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# Enantioselective Synthesis of Ferrocenyl Nucleoside Analogues with Apoptosis-Inducing Activity

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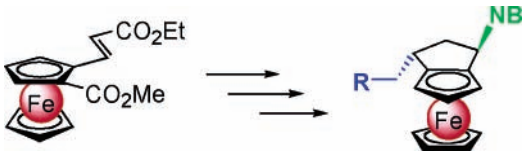
## ABSTRACT




**As a contribution to bioorganometallic chemistry, an enantioselective synthesis of novel carbocyclic nucleoside analogues with a ferrocenocyclopentene backbone was developed. Diastereoselective cuprate 1,4-addition or Mukaiyama–Michael addition to a planar-chiral enoate (ethyl (*E*)-2-[2-methoxycarbonyl-ferrocenyl]-acrylate) allowed for the introduction of different side chains ($RCH_2$). Other important steps include a Dieckmann cyclization and the attachment of the nucleobase (NB) in an iron-assisted $S_N1$ reaction. Some of the target compounds were shown to exhibit significant apoptosis-inducing activity ($LD_{50}$ = 10–20 $\mu$M) against tumor cells.**

Nucleosides are of fundamental importance for all living systems, for instance, as structural modules of nucleic acids, cofactors, and messenger substances.[1] It is thus not surprising that they have been a source of inspiration in the discovery of new drugs for many decades. Variation of both the carbohydrate and the nucleobase led to the development of important antiviral and antitumoral agents.[2]

A few years ago, concurrently with the emergence of the field of bioorganometallic chemistry,[3] we discovered that nucleoside analogues such as **1** (Figure 1), bearing an

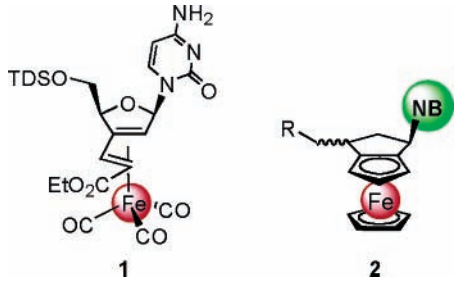



**Figure 1.** Iron-containing nucleoside analogues.

$Fe(CO)_3$ fragment, exhibit pronounced apoptosis-inducing properties against BJAB tumor cells ($LD_{90}$ = 20 $\mu$M)[4] and, most remarkably, are also active against leukemia cells resistant to common cytostatic drugs.

To explore the biological potential of such metal-containing nucleosides and to better understand the role of the metal

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(1) See, for instance: Voet, D.; Voet, J. G. *Biochemistry*, 2nd ed.; John Wiley & Sons: New York, 1995.

(2) See, for instance: (a) El Kouni, M. H. *Curr. Pharm. Des.* **2002**, *8*, 581. (b) Richman, D. D. *Nature* **2001**, *410*, 995. (c) Larder, B. A.; Stammers, D. K. *Nat. Struct. Biol.* **1999**, *6*, 103. (d) Huryn, D. M.; Okabe, M. *Chem. Rev.* **1992**, *92*, 1745. (e) *Recent Advances in Nucleosides: Chemistry and Chemotherapy;* Chu, C. K., Ed.; Elsevier: New York, 2002.

(3) (a) Fish, R. H.; Jaouen, G. *Organometallics* **2003**, *22*, 2166. (b) Schatzschneider, U.; Metzler-Nolte, N. *Angew. Chem., Int. Ed.* **2006**, *45*, 1504. (c) *Bioorganometallics*; Jaouen, G., Ed.; Wiley-VCH: New York, 2005.

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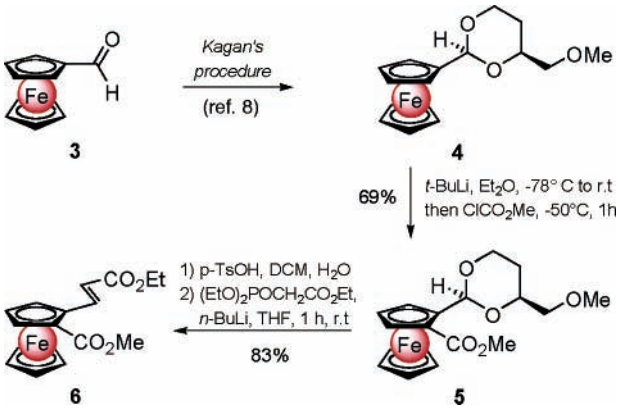
fragment, we envisaged synthesizing ferrocenyl nucleoside derivatives of type **2**.[5] In such structures, the butadiene−$Fe(CO)_3$ unit of **1** would be formally replaced by a ferrocene, i.e., a stable metallocene moiety, which had successfully been incorporated before into other bioactive molecules (such as ferrocifen, a tamoxifen analogue).[6]

As a key intermediate for the enantioselective synthesis of ferrocenyl nucleosides of type **2**, we took the planar chiral complex **6**, which was prepared from ferrocene-carboxaldehyde **3**[7] as shown in Scheme 1.

**Scheme 1.** Enantioselective Preparation of **6**




The synthesis of **6** follows the protocol of Kagan and coworkers who had shown that the chiral acetal **4** can be functionalized through highly diastereoselective ortho-lithiation.[8] When the lithiated species, derived from **4** by treatment with *tert*-BuLi in diethyl ether, was added to an excess of methylchloroformate at −50 °C (reverse addition), the methoxycarbonylated product **5** was obtained as a virtually pure diastereomer in 69% yield. Removal of the chiral auxiliary group by acidic acetal hydrolysis and subsequent Horner−Wadsworth−Emmons olefination of the intermediate aldehyde with triethyl-phosphonoacetate gave the ($S_p$)-(*E*)-configured diester **6** ($[\alpha]_D = +1297$ in $CHCl_3$) as a pure stereoisomer.

As revealed by an X-ray crystal structure analysis (Figure 2), compound **6** minimizes allylic strain by adopting a conformation in which the α,β-unsaturated ester chain is pointing away from the methyl ester group, whereas both

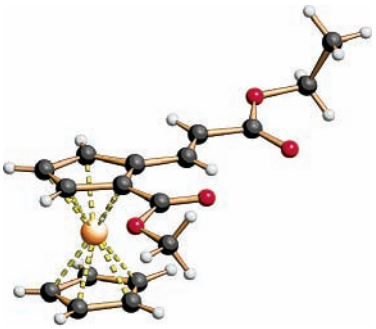
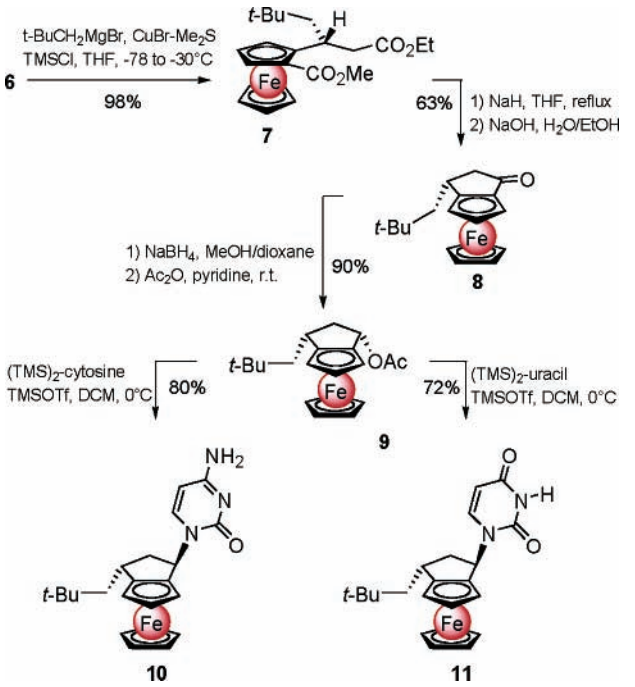


**Figure 2.** X-ray crystal structure of **6**.

substituents lie within the plane of the ferrocene ring to benefit from conjugation with the electron-rich π-system.

On the way toward nucleosides of type **2**, we planned to introduce a side chain ($RCH_2$) through conjugate addition to the enoate substructure. The reaction of **6** with the cuprate derived from neopentylmagnesium bromide and CuBr−$SMe_2$ (Scheme 2) proceeded smoothly to afford the product **7** with

**Scheme 2.** Conversion of **6** into the Nucleosides **10** and **11**




high diastereoselectivity (dr > 98:2). The relative configuration of **7** was assigned on the basis of the assumption that **6** would exist in its preferred conformation (Figure 2) while being attacked by the nucleophile from the less-hindered face (opposite to the FeCp unit).

To close the five-membered ring by Dieckmann condensation,[9] **7** was treated with sodium hydride in refluxing THF. Without purification, the resulting β-keto ester was subjected to a saponification−decarboxylation sequence to give the

(4) (a) Schmalz, H.-G.; Prokop, A.; Wieder, T.; Daniel, P. PCT Int. Appl. WO 02/80923A1, 2002; *Chem. Abstr.* **2002**, *137*, 295194; AN 2002: 793423. (b) Schlawe, D.; Majdalani, A.; Velcicky, J.; Hessler, E.; Wieder, T.; Prokop, A.; Schmalz, H.-G. *Angew. Chem., Int. Ed.* **2004**, *43*, 1731.

(5) For the preparation of nucleosides equipped with a (conformationally flexible) side chain containing a ferrocene moiety, see: De Champdoré, M.; Di Fabio, G.; Messere, A.; Montesarchio, D.; Piccialli, G.; Loddo, R.; La Colla, M.; La Colla, P. *Tetrahedron* **2004**, *60*, 6555 and references cited therein.

(6) For recent work, see: Hillard, E.; Vessières, A.; Thouin, L.; Jaouen, G.; Amatore, C. *Angew. Chem., Int. Ed.* **2006**, *118*, 291.

(7) For a superior synthesis of compound **3**, see: Neto, A. F.; Miller, J.; Faria de Andrade, V.; Fujimoto, S. Y.; Maisa de Freitas Afonso, M.; Archanjo, F. C.; Darin, V. A.; Andrade e Silva, M. L.; Donizete Lanchote Borges, A.; Del Ponte, G. *Z. Anorg. Allg. Chem.* **2002**, *628*, 209.

(8) Riant, O.; Samuel, O.; Flessner, T.; Taudien, S.; Kagan, H. B. *J. Org. Chem.* **1997**, *62*, 6733.

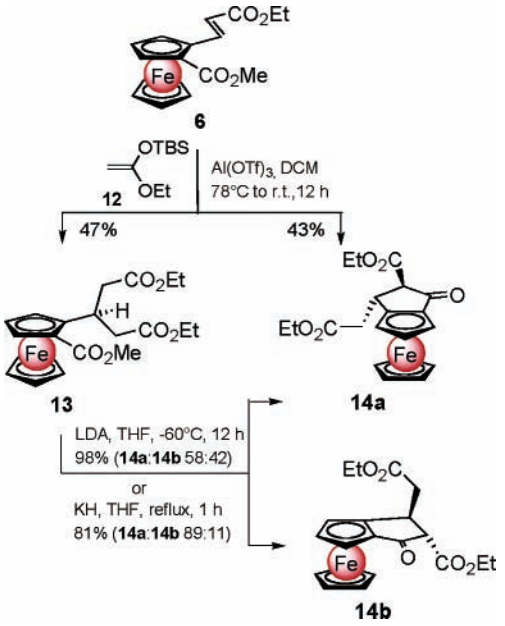
ferroceno-cyclopentenone **8** ($[\alpha]_D = +213.6$ in $CHCl_3$) in 63% yield (two steps). At this stage, the predicted configuration of the chirality center formed in the 1,4-addition step was proven by NOE NMR spectroscopy (contact of the neopentyl $CH_2$ group to the lower Cp ring).

To prepare for the introduction of the nucleobase, compound **8** was first reduced with $NaBH_4$ and the crude alcohol formed was directly acetylated to afford the *endo*-acetate **9** as a pure diastereomer after flash chromatography.

The synthesis of the ferrocenyl nucleosides **10** and **11** (Scheme 2) was completed by reaction of **9** with the respective silylated nucleobase in the presence of TMS−triflate.[4,10] We assume that these ($S_N1$-type) reactions proceed via a stabilized ferrocenyl cation,[11] which is diastereoselectively attacked from the unhindered face. Starting from ferrocene, we obtained the nucleoside analogues **10** and **11** in ca. 12% overall yield (over 13 steps).

As an alternative for the conversion of the key intermediate **6** into suitable nucleoside precursors, we also explored the possibility to introduce an ethyl acetate side chain through conjugate addition (Scheme 3). As the resulting triester **13**

**Scheme 3.** Mukaiyama−Michael Addition to Diester **6**

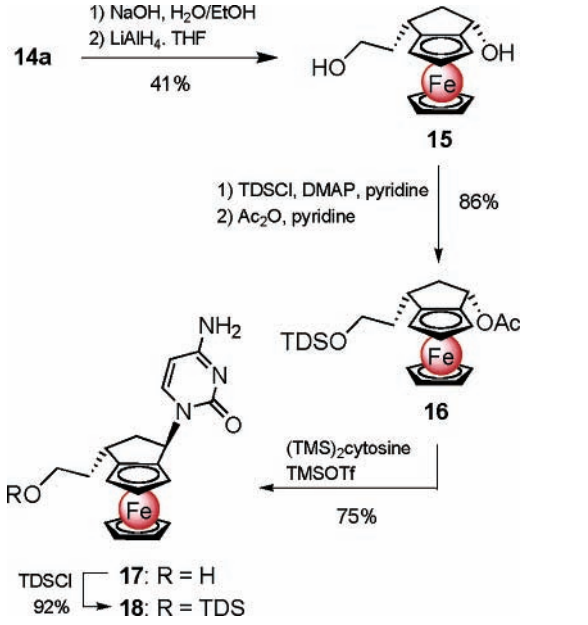



possesses two diastereotopic C−H acidic ester functions, we were curious whether a group-selective Dieckmann condensation could be achieved to place the $CH_2R$ group (compare general structure **2**) in either the endo or the exo position.

By treatment of **6** with the *O*-silyl ketene acetal **12**[12] in the presence of $Al(OTf)_3$ as an in situ generated Lewis acid catalyst,[13] **13** was isolated in 47% yield as the expected product of a Mukaiyama−Michael reaction.[14] In addition, significant amounts (43%) of the already cyclized product **14a** were obtained, remarkably, as a pure diastereomer (Scheme 3). Obviously, the O-silylated ketene acetal formed diastereoselectively from **6** and **12**, as the initial product was able to undergo a Dieckmann-type 5-exo-trig-cyclization to give **14a** in a tandem process. The trans configuration of the two ester substituents in **14a** was determined by means of an NOE experiment. All our attempts to improve the yield of **14a** in this reaction, e.g., by using longer reaction times, were not successful so far. Treatment of **13** with LDA (1.1 equiv) at −60 °C resulted in a 58:42 mixture of the two diastereoisomers **14a** and **14b** (98% combined yield). In contrast, reaction of **13** with KH in refluxing THF led to the preferential formation of diastereomer **14a** with good selectivity (dr = 89:11).

The conversion of **14a** into the nucleoside analogues **17** and **18** is shown in Scheme 4. At first, ester hydrolysis and

**Scheme 4.** Synthesis of Nucleoside Analogues **17** and **18**




decarboxylation led to a keto acid, which was directly reduced with $LiAlH_4$ to yield the endo diol **15**. A recrystallized sample of this compound was analyzed by X-ray crystallography (see Supporting Information) revealing its relative and absolute configurations. After selective protection of the primary alcohol function using exactly 1 equiv of chlorodimethylthexylsilane (TDSCl), the remaining secondary alcohol was acetylated to afford compound **16**. The introduction of the nucleobase was finally achieved using the di-TMS-protected cytosine under the same conditions as before (compare Scheme 2). Surprisingly, the desilylated

(9) Shirafuji, T.; Odaira, A.; Yamamoto, Y.; Nozaki, H. *Bull. Chem. Soc. Jpn.* **1972**, *45*, 2884.
(10) (a) Niedballa, U.; Vorbrüggen, H. *J. Org. Chem.* **1974**, *39*, 3654. (b) Vorbrüggen, H.; Krolikiewicz, K.; Bennua, B. *Chem. Ber.* **1981**, *114*, 1234. For a extensive review on nucleoside synthesis, see also: (c) Vorbrüggen, H.; Ruh-Polenz, C. *Org. React.* **2000**, *55*, 1−630.
(11) Gokel, G.; Marquarding, D.; Ugi, I. *J. Org. Chem.* **1972**, *37*, 3052.
(12) Kita, Y.; Segawa, J.; Haruta, J.-i.; Yasuda, H.; Tamura, Y. *J. Chem. Soc., Perkin Trans. 1* **1982**, 1099.
(13) Minowa, N.; Mukaiyama, T. *Chem. Lett.* **1987**, 1719.
(14) Saigo, K.; Osaki, M.; Mukaiyama, T. *Chem. Lett.* **1976**, 163.

product (**17**) was obtained which, however, could easily be reprotected in good yield to give the nucleoside analogue **18**.

The cytotoxic activity of the new ferrocenyl nucleosides **10**, **11**, **17**, and **18** was evaluated in vitro and ex vivo using BJAB tumor cells (Burkitt-like lymphoma cells) and primary lymphoblasts from children with acute lymphoblastic leukemia (ALL).[15] It was found that compounds **10** and **18** both exhibit significant apoptosis-inducing activity in the lower micromolar range ($LD_{50} = 10-20\ \mu M$), whereas compounds **11** and **17** are not active. Apoptosis induction was determined by measuring DNA fragmentation (see Figure 3). Cells

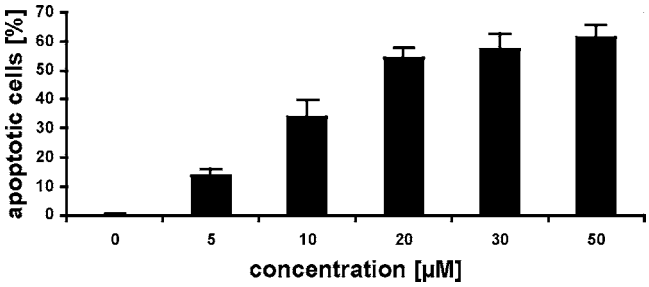



**Figure 3.** Concentration dependency of apoptosis induction. Percentage of apoptotic cells as determined by DNA fragmentation via flow cytometry after treatment of BJAB cells for 72 h with different concentrations of **10**.

treated with **10** or **18** also showed cell shape changes, cytoplasm condensation, nuclear envelope changes, and blebbing characteristic for apoptosis (Figure 4).

Moreover, it was found in an ex vivo experiment using primary lymphoblasts isolated from children with a relapse of ALL that **10** is able to overcome resistance.[16]

These results convincingly demonstrate that ferrocenyl nucleosides of type **2** exhibit pronounced apoptosis-inducing activity with a structure−activity dependency similar to that for the butadiene-$Fe(CO)_3$-derived nucleosides (e.g., **1**).[4] Again, a lipophilic side chain ($CH_2R$) seems to be important

(15) For a description of the methods used, see ref 4b as well as: (a) Wieder, T.; Prokop, A.; Bagci, B.; Essmann, F.; Bernicke, D.; Schulze-Osthoff, K.; Dörken, B.; Schmalz, H. G.; Daniel, P. T.; Henze, G. *Leukemia* **2001**, *15*, 1735.

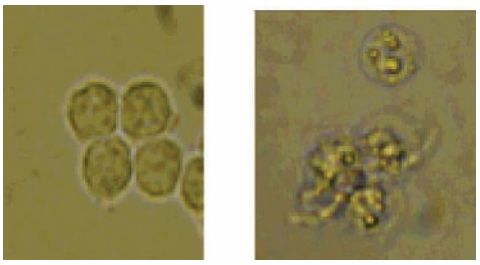


**Figure 4.** Morphology of BJAB cells under the microscope. Left: negative control after 45 h (intact cells). Right: cells treated with **10** (50 $\mu$M) for 45 h. The progress of apoptosis is indicated by typical morphological changes.

for activity, with cytosine belonging to the preferred nucleobases.

In conclusion, we have elaborated an efficient, stereoselective, and modular synthetic route to novel ferrocene-derived nucleosides of type **2** which were identified as a new promising class of organometallic antitumoral agents.

The work described herein represents an important basis for the future synthesis of various substituted ferrocenocyclopentenes as potentially bioactive compounds. Further studies currently being performed in these laboratories are aimed at the elucidation of the mechanism of action of the new compounds and also explore the specific role of the metal fragment for biological activity.

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**Supporting Information Available:** Experimental procedures and analytical data, including X-ray data for compounds **6** and **15**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(16) These cells were fully resistant to common cytostatic drugs such as daunorubicin, doxorubicin, vincristin, or cytarabin but underwent apoptosis on treatment with **10** (concentration used: $LD_{50}$ against BJAB).