biomolecules.

Acknowledgement

We thank Dr. Sławomir Domagała (Department of General and Inorganic Chemistry, University of Łódź) for preliminary cyclovoltammetric data for **3**.

References and notes

1. (a) Van Staveren, D.; Metzler-Nolte, N. *Chem. Rev.* **2004**, *104*, 5931–5985; (b) Zatsepin, T. S.; Andreev, S. Yu.; Hianik, T.; Oretskaya, T. S. *Russ. Chem. Rev.* **2003**, *72*, 537–554; (c) Neuse, E. W. *J. Inorg. Organomet. Polym. Mat.* **2005**, *15*, 2371–2379; (d) Kraatz, H.-B. *J. Inorg. Organomet. Polym. Mat.* **2005**, *15*, 2380–2388; (e) Jaouen, G.; Top, S.; Vessières, A. In *Bioorganometallics*; Jaouen, G., Ed.; Wiley-VCH Weinheim, 2006; pp 65–95; (f) Fouda, M. F. R.; Abd-Elzaher, M. M.; Abdelsamaia, R. A.; Labib, A. A. *Appl. Organomet. Chem.* **2007**, *21*, 613–615; (g) Seiwert, B.; Karst, U. *Anal. Bioanal. Chem.* **2008**, *390*, 181–200.
2. (a) Brázdilová, P.; Vrábel, M.; Pohl, R.; Pivoňková, H.; Havran, L.; Hocek, M.; Fojta, M. *Chem. Eur. J.* **2007**, *13*, 9527–9533; (b) Hocek, M.; Štepnička, P.; Ludvík, J.; Cisařová, I.; Votruba, I.; Řeha, D.; Hobza, P. *Chem. Eur. J.* **2004**, *10*, 23–27.
3. (a) Hasegawa, T.; Umeda, M.; Numata, M.; Li, C.; Bae, A.-H.; Fujisawa, T.; Haraguchi, S.; Sakurai, K.; Shinkai, S. *Carbohydr. Res.* **2006**, *341*, 35–40; (b) Hasegawa, T.; Umeda, M.; Numata, M.; Fujisawa, T.; Haraguchi, S.; Sakurai, K.; Shinkai, S. *Chem. Lett.* **2006**, *35*, 82–83; (c) Casas-Solvas, J. M.; Vargas-Berenguel, A.; Capitán-Vallvey, L. F.; Santoyo-Gonzáles, F. *Org. Lett.* **2004**, *6*, 3687–3690.
4. Kosiova, I.; Janicova, A.; Kois, P. *Beilstein J. Org. Chem.* **2006**, *2*, 23–27.
5. For recent reviews of the Mitsunobu reaction, see: (a) But, T. Y. S.; Toy, P. *Chem. Asian J.* **2007**, *2*, 1340–1355; (b) Dembinski, R. *Eur. J. Org. Chem.* **2004**, 2763–2772.
6. (a) Patwa, A. N.; Gupta, S.; Gonnade, R. J.; Kumar, V. K.; Bhathabe, M. M.; Ganesh, K. N. *J. Org. Chem.* **2008**, *73*, 1508–1515; (b) Bucci, E.; De Napoli, L.; Di Fabio, G. D.; Messere, A.; Montesarchio, D.; Romanelli, A.; Picialli, G.; Vara, M. *Tetrahedron* **1999**, *55*, 14435–14450; (c) Baldoli, C.; Falciola, L.; Licandro, E.; Maiorana, S.; Mussini, P.; Ramani, P.; Rigamonti, C.; Zinzalla, G. *J. Organomet. Chem.* **2004**, *689*, 4791–4802.
7. Plażuk, D.; Zakrzewski, J.; Rybarczyk-Pirek, A.; Domagała, S. *J. Organomet. Chem.* **2005**, *690*, 4302–4308.
8. **Experimental procedure**: Compound **1** (80 mg, 0.25 mmol), triphenylphosphine (112 mg, 0.375 mmol), the appropriate alcohol (0.25 mmol), and diethyl azodicarboxylate (0.06 ml, 0.375 mmol) were dissolved in 0.2 ml of dry THF under argon. The reaction mixture was stirred at room temperature for the time indicated below, concentrated in vacuo and chromatographed on silica gel using dichloromethane as eluent.
Compound **2a**. Reaction time 4 h. Yield 56%. Red oil. Identified by comparison with an authentic sample.[9]
Compound **2b**. Reaction time 4 h. Yield 87%. Red plates. Identified by comparison with an authentic sample.[9]
Compound **3**. Reaction time 3 days. Yield 83%. Orange powder. ^{1}H NMR (700 MHz, $CDCl_3$, δ): 5.39 (d, J = 4.8 Hz, 1H, H-6), 4.67 (s, 1H, Cp), 4.65 (s, 1H, Cp), 4.39 (s, 2H, Cp), 4.25 (s, 5H, Cp′), 4.22 (q, J = 7.0 Hz, 2H, CH_2CH_3), 4.11 (m, 1H, H-3), 2.77 (d, J = 14 Hz, 1H), 2.26 (d, J = 14 Hz, 1H), 1.98 – 2.04 (m, 3H), 1.81 – 1.91 (m, 2H), 1.54–1.75 (m, 4H), 1.42–1.53 (m, 4H), 1.34–1.40 (m, 3H), 1.31 (t, J = 7.0 Hz, 3H, CH_2CH_3), 1.24–1.27 (m, 1H), 1.04–1.20 (m, 10H), 1.03 (s, 3H, CH_3), 0.98–1.02 (m, 2H), 0.92 (d, J = 7.0 Hz, 3H, CH_3), 0.87 (d, J = 7.0 Hz, 3H, CH_3), 0.86 (d, J = 7.0 Hz, 3H, CH_3). ^{13}C NMR (175 MHz, $CDCl_3$): δ 172.6, 161.9, 139.2, 122.6, 78.5, 70.9, 70.8, 69.3, 68.8, 62.2, 56.8, 56.2, 50.2, 44.2, 42.3, 39.8, 39.5, 37.2, 36.2, 35.8, 35.6, 31.85, 31.8, 28.2, 28.0, 26.9, 24.3, 23.9, 22.8, 22.6, 20.8, 19.2, 18.7, 14.4, 11.9. IR (KBr, cm^{-1}): 2934, 2903, 2868, 2851, 1710, 1616, 1249, 1232, 1198. Anal. calcd for $C_{41}H_{59}FeNO_2S$: C, 71.80; H, 8.67; N, 2.04; S, 4.68. Found: C, 71.73; H, 8.59; N, 2.11; S, 4.72.
Compound **4**. Reaction time 3 days. Yield 87%. Orange oil. ^{1}H NMR (200 MHz, $CDCl_3$): δ 5.38 (d, J = 4.1 Hz, 1H, CH), 5.02–5.14 (m, 2H), 4.64 (t, J = 1.8 Hz, 2H, Cp), 4.37 (t, J = 1.8 Hz, 2H, Cp), 4.23 (s, 5H, Cp′), 4.20 (q, J = 8.0 Hz, 2H, CH_2CH_3), 2.71–2.80 (m, 1H), 1.94–2.28 (m, 6H), 1.42–1.76 (m, 12H), 1.29 (t, J = 6.9 Hz, 3H, CH_2CH_3), 1.02–1.22 (m, 12H), 0.77–0.85 (m, 10H), 0.69 (s, 3H, CH_3). IR (KBr, cm^{-1}): 2956, 2934, 2904, 2869, 1710, 1617, 1249, 1230, 1198.Anal. calcd for $C_{43}H_{61}FeNO_2S$: C, 72.55; H, 8.64; N, 1.97. Found: C, 72.49; H, 8.68; N, 2.01.
Compound **5**. Reaction time 24 h. Yield 91%. Orange powder. ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.38 (s, 1H, adenine), 7.94 (s, 1H, adenine), 6.08 (d, J = 2.0 Hz, 1H), 5.72 (s, 2H, NH_2), 5.55 (m, 1H), 5.09 (dd, J_1 = 3.0 MHz, J_2 = 6.2 MHz, 1H), 4.64 (t, J = 1.9 Hz, 2H, Cp), 4.53 (dd, J_1 = 2.0 MHz, J_2 = 6.4 MHz, 1H), 4.41 (t, J = 1.9 Hz, 2H, Cp), 4.24 (s, 5H, Cp′), 4.21–4.28 (m, 2H, CH_2CH_3), 3.35–3.40 (m, 2H), 1.61 (s, 3H, CH_3), 1.39 (s, 3H, CH_3), 1.29 (t, J = 7.1 Hz, 3H, CH_2CH_3). IR (KBr, cm^{-1}): 3355, 3262, 3183, 3112, 2984, 2936,1709, 1635, 1601. Anal. calcd for $C_{27}H_{30}FeN_6O_5S$: C, 53.47; H, 4.99; N, 13.86. Found: C, 53.21; H, 4.97; N, 13.92. MS (EI): 606 (M^+), HRMS: Found: 606.134782, Calcd. for $C_{27}H_{30}FeN_6O_5S$: 606.13478.
Compound **6**. Reaction time 24 h. Yield 72%. Orange powder. ^{1}H NMR (700 MHz, DMSO): δ 8.34 (s, 1H, adenine), 8.15 (s, 1H, adenine), 7.26 (s, 2H, NH_2), 6.35(m, 1H), 5.50 (d, J = 7.0 Hz, 1H, OH), 4.58 (t, J = 2.0 Hz, 2H, Cp), 4.53 (t, J = 2.0 Hz, 2H, Cp), 4.44 (m, 1H), 4.22 (s, 5H, Cp′), 4.14 (q, J = 7.0 Hz, 2H, CH_2CH_3), 3.37 (dd, J_1 = 3.0 MHz, J_2 = 6.1 MHz, 1H), 3.29 (m, 2H), 2.92(m, 1H), 2.29 (m, 1H), 1.21 (t, J = 7.0 Hz, 3H, CH_2CH_3). ^{13}C NMR (175 MHz, $CDCl_3$): δ 171.3, 161.2, 156.6, 153.1, 149.6, 140.2, 139.9, 119.8, 85.2, 84.2, 79.7, 77.4, 73.6, 71.8, 71.2, 69.1, 69.0, 62.5, 38.5, 33.6, 14.6. IR (KBr, cm^{-1}): 3375, 3327, 3118, 2980, 2927, 1709, 1620, 1578, 1598, 1254, 1234, 1200. Anal. calcd for $C_{24}H_{26}FeN_6O_4S$: C, 52.37; H, 4.76; N, 15.27. Found: C, 52.11; H, 4.63; N, 15.12.
Compound **7**. Reaction time 3 days. Yield 93%. Orange powder. ^{1}H NMR (700 MHz, DMSO-d_6): δ 8.37 (s, 1H, adenine), 8.16 (s, 1H, adenine), 7.30 (bs, 2H, NH_2), 5.90 (d, J = 6.0 Hz, 1H, OH), 5.53 (d, J = 5.9 Hz, 1H, OH), 5.42 (d, J = 5.0 Hz, 1H, OH), 4.83 (m, 1H), 4.59 (t, J = 1.8 Hz, 2H, Cp), 4.55 (t, J = 1.8 Hz, 2H, Cp), 4.24 (s, 5H, Cp), 4.17 (m, 4H), 3.34 (m, 1H), 3.33 (m, 1H), 1.21 (t, J = 7.0 Hz, 3H, CH_2CH_3). ^{13}C NMR (50 MHz, DMSO-d_6): δ 171.2, 161.1, 156.5, 153.0, 149.8, 140.4, 119.8, 87.8, 82.6, 77.2, 73.1, 72.7, 71.6, 70.9, 68.9, 68.8, 62.2, 33.2, 14.3. IR (KBr, cm^{-1}): 3371, 3326, 3115, 2971, 2927, 1712, 1623, 1574, 1587, 1247, 1234. Anal. calcd for $C_{24}H_{26}FeN_6O_5S$: C, 50.89; H, 4.63; N, 14.84. Found: C, 50.75; H, 4.77; N, 14.73.
9. Wrona, A.; Palusiak, M.; Zakrzewski, J. *J. Organomet. Chem.* **2008**, *693*, 4302–4308.
10. For an example, see: Cadenas, R. A.; Gelpi, M. E.; Mosettig, J. *J. Heterocycl. Chem.* **2005**, *42*, 1–3.
11. For an example, see: Volante, R. P. *Tetrahedron Lett.* **1981**, *22*, 3119–3122.