Pergamon

SCIENCE DIRECT®

Tetrahedron Letters 44 (2003) 1685–1690

TETRAHEDRON LETTERS

Synthesis of an ethidium nucleoside and its acyclic analog

Nicole Amann and Hans-Achim Wagenknecht*

Institute for Organic Chemistry and Biochemistry, Technical University of Munich, Lichtenbergstr. 4, D-85747 Garching, Germany

Received 24 October 2002; accepted 19 December 2002

Abstract—The protected ethidium nucleosides 8-(3′,5′-di-*O*-benzoyl-2′-deoxy-D-ribofuranosyl)-3-acetamido-5-ethyl-6-phenyl-phenanthridinium (**5**), 8-(3′,5′-di-*O*-acetyl-2′-deoxy-D-ribofuranosyl)-3-acetamido-5-ethyl-6-phenyl-phenanthridinium (**6**), and the acyclic analog 8-[(3*R*)-1,3-dihydroxy-4-yl]-acetamido-3-amino-5-ethyl-6-phenyl-phenanthridinium (**3**) were prepared. Based on to their different stability, only the acyclic derivative **3** seems to be suitable for oligonucleotide synthesis. Furthermore, the acyclic ethidium nucleoside analog **3** exhibits comparable absorption and emission properties of the underivatized ethidium (**1**).

1. Introduction

One of the interesting properties of planar polycyclic aromatic molecules is their ability to intercalate between two adjacent base pairs in duplex DNA which was first proposed by Lerman.[1] This type of DNA binding is mediated by non-covalent stacking interactions with nucleobases and often combined with hydrogen bonding or other types of interactions. Among these intercalators, the highly polar or even charged systems are the most potent ones with respect to drug positioning and sequence preferences. Thus, it is believed that the electrostatic energy plays a major role in the intercalation process.[2]

3,8-Diamino-5-ethyl-6-phenylphenanthridium ('ethidium', **1**) is one of the well known positively charged intercalators for duplex DNA and has been widely used as a fluorescent staining agent due to the significant fluorescence enhancement which it exhibits upon intercalation.[3] Ethidium (**1**) and its derivatives are also well known as potent trypanocidal drugs.[4] In order to evaluate the specific features of ethidium (**1**) such as binding affinity, base sequence specifity and fluorescence properties, different experimental approaches were tried in the past. Among these are the synthesis and application of bifunctional DNA intercalators bearing ethidium derivatives,[5] theoretical calculations,[2] and the application of the photochemical reactivity beyond the absorption of the nucleic acids (>300 nm).[6] Ethidium (**1**) also plays an important role with respect to the studies of photoinduced processes in DNA.[7] In order to investigate the distance dependence of charge transfer in DNA, modified oligonucleotides have been used containing ethidium covalently attached to the 5′-end via an alkyl linker.[8] Herein, we want to present the synthesis and properties of the ethidium nucleoside **2** and its acyclic analog **3** (Scheme 1) towards potential building blocks for oligonucleotide synthesis.

2. Results and discussion

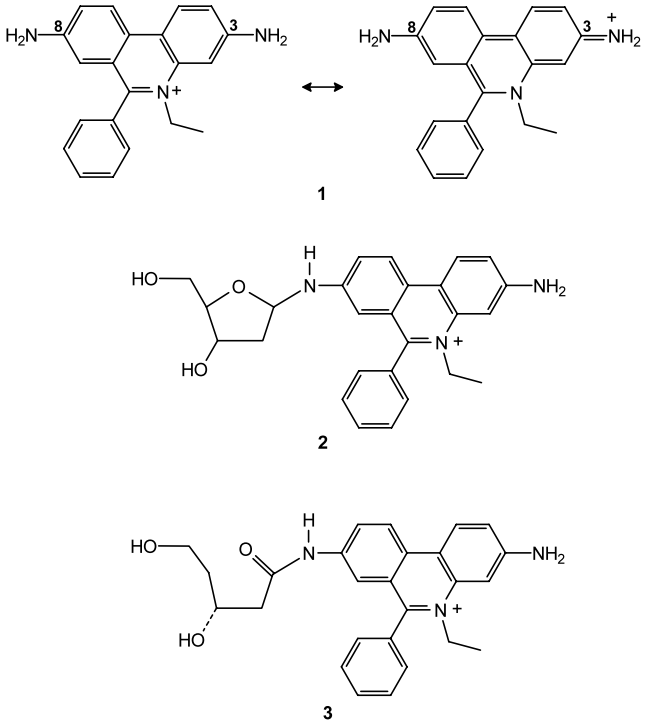
Early steps in the synthesis of ethidium (**1**) and its derivatives were made by Walls et al.,[9] Watkins et al.,[10] and Berg et al.[11] More recently, work by Schacht et al. showed clearly that the amino group in position C-3 of ethidium (**1**) is deactivated in comparison to the C-8 amino group due to the mesomeric stabilization of the positive charge (Scheme 1).[12] Thus in general, the C-8 amino group exhibits a higher reactivity towards any modification. Hence, we expect that a regioselective reaction of the C-8 amino group should be possible in order to prepare the ethidium derivatives **2** and **3**.

8-(2′-Deoxy-D-ribofuranosyl)-3-acetamido-5-ethyl-6-phenyl-phenanthridinium (**2**) contains the ethidium moiety glycosidically linked to the furanose form of 2′-deoxyribose. Glycosides with ethidium as an aglycon, e.g. 3- and 8-*N*-glucuronosylethidium, have been previously identified as the major metabolites of ethidium.[13] Although the structure of **2** represents an N,O-acetal, or a glycosylamine, respectively, the stability of

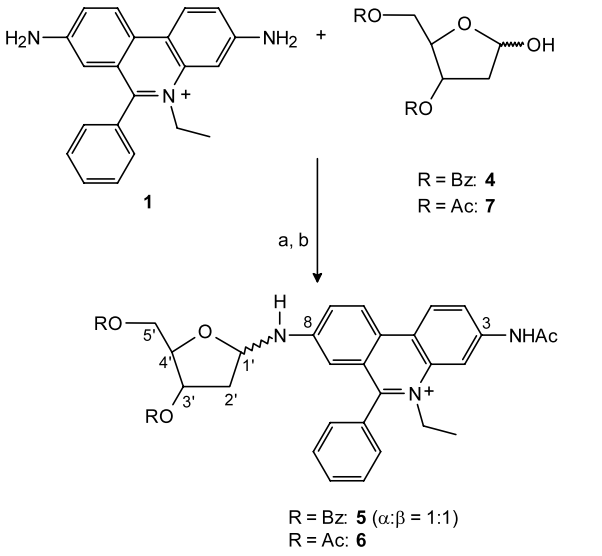
Keywords: ethidium; nucleoside; stacking; intercalation.

* Corresponding author. Fax: +49-(89)-289-13210; e-mail: wagenknecht@ch.tum.de

0040-4039/03/$ - see front matter
PII: S0040-4039(03)00025-X



Scheme 1. Deactivation of the amino group at C-3 of ethidium (**1**) due to mesomeric stabilization of the positive charge. Ethidium nucleoside **2** and its acyclic analog **3**.



Scheme 2. Synthesis of the protected ethidium nucleosides **5** and **6**: (a) **1** (1.3 equiv.), *p*TsOH (0.1 equiv.), MeOH, 60°C, 24 h; (b) Ac_2O (3.3 equiv.), pyridine, rt, 6 h, 10–15% (a+b).

2 towards acid and base hydrolysis should be high enough to guarantee the later incorporation into oligonucleotides. This expectation is mainly based on the assumption that a significant mesomeric stabilization exists within the ethidium aromatic system. We started the preparation of the 2′-deoxyribofuranoside **2** from the precursors 1,3,5-tri-*O*-acetyl-2-deoxy-D-ribofuranose,[14] 1-chloro-3,5-di-*O*-benzoyl-2-deoxy-D-ribofuranose,[15] and 3,5-di-*O*-benzoyl-2-deoxy-D-ribofuranose.[16] A whole variety of different reactions conditions were tried to perform this preparation including the *Vorbrueggen* conditions for the preparation of nucleosides,[17] the *Koenigs–Knorr* conditions[18] or *Lewis*-acid catalyzed conditions[19] for the preparation of glycosides, and, finally, special reaction conditions for the preparation of aromatic glycosylamines.[20] In all of these experiments none or only traces of the product could be detected by different analytical techniques, mainly HPLC-MS.

In conclusion, only the reaction of 3,5-di-*O*-benzoyl-2-deoxy-D-ribofuranose (**4**) with ethidium (**1**) by treatment with the weak acid NH_4Cl in MeOH[21] gave the desired product in a yield of 10–15% (Scheme 2) which was high enough in order to characterize and test the stability of this ethidium nucleoside. Detailed investigations of the conditions of this reaction mainly by HPLC showed that the maximum amount of the desired product **4** could be found after 24 h reaction time under reflux. The yield could not be improved since degradation of the product was detected after 24 h by HPLC. In order to facilitate the chromatographic purification, the coupling product was acetylated directly after the preparation. The ethidium glycoside **5** was characterized by UV/vis spectroscopy and ESI mass spectrometry.[22] The structure of **5** was confirmed by NMR spectroscopy, including the 2D techniques HMQC and DQF-COSY. These NMR experiments clearly revealed that the protected 2′-deoxyribofuranoside moiety is covalently attached to the amino group in the C-8 position of the ethidium part. The α-/β-anomers of the glycoside **5** showed a slightly different R_f value on TLC. Additionally, the NH-HSQC spectrum of **5** (Fig. 1) showed two cross-peaks for the NH-protons in the C-8-position of the ethidium group representing the two anomers, whereas the NH-protons in the C-3 position which is acetylated showed only one cross-peak.

It turned out that a complete interpretation of the aromatic ethidium resonances in the ^1H NMR spectrum is impossible due to the overlay with the resonances of the protons of the two benzoyl groups in the positions 3′ and 5′ of the 2′-deoxyribose moiety of **5**. Hence, we synthesized additionally the corresponding ethidium glycoside **6** from 3,5-di-*O*-acetyl-2′-D-deoxyribofuranose (**7**) again by treatment with NH_4Cl in MeOH and subsequent acetylation. The yield of **6** was comparable to the synthesis of **5**. Using this compound, the substitution pattern of the ethidium group could be investigated through 2D NMR experiments (DQF-COSY) and was confirmed.[23]

We checked the stability of the protected ethidium nucleosides **5** and **6** towards the typical reaction conditions during automated DNA synthesis and workup. Among these, the most critical ones for the glycosides **5** and **6** were expected to be the treatment with acids and strong bases. As expected due to the mesomeric resonance stabilization, both compounds, **5** and **6**, stayed stable towards the presence of trifluoroacetic acid in

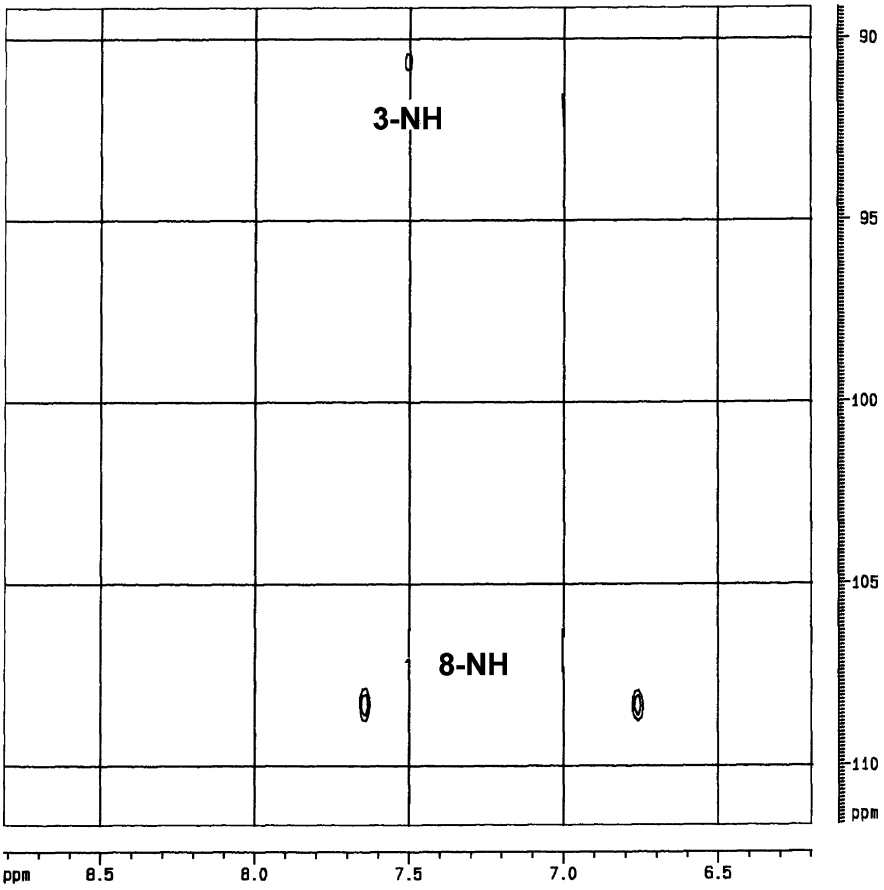


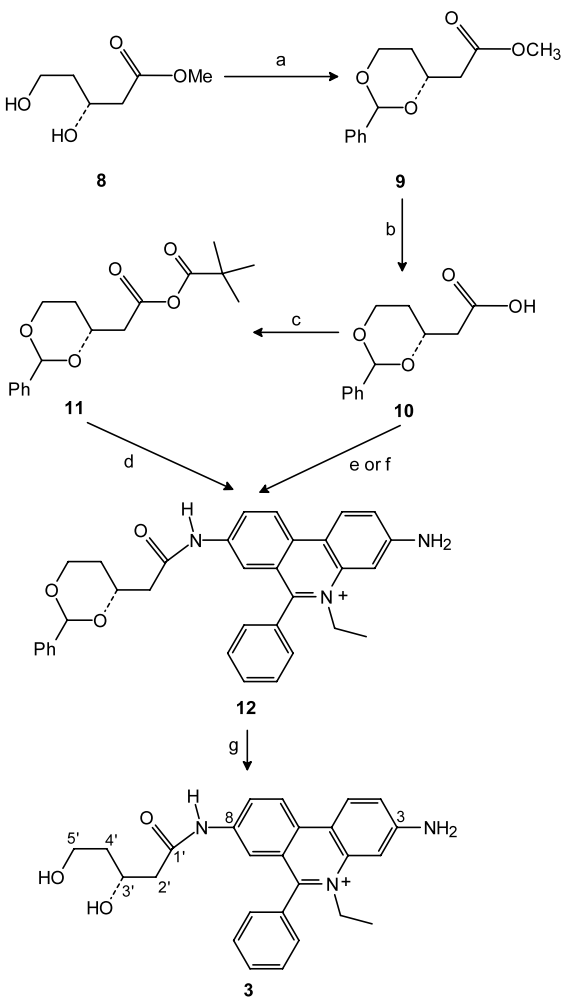
Figure 1. NH–HSQC of the ethidium nucleoside **5**.

MeCN over at least 3 h which was investigated by HPLC analysis. In contrast, treatment of **5** and **6** with aq. NH_4OH solution completely degraded the products within 3 h by cleaving the glycosidic bond between the ethidium and the 2′-deoxyribofuranose part. This fragments could be identified by HPLC-MS. This observed instability of the glycosylamines **5** and **6** is in agreement with results by Carell et al.[21] and clearly indicates that they are not suitable as building blocks for the preparation of oligonucleotides with ethidium as an artificial DNA base.

As an alternative, we designed 8-[(3′*R*)-1′,3′-dihydroxy-4′-yl]-acetamido-3-amino-5-ethyl-6-phenyl-phenanthridinium (**3**) which represents an acyclic analog of the ethidium nucleoside **2**. The (3*R*),5-dihydroxy pentanoic acid part mimics the 2′-deoxyribose moiety of common nucleosides. Ethidium derivatives with an amide bond to the exocyclic amino function at C-8 have proven to be stable during DNA workup conditions as shown by Barton et al.[24] We started the synthesis (Scheme 3) with the enantiomerically pure (3*R*),5-dihydroxypentanoic acid methyl ester **8** which can be prepared in a multistep process according to literature procedures.[25] The two hydroxy functions of **8** were protected by a benzylidene group and the ester function of **9**[26] was hydrolyzed in aq. LiOH.[27]

The coupling of the carboxylic acid **10**[28] with ethidium (**1**) was performed by three different ways. First, the carboxylic acid of **10** was activated with *iso* butyl chloroformiat.[29] Subsequent treatment of the mixed anhydride **11**[30] with ethidium (**1**) in DMF at elevated temperature gave the coupling product **12**[31] in good yield (70%). Using this synthetic pathway, only 3% of 3,8-disubstituted ethidium side product were detected by HPLC-MS. Alternatively, the direct coupling of the carboxylic acid **10** with ethidium (**1**) via typical and common peptide coupling procedures gave **12** in comparable yields (60–80%). We tried this coupling using HATU/HOAt,[32] and in the presence of DCC/DMAP[12]. Using the latter method, the 3,8-disubstituted ethidium side product was formed only in traces whereas the HATU/HOAt-catalyzed coupling gave this side product in higher amounts (14%). Finally, the benzylidene group of **12** was removed in aq. HCl yielding the ethidium derivative **3**.

We confirmed the structure of the ethidium derivative **3**[33] by 2D NMR experiments (DQF-COSY and HMQC) and by mass spectrometry. Most importantly, in contrast to the glycosides **5** and **6**, the acyclic derivative **3** stays stable for hours upon treatment with acids and bases presenting the conditions of the DNA syn-



Scheme 3. Synthesis of the acyclic analog **3**: (a) Ph-$CH(OCH_3)_2$ (1.0 equiv.), *p*TsOH (0.1 equiv.), CH_2Cl_2, 40°C, 2 h, 63%; (b) LiOH, THF:H_2O = 1:1, rt, 12 h, 64%; (c) 4-ethylmorpholine (1.1 equiv.), *iso*butyl chloroformiate (1.1 equiv.), THF, rt, 10 min, quant; (d) **1** (1.0 equiv.), DMF, 70°C, 48 h, 70%; (e) **1** (0.9 equiv.), HATU (1.0 equiv.), HOAt (1.0 equiv.), DMF, rt, 12 h, 80%; (f) **1** (1.0 equiv.), DCC (2.4 mmol), DMAP (0.5 equiv.), DMF, 70°C, 48 h, 60%; (g) *p*TsOH (cat.), MeOH, rt, 30 min, 89%.

thesizer cycle and workup. With respect to this stability, the ethidium derivative **3** is suitable in terms of a potential building block for oligonucleotide synthesis.

In order to evaluate the absorption and emission properties of the ethidium derivative **3**, we finally measured the UV/vis absorption and the steady-state fluorescence of **3** in MeCN and MeOH, representing two typical organic solvents without or with hydrogen-bonding capabilities, respectively (Fig. 2). Whereas the UV/vis absorption is very similar in both solvents, the fluorescence intensity is significantly different. In MeCN, the integrated emission of **3** is 3.7 fold higher than in MeOH, although the emission maxima reflect only a small difference. This result stands in agreement with the well-known electronic properties of ethidium (**1**) meaning that the quantum yield of the ethidium

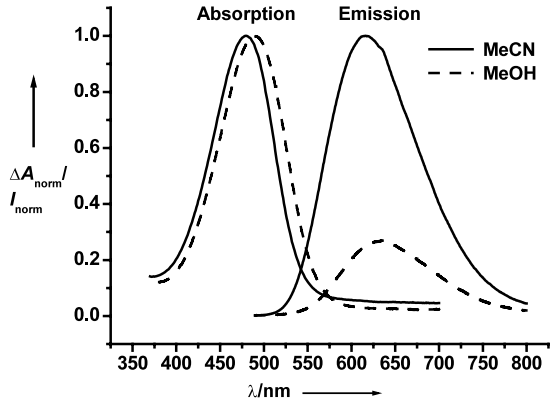


Figure 2. UV/vis absorption and steady-state emission spectra of the ethidium derivative **3** in MeCN (—) and MeOH (---).

fluorescence in MeOH is significantly lower due to quenching. In addition to the NMR characterization, the absorption maximum of **3** in the area of 480–490 nm clearly indicates that only one exocyclic amino group of the ethidium moiety has been derivatized. The additional derivatization of the exocyclic amino group in position C-3 of the ethidium part in **3** would result in a hypsochromic shift of the absorption spectrum to the area of 430–440 nm.[34]

3. Conclusion

In conclusion, we presented here the preparation of the ethidium nucleosides 8-(3′,5′-di-*O*-benzoyl-2′-deoxy-D-ribofuranosyl)-3-acetamido-5-ethyl-6-phenyl-phenanthridinium (**5**), 8-(3′,5′-di-*O*-acetyl-2′-deoxy-D-ribofuranosyl)-3-acetamido-5-ethyl-6-phenyl-phenanthridinium (**6**), and the acyclic analog 8-[(3′*R*)-1′,3′-dihydroxy-4′-yl]-acetamido-3-amino-5-ethyl-6-phenyl-phenanthridinium (**3**). Due to their different stability, only the acyclic derivative **3** is suitable as a potential building block for oligonucleotide synthesis. Furthermore, the acyclic ethidium nucleoside **3** exhibits the comparable absorption and emission properties of the underivatized ethidium (**1**).

Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft and the Volkswagen-Stiftung. N.A. and H.A.W. are grateful to Professor Horst Kessler, Technical University of Munich, for the generous support. We thank Melina Haupt for measuring the NH-HSQC of **5**.

References

1. Lerman, L. S. *J. Mol. Biol.* **1961**, *3*, 18–30.
2. (a) Misra, V. K.; Honig, B. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 4691–4695; (b) Patterson, S. E.; Coxon, J. M.; Stekowski, L. *Bioorg. Med. Chem.* **1997**, *5*, 277–281; (c) Medhi, C.; Mitchell, J. B. O.; Price, S. L.; Tabor, A. B.

Biopolymers **1999**, *52*, 84–93; (d) Reha, D.; Kabelác, M.; Ryjácek, F.; Šponer, J.; Šponer, J. E.; Elstner, M.; Suhai, S.; Hobza, P. *J. Am. Chem. Soc.* **2002**, *124*, 3366–3376.
3. (a) Waring, M. J. *J. Mol. Biol.* **1965**, *13*, 269–282; (b) LePecq, J.-B.; Paleotti, C. *J. Mol. Biol.* **1967**, *27*, 87–106; (c) Olmsted, J.; Kearns, D. *Biochemistry* **1977**, *16*, 3647–3654.
4. (a) Tettey, J. N. A.; Skellern, G. G.; Grant, M. H.; Midgley, J. M. *J. Pharm. Biomed. Anal.* **1999**, *21*, 1–7; (b) Tettey, J. N. A.; Skellern, G. G.; Midgley, J. M.; Grant, M. H.; Pitt, A. R. *Chemico-Biol. Interact.* **1999**, *123*, 105–115.
5. (a) Gaugain, B.; Barbet, J.; Oberlin, R.; Roques, B. P.; Le Pecq, J.-B. *Biochemistry* **1978**, *17*, 5071–5078; (b) Gaugain, B.; Barbet, J.; Capelle, N.; Roques, B. P.; Le Pecq, J.-B. *Biochemistry* **1978**, *17*, 5078–5088; (c) Kuhlmann, K. F.; Mosher, C. W. *J. Med. Chem.* **1981**, *24*, 1333–1337; (d) Lopp, I. G.; Wilson, W. D.; Boykin, D. W. *J. Heterocycl. Chem.* **1982**, *19*, 695–696.
6. (a) Benimetskaya, L.; Bulychev, N. V.; Kozionov, A. L.; Koshkin, A. A.; Lebedev, A. V. M.; Novozhilov, S. Y.; Stockman, M. I. *Biopolymers* **1989**, *28*, 1129–1147; (b) Krishnamurthy, G.; Polte, T.; Rooney, T.; Hogan, M. E. *Biochemistry* **1990**, *29*, 981–988; (c) Gritsan, N. P.; Koshkin, A. A.; Denisov, A. Y.; Markushin, Y. Y.; Cherepanova, E. V.; Lebedev, A. L. *J. Photochem. Photobiol. B: Biol.* **1997**, *37*, 40–51.
7. (a) Fromherz, P.; Rieger, B. *J. Am. Chem. Soc.* **1986**, *108*, 5361–5362; (b) Atherton, S. J.; Beaumont, P. C. *J. Phys. Chem.* **1987**, *91*, 3993–3997; (c) Pasternack, R. F.; Caccam, M.; Keogh, B.; Stephenson, T. A.; Williams, A. P.; Gibbs, E. J. *J. Am. Chem. Soc.* **1991**, *113*, 6835–6840; (d) Dunn, D. A.; Lin, V. H.; Kochevar, I. E. *Biochemistry* **1992**, *31*, 11620–11625; (e) Brun, A. M.; Harriman, A. *J. Am. Chem. Soc.* **1992**, *114*, 3656–3660; (f) Atherton, S. J.; Beaumont, P. C. *J. Phys. Chem.* **1995**, *99*, 12025–12029; (g) Jin, W. J.; Wei, Y. S.; Liu, C. S.; Shen, G. L.; Yu, R. Q. *Spectrochim. Acta A* **1997**, *53*, 2701–2707; (h) Kononov, A. I.; Moroshkina, E. B.; Tkachenko, N. V.; Lemmetyinen, H. *J. Phys. Chem. B* **2001**, *105*, 535–541; (i) Henderson, P. T.; Boone, E.; Schuster, G. B. *Helv. Chim. Acta* **2002**, *85*, 135–151.
8. (a) Kelley, S. O.; Holmlin, R. E.; Stemp, E. D. A.; Barton, J. K. *J. Am. Chem. Soc.* **1997**, *119*, 9861–9870; (b) Hall, D. B.; Kelley, S. O.; Barton, J. K. *Biochemistry* **1998**, *37*, 15933–15940; (c) Kelley, S. O.; Barton, J. K. *Chem. Biol.* **1998**, *5*, 413–425; (d) Wan, C.; Fiebig, T.; Kelley, S. O.; Treadway, C. R.; Barton, J. K.; Zewail, A. H. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 6014–6019.
9. (a) Morgan, G.; Walls, L. P. *J. Chem. Soc.* **1938**, 389–397; (b) Walls, L. P. *J. Chem. Soc.* **1945**, 294–300.
10. Watkins, T. I. *J. Chem. Soc.* **1952**, *3*, 3059–3064.
11. Berg, S. S. *J. Chem. Soc.* **1963**, *14*, 3635–3640.
12. Loccufier, J.; Schacht, E. *Tetrahedron* **1989**, *45*, 3385–3396.
13. Tettey, J. N. A.; Skellern, G. G.; Midgley, J. M.; Grant, M. H.; Wilkinson, R.; Pitt, A. R. *Xenobiotica* **1999**, *29*, 349–360.
14. 1,3,5-Tri-*O*-acetyl-2-deoxy-D-ribofuranose was prepared via a multi-step synthesis according to the following procedures: (a) Recondo, E. F.; Rinderknecht, G. L. *Helv. Chim. Acta* **1959**, *42*, 1171–1173; (b) Kline, P. C.; Serianni, A. S. *J. Am. Chem. Soc.* **1990**, *112*, 7373–7381; (c) Bhat, C. C. In *Synthetic Procedures in Nucleic Acids Chemistry*: *Vol. 1*; Zorbach, W. W.; Tipson, R. S., Eds.; John Wiley and Sons: New York, 1968; pp. 521–522; (d) Gold, A.; Sangaiah, R. *Nucleosides & Nucleotides* **1990**, *9*, 907–912.
15. Hoffer, M. *Chem. Ber.* **1960**, *93*, 2777–2781.
16. Marriott, D. P.; Bantick, J. R. *Tetrahedron Lett.* **1981**, *22*, 3657–3658.
17. (a) Vorbrüggen, H.; Krolikiewicz, K.; Bennua, B. *Chem. Ber.* **1981**, *114*, 1234–1255; (b) Vorbrüggen, H.; Höfle, G. *Chem. Ber.* **1981**, *114*, 1256–1268; (c) Vorbrüggen, H.; Bennua, B. *Chem. Ber.* **1981**, *114*, 1279–1286.
18. Whistler, R. L.; Wolfrom, M. L., Eds. *Methods in Carbohydrate Chemistry*: *Vol. 2*; Academic Press: New York, 1963; pp. 221–222, 332–335.
19. For reference example see: Rokos, H.; Pfleiderer, W. *Chem. Ber.* **1971**, *104*, 748–769. Other Lewis acids, such as BF_3, $SnCl_4$, and TMSOTf, were also tried.
20. Takeuchi, Y.; Chang, M.-r.; Hashigaki, K.; Yamato, M. *Chem. Pharm. Bull.* **1991**, *39*, 1629–1631.
21. Schwögler, A.; Gramlich, V.; Carell, T. *Helv. Chim. Acta* **2000**, *83*, 2452–2463.
22. Data of **5**: R_f=0.75 (silica gel, EtOAc:MeOH:H_2O=6:2:1). UV/vis (MeCN): λ_{max}=219, 292, 472 nm. ^{1}H signals were assigned by 2D experiments (DQF-COSY). ^{1}H NMR (500 MHz, [d_4]-MeOH): d=1.55 (t, 3 H, CH_2CH_3), 1.90 (s, 3 H, $NHCOCH_3$), 2.35–2.10 (m, 2 H, H-2/2′), 4.20–4.05, 3.80–3.70, 3.65–3.50 (m, 3 H, H-4/5/5′), 4.65 (q, 2 H, CH_2CH_3), 4.85–4.25 (m, 2 H, H-3′), 6.62, 6.54 (2d, 1 H, H-1′), 8.00–7.20 (m, 15 H, aromatic H), 7.70 (d, 1 H, H-7), 7.90 (s, 1 H, H-2), 8.15 (d, 1 H, H-9), 8.65 (d, 1 H, H-10), 8.75 (d, 1 H, H-1), 9.00 ppm (s, 1 H, H-4). NH-HSQC (500 MHz, [d_3]-MeOH): d=7.64/108.4 (8-N*H*), 7.51/90.7 (3-N*H*), 6.76/108.4 ppm (8-N*H*). ESI-MS: m/z=680.5 (100%) [M]$^+$.
23. Data of **6**: R_f=0.70 (silica gel, EtOAc:MeOH:H_2O=6:2:1). UV/vis (MeCN): λ_{max}=217, 291, 474 nm. ^{1}H signals were assigned by 2D experiments (DQF-COSY). ^{1}H-NMR (500 MHz, [d_4]-MeOH): d=1.55 (t, 3 H, CH_2CH_3), 2.05, 2.00, 1.85, 1.80 (2s, 6 H, $COCH_3$), 2.20 (s, 3 H, $NHCOCH_3$), 3.30–3.15 (m, 2 H, H-2/2′), 4.15–3.80, 3.65–3.45 (m, 3 H, H-4/5/5′), 4.65 (q, 2 H, CH_2CH_3), 5.20–4.95 (m, 2 H, H-3′), 6.56, 6.48 (2d, 1 H, H-1′), 7.80–7.55 (m, 5 H, aromatic H), 7.65 (d, 1 H, H-7), 7.90 (s, 1 H, H-2), 8.10 (d, 1 H, H-9), 8.65 (d, 1 H, H-10), 8.75 (d, 1 H, H-1), 9.00 ppm (s, 1 H, H-4). ESI-MS: m/z=557.5 (100%) [M]$^+$.
24. Kelley, S. O.; Holmlin, R. E.; Stemp, E. D. A.; Barton, J. K. *J. Am. Chem. Soc.* **1997**, *119*, 9861–9870.
25. Loubinoux, B.; Sinnes, J.-L.; O'Sullivan, A. C.; Winkler, T. *Tetrahedron* **1995**, *51*, 3549–3558.
26. Preparation of the starting material **9** was performed according to the procedure for the protection of the starting material **8** by an isopropylidene group: Blechert, S.; Dollt, H. *Liebigs Ann.* **1996**, 2135–2140. Data of **9**: R_f=0.95 (silica gel, MeOH). ^{1}H NMR (250 MHz, $CDCl_3$): d=1.55–1.61, 1.76–1.90 (m, 2 H, H-5), 2.45–2.69 (m, 2 H, H-2), 3.65 (s, 3 H, OCH_3), 3.93–3.98 (m, 2 H, H-4), 4.17–4.29 (m, 1 H, H-3), 5.49 (s, 1 H, Ph-C*H*), 7.27–7.58 ppm (m, 5 H, aromatic H).
27. Tester, R. W.; West, F. G. *Tetrahedron Lett.* **1998**, *39*, 4631–4634.

28. Data of **10**: $R_f = 0.95$ (silica gel, MeOH). ^{1}H NMR (250 MHz, $CDCl_3$): $d = 1.58$–1.63, 1.79–1.81 (m, 2 H, H-5), 2.50–2.77 (m, 2 H, H-2), 3.90–3.94 (m, 1 H, H-4), 3.95–3.99 (m, 1 H, H-4), 4.20–4.30 (m, 1 H, H-3), 5.50 (s, 1 H, Ph-C*H*), 7.27–7.56 ppm (m, 5 H, aromatic H).
29. Diederichsen, U.; Weicherding, D. *Synlett* **1999**, 917–920.
30. The pale yellow solid of **11** was used directly without characterization.
31. Data of **12**: $R_f = 0.60$ (silica gel, EtOAc:MeOH:H_2O = 6:2:1). UV/vis (MeOH): $\lambda_{max} = 214$, 291, 479 nm. ^{1}H and ^{13}C NMR signals were assigned according to 2D experiments (DQF-COSY and HMQC). ^{1}H NMR (500 MHz, [d_4]-MeOH): $d = 1.53$ (t, 3 H, CH_2CH_3), 1.64, 1.81 (m, 2 H, H-4′), 2.56, 2.75 (m, 2 H, H-2′), 4.01, 4.21 (m, 2 H, H-5′), 4.30 (m, 1 H, H-3′), 4.71 (q, 2 H, CH_2CH_3), 5.57 (s, 1 H, Ph-C*H*), 7.37 (s, 1 H, H-2), 7.36–7.38, 7.45–7.47, 7.50 (m, 5 H, Ph-H), 7.52 (s, 1 H, H-4), 7.70–7.73, 7.85–7.89 (m, 5 H, ethidium aromatic H), 7.94 (s, 1 H, H-7), 8.30 (d, 1 H, H-9), 8.81 (d, 1 H, H-10), 8.83 ppm (d, 1 H, H-1). ^{13}C NMR (125.8 MHz, [d_4]-MeOH): $\delta = 13.2$ (CH_2CH_3), 30.8 (C-4′), 43.3 (C-2′), 49.7 (CH_2CH_3), 66.6 (C-5′), 74.1 (C-3′), 98.5, 101.0 and 101.1 (Ph-C), 108.1, 110.2, 119.2, 120.0, 122.2, 123.3, 124.1, 125.4, 125.7, 125.8, 127.4, 127.5, 127.6, 128.0, 128.1, 128.2, 128.3, 128.4, 129.2, 129.3, 129.4, 129.9, 131.0, 131.7, 203.8 (C-1′). ESI-MS m/z (%): 518.4 (100) $[M]^+$.
32. Alberico, F.; Bofill, J. M.; El-Faham, A.; Kates, S. A. *J. Org. Chem.* **1998**, *63*, 9678–9683.
33. Data of **3**: $R_f = 0.20$ (silica gel, EtOAc:MeOH:H_2O = 6:2:1). UV/vis (MeOH): $\lambda_{max} = 215$, 295, 487. ^{1}H and ^{13}C NMR signals were assigned according to 2D experiments (DQF-COSY and HMQC). ^{1}H NMR (500 MHz, [d_4]-MeOH): $d = 1.56$ (t, 3 H, CH_2CH_3), 1.72, 1.83 (m, 2 H, H-4′), 2.51, 2.71 (m, 2 H, H-2′), 3.71, 3.77 (m, 2 H, H-5′), 4.21, 4.39 (m, 1 H, H-3′), 4.77 (q, 2 H, CH_2CH_3), 7.47 (s, 1 H, H-2), 7.49 (s, 1 H, H-4), 7.66–7.69, 7.81–7.85 (m, 5 H, aromatic H), 7.95 (s, 1 H, H-7), 8.31 (d, 1 H, H-9), 8.77 (d, 1 H, H-10), 8.79 ppm (d, 1 H, H-1). ^{13}C NMR (125.8 MHz, [d_4]-MeOH): $\delta = 13.2$ (CH_2CH_3), 39.1 (C-4′), 44.8 (C-2′), 49.7 (CH_2CH_3), 58.1 (C-5′), 65.8 (C-3′), 98.5, 108.2, 110.0, 119.0, 119.9, 122.1, 122.5, 123.3, 123.9, 125.4, 127.4, 128.0, 128.1, 129.2, 129.3, 130.0, 130.9, 131.0, 131.7, 198.4 ppm (C-1′). ESI-MS m/z (%): 430.4 (100) $[M]^+$.
34. According to the comparison of the UV/vis spectra (MeCN) of 8-acetamido-3-amino-5-ethyl-6-phenyl-phenanthridium ($\lambda_{max} = 216$, 291, 477 nm) and 3,8-diacetamido-5-ethyl-6-phenyl-phenanthridium ($\lambda_{max} = 213$, 284, 431 nm).