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Nucleoside Analogues with a 1,3-Diene–$Fe(CO)_3$ Substructure: Stereoselective Synthesis, Configurational Assignment, and Apoptosis-Inducing Activity

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Dedicated to Professor Stefan Toma on the occasion of his 76th birthday

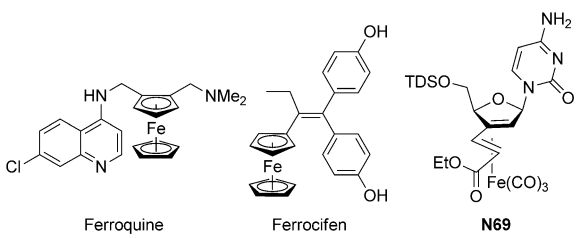
Abstract: The synthesis and stereochemical assignment of two classes of iron-containing nucleoside analogues, both of which contain a butadiene–$Fe(CO)_3$ substructure, is described. The first type of compounds are $Fe(CO)_3$-complexed 3′-alkenyl-2′,3′-dideoxy-2′,3′-dehydro nucleosides (2,5-dihydrofuran derivatives), from which the second class of compounds is derived by formal replacement of the ring oxygen atom by a CH_2 group (carbocyclic nucleoside analogues). These compounds were prepared in a stereoselective manner through the metal-assisted introduction of the nucleobase. Whilst the furanoid intermediates were prepared from carbohydrates (such as methyl-glucopyranoside), the carbocyclic compounds were obtained by using an intramolecular Pauson–Khand reaction. Stereochemical assignments based on NMR and CD spectroscopy were confirmed by X-ray structural analysis. Biological investigations revealed that several of the complexes exhibited pronounced apoptosis-inducing properties (through an unusual caspase 3-independent but ROS-dependent pathway). Furthermore, some structure–activity relationships were identified, also as a precondition for the design and synthesis of fluorescent and biotin-labeled conjugates.

Keywords: antitumor agents · apoptosis · carbonyl ligands · iron · nucleosides

Introduction

In recent years, the field of bio-organometallic chemistry has enjoyed increasing attention because several transition-metal–organometallic compounds have been shown to exhibit significant biological activities.[1] Among the most-prominent examples are ferrocene analogues of well-established drugs, such as ferrocifene[2] (derived from the breast-cancer agent tamoxifen) and ferroquine[3] (related to the anti-malaria drug chloroquine). These and many other[4] examples demonstrate the still-underestimated potential of metal-containing compounds for future pharmaceutical applications.

In 2004, we introduced a new class of iron-containing nucleoside analogues with a butadiene–$Fe(CO)_3$ substructure.[5] Some of these agents were found to induce apoptosis in BJAB tumor cells at low micromolar concentrations. Furthermore, the cytosine derivative **N69** (Figure 1), a particularly active compound, was found to target new caspase-independent but ROS-dependent apoptosis pathways in melanoma cells.[6] The surprising biological properties and the fact that the metal-free congener of **N69** were found to be more or less inactive prompted us to further explore this class of iron-containing nucleoside analogues.[7] Herein, we



Ferroquine Ferrocifen **N69**

Figure 1. Structures of ferrocifen, ferroquine, and **N69**.

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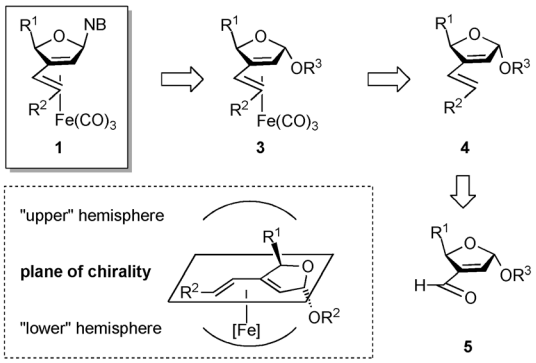
report the stereoselective synthesis and configurational assignment of a variety of $Fe(CO)_3$-containing nucleoside analogues of type **1** and their carbocyclic relatives of type **2** (Figure 2). Moreover, we report the results of biological investigations, which have led to the identification of structure–activity relationships concerning the apoptosis-inducing activities of these compounds.

Figure 2. Two types of nucleoside analogues with a 1,3-diene–$Fe(CO)_3$ substructure as target structures. NB = nucleobase.

Results and Discussion

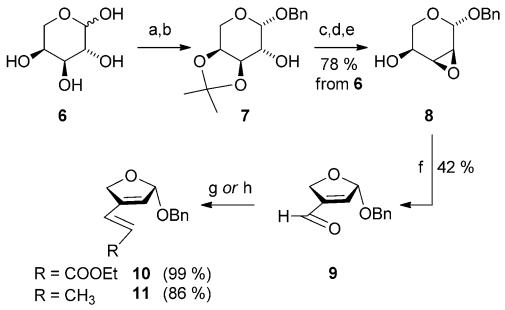
Synthesis of vinyl–dihydrofuran-derived Fe-containing nucleosides of type 1: Our strategy for the synthesis of nucleoside analogues of type **1** is outlined in Scheme 1. We intend-



Scheme 1. Planned synthesis of iron-containing nucleoside analogues of type **1**.

ed to introduce the nucleobase (NB) in a diastereoselective fashion through Lewis acid-mediated S_N reactions, such as by applying the Vorbrüggen variation[8a] of the Hilbert–Johnson method.[8b–d] As a key feature, we expected the $Fe(CO)_3$ fragment to shield the complexed face ("lower hemisphere") of the π ligand, thus forcing the nucleobase to preferentially approach from the less-shielded "upper hemisphere" (Scheme 1). Therefore, the stereoselective generation of the "planar chiral" diene–$Fe(CO)_3$ substructure through diastereoselective complexation (**3**→**4**) and a reliable assignment of the configuration of the resulting complexes were pivotal. Two factors were proposed to control the diastereoselective complexation of compound **4**: 1) Steric shielding of the "upper hemisphere" by a bulky substituent (R^1) and 2) pre-coordination of the $Fe(CO)_x$ reagent by the alkoxy group (OR^3), thus directing it towards the "lower hemisphere" of the diene ligand. The required diene precursors of type **4** were considered to be prepared by a Wittig reaction from aldehydes of type **5**, which were accessible from suitable carbohydrate building blocks, as described by Rehnberg and Magnusson.[9]

We started our investigation with the synthesis of simplified dienes **10** and **11**, which lacked the 5′-substitutent. As we had previously communicated,[10] the synthesis (Scheme 2) started with the conversion of (+)-L-arabinose

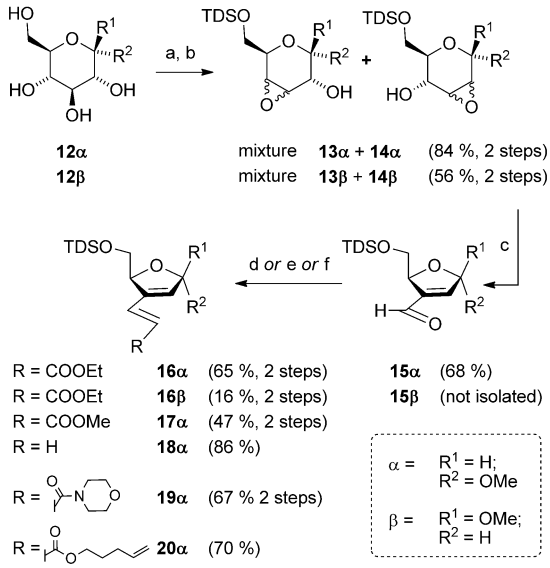


Scheme 2. Synthesis of vinyl–dihydrofurans **10** and **11**: a) BnOH, $HCl_{(g)}$, 0 °C, 5 h; b) $(CH_3)_2C(OMe)_2$, benzene, *p*-TsOH, reflux, 105 min; c) pyridine, *p*-TsCl, RT, 48 h; d) 1 M HCl, THF, 50 °C, 12 h; e) NaOMe, MeOH, RT, 16 h; f) LiBr, TMU, toluene, reflux, 1.5 h; g) for compound **10**: $Ph_3P{=}CHCO_2Et$, THF, 50 °C, 5 h; h) for compound **11**: $[Ph_3PCH_3]^+Br^-$, *n*BuLi, −90 °C to −5 °C, 4 h. Bn = benzyl, *p*-Ts = *para*-toluenesulfonyl.

(**6**) into the benzyl glycoside under acidic conditions to exclusively afford the thermodynamically favored α-product, which was subsequently transformed into *cis*-acetonide **7**. Tosylation of the remaining OH group, acetal cleavage, and subsequent base-induced epoxide formation delivered compound **8**. Upon treatment with LiCl and tetramethylurea (TMU) in toluene, the epoxide underwent a ring-contractive rearrangement (and subsequent water elimination) to yield unsaturated aldehyde **9**. Wittig olefination with the appropriate reagents finally afforded dienes **10** and **11**, respectively.

The synthesis of the corresponding diene ligands with an additional (silyl-protected) CH_2OH substituent (**16**–**20**; Scheme 3) started from commercially available methyl-(D)-glucopyranosides, that is, compounds **12α** and **12β**, respectively.

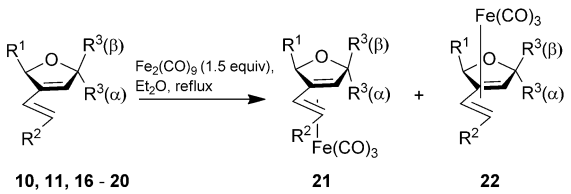
In both the α- and β-series, the selective silylation of the primary alcohol function[11] and subsequent Mitsunobu reaction yielded mixtures of diastereomeric epoxides **13** and **14**.[12] Without separation, the diastereomer mixtures (**13**/**14**) were subjected to the ring-contractive rearrangement/condensation conditions (LiCl, TMU, toluene, reflux, slow addition of the substrate) to yield the expected aldehydes of type **15**. The yield of this transformation was found to be highly dependent on the quality of the reagents that were used (in particular TMU).[13] Finally, Wittig olefination reactions to afford compounds **16α**, **16β**, **17α**, and **19α** were performed by directly adding the appropriate Wittig reagents (ylenes) to the crude reaction mixture of aldehyde **15α** or **15β**. The corresponding olefinations that yielded



Scheme 3. Synthesis of 4-substituted 3-alkenyl-2,5-dihydrofuran ligands: a) TDSCl, pyridine, RT, 18 h to 60 h; b) for compounds **13α/14α**: DIAD, PPh_3, toluene, RT, 1 h, then reflux, 2 h; for compounds **13β/14β**: DEAD, PPh_3, benzene, RT, 0.5 h, then reflux, 4 h; c) LiBr, TMU, toluene, reflux, 3 h; d) for compounds **16α**, **16β**, **17α**, and **19α**: starting from an appropriate mixture of compounds **13/14**: LiBr, TMU, toluene, reflux, 3 h, then $Ph_3P{=}CH(C{=}O)R'$, RT, 0.5 h, reflux 1.5 h; e) $[Ph_3PCH_3]^+Br^-$, *n*BuLi, −90 °C to −5 °C, 4 h; f) NaH, $H(P{=}O)(OEt)_2$, DME, RT, 5 min, then $CH_2{=}CH(CH)_3O(C{=}O)CH_2Cl$, RT, 15 min, then compound **15α**, RT, 0.5 h. TDS = thexyldimethylsilyl.

compounds **18α** and **20α** were carried out by employing isolated aldehyde **15α**. In the latter case, a Wittig–Horner–Emmons reagent that was prepared from 4-pentenyl chloroacetate was used.[14]

With a range of dienes of type **4** in hand, we next investigated the complexation of these ligands to $Fe(CO)_3$ by using $Fe_2(CO)_9$ as the reagent of choice.[15] Of particular interest was the evaluation of the factors that were responsible for the diastereoselectivity. Diastereomeric ratios (d.r.) were determined by ^{1}H NMR spectroscopy of the mixtures after column chromatography on silica gel (see below for assignment of the diastereomers). As shown in Table 1, significant diastereoselectivity in favor of the desired complexes of type **21** was observed for the R^1-unsubstituted substrates. Thus, the complexation of compound **10** afforded compounds **21a/22a** in a diastereomeric ratio (d.r.) of 3.0:1, whilst compound **11** gave rise to compounds **21b/22b** with a d.r. of 2.1:1 (Scheme 4). The (α-configured) substrates that



Scheme 4. Complexation of dienes **10**, **11**, and **16**–**20**. Yields and selectivities are listed in Table 1.

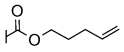
contained an additional R^1 substituent (**16α**, **17α**, **18α**, and **20α**) reacted with increased diastereoselectivity, again in favor of the desired complexes of type **21**. Amide **19α** displayed lower diastereoselectivity; reversed diastereoselectivity was only observed in the case of substrate **16β** and complexes **21d** and **22d** were formed in a ratio of 1:2.7.

From these results, we concluded that two factors (as mentioned above) contributed to the observed diastereoselectivities: 1) Pre-coordination of the incoming $Fe(CO)_x$ reagent to the alkoxy (OR^3) functionality directed its delivery to the diene from the same hemisphere,[16] as evidenced by the complexation of compounds **10** and **11**. 2) The steric shielding of the "upper hemisphere" by the R^1 substituent directed the incoming iron fragment towards the opposite face of the ligand, as confirmed by the increased diastereoselectivity in the complexation of compounds **16α**, **17α**, **18α**, and **20α**. The outcome of the complexation of compound **16β**, in which the repulsive effect of the R^1 and the attractive (pre-coordination) effect of the R^3 substituent are opposed to each other, indicates that the pre-coordination is the dominant effect (Figure 3).

It must be mentioned that separation of the diastereomeric complexation products by column chromatography on silica gel was often difficult, in particular in the case of ester-substituted compounds, such as **21c/22c**, which were only separable by HPLC. However, for the larger-scale syntheses of such complexes, the separation was achieved by temporary desilylation (TBAF, THF/water), flash column chromatography on silica gel of the resulting alcohols, and subsequent re-protection.[5] More conveniently, separation of the diastereomers was achieved by column chromatography on silica gel at a later stage, that is, after the introduction of the nucleobase.

The main remaining goal was the introduction of the nucleobase. As our initial studies had shown, the Fe-assisted nucleophilic substitution reactions at the acetal center of complexes of type **3** occurred with significant levels of diastereoselectivity.[17] In particular, nucleoside formation by applying the

Table 1. Complexation of different dienes according to Scheme 4.

Diene	Products	R^1	R^2	R^3(α)	R^3(β)	**21/22**	Yield [%]
10	**21a/22a**	H	COOEt	OBn	H	3.0:1.0[a]	79
11	**21b/22b**	H	H	OBn	H	2.1:1.0[a]	77
16α	**21c/22c**	CH_2OTDS	COOEt	OMe	H	4.3:1.0[a]	77
16β	**21d/22d**	CH_2OTDS	COOEt	H	OMe	1.0:2.7[a]	65
17α	**21e/22e**	CH_2OTDS	COOMe	OMe	H	3.8:1.0[a]	86
18α	**21f/22f**	CH_2OTDS	H	OMe	H	3.3:1.0[a]	72
19α	**21g/22g**	CH_2OTDS		OMe	H	2.1:1.0[b]	74
20α	**21h/22h**	CH_2OTDS		OMe	H	4.0:1.0[a]	52

[a] Ratio determined by ^{1}H NMR spectroscopy. [b] Ratio determined after isolation.

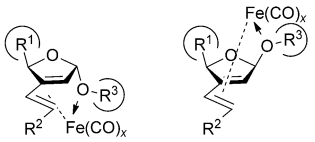
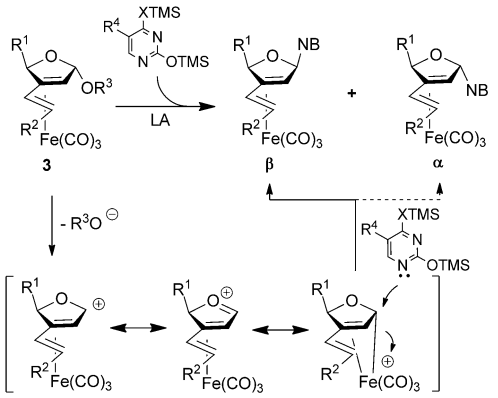


Figure 3. The pre-coordination of the incoming $Fe(CO)_x$ reagent to the alkoxy substituent mainly determines the diastereoselectivity of the complexation step.

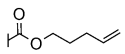
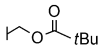
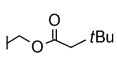
Vorbrüggen variation of the Hilbert–Johnson method[8] afforded mixtures of diastereomeric nucleoside analogues with a significant preference for the β product. This reaction can be best rationalized in terms of a S_N1-type mechanism, as shown in Scheme 5. We suggest that the cationic intermediate that is generated from compound **3** is not only stabilized by the anomeric oxygen atom, but also by the butadiene–$Fe(CO)_3$ fragment.[18] Then, the steric influence of the iron fragment directs the nucleophilic attack to the "upper hemisphere", thereby resulting in the predominant formation of the desired β diastereomer. The antagonizing steric effect of the R^1 substituent becomes evident by comparing the reactions of compounds **21b** and **21f**. Whilst compound **21b** reacted under the conditions given in Table 2 (bis-TMS-uracile, $SnCl_4$, TMS = trimethylsilyl) to yield compounds **23α** and **23β** in a 19:1 ratio, the corresponding reaction of compound **21f** (Table 2, entry 2) produced compounds **24α** and **24β** in only a 5.6:1 ratio. This decrease in stereoselectivity for the desired β product can clearly be attributed to the steric demand of the R^1-CH_2OTDS substituent of compound **21f**, which partially impedes the nucleophilic attack from the "upper hemisphere". As the various reactions summarized in Table 2 demonstrate, the β products were generally formed with significant levels of diastereoselectivity, independent of the nucleophile and the Lewis acid that were employed. This result indicates the dominance of the directing effect of the $Fe(CO)_3$ fragment.



Scheme 5. Reaction of complexes of type **3** with silylated nucleobases that were derived from cytosine (C*; X = NH, R^4 = H), uracil (U*; X = O, R^4 = H), thymine (T*; X = O, R^4 = CH_3), or 5-bromo-uracil (BrU*; X = O, R^4 = Br) under Lewis acidic conditions (LA, Table 2).

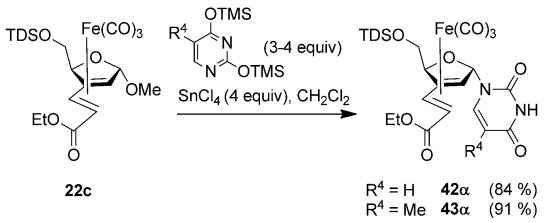
The arguments discussed above are supported by the outcome of reactions that were performed with complex **22c**, in which the $Fe(CO)_3$ fragment and the sterically demanding silyl group were located in the same ("upper") hemisphere. In this case, the Lewis-acid-mediated introduction of uracil

Table 2. Introduction of the nucleobase (NB) according to Scheme 5.

Entry	SM	R^1	R^2	OR^3	NB[a]	Lewis acid (equiv)	T [°C]	Products	β/α	Yield [%]
1	**21b**	H	H	OBn	U	$SnCl_4$ (2.9)[c]	40	**23β/23α**	19:1[d]	99
2	**21f**	CH_2OTDS	H	OMe	U	$SnCl_4$ (2.4)[c]	40	**24β/24α**	5.6:1[d]	79
3	**21f**	CH_2OTDS	H	OMe	T	$SnCl_4$ (3.0)[c]	40	**25β/25α**	1.6:1[d]	82
4	**21f**	CH_2OTDS	H	OMe	C	TMSOTF (6)	RT	**26β/26α**	2.3:1[d]	96
5	**21c**	CH_2OTDS	COOEt	OMe	U	$SnCl_4$ (3.4)[c]	40	**27β/27α**	6.6:1[d]	84
6	**21c**	CH_2OTDS	COOEt	OMe	T	$SnCl_4$ (4.5)[c]	40	**28β/28α**	2.4:1[d]	81
7	**21c**	CH_2OTDS	COOEt	OMe	BrU	$SnCl_4$ (5.6)[c]	40	**29β/29α**	4.5:1[d]	72
8	**21c**	CH_2OTDS	COOEt	OMe	C	$SnCl_4$ (4.5)[c]	40	**30**	n.d.	18
9	**21c**	CH_2OTDS	COOEt	OMe	C	TMSOTF (6)	RT	**N69/31α**	3.4:1[d]	77
10	**21e**	CH_2OTDS	COOMe	OMe	C	TMSOTF (6)	RT	**32β/32α**	4.5:1[d]	67
11	**21g**	CH_2OTDS		OMe	C	TMSOTF (6)	RT	**33β/33α**	2.3:1[e]	96
12	**21h**	CH_2OTDS		OMe	C	TMSOTF (6)	RT	**34β/34α**	n.d.[f]	57
13	**21i**[b]	CH_2OAc	COOEt	OMe	C	TMSOTF (6)	RT	**35β/35α**	11.5:1[d]	75
14	**21j**[b]	CH_2OAc	COOEt	OMe	BrU	$SnCl_4$ (5.5)[c]	RT	**36β/36α**	3.3:1[d]	69
15	**21k**[b]	CH_2OTBS	COOEt	OMe	C	TMSOTF (6)	RT	**37β/37α**	6.7:1[d]	77
16	**21l**[b]	CH_2OTBS	COOEt	OMe	BrU	$SnCl_4$ (5.5)[c]	RT	**38β/38α**	4.7:1[d]	51
17	**21m**[b]	$CH_2OTBDPS$	COOEt	OMe	C	TMSOTF (6)	RT	**39β/39α**	8.2:1[d]	55
18	**21n**[b]		COOEt	OMe	C	TMSOTF (6)	RT	**40β/40α**	3.5:1[d]	58
19	**21o**[b]		COOEt	OMe	C	TMSOTF (6)	RT	**41β/41α**	5.1:1[d]	85

Reaction conditions: $(TMS)_2NB$, Lewis acid, CH_2Cl_2, 2 h to 20 h. [a] C = cytosine, U = uracil, T = thymine, BrU = 5-bromo-uracil. [b] Complexes **21i–21o** were prepared by the desilylation of compound **21c** and subsequent re-protection (see the Supporting Information). [c] $SnCl_4$ was added to the refluxing reaction mixture over 2 h. [d] d.r. was calculated from the yields of the isolated diastereomers. [e] d.r. determined by ^1H NMR spectroscopy. [f] compound **34α** was not isolated. n.d. = not determined.

and thymine proceeded with the complete retention of configuration to give the products **42α** and **43α**, respectively, in high yields (Scheme 6).



Scheme 6. Fully diastereoselective introduction of the nucleobase by using complex **22c**.

As Table 2 shows, this method (which was originally developed during the course of a mechanistic investigation)[10] was applicable for the efficient introduction of different nucleobases. Thus, a reliable synthetic access to various nucleoside analogues of type **1** was established, as a precondition for investigating the key structural features that are responsible for the biological activity of these compounds. For the introduction of uracil, 5-bromo-uracil, or thymine, substrates of type **21** were reacted with the silylated nucleobases by using $SnCl_4$ as a Lewis acid in refluxing CH_2Cl_2 to yield the corresponding nucleosides (as an **α**/**β** mixture) in which the pyrimidine unit was bound through the N-1 atom. Interestingly, attempts to introduce cytosine under these conditions (Table 1, entry 3) primarily afforded the N-4′-bound cytosine derivative (**30**), which was an isomer of **N69** (Figure 4).

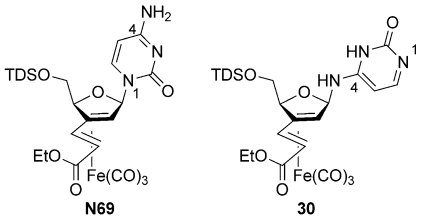
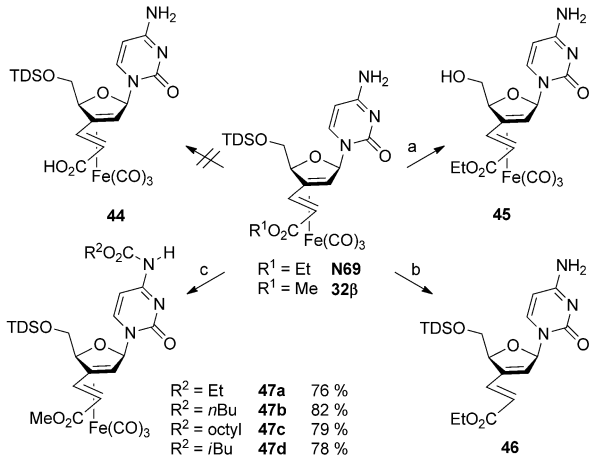


Figure 4. Structures of the desired cytosine derivative **N69** (Table 2, entry 9) and its *N*-4′-linked isomer (**30**; Table 2, entry 8).

However, this undesired reaction pathway could be suppressed by using an excess of TMSOTf instead of $SnCl_4$. Whilst $SnCl_4$ facilitates desilylation at the N-4′ position, the beneficial effect of TMSOTf might be associated to its ability to keep the NH_2 group TMS-protected during the reaction, thus guaranteeing the N-1-selective formation of cytosine nucleosides (Table 2, entries 4, 9–13, 15, and 17–19).[19]

For the biological investigations, additional analogues of lead compound **N69** and its methyl ester congener (**32β**) were prepared (Scheme 7). Removal of the TDS group proceeded smoothly with TBAF under standard conditions (THF, water) to give the C-5′-unprotected nucleoside (**45**). It should be mentioned that the synthesis of various O-5′-de-



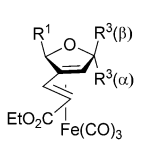
Scheme 7. Further derivatization of compounds **N69** and **32β**: a) TBAF, THF (containing 0.1–0.5 % water), RT, 3 h, 37 % yield; b) TMANO, toluene, 0 °C, 1.5 h, 74 % yield; c) $R^2O(C{=}O)Cl$, Et_3N, DMAP, CH_2Cl_2, 0 °C to RT, 16 h. TBAF = tetra-*n*-butylammonium fluoride, TMANO = trimethylamine *N*-oxide, DMAP = 4-dimethylaminopyridine.

rivatives of compound **45** required O-5′ functionalization prior to the introduction of the nucleobase. Thus, starting from compound **21c**, the TDS group was first cleaved off and the resulting alcohol was reacted with an appropriate chlorosilane or activated carboxylic acid derivative before the nucleobase was introduced under the optimized conditions (Table 2, entries 13–19; also see the Supporting Information). Remarkably, all of our attempts to cleave the ester function in compounds **N69** or **32β** (to give acid **44**) completely failed under a variety of conditions (see the Supporting Information). Therefore, the preparation of different carboxylic acid derivatives (such as amide **33β** and ester **34β**) required the introduction of these moieties earlier in the synthesis, that is, at the stage of the Wittig reaction (Scheme 3). "Metal-free" **N69** derivative **46** was prepared by oxidative decomplexation with TMANO. Finally, inspired by carbamate-protected nucleoside prodrugs, such as capecitabine,[20] we also prepared some carbamate derivatives of **N69**, that is, compounds **47a**–**47d**, by reacting compound **32β** with commercially available chloroformates under standard conditions (Scheme 7).

Configurational assignment of substituted vinyl–dihydrofuran–$Fe(CO)_3$ complexes: The configurational assignment of various diene–$Fe(CO)_3$ complexes, that is, the position of the $Fe(CO)_3$ fragment with respect to the plane of chirality (Scheme 1), was not trivial at all and deserves some detailed discussion. The relative configuration could not be determined by NMR spectroscopy, although characteristic NMR patterns were observed for stereochemically related complexes. Therefore, additional analytical methods had to be employed. We considered circular dichroism (CD) as a suitable method, which allows for a credible assignment of the absolute configuration, at least where reference spectra of comparable complexes with confirmed configuration are

available. Martelli, Bolard, and co-workers[21] found a strong correlation between the absolute configuration of acyclic diene–$Fe(CO)_3$ complexes with a terminal electron-withdrawing substituent (ester, aldehyde, or ketone) and the sign of the Cotton effects at longer wavelengths (about 400 nm). This relationship allowed us to tentatively assign the configurations of various complexes of type **21** and **22** with an ester group at the R^2 position (Table 3). Whilst all of the complexes of type **21** showed a positive Cotton effect at about 400 nm (Table 3, entries 1–13), their diastereomeric

Table 3. Cotton effects (θ) of various complexes at about 400 nm.



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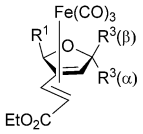

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Entry	Complex	Type	R^1	$R^3(\alpha)$	$R^3(\beta)$	θ (max)	λ [nm]
1	**21a**	**21**	H	OBn	H	+2793	395
2	**21c**	**21**	CH_2OTDS	OMe	H	+1566	402
3	**21d**	**21**	CH_2OTDS	H	OMe	+2690	390
4	**27α**	**21**	CH_2OTDS	U	H	+1937	398
5	**27β**	**21**	CH_2OTDS	H	U	+3878	397
6	**28α**	**21**	CH_2OTDS	T	H	+2000	394
7	**28β**	**21**	CH_2OTDS	H	T	+3990	397
8	**29α**	**21**	CH_2OTDS	BrU	H	+1891	398
9	**29β**	**21**	CH_2OTDS	H	BrU	+3140	397
10	**30**	**21**	CH_2OTDS	H	C[a]	+1220	384
11	**31α**	**21**	CH_2OTDS	C	H	+1020	389
12	**N69**	**21**	CH_2OTDS	H	C	+920	391
13	**21c-1**	**21**	CH_2OH	OMe	H	+770	394
14	**22a**	**22**	H	OBn	H	−2938	390
15	**22c**	**22**	CH_2OTDS	OMe	H	−3689	387
16	**22d**	**22**	CH_2OTDS	H	OMe	−620	395
17	**42α**	**22**	CH_2OTDS	U	H	−4267	388
18	**43α**	**22**	CH_2OTDS	T	H	−4520	387
19	**22c-1**	**22**	CH_2OH	OMe	H	−1740	384

[a] The cytosine was bound at the N-4 position.

counterparts (**22**) exhibited a negative Cotton effect within the same range (Table 3, entries 14–19). Conclusive evidence was obtained from the crystal structure of desilylated complex **22c-1** (Figure 5).

The stereostructural assignment of complexes that lacked an electron-withdrawing ester substituent on the diene unit

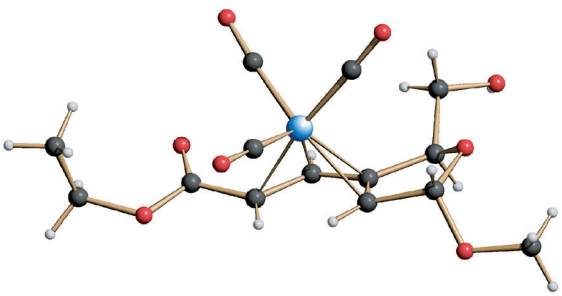


Figure 5. Crystal structure of compound **22c-1**, which was obtained after the desilylation of complex **22c**.

(for which no CD-based assignment was possible) was accomplished by comparison of the ^1H NMR spectra. As shown in Figure 6, the compounds showed distinct signal patterns, in particular within the range $\delta = 4.5$–6.0 ppm. Thus, the configuration of all of the compounds that were investigated could be unambiguously assigned.

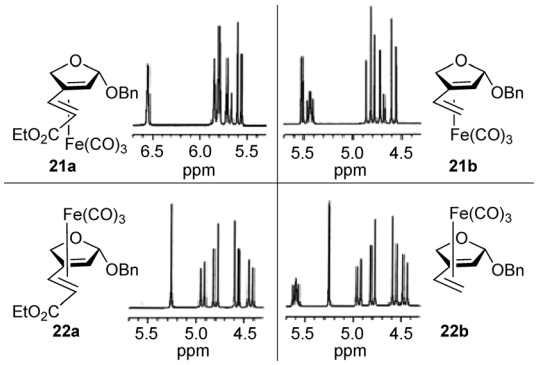
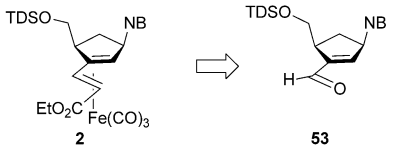


Figure 6. Section of the ^1H NMR spectra of complexes of type **21a/b** and **22a/b**, which show the characteristic patterns of both stereochemical series.

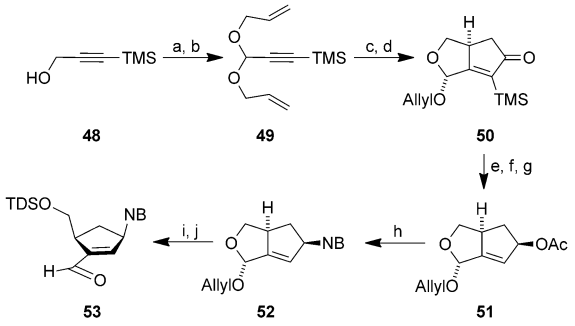
Synthesis of carbocyclic nucleosides of type 2: Although the dihydrofuran-derived nucleoside analogues of type **1** were found to be reasonably stable against air and moisture,[22] we envisioned that carbocyclic analogues of type **2** might exhibit even higher stability. Moreover, carbocyclic nucleosides often display different biological properties, such as increased resistance towards enzymatic degradation, as well as decreased toxicity.[23] For the synthesis of carbocyclic nucleosides of type **2**, we decided to introduce the $Fe(CO)_3$ fragment in the final step through the diastereoselective complexation of diene precursors of type **54** because these enoates should be readily prepared through Wittig olefination reactions.

A synthesis of the required aldehyde precursors (**53**) has previously been developed in our laboratories and shall only be discussed briefly here (Scheme 8).[24] This synthesis started with the PCC oxidation of C-silylated propargyl alcohol **48** and the subsequent formation of diallyl–acetal **49**, which was further transformed into furan derivative **50** through a Pauson–Khand reaction[25] and subsequent kinetic resolution by means of CBS reduction.[26] After the Luche reduction of compound **50**,[27] alkoxide-induced desilylation, and acetylation (to afford compound **51**), the diastereoselective intro-



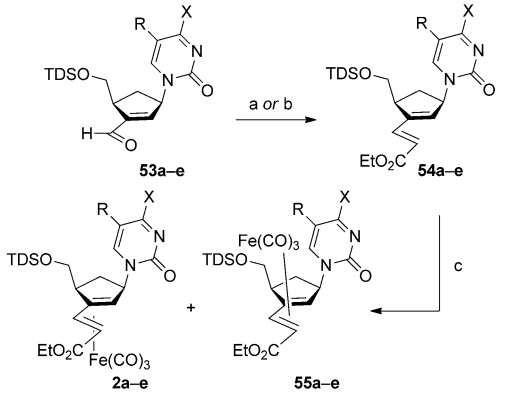
Scheme 8. Projected synthesis of carbocyclic nucleoside analogues of type **2** through diastereoselective complexation.

duction of the nucleobase was achieved through a Pd-catalyzed allylic substitution reaction. Finally, the resulting compounds of type **52** were converted into aldehydes **53** through acid acetal hydrolysis and subsequent silylation of the OH function (Scheme 9).

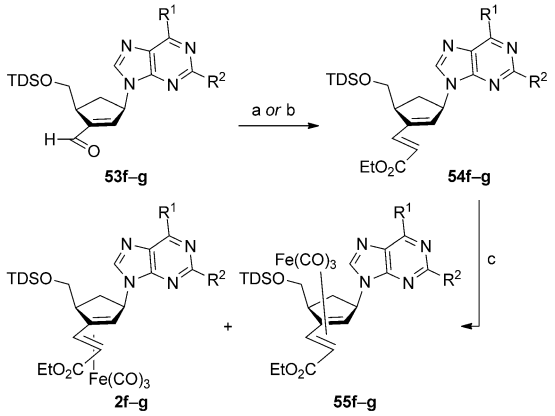


Scheme 9. Synthesis of the aldehyde precursors of carbocyclic nucleosides of type **2**: a) PCC (1.5 equiv), CH_2Cl_2, RT, 3 h; b) allyl-OH (excess), *p*-TsOH (cat.), benzene, reflux, 15 h, 88% yield from compound **48**; c) $[Co_2(CO)_8]$ (1.1 equiv), CH_2Cl_2, 4 Å molecular sieves, RT, 2 h, then TMANO (8.8 equiv), air, 0 °C to RT, 15 h, 76% yield; d) kinetic resolution through CBS reduction (34% yield from compound **50**); e) $NaBH_4$, $CeCl_3$, MeOH, 0 °C, 0.5 h, quantitative yield; f) *t*BuOK, DMSO/water (19:1), RT, 1 h, 87% yield; g) Ac_2O, Et_3N, DMAP (cat.), CH_2Cl_2, RT, 1 h, 99% yield; h) NB or derivative, Pd^0 (cat.); i) *p*-TsOH, acetone/water, reflux, 3 h; j) TDSCl, (1.5 equiv), pyridine, RT, 16 h. PCC = pyridinium chlorochromate.

The Wittig olefination of aldehydes **53** to afford their corresponding enoates (**54**) proceeded smoothly. The final complexation was performed with $Fe_2(CO)_9$ to give the desired nucleosides of type **2** with a decent degree of diastereoselectivity (Scheme 10, Scheme 11, Table 4, and Table 5). However, the diastereomeric by-products (**55**) could be separated off by means of flash column chromatography on silica gel in all cases. Because the kinetic resolution of compound *rac*-**50** gave rise to both enantiomers, we also conducted the synthesis of a set of "unnatural", that is enantiomeric target nucleosides (*ent*-**2**).[28] In most cases, Wittig olefination of the aldehydes (**53**) was efficiently achieved by using the commercial reagent $Ph_3P{=}CH_2COOEt$ (method A).



Scheme 10. Synthesis of carbocyclic nucleosides of type **2**: a) $Ph_3P{=}CHCOOEt$ (1.2 equiv), THF, 50 °C, 5 h, to RT, 16 h; b) $(EtO)_2OPCH_2COOEt$ (1.75 equiv), *n*BuLi (1.55 equiv in hexanes), THF, 0 °C, then compound **53**, 0 °C to RT, 16 h; c) $Fe_2(CO)_9$, Et_2O, reflux, 24 h.



Scheme 11. Synthesis of nucleosides of type **2** continued: a) $Ph_3P{=}CHCOOEt$ (1.2 equiv), THF, 50 °C, 5 h, to RT, 16 h; b) $(EtO)_2OPCH_2COOEt$ (1.75 equiv), *n*BuLi (1.55 equiv in hexanes), THF, 0 °C, then compound **53**, 0 °C to RT, 16 h; c) $Fe_2(CO)_9$, Et_2O, reflux, 24 h.

Table 4. Compounds that were synthesized according to Scheme 10.

Series	R	X	Wittig method[a]	Yield of **54** [%]	Yield of **2** [%]	Yield of **55** [%]	d.r. (**2**/**55**)
a	H	OH	A	99	47	17	2.8:1
b	F	OH	A	95	57	27	2.2:1
c	Br	OH	A	99	73	20	3.8:1
d	Me	OH	A	99	57	n.d.	n.d.
e-1	H	NHBz	B	81	55	n.d.	n.d.
e	H	NH_2	–	98[b]	42	n.d.	n.d.

[a] Method A: $Ph_3P{=}CHCOOEt$, THF, 50 °C; method B: $(EtO)_2OPCH_2COOEt$, *n*BuLi in hexanes, THF, 0 °C to RT. [b] Compound **54e** was prepared from compound **54e-1** (2 M NH_3 in water, 40 equiv, RT, 16 h).

However, in the case of (protected) cytosine- and adenine-derivatives **53e** and **53g** (Scheme 10, Scheme 11, Table 4, and Table 5), a Wittig–Horner–Emmons procedure (method B) was applied to circumvent the difficult removal of triphenylphosphine-oxide contaminants from the products (**54e-1** and **54g-1**, respectively). Not unexpectedly, the complexation of dienes of type **54** occurred preferentially from the less-hindered hemisphere (d.r. between 2.2:1 and 3.8:1).

Table 5. Compounds that were synthesized according to Scheme 11.

Series	R^2	R^2	Wittig method[a]	Yield of **54** [%]	Yield of **2** [%]	Yield of **55** [%]	d.r. (**2**/**55**)
f (*rac*)	NH_2	H	B	99	45	11	4.1:1
g-1	$R^{2[b]}$	NHAc	A	95	28	n.d.	n.d.
g	OH	NH_2	–	99[c]	25	11	2.3:1

[a] Method A: $Ph_3P{=}CHCOOEt$, THF; method B: $(EtO)_2OPCH_2COOEt$, *n*BuLi in hexanes, THF. [b] For compounds **53g-1** and **54g-1**, R^1 = OCbz; for compounds **2g-1** and **55g-1**, R^1 = OH. [c] Compound **54g** was prepared from compound **54g-1** (2 M NH_3 in water, 40 equiv, RT, 16 h). Cbz = carboxybenzyl.

Uracil-nucleoside analogues **2a**–**2d** were obtained in 40–70% yield after purification; minor diastereomers **55a**–**55d** were also isolated in most cases. For the synthesis of cytosine-derivative **2e**, we employed benzoyl-protected derivative **53e**,[29] which was olefinated to give compound **54e-1**. However, after complexation to give compound **2e-1**, nucleophilic deprotection with ethoxide[30] was unsuccessful. Nevertheless, diene **54e-1** could be deprotected in quantitative yield by using ammonia (2 M in MeOH, 16 h, RT), thus delivering deprotected compound **54e**, which, on complexation, afforded the desired nucleoside analogue (**2e**) in 42% yield.

A related strategy was employed in the synthesis of guanine derivative **2g**. In this case, the deprotection of diene precursor **54g-1** was again achieved with ammonia. Then, subsequent complexation afforded the desired product (**2g**) in only 25% yield. The synthesis of the corresponding adenine derivative was conducted both in the racemic (*rac*-**2f**) and the enantiomerically pure series (*ent*-**2f**).

Configurational assignment of nucleoside analogues of Type 2: The assigned relative configuration of these complexes was confirmed by CD spectroscopy, in comparison with the data for the dihydrofuran series (see above). Because the methylene group in the carbocyclic compounds was isosteric with the oxygen atom in the corresponding furanoids, related chiroptical properties were expected. Indeed, the recorded CD spectra of compounds **2a** and **2c**–**2e** were similar to those of their corresponding dihydrofuran derivatives, as shown in Figure 7. Moreover, in the cases of fluorouracil derivative **2b** and purines *ent*-**2f** and **2g** (for which no reference data were available), the characteristic curves

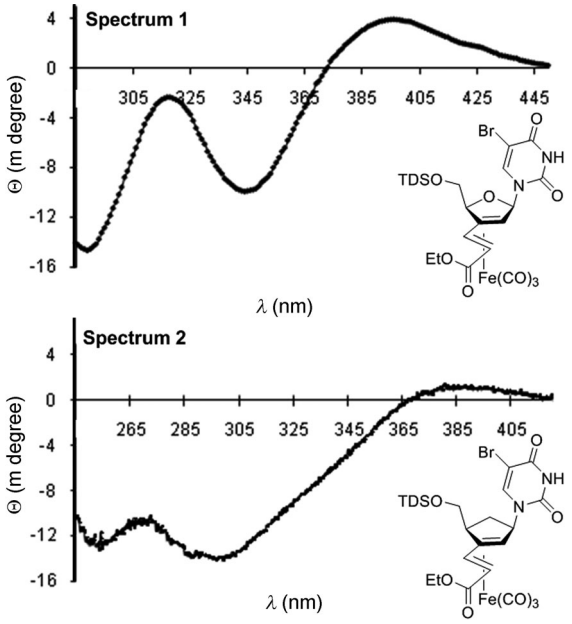


Figure 7. CD spectra of compounds **29β** (spectrum 1) and **2c** (spectrum 2). The positive Cotton effect at about 390 nm is indicative of the absolute configuration shown (cf. Table 3).

associated to Cotton effects at about 390 nm confirmed the configurational assignments.

NMR spectroscopy was used as a second method to probe the configurational assignments. As observed in the furanose series, diastereomeric complexes of type **2** and type **55** showed characteristic differences in their ^{1}H NMR spectra (Figure 8). In compounds of type **2**, the $Fe(CO)_3$ fragment distorts the diene ligand in such a fashion that the olefinic H-2′ proton moves out of the plane, thereby increasing the dihedral angle between the C–H-2 and C–H-3′ bonds to approximately 90°. Consequently, no coupling between these two protons is observed, as shown for compound **2c** (Figure 8, spectrum 1). Owing to the lack of other coupling partners, the H-2′ atom ($\delta = 1.85$ ppm) is observed as a singlet and the H-3′ atom ($\delta = 5.09$ ppm) as a doublet of doublets (dd). In the case of the corresponding diastereomer (**55c**), the angle between the H-2′ and H-3′ atoms is smaller than 90° (Figure 8, Spectrum 2), which results in significant coupling (4.2 Hz) between the H-2′ and H-3′ signals. Accordingly, the H-2′ signal appears as a doublet and the H-3′ signal appears as a doublet of doublet of doublets (ddd).[31]

Cytotoxicity data and structure–activity relationships: The cytotoxic properties of the various iron-containing nucleoside analogues were tested in vitro by using an established cell assay. For this purpose, Burkitt-like lymphoma (BJAB) cells were grown in a culture medium that contained fetal calf serum. After incubation with the potential cytotoxic agents for 48 h, the release of lactate dehydrogenase (LDH) was measured as a quantitative indicator of deceased cells.[32]

A comparison of the LD_{50} values of various nucleoside analogues of type **1** (Table 6) indicated certain structural features that were connected to potent cytotoxicity: Firstly, a lipophilic R^1 substituent seemed to be necessary. Whereas other silyl ether congeners of **N69**, such as compounds **37β** and **39β**, still showed significant cytotoxic activity, deprotected derivative **45**, as well as ester-substituted analogues **35β**, **40β**, and **41β**, were practically inactive. Secondly, the R^2 substituent appeared to be a variable position tolerant of various substituents, because all of the tested **N69** derivatives that were modified at this position (**26β**, **32β**, **33β**, **34β**) exhibited cytotoxicity, although morpholine derivative **33β** was less active. Thirdly, the use of cytosine as a nucleobase gave rise to the highest cytotoxicities (which was why most of the compounds that were prepared were cytosine derivatives). However, the functionalization of the cytosine amino group as a carbamate resulted in a loss of activity. As we had reported previously,[5] decomplexation (removal of the $Fe(CO)_3$ fragment) also led to a greatly diminished activity. Closer investigations were undertaken for **N69**, as the most potent compound of type **1**, and for morpholine derivative **33β**, as a compound with improved water solubility. The cytostatic activity of these compounds, as determined by counting the living cells (again by using BJAB cells), was significant in both cases, with **N69** several times more active than compound **33β** (Figure 9). In addition, the apoptosis-

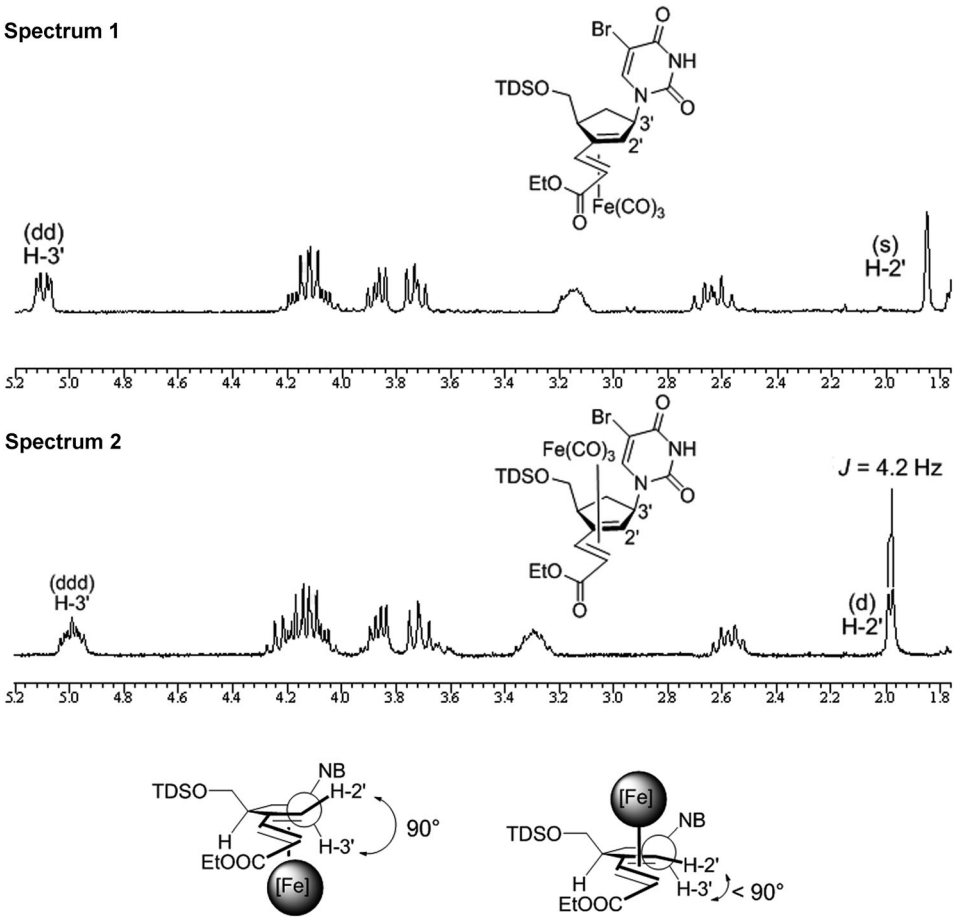


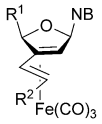
Figure 8. ^{1}H NMR spectra of compounds **2c** (spectrum 1) and **55c** (spectrum 2) and conformational analysis.

inducing activity of these two compounds was demonstrated (and quantified) by measuring the DNA fragmentation after 72 h. These results were in accordance with characteristic morphological changes (blebbing) that were observed by microscopy. In the case of **N69**, Western Blot analysis also showed that apoptosis induction was connected with proteolytic processing and activation of caspase-3. Furthermore, we clearly demonstrated that **N69** neither decreased the viability of healthy, primary leukocytes nor induced apoptosis, as determined by DNA fragmentation in NIH 3T3 fibroblasts (data not shown). Thus, nucleoside analogues that possess a characteristic butadiene–Fe(CO)$_3$ substructure are promising candidates for the development of iron-containing anti-cancer drugs with pro-apoptotic activity.

The cytotoxic properties of a series of carbocyclic compounds of type **2** (and their enantiomers *ent*-**2**) were also determined. The results (Table 7) reveal that the "natural" enantiomers (**2**) showed similar cytotoxic properties to their oxacyclic congeners. Again, cytosine derivative **2e** (Table 7, entry 9) was more potent than the thymine-, uracil-, and halo-uracil-substituted compounds, although it was less potent than **N69**. Again, functionalization of the cytosine amine (**2e**-**1**; Table 7, entry 11) quenched its activity. A notable result was the increased activity of the "unnatural" enantiomers (*ent*-**2**) in comparison to their "natural" counterparts. This difference was the most pronounced in the case of fluorouracil derivative **2b** (Table 7, entries 3 and 4). Both purine derivatives, that is, compounds **2f** and *ent*-**2g**, also showed significant cytotoxic activity (Table 7, entries 12 and 13).[33] DNA-fragmentation was quantified for compounds **2a**, *ent*-**2a**, **2c**, **2d**, and *ent*-**2d** at selected concentrations after 72 h. The values that were obtained corresponded to their respective cytotoxicity levels, thereby confirming that cell death was due to apoptosis in these cases as well.

Synthesis of N69 conjugates: The data presented above demonstrate the biological potential of **N69** and its related Fe-containing nucleoside analogues. Most interestingly, the clear structure–activity relationships (including stereochemical aspects) suggest that these compounds do not act as "normal" nucleoside analogues. Therefore, it is likely that the compounds act through an (unknown) protein-based mechanism, which constitutes a challenge to future biological and biochemical investigations to elucidate the protein targets that are involved. Against this background, suitable procedures for the synthesis of labeled derivatives have been developed. Based on the structure–activity relationships discussed above, **N69** conjugates of type **56** with biotin, fluorescein, or 7-dimethylaminocoumarin-4-acetic acid (DMAC)[34] have emerged as desirable targets, in which the label is attached through a flexible alkyl linker onto the ester unit of the **N69** core. As indicated in Figure 10, three synthetic approaches were evaluated. Initially, it seemed feasible to synthesize compounds of type **56** directly from **N69** or its methyl-ester analog (**32β**) by cleavage of the ester and subsequent coupling with a labeled linker. However, we did not succeed in obtaining the free acid (**44**, Scheme 7; for the tested conditions, see the Supporting Information). Also, all attempts to synthesize conjugates of type **56** by cross-metathesis (by employing pentenyl ester **34β**) and subsequent hydrogenation failed (see the Supporting Information). However, the synthesis of the labeled derivatives (**56**, $n=0$) was achieved by introducing a masked 1,6-aminohexanol linker early in the synthesis (Scheme 12).

Table 6. Cytotoxicity of nucleoside analogues of type **1** against BJAB cells.



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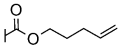
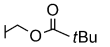
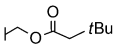
Entry	Compound	R^1	R^2	NB	LD_{50}[b] [μM]	LD_{50}[c] [μM]
1	**N69**	CH_2OTDS	COOEt	C	10	18
2	**32β**	CH_2OTDS	COOMe	C	–	24
3	**26β**	CH_2OTDS	H	C	18	–
4	**33β**	CH_2OTDS		C	88	91
5	**34β**	CH_2OTDS		C	–	23
6	**37β**	CH_2OTBS	COOEt	C	30	–
7	**39β**	$CH_2OTBDPS$	COOEt	C	14	–
8	**35β**	CH_2OAc	COOEt	C	>100	–
9	**40β**		COOEt	C	>100	–
10	**41β**		COOEt	C	>100	–
11	**45**	CH_2OH	COOEt	C	>100	–
12	**29β**	CH_2OTDS	COOEt	BrU	>100	–
13	**38β**	CH_2OTBS	COOEt	BrU	72	–
14	**28β**	CH_2OTDS	COOEt	T	>100	–
15	**47a**	CH_2OTBS	COOMe	C-a[a]	>100	–
16	**47b**	CH_2OTBS	COOMe	C-b[a]	>100	–
17	**47c**	CH_2OTBS	COOMe	C-c[a]	>100	–
18	**47d**	CH_2OTBS	COOMe	C-d[a]	>100	–

[a] Carbamoyl-protected cytosine (c.f. Scheme 7). [b] based on LDH concentration after 48 h. [c] based on DNA fragmentation after 72 h. TBS = *tert*-butyldimethylsilyl.

Phthalimide- and tetrachlorophthalimide (TCP)-protected linkers **57a** and **57b** were transformed into the corresponding Wittig salts and coupled with aldehyde **15β** to yield dienes **58a** and **58b**, respectively. Following the established route, these dienes were reacted with $Fe_2(CO)_9$ to yield complexes of type **59**, which were subsequently transformed into the corresponding nucleoside analogues of type **60**. Although the deprotection of the TCP derivative **60b** with 1,2-diaminoethane did deliver the desired amine (**61**), contamination of the product with excess 1,2-diaminoethane presented a problem. However, the facile deprotection of phthalimide derivative **60a** with hydrazine in EtOH at 60 °C yielded compound **61** in high purity (75 % yield after aqueous workup). Labeling with biotin (**56a**) and DMAC (**56c**) was achieved under standard peptide-coupling conditions. The reaction of compound **61** with fluorescein isothiocyanate (FITC) in DMF directly afforded compound **56b**. The regioselectivity of these reactions was confirmed by heteronuclear multiple-bond correlation (HMBC) spectroscopy, which indicated that no by-products had been formed that would result from electrophile attack at the (less nucleophilic) cytosine-NH_2 function. Thus, reliable procedures for the synthesis of labeled **N69** derivatives have been established, which constitute a valid basis for future biological studies. An initial cytotoxicity assay revealed pleasing activity for biotin conjugate **56a** (LD_{50} = 17 μM), whilst fluorescein derivative **56b** and the (much-smaller and less-polar) DMAC conjugate **56c** were inactive.[35]

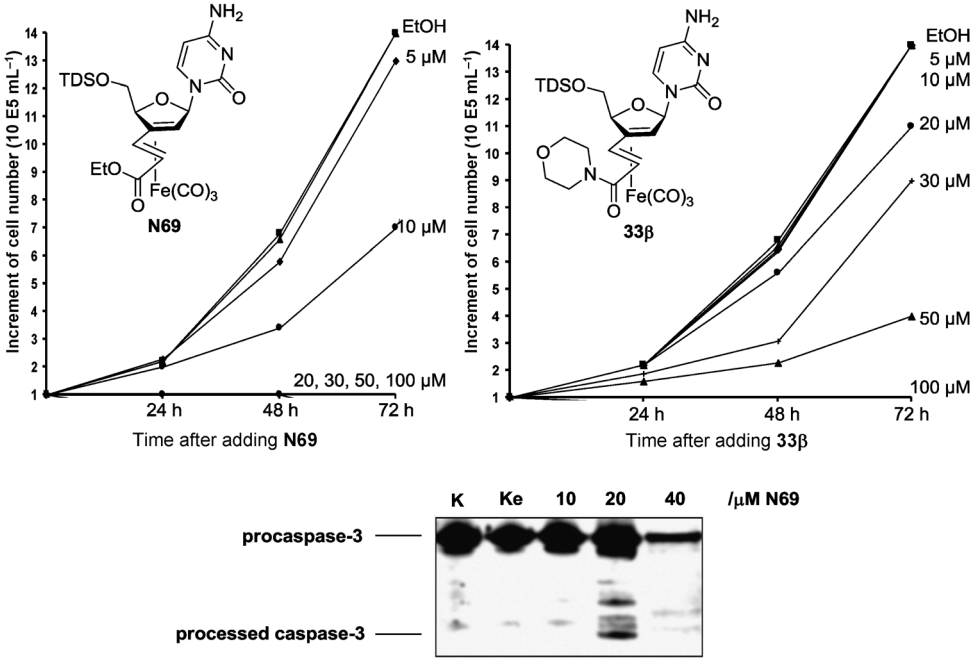
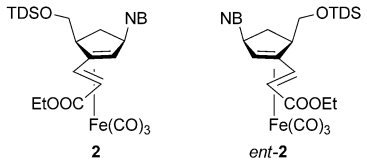


Figure 9. Cytostatic activity of compounds **N69** and **33β**, as well as **N69**-induced cleavage of caspase-3.

Conclusion

As a contribution to the field of bio-organometallic chemistry, we have developed reliable schemes for the synthesis of enantiomerically pure nucleoside analogues that possess a characteristic butadiene–$Fe(CO)_3$ substructure. The stereoselective generation of the planar chiral butadiene–$Fe(CO)_3$ unit was achieved by the diastereoselective complexation of appropriate chiral diene ligands, governed both by (repulsive) steric factors and by attractive pre-coordination effects. The introduction of the nucleobase also proceeded with good-to-excellent levels of diastereoselectivity

Table 7. Cytotoxicity of carbocyclic Fe-containing nucleoside analogues of type **2**.



Entry	Compound	Type	NB	LD_{50}[a] [μM]
1	**2a**	**2**	U	54
2	*ent*-**2a**	*ent*-**2**	U	23
3	**2b**	**2**	FU	>100
4	*ent*-**2b**	*ent*-**2**	FU	28
5	**2c**	**2**	BrU	>100
6	*ent*-**2c**	*ent*-**2**	BrU	81
7	**2d**	**2**	T	79
8	*ent*-**2d**	*ent*-**2**	T	33
9	**2e**	**2**	C	40
10	*ent*-**2e**	*ent*-**2**	C	39
11	**2e-1**	**2**	Bz-C	>100
12	**2f**	**2**	G	28
13	*ent*-**2g**	*ent*-**2**	A	32

[a] Based on LDH release after 48 h. FU = 5-fluoro-1H,3H-pyrimidine-2,4-dione BrU = 5-bromo-1H,3H-pyrimidine-2,4-dione.

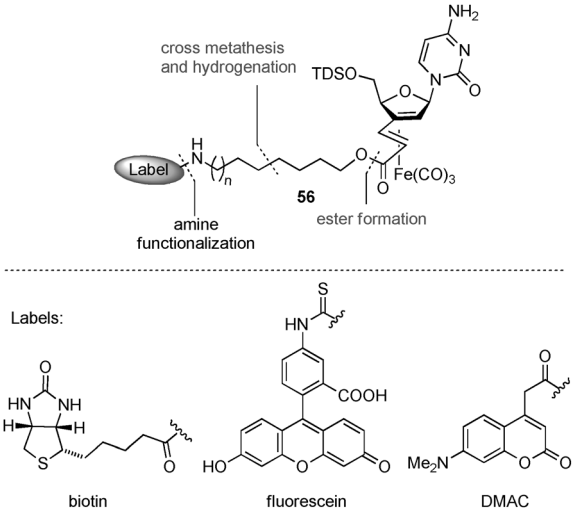
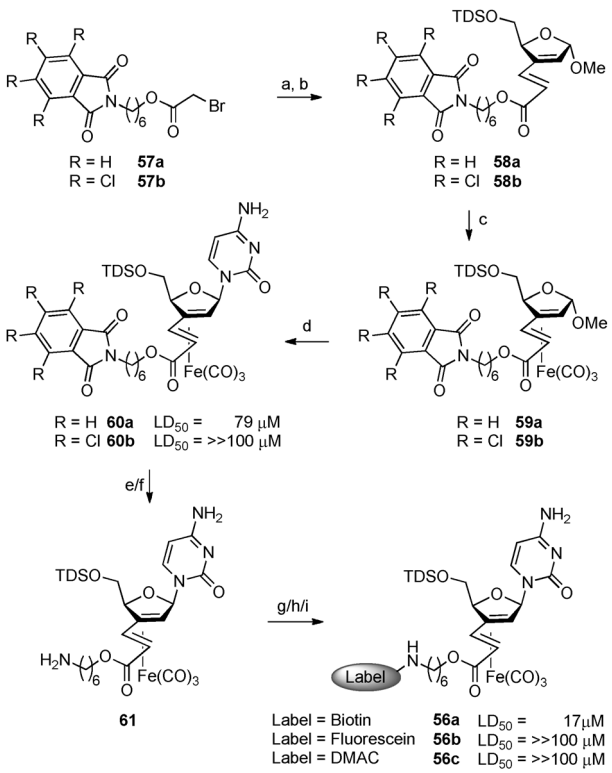


Figure 10. **N69** conjugates with biological labels and our attempted synthetic approaches (DMAC = 7-dimethylaminocoumarine).



Scheme 12. Synthesis of conjugates of **N69** with different molecular labels: a) PPh_3 (1.1–1.4 equiv), toluene, RT, 0.5–4 days, for R = H: 66% yield, for R = Cl: 95% yield; b) (i) Wittig salt (1.8 equiv–2.0 equiv), LiHMDS (1.3 equiv–1.7 equiv), THF, RT, 0.5 h, (ii) compound **15α** (1 equiv), reflux, 2 h, for R = H: 95% yield, for R = H: 85% yield; c) (i) $Fe_2(CO)_9$ (1.3 equiv), Et_2O, RT 2 h, reflux, 2 h, for R = H: 77% yield, d.r. 4.0:1, for R = Cl: 71% yield, d.r. 3.1:1 (diastereomers not separated); (ii) isolation of compound **59a** by temporary desilylation: TBAF (1.2 equiv), THF (containing 0.25% water), −15 °C to 10 °C, 3 h, 92% yield; (iii) TDSCl, pyridine, 0 °C to RT, 12 h, 89% yield; d) $(TMS)_2Cyt$ (4 equiv), TMSOTf (6 equiv), CH_2Cl_2, RT, 5–24 h, for R = H: 60% yield, for R = Cl: 27% yield; e) for R = H: $H_2N{-}NH_2$, EtOH, 60 °C, 2 h, 75% yield; f) for R = Cl: $(H_2NCH_2)_2$ (4 equiv), EtOH/THF (1.3:1), 60 °C; g) biotin, EDCI·HCl (2 equiv), HOBT, DiPEA (2 equiv), DMF, 0° to RT overnight, 63% yield; h) FITC, DMF, −10 °C to 8 °C overnight, 84% yield; i) DMAC-carboxylic acid, HBTU (2 equiv), DiPEA (2 equiv), DMF, −10 °C to RT overnight, 36% yield. LiHMDS = lithium bis(trimethylsilyl)amide, TMSOTf = trimethylsilyl trifluoromethanesulfonate, EDCI = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, HOBT = hydroxybenzotriazole, DiPEA = *N*,*N*-diisopropylethylamine, HBTU = 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluroniumhexafluorophosphate.

from the face opposite the bulky $Fe(CO)_3$ fragment. The relative configuration of the iron-complexes was assigned by means of NMR and CD spectroscopy and confirmed through X-ray crystallography in one case.

Whilst the lead compound, that is, **N69**, had previously been shown to exhibit pronounced apoptosis-inducing properties (even against highly resistant tumor cells), cytotoxicity studies of the various complexes that were prepared during the course of this study revealed that compounds of both series (i.e., compounds of type **1** and their carbocyclic analogues of type **2**) were able to induce apoptosis in BJAB tumor cells, in particular if cytosine was present as a nucleobase. Also, a lipophilic substituent at the R^1 position was shown to be essential for activity. In contrast, variation of the substituent at the R^2 position was possible and this finding was exploited in the synthesis of labeled **N69** derivatives in which a fluorescent or a biotin unit was connected to the ether function through a 6-aminohexyl linker. Therefore, we have paved the way for further biological investigations of **N69** and related iron-containing nucleoside analogues, with the aim of identifying molecular targets and other aspects of their clearly unusual mechanism of action.

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[29] Benzoyl (Bz) protection of the cytosine amine atom was necessary for the palladium-catalyzed introduction of the nucleobase (Scheme 8, **51**→**52**).

[30] Deprotection of such substrates has been described with ammonia or alkoxides. Because the use of ammonia was expected to be incompatible with the $Fe(CO)_3$ fragment, we decided in favor of the alkoxide method, by using ethoxide to avoid the formation of trans-

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