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Synthesis and DNA binding properties of novel benzo[*b*]isoquino[2,3-*h*]-naphthyridines†‡

Oliver Koepler,[a] Stefania Mazzini,*[b] Maria Cristina Bellucci,[b] Rosanna Mondelli,[b] Angelika Baro,[c] Sabine Laschat,*[c] Marc Hotfilder,[d] Christophle Viseur[d] and Wolfgang Frey[e]

[a] *Institut für Organische Chemie, Technische Universität Braunschweig, Hagenring 30, D-38106, Braunschweig, Germany*

[b] *Dipartimento di Scienze Molecolari Agroalimentari, Università di Milano, Via Celoria 2, 20133, Milano, Italy. E-mail: rosanna.mondelli@unimi.it.; Fax: +02-503-16801; Tel: +02-503-16810*

[c] *Institut für Organische Chemie, Universität Stuttgart, Pfaffenwaldring 55, D-70569, Stuttgart, Germany. E-mail: sabine.laschat@oc.uni-stuttgart.de; Fax: +49(0)711 685 4285; Tel: +49 (0)711 685 4565*

[d] *Klinik und Poliklinik für Kinderheilkunde, Pädiatrische Hämatologie/Onkologie, Domaghstr. 9a, D-48149, Münster, Germany*

[e] *Institut für Anorganische und Analytische Chemie, Technische Universität Braunschweig, Hagenring 30, D-38106, Braunschweig, Germany*

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Several benzo[*b*]isoquino[2,3-*h*]-naphthyridines have been prepared *via* formal hetero-Diels Alder reaction of *N*-aryl imines as a key step. These compounds have different side chains at C-11, and a *cis* or *trans* configuration at the C-8a,C-14a ring junction. Binding constants for the interaction with oligonucleotides and polynucleotides were determined by UV absorption and melting experiments. NMR experiments (NOE) revealed that the *cis* isomers, showing a slightly folded structure, preferentially bind to the minor groove of AT-rich oligomers. In contrast, the *trans* isomers prefer the CG-rich sequences, leading to *cap*-complexes with the isoquinoline moiety stacked at the top of the double helix, in agreement with the flatter shape, and with a preference for the 3′-terminals, as found for camptothecins. Models of the complexes were built up by molecular dynamics (MD) calculations, by using the inter-proton distances derived from the NOE values. Cytotoxicity assays against human Ewing sarcoma cell lines RD-ES and CAD-ES1 were performed.

Introduction

Recent studies from our laboratory have shown that isoquinoline derivatives of type **1** (Scheme 1), exhibit *in vitro* cytotoxic activity against three different human brain tumour cell lines.[1] In order to improve the activity, we synthesized some benzo[*b*]isoquino[2,3-*h*]naphthyridines (*e.g.* **2**), which have less flexible conformations and an increased cytotoxicity against the same cell lines.[2]

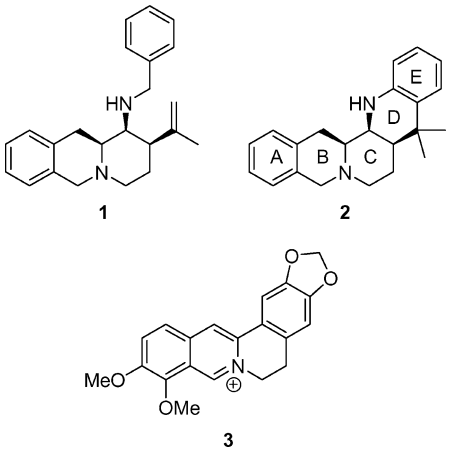
The aim of this work is to find out whether or not these molecules can bind to DNA, as well as the mode of binding to DNA and their specificity for particular DNA sequences. As DNA remains the main target of antitumour drugs,[3–5] knowledge of the mode of binding at a molecular level is the first step in rational drug design. For these studies, the interaction of small molecules with oligonucleotides is a good model of DNA interaction, because a relatively short sequence is generally involved in the recognition process. We selected some of these compounds for a spectroscopic study, comparing them with camptothecins[6] and berberine **3** (Scheme 1), an isoquinoline plant alkaloid belonging to the structural class of protoberberines, known to be DNA binders[5,7,8] and to show antitumour activity.[9]

We synthesized analogues of **2** with different side chains on the benzene ring E (in order to modulate the solubility in water) and with *cis* and *trans* configurations at the C-8a,C-14a ring junction (Scheme 2). In order to allow convenient synthesis of analogues, the pentacyclic benzo[*b*]isoquino[2,3-*h*]-naphthyridine system was prepared *via* a convergent one-pot imine condensation/hetero-Diels–Alder reaction[10] using functionalised aniline derivatives, which could be further modified.

We studied the *in vitro* cytotoxicity of these compounds and their interaction with DNA sequences by using UV and NMR spectroscopy, specifically nuclear Overhauser experiments.[11] Poly(dA–dT)$_2$, poly(dG–dC)$_2$ and salmon sperm DNA were used for UV spectra. The self-complementary duplexes

† Electronic supplementary information (ESI) available: NMR spectral data and crystal structure data. See http://dx.doi.org/10.1039/b503281d

‡ Dedicated to Professor Horst Kunz on occasion of his 65th birthday.



Scheme 1

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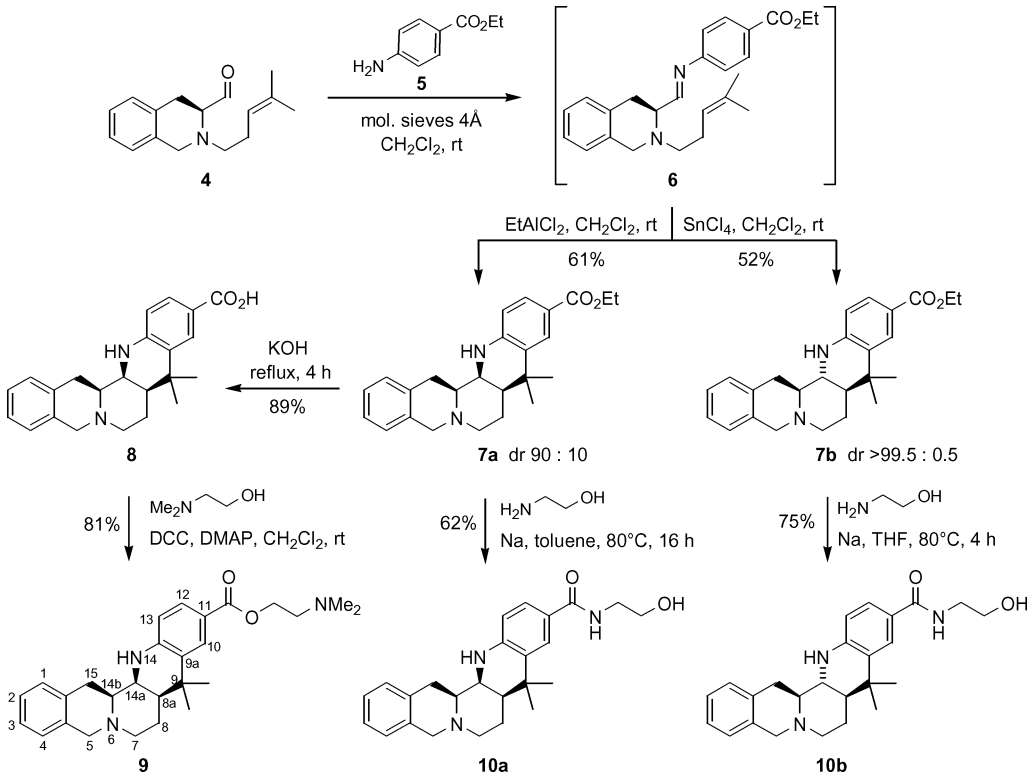


Scheme 2 Preparation of water-soluble benzo[*b*]isoquino[2,3-*h*]-1,7-naphthyridine derivatives **9** and **10a**, as well as the corresponding *trans*-configured derivative **10b** starting from tetrahydroisoquinoline-3-carbaldehyde **4**.

d(AAGAATTCTT)$_2$ (AATT), d(CGACGTCG)$_2$ (ACGT), d(GCGATCGC)$_2$ (GATC), d(GCATCGCGATGC)$_2$ (CGCG) and d(CGTATACG)$_2$ (TATA) were used for the NMR study.

Results and discussion

Syntheses

Tetrahydroisoquinoline-3-carbaldehyde **4** (*S* enantiomer) was obtained in four steps starting from L-phenylalanine in 54% overall yield.[12] As outlined in Scheme 2, condensation of aldehyde **4** with ethyl 4-aminobenzoate (**5**) in the presence of 4 Å molecular sieves yielded the corresponding intermediate imine **6**, which was directly treated with $EtAlCl_2$ to give the pentacyclic product **7a** in 61% yield with high *cis* diastereoselectivity (*cis*/*trans* = 90 : 10).

The cyclization can be considered to be a formal hetero-Diels–Alder reaction, although previous mechanistic studies with related systems clearly indicated a stepwise mechanism.[13,14] Hydrolysis of ethyl ester **7a** with KOH gave the carboxylic acid **8** in 89% yield. Conversion of **8** with *N*,*N*-dimethylaminoethanol and DCC afforded ester **9** in 81% yield. Then amide **10a** was prepared in 62% yield from ethyl ester **7a** by reaction with aminoethanol.

The structure of benzo[*b*]isoquino[2,3-*h*]naphthyridines **7a**, **8**, **9**, and **10a** was confirmed by ^{1}H and ^{13}C NMR spectroscopy. The all-*cis* configuration follows from the values of the coupling constants involving the protons at C-8a, C-14a, C-14b, and C-15 (see Table 1, and the discussion in the next paragraph).

Compounds with an all-*trans* configuration would have a flatter shape, which might induce a different mode of interaction to the double helix. In order to get access to the *trans* series, imine **6** was treated with $SnCl_4$ to give the pentacyclic *trans* diastereomer **7b** in 52% yield. The diastereomeric ratio of the crude mixture was **7a** : **7b** = 0.5 : 99.5 (Scheme 2). Treatment of ester **7b** with aminoethanol and sodium in THF at 80 °C gave the amide **10b** in 75% yield. An X-ray crystal structure analysis of **7b** and the coupling constants measured for **7b** and **10b** (Table 1) proved the relative configuration and the overall flat structure for the compounds of the *trans* series, as shown in Fig. 1.[15] As the stereocentre C-14b is *S*, the absolute configuration must be 8a*R*,14a*R*,14b*S*.

Table 1 Coupling constant values for the B and C rings of two selected benzo[*b*]isoquino[2,3-*h*]-naphthyridines with all-*cis* (**9**) and all-*trans* (**10b**) configuration[a]

J(H,H)	**9**	**10b**
5ax,5eq	15.3	15.1
7ax,7eq	11.6	11.5
8ax,8eq	13.3	13.1
7ax,8ax	12.8	12.2
7ax,8eq	3.3	2.5[b,c]
7eq,8ax	4.1	3.8
7eq,8eq	2.7	3.0
8a,8ax	12.3 (ax,ax)	12.3 (ax,ax)
8a,8eq	3.4 (ax,eq)	3.3 (ax,eq)
8a,14a	3.1 (**ax**,**eq**)	10.7 (**ax**,**ax**)
14a,14b	2.3 (**eq**,**ax**)	8.5 (**ax**,**ax**)
14a,NH[b,c,d]	~1.5	0.7
14b,15ax	11.3 (ax,ax)	10.5 (ax,ax)
14b,15eq	4.8 (ax,eq)	4.3 (ax,eq)
15ax,15eq	16.7	15.9
^5J(5ax,15ax)[b,c]	1.1	1.3
^4J(8eq,14a)[b,c]	1.3	0
^4J(8a,NH)[b,c]	1.5	0

[a] Measured in Hz by 1D experiments, solvent $CDCl_3$, estimated accuracy within ±0.05 Hz, unless otherwise specified. **7b** shows the same values as **10b** (see the Supplementary material). [b] Estimated accuracy ±0.1 Hz. [c] Proved by decoupling experiments. [d] These values may be affected by exchange due to the small amount of acid, present in the unstabilised $CDCl_3$.

Next we investigated the cyclization of the *N*-arylimine **11**, which was formed *in situ* from aldehyde **4** and 3,4-methylenedioxyaniline. As shown in Scheme 3, treatment of imine **11** with $SnCl_4$ followed by aqueous workup gave a diastereomeric mixture of the all-*cis* product **12a** (22%) and the all-*trans* product **12b** (37%), which could be separated by column chromatography. The decrease in the *cis* diastereoselectivity is

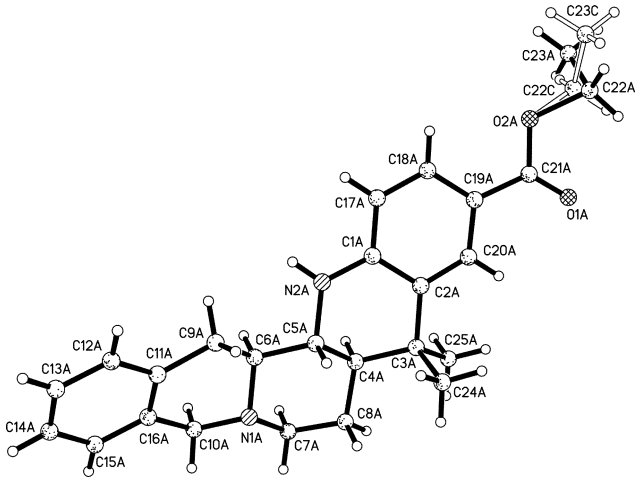
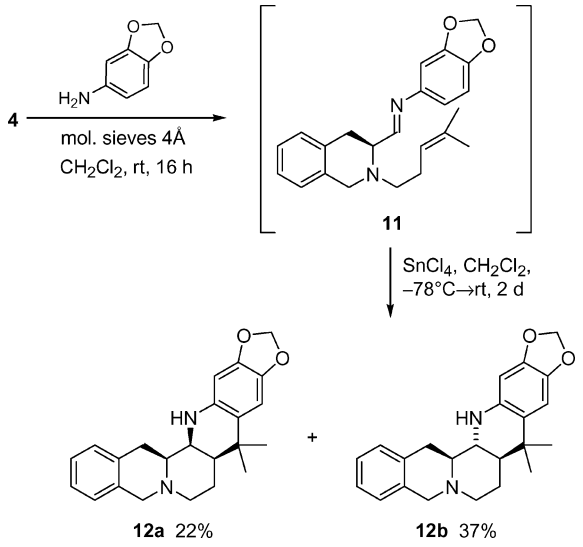


Fig. 1 ORTEP view of **7b**, confirming the relative *trans* configuration. Please note that the X-ray numbering is different from the IUPAC numbering used in Scheme 2 and in the remaining text. Only one of the two unique molecules in the asymmetric unit it shown.

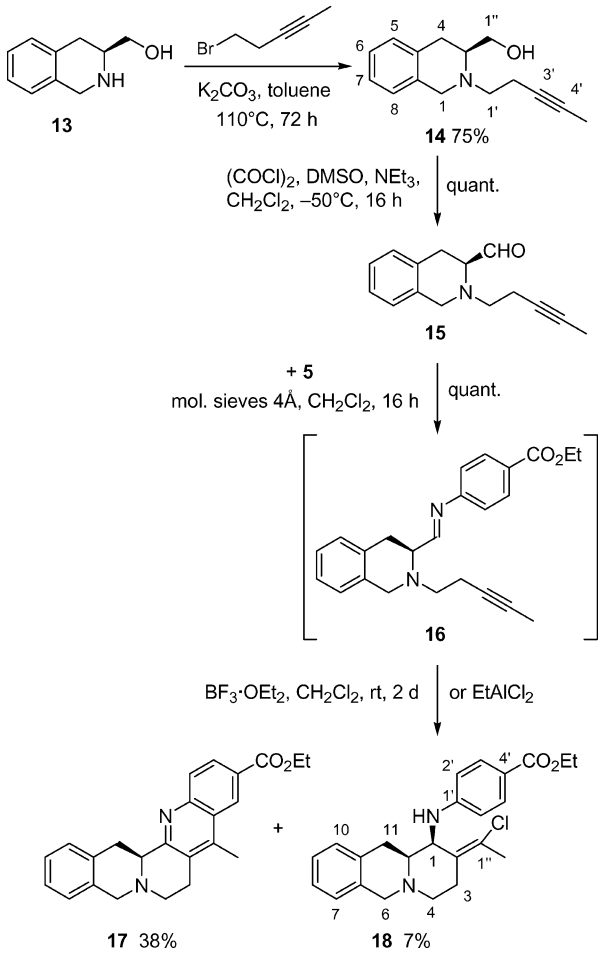


Scheme 3 Preparation of diastereomers **12a** and **12b**.

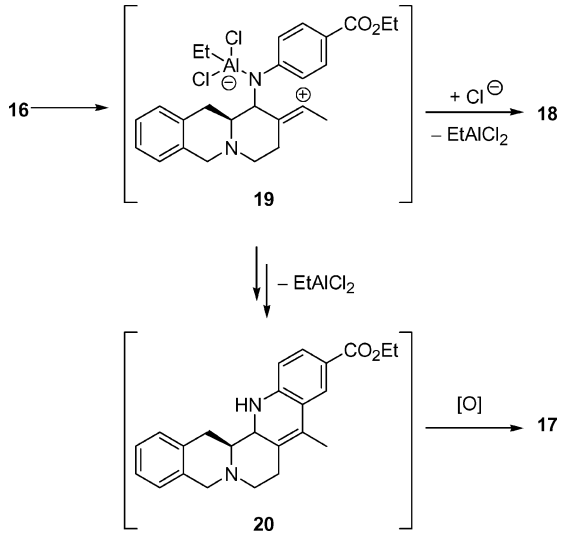
probably due to the presence of two additional electron-donating ether moieties, which are capable of binding the Lewis acid and thus of interfering with a chelation-controlled cyclization mechanism.[13,14]

In order to improve the flatness of the C-ring in the benzo[*b*]isoquino[2,3-*h*]naphthyridines, we attempted to cyclize a tethered alkyne moiety instead of an alkene. Tetrahydroisoquinoline alcohol **13** was therefore alkylated with 1-bromo-3-pentyne in the presence of K_2CO_3 in refluxing toluene to afford the alcohol **14** in 75% yield (Scheme 4). Compound **14** was submitted to Swern oxidation giving aldehyde **15**,[16] which was treated with ethyl 4-aminobenzoate (**5**) under the usual conditions without further purification. Subsequent addition of $BF_3{\cdot}OEt_2$ and aqueous workup after 2 days reaction time resulted in the formation of the quinoline ester **17** in 38% yield. When $BF_3{\cdot}OEt_2$ was replaced by $EtAlCl_2$, the chloro compound **18** was isolated in 7% yield as a minor by-product.

The isolation of by-product **18** further supports the cationic cyclization mechanism depicted in Scheme 5. Thus, in presence of $EtAlCl_2$, the cyclization of imine **16** should give carbenium ion **19**, which either can be trapped by external chloride to yield compound **18** or can undergo Friedel–Crafts-type electrophilic aromatic substitution, followed by tautomerization to give **20**. Oxidation of **20** during workup finally results in aromatization of the C-ring.



Scheme 4 Preparation of benzo[*b*]isoquino[2,3-*h*]naphthyridine **17** with enhanced flatness in the C-ring.



Scheme 5 Proposed cationic cyclization mechanism.

Conformational analysis and relative stereochemistry

The conformation and the relative configuration of **7a**, **7b**, **9**, **10a**, and **10b** were derived from the H,H coupling constants, with the help of 2D NOESY and COSY experiments. The values for two compounds, **9** and **10b** as examples of *cis* and *trans* configurations, are reported in Table 1; the data for all compounds are provided in the Supplementary material†. The axial orientation of 14b-H and 8a-H is proved for both compounds by the high *J* values for the coupling with the axial protons of the vicinal methylene groups, 15-CH_2 and 8-CH_2, respectively, *i.e.* 12.3–12.5 Hz for *J*(8a,8ax) and 11.3–11.8 Hz for *J*(14b,15ax).

The small coupling constants with the vicinal protons shown by H-14a prove the equatorial orientation of this proton and thus, the all-*cis* configuration of **7a**, **9** and **10a**. In contrast, the values of 10.6 and 8.5 Hz found for J(8a,14a) and J(14a,14b) in the case of **7b** and **10b** prove the axial orientation of these protons, and consequently the all-*trans* configuration. The lower value of J(14a,14b) with respect to J(8a,14a), is due to the electronegativity effect of the nitrogen atom bound to C-14b. To the same effect are due the low values of J(14a,14b) in the all-*cis* stereoisomers, *i.e.* 1.5–2.0 Hz, compared with the coupling between equatorial and axial protons in cyclohexanes.[17]

The conformation of ring C is a perfect chair, while rings B and D are half-chairs, for all compounds. In the case of the half-chair conformation, the axial and equatorial orientations stand for *pseudo*-axial and *pseudo*-equatorial; the dihedral angles for axial–axial protons are 160–180°, and those for axial–equatorial are 50–60°. The molecules are quite rigid and have a folded structure in the case of the all-*cis* series, whereas the all-*trans* compounds **7b** and **10b** are rather flat (Fig. 2).

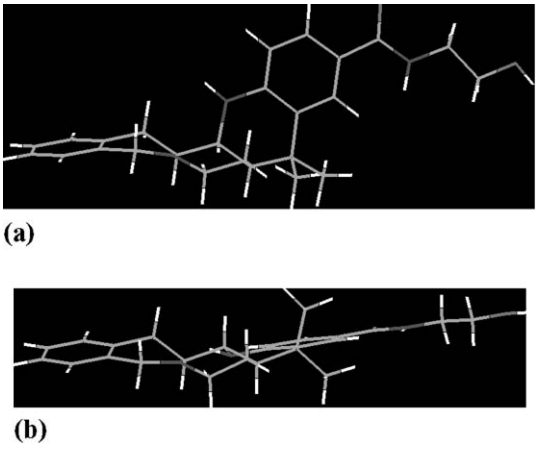


Fig. 2 Energy minimized structures of: (a) the all-*cis* **10a**, and (b) the all-*trans* **10b** stereoisomers.

DNA binding properties

We used UV spectroscopy as a first approach for studying the DNA binding properties of these compounds. The binding constant, obtained by both UV wavelength variations and UV melting experiments (Table 2), show that in the case of the *cis* isomers **7a**, **9** and **10a** the interaction with poly(dA–dT)$_2$ is preferred (K of the order of 10^4 M^{-1}) over poly(dG–dC)$_2$ (K of the order of 10^3 M^{-1}). In contrast, the molecules with a *trans* configuration, **7b** and **10b**, appear to prefer the CG sequence (K values *ca.* 10^4 M^{-1}), whereas the constants for the AT-rich sequences are of the order of 10^2 M^{-1}. The K values obtained with oligonucleotides of different sequences confirm these results and allowed us to select the oligomers for the NOE experiments.

The UV melting experiments were performed with the pair of stereoisomers **10a** and **10b** (see Table 2), just to confirm the results described above. The increase of the melting temperature of the duplex is 2 °C, which means that stabilization (albeit poor) of the double helix occurs, as found for berberine[8] and for other minor-groove binders.[18,19]

The aggregation process is of the same order of magnitude observed for campthotecins;[6] the dimerization constants K_D (10^3 M^{-1}) were included in the calculation of the binding constants.

The preference for the AT-rich sequence by the *cis* compounds and for the CG-rich sequences by the *trans* isomers was also suggested by the variations of ^{1}H chemical shift, found in the titration experiments performed with the same oligonucleotides. The data are reported in the Supplementary material.† Upfield shifts of 0.1–0.2 ppm were found for **9** and **10a** with d(AAGAATTCTT)$_2$, but of less than 0.1 ppm with the CG-rich oligomer d(GCGATCGC)$_2$. The inverse occurs for **10b**: an upfield shift of 0.1–0.2 ppm with d(GCGATCGC)$_2$ and the other CG-rich oligomers, but of less than 0.1 ppm with d(AAGAATTCTT)$_2$. A small shift variation was observed for the protons of the duplexes (upfield $\Delta\delta$ in the range 0.05–0.1). In the case of the complexes **10b**/d(GCGATCGC)$_2$ and **10b**/d(CGTATACG)$_2$, the imino NH protons of the terminal CG base-pairs appear shielded by *ca.* 0.3 ppm, as found for campthotecines;[6] because their signals become very broad, a more accurate value could not be obtained.

The binding constants, as well as the proton shift variation of the ligand, are not diagnostic for the mode of binding to DNA, because their values are the sum of contributions derived from specific and non-specific interactions. In addition, the external non-specific ionic interactions of a positively charged drug with the negatively charged ionic surface of the oligonucleotide are the main factors responsible for such effects. However, they can suggest, as in this case, a preference for the AT or CG sequence.

The variation of ^{31}P chemical shifts is negligible, *i.e.* less than 0.1 ppm, for all the complexes. This is the first evidence for excluding the intercalation of these molecules into the double helix.[20,21] One example is reported in the Supplementary material.†

NOE Experiments and structure derivation of the complexes with d(AAGAATTCTT)$_2$ and d(CGACGTCG)$_2$

^{1}H NOE experiments, which allow the detection of specific contacts between protons of the ligand and protons of the duplex, were performed in order to recognize the preferred interaction sites. The resonances of the oligomers were assigned, for both free and bound species, following well-established procedures[11] for the analysis of double-stranded oligonucleotides. The sequential assignment of the nucleotide units was performed by detecting the NOE cross-peaks between the aromatic protons of the bases and the 1′,2′,2″-ribose protons of the 5′-neighbour unit, thus allowing the recognition of the B-DNA structure of the

Table 2 Binding constants (K) for the interaction with oligonucleotides and self-association constants (K_D) for benzo[*b*]isoquino[2,3-*h*]naphthyridines[a]

K/M^{-1}	**7a** (all-*cis*)	**7b** (all-*trans*)	**9** (all-*cis*)	**10a** (all-*cis*)	**10b** (all-*trans*)	Topotecan[6]
Salmon sperm DNA	$(3 \pm 0.2) \times 10^3$	$(3 \pm 0.1) \times 10^4$	$(5 \pm 0.2) \times 10^4$	—	$(6 \pm 0.3) \times 10^4$	—
Poly(dA–dT)$_2$	$(1 \pm 0.2) \times 10^4$	$(8 \pm 0.2) \times 10^2$	$(5 \pm 0.1) \times 10^4$	$(8 \pm 0.3) \times 10^4$	$(7 \pm 0.3) \times 10^2$	$(2 \pm 0.3) \times 10^3$
Poly(dG–dC)$_2$	$(1 \pm 0.1) \times 10^3$	$(2 \pm 0.3) \times 10^4$	$(7 \pm 0.2) \times 10^3$	$(4 \pm 0.1) \times 10^3$	$(8 \pm 0.1) \times 10^4$	$(7 \pm 0.1) \times 10^4$
d(AAGAATTCTT)$_2$	$(9 \pm 0.1) \times 10^2$	$(3 \pm 0.3) \times 10^2$	$(1 \pm 0.1) \times 10^4$	$(1 \pm 0.2) \times 10^4$[b]	$(9 \pm 0.2) \times 10^2$	$(9 \pm 0.5) \times 10^2$
d(GCGATCGC)$_2$	$(1 \pm 0.1) \times 10^2$	$(5 \pm 0.2) \times 10^3$	$(1 \pm 0.1) \times 10^3$	$(6 \pm 0.1) \times 10^3$	$(9 \pm 0.1) \times 10^3$[c]	$(1 \pm 0.1) \times 10^4$
d(CGACGTCG)$_2$	—	$(6 \pm 0.1) \times 10^3$	—	—	$(2 \pm 0.1) \times 10^4$	—
d(GCATCGCGATGC)$_2$	$(7 \pm 0.1) \times 10^2$	$(1 \pm 0.1) \times 10^4$	$(2 \pm 0.2) \times 10^3$	$(3 \pm 0.2) \times 10^3$	$(3 \pm 0.1) \times 10^4$	—
d(CGTATACG)$_2$	—	$(7 \pm 0.2) \times 10^2$	—	—	—	—
K_D (M^{-1})	$(7 \pm 0.2) \times 10^3$	$(9 \pm 0.2) \times 10^2$	$(1 \pm 0.2) \times 10^3$	$(2 \pm 0.1) \times 10^3$	$(1 \pm 0.1) \times 10^3$	$(2 \pm 0.1) \times 10^3$

[a] Measured by UV absorption at 20 °C, pH 5.8 and with 0.02 M NaCl, unless specified. The values were derived from the experimental data by solving a system of a non-linear equations and using MATLAB software (v. 5.1), reported in ref. 8. The dimerization costant was included in the calculations. [b] $K = (4 \pm 0.2) \times 10^4$ M^{-1} by UV melting experiments, $\Delta T = 2.0$ °C. [c] Measured by UV melting experiments, $\Delta T = 1.5$ °C.

Table 3 Inter-molecular NOE interactions and inter-proton distances in the complexes **9** or **10a**/d(AAGAATTCTT)$_2$ and **10b**/d(CGACGTCG)$_2$[a]

			Distances/Å[b]	
AATT	**9** or **10a**	NOE_{exp}	Strand I	Strand II[c]
H-2[d]	H-2 A_4	w[e]	4.77	13.81
H-1	H-2 A_5	m	4.66	6.78
H-2	H-2 A_5	m	3.19	11.68
H-2	H-1′ A_5	w	4.75	10.88
H-1	H-1′ T_6	m	3.10	6.21
H-2	H-4′ T_6	m	3.95	5.84
H-1	H-1′ T_7	w	7.20	4.12
H-2	H-1′ T_7	s	8.44	2.50
H-3	H-4′ A_4	w	4.95	15.45
H-3	H-1′ T_6	zero NOE	5.47	8.09
H-3	H-1′ T_7	m	10.71	3.00
H-3	H-1′ C_8	w	10.03	4.34
H-4	H-2 A_5	zero NOE	6.75	10.81
H-4	H-1′ A_5	w	4.37	15.11
H-4	H-4′ A_5	w	4.37	14.02
H-4	H-1′ T_6	zero NOE	6.23	10.07
H-4	H-2 A_4	w	5.00	11.58
H-10	H-4′ T_6	zero NOE	8.92	16.38
H-13	H-4′ T_6	w	4.49	12.51
ACGT	**10b**	**NOE_{exp}**	**Distances/Å[b]**	
H-1[f]	H-8 G_8	m	3.54	
H-2	H-8 G_8	m	3.35	
H-3	H-8 G_8	w	4.68	
H-1	H-2″ G_8	m	3.18	
H-2	H-2″ G_8	m	4.14	
H-3	H-2′ G_8	w	4.95	
H-1	H-1′ G_8	w	4.51	
H-2	H-1′ C_7	w	5.00	

[a] Acquired at 20 °C, in D_2O, at pH 5.8,and with 0.02 M NaCl. [b] Obtained from the final structure of the complex after MD calculations. [c] See Fig. 4. [d] 1-H and 2-H have close chemical shifts. [e] Intensity of the NOE signals, estimated as follows: w = 4.5–5.5, m = 3.5–4.5, s = 2.5–3.5, by using as a reference the cross-peak of 5-H/6-H of cytidines (2.5). [f] 1-H and 2-H are coincident.

double helix. The formation of the complex was monitored by titration experiments of the drug against the nucleotide solution, and the NOESY spectra were acquired at $R = [drug]/[DNA] = 1$, 1.5 and 2. An example is shown in Fig. 3 and the results are reported in Table 3.

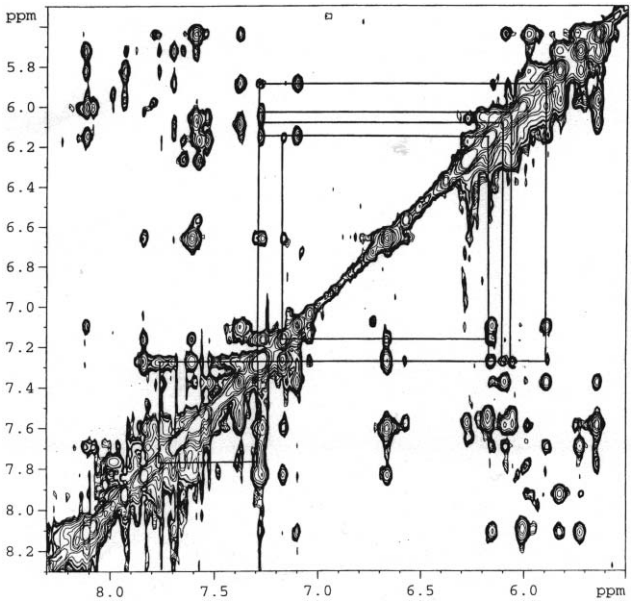


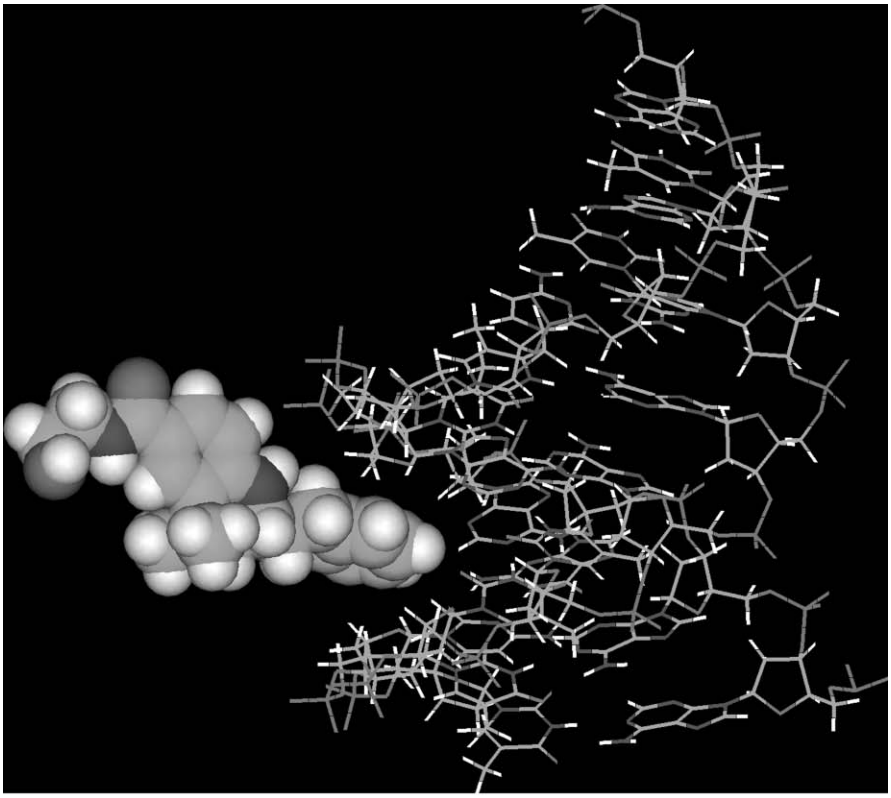
Fig. 3 2D NOESY spectrum of the complex **10a**/d(AAGAATTCTT)$_2$, $R = 1$ (aromatic and H-1′ region, 20 °C, t_{mix} 300 ms, in D_2O, pH 5.8, 0.02 M NaCl).

The very low solubility in water of **7a** and **7b** precluded any NMR experiment in this solvent, but the analogues with amino or hydroxyalkyl side-chains are enough soluble at pH 5.8–6.0 to perform NOE experiments. The interaction of **9** and **10a** with d(AAGAATTCTT)$_2$ led to NOE signals involving the aromatic protons of ring A and protons of the nucleotide located in the minor groove of the double helix, specifically of the adenines A_4, A_5 and of the thymines T_6 and T_7. The aromatic protons 1-H and 2-H on ring A of the ligand have very close chemical shifts and cannot be distinguished in the NOE experiments, but this does not invalidate the results, because they are vicinal and their partner on the same ring, 3-H, shows interactions with the same units, *i.e.* 4′-H of A_4 and 1′-H of T_7 and C_8. In addition, 4-H has interactions with 2-H of adenine A_4 and with the ribose protons 1′-H and 4′-H of A_5. The aromatic proton 13-H located on ring E shows an interaction with 4′-H of T_6, which indicates that the 14-NH group is oriented towards the helix.

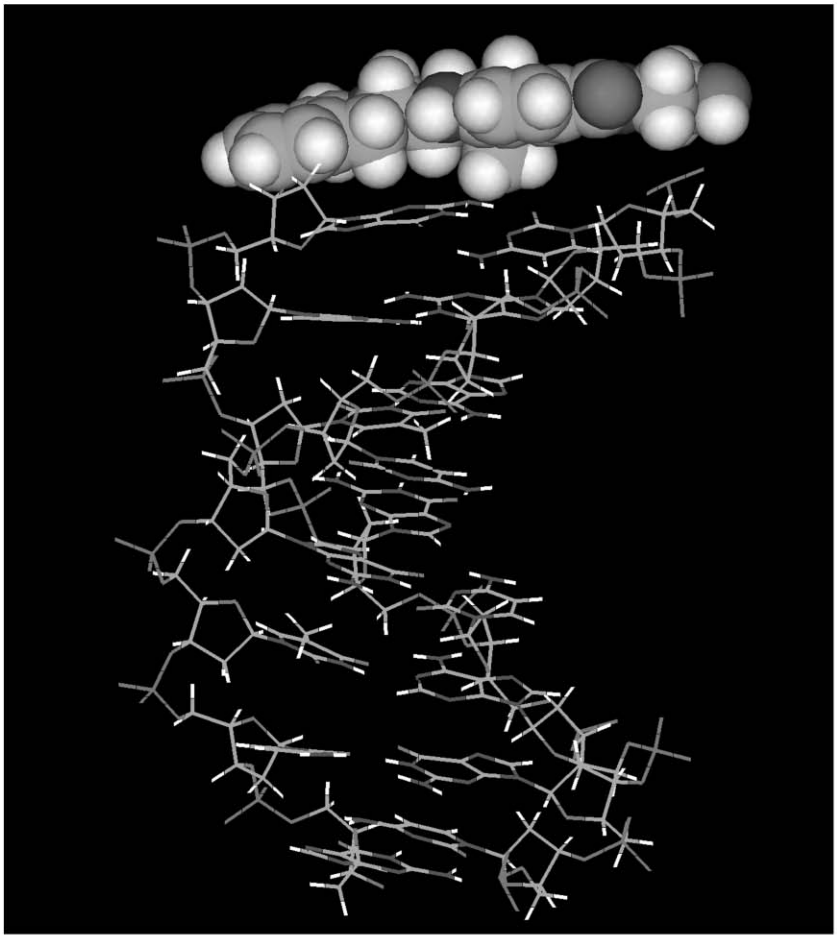
From the structural model of d(AAGAATTCTT)$_2$ we developed models for the complexes with **9** and **10a**, by docking the molecules into the DNA in an orientation suggested by the experimental NOEs. The MD study of the complexes was performed by using the simulations with CVFF force field on the picosecond time scale, in presence of a sphere of water molecules. After a first minimization, a 10 ps simulation was performed, with no restraints except for the NOE contacts and the hydrogen bonds between the base pairs. The models of the complexes with **9** and **10a** are very similar, as appears from the inter-proton distance values; therefore, only the data for **10a** are reported in Table 3, those for the other compounds are in the Supplementary material†. The result of the interaction of **9** and **10a** with d(AAGAATTCTT)$_2$ shows that these molecules are partial minor-groove binders, as the planar moiety of the molecule (ring A) is deeply inserted in the groove, ring B is partially inserted, while the other rings C, D and E are more external, but with 13-H on ring E relatively close to the phosphate oxygen atoms of T_6 and with the 14-NH group facing the helix (Fig. 4a). This is a consequence of the folded shape (Fig. 2), due to the all-*cis* configuration.

No NOE contacts were detected for **9** and **10a** with the CG-rich oligonucleotides examined. The wider minor groove of these duplexes might not allow contacts between the ligand and the walls of the groove, but on the other hand, the AT-rich minor groove has a relatively negative electrostatic potential,[22] which significantly enhances its ability to bind positively charged molecules. Actually, the binding constant values show that the interaction of *cis* stereoisomers with poly(dG–dC)$_2$ and with CG-rich sequences is poor.

In contrast, the all-*trans* compounds **7b** and **10b** with a flatter shape, not suitable for minor-groove binding, did not show any NOE signals with d(AAGAATTCTT)$_2$, but preferred interactions with the CG-rich sequences. Compound **7b** is not soluble enough for NMR experiments, but when **10b** is added to d(CGACGTCG)$_2$, NOE signals involving the aromatic protons of ring A appear. In fact, 1-H, 2-H and 3-H interact with protons of the CG base pairs at the terminal ends of the oligomers, specifically with 8-H of G_8 and with ribose protons of G_8 and C_7, whereas zero NOEs were found for 4-H. The bases located in the inner part of the duplex did not show any contact. The structural model obtained for this complex shows the interacting molecule located at the top of the helix, with the A and B rings stacked with the guanine of the 3′-terminal ends (Fig. 4b). Similar *cap*-complexes were found for topotecan and other camptothecins,[6] with the same preference for the 3′-terminal end sites. In order to confirm this preference, we examined the interaction with d(GCGATCGC)$_2$: in this case the NOE contacts were found between the above-mentioned protons of ring A and 6-H of C_8 and 2″-H of G_7, but not with protons of C_1 and G_2, thus confirming the preference for the 3′-terminal ends. The formation of *cap*-complexes might stabilize the double helix of oligonucleotides, as occurs for the dangling ends, which can



(a)



(b)

Fig. 4 Energy minimized molecular models of the complexes: (a) **10a**/d(AAGAATTCTT)$_2$: the planar moiety of the molecule (ring A) is deeply inserted in the groove (contacts with A_4, A_5, T_6 and T_7), ring B is partially inserted, while the other rings C, D and E are more external, but 13-H on ring E is relatively close to the phosphate oxygen atoms of T_6; (b) **10b**/d(CGACGTCG)$_2$: the A and B rings are stacked with the guanine G_8 of one of the two 3′-terminal ends (only one ligand molecule is drawn).

Table 4 LC_{50} values [μmol L^{-1}] of benzo[*b*]isoquino-[2,3-*h*]-naphthyridines **7a,b**, **9**, **10a,b**, **12a,b** and **17** against two human Ewing sarcoma cell lines[a]

Cell line	**7a**	**7b**	**9**	**10a**	**10b**	**12a**	**12b**	**17**
RD-ES	190	197	128	353	318	183	427	831
CAD-ES1	—[b]	21	138	1029	287	145	1550	2216

[a] LC_{50} values refer to concentrations at which 50% of the tumour cells survived. For details see ref. 22. [b] The observed LC_{50} value was >1000 μmol L^{-1}.

stabilize duplexes as much as an additional base-pair,[23] but this should not be relevant in the case of polynucleotides. However, the capping binding mode of small molecules to nucleotides has received recent attention for many reasons, for instance because many biochemical processes involve steps occurring at the interface between a terminus and a ligand protein.[24]

Cytotoxicity studies

Compounds **7a,b**, **9**, **10a,b**, **12a,b**, and **17b** were used for *in vitro* cytotoxicity tests against two different human tumour cell lines, RD-ES and CAD-ES1 (Ewing's sarcoma) (Table 4).[25] The LC_{50} values for both cell lines are high, except for compound **7b** against CAD-ES1 cells (LC_{50} = 21 μM), however its activity appears to be modest. As the binding properties of **7b** and **10b** are similar, the lower LC_{50} of **7b** with respect to **9** must be due to other factors, probably to the easier transport through the membranes due to its lipophilic characteristics. The same reasons could explain the lower activity of the present compounds with respect to the preceding series[2] of more lipophilic analogues. Consequently, further studies are needed in order to improve and explain the biological activity of these molecules.

Conclusions

Lewis acid catalyzed hetero-Diels–Alder reaction and subsequent functionalization provides a convenient access to novel benzo[*b*]isoquino[2,3-*h*]naphthyridines. DNA binding experiments with polynucleotides and model oligonucleotides revealed a preference of the *cis* diastereomers for AT-rich sequences and a preference of the *trans* diastereomers for CG-rich sequences. NOE experiments showed that the *cis* isomers bind to the minor groove of $d(AAGAATTCTT)_2$, whereas the *trans* isomers lead to *cap*-complexes with CG-rich oligonucleotides, with a preference for the 3′-terminals, as found for camptothecins. The compounds showed modest cytotoxicity against two different human tumour cell lines, RD-ES and CAD-ES1 (Ewing's sarcoma).

Experimental

General

The self-complementary oligonucleotides $d(AAGAATTCTT)_2$, $d(CGACGTCG)_2$, $d(GCGATCGC)_2$ and d(GCATCGCG-ATGC)$_2$, as free acids, were purchased from Roche Diagnostics: they were synthesized on a 10 μmol scale by solid-phase techniques and purified by gel filtration. Poly(dA–dT)$_2$, poly(dG–dC)$_2$ and salmon sperm DNA were purchased from Sigma. The oligonucleotides were dissolved in D_2O with low paramagnetic impurities (99.99% isotope, Aldrich) or in H_2O–D_2O (90 : 10) HPLC grade (Aldrich), in the presence of a minimum amount of NaCl (0.02 M) in order to stabilize the double helix. The pH was adjusted to 5.8–6.0. Assays for cytotoxicity experiments were carried out according to the literature.[1,2,25] Compound **13** was prepared following a literature method.[12]

Ethyl (8a*R*,14a*S*,14b*S*)-9,9-dimethyl-7,8,8a,9,14,14a,14b,15-octahydro-5*H*-benzo[*b*]isoquino[2,3-*h*]-1,7-naphthyridine-11-carboxylate (7a)

A solution of (*S*)-*N*-(4-methyl-3-pentenyl)-1,2,3,4-tetrahydroisoquinoline-3-carbaldehyde **4** (3 g, 14 mmol) in CH_2Cl_2 (15 mL) and ethyl 4-aminobenzoate **5** (2.31 g, 14 mmol) in CH_2Cl_2 (10 mL) was added to a suspension of 4 Å molecular sieves (20 g) in dry CH_2Cl_2 under an inert gas atmosphere. After stirring at room temperature for 12 h, the reaction mixture was filtered under an inert gas atmosphere through Celite with dry CH_2Cl_2 as eluent. The filtrate was concentrated to 25 mL, and at −78 °C a 1 M solution of $EtAlCl_2$ in hexane (18 mL, 18 mmol) was added. The reaction mixture was allowed to warm up to room temperature, stirred for a further 2 days, and hydrolyzed with 2 M NH_4F solution (300 mL). The aqueous layer was extracted with CH_2Cl_2 (4 × 100 mL), and the combined organic layers were dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography (R_f 0.41, SiO_2, pentane–CH_2Cl_2–NEt_3, 15 : 3 : 1) to give **7a** (3.37 g, 8.60 mmol, 61%) as colourless needles, $[a]_D^{22}$ +218.76 (*c* 1.13, CH_2Cl_2). $\tilde{\nu}_{max}$ (KBr)/cm^{-1} 3363 (s), 3070, 3045, 3024, 3001 (m), 2976, 2966, 2949, 2935, 2908, 2895, 2855, 2749 (m), 1682 (s), 1606, 1513, 1501 (s), 1296, 1251 (s), 772, 739 (m). δ_H (600 MHz, $CDCl_3$) 7.86 (d, *J* 2.0, 10-H), 7.65 (dd, *J* 8.4 and 2.0, 12-H), 7.17–7.12 (m, 1,2,3-H), 7.03 (dd, 4-H), 6.46 (d, *J* 8.4, 13-H), 4.51 (br dd, NH), 4.30 (q, *J* 7.1, O–CH_2), 3.95 (d, *J* 15.2, 5-Heq), 3.71 (br m, 14a-H), 3.38 (dd, *J* 15.2 and ^5J 1.0, 5-Hax), 3.16 (ddd, *J* 16.7, 11.3 and ^5J 1.0, 15-Hax), 3.13 (ddd, *J* 11.6, 4.1 and 3.0, 7-Heq), 2.84 (dd, *J* 16.7 and 4.7, 15-Heq), 2.56 (ddd, *J* 11.3, 4.7 and 2.1, 14b-H), 2.17 (ddd, *J* 11.6, 12.6, 3.2, 7-Hax), 1.61 (dqd, *J* 13.2, 3.2, 3.2, 3.0, $^4J_{8eq,14a}$ 1.2, 8-Heq), 1.51 (dtd, *J* 12.5, 3.2, 3.2, $^4J_{8a,NH}$ 1.3, 8a-H), 1.40 (qd, *J* 13.2, 12.5, 12.5, 4.1, 8-Hax), 1.40 (s, 9-Me), 1.36 (t, *J* 7.1, Me), 1.27 (s, 9-Me). δ_C (100 MHz, $CDCl_3$) 167.1 (C=O), 146.6 (C-13a), 133.5 (C-4a), 132.6 (C-15a), 128.7 (C-12), 128.4 (C-10), 128.1, 126.3, 125.9 (C-1,2,3), 127.6 (C-9a), 126.0 (C-4), 125.9 (C-1), 118.6 (C-11), 113.8 (C-13), 60.6 (C-14b), 60.1 (OCH_2), 58.0 (C-5), 56.2 (C-7), 49.6 (C-14a), 44.6 (C-8a), 35.3 (C-9), 33.1 (9-Me), 30.9 (C-15), 25.6 (9-Me), 22.1 (C-8), 14.5 (Me). HRMS $C_{25}H_{30}N_2O_2$: calcd. 390.2307, found 390.229.

Ethyl (8a*R*,14a*R*,14b*S*)-9,9-dimethyl-7,8,8a,9,14,14a,14b,15-octahydro-5*H*-benzo[*b*]isoquino[2,3-*h*]-1,7-naphthyridine-11-carboxylate (7b)

As described above for **7a**, using **4** (300 mg, 1.23 mmol), **5** (224 mg, 1.39 mmol), 4 Å molecular sieves (2 g), and a 1 M solution of $SnCl_4$ (1.6 mL, 1.6 mmol), with purification by flash chromatography (R_f 0.30, SiO_2, pentane–CH_2Cl_2–NEt_3, 15 : 3 : 1) was obtained **7b** (180 mg, 0.46 mmol, 37%) as a colourless amorphous solid, $[a]_D^{22}$ −271.5 (*c* 1.20, CH_2Cl_2). Found: C, 76.84; H, 8.01; N, 7.18. $C_{25}H_{30}N_2O_2$ (390.5) requires C, 76.89; H, 7.74; N, 7.1. $\tilde{\nu}_{max}$ (KBr)/cm^{-1} 3387 (s), 3066, 3028 (m), 2970, 2968, 2930, 2904 (m), 1704 (s), 1607, 1515, 1458 (s), 1296, 1251 (s), 771, 742 (m). δ_H (600 MHz, $CDCl_3$) 7.96 (d, *J* 2.0, 10-H), 7.68 (dd, *J* 8.4 and 2.0, 12-H), 7.17–7.12 (m, 1,2,3-H), 7.05 (dd, 4-H), 6.49 (d, *J* 8.4, 13-H), 4.45 (br s, NH), 4.31 (q, *J* 7.1, O–CH_2), 3.92 (d, *J* 15.2, 5-Heq), 3.45 (br d, *J* 15.2 and ^5J 1.2, 5-Hax), 3.22 (dt, *J* 11.4, 3.8 and 3.0, 7-Heq), 3.19 (dd, *J* 15.9 and 4.3, 15-Heq), 3.10 (dd, *J* 10.6 and 8.5, 14a-H), 2.88 (br dd, *J* 15.9, 10.5 and ^5J 1.2, 15-Hax), 2.29 (ddd, *J* 11.4, 12.4 and 2.6, 7-Hax), 2.28 (ddd, *J* 10.5, 4.3 and 8.5, 14b-H), 1.92 (dq, *J* 13.0, 3.0 and 2.6, 8-Heq), 1.65 (qd, *J* 13.0, 12.4, 12.3 and 3.8, 8-Hax), 1.53 (ddd, *J* 10.6, 12.3 and 3.3, H-8a), 1.41 (s, 9-Me), 1.21 (s, 9-Me), 1.36 (t, *J* 7.1, Me). δ_C (100 MHz, $CDCl_3$) 167.1 (C=O), 146.6 (C-13a), 133.7 (C-4a), 132.1 (C-15a), 129.8 (C-9a), 128.9 (C-12), 128.4 (C-10), 128.2, 126.5, 125.9 (C-1,2,3), 126.0 (C-4), 118.9 (C-11), 113.4 (C-13), 62.9 (C-14b), 60.2 (OCH_2), 57.7 (C-5), 56.0 (C-14a), 55.8 (C-7), 44.8 (C-8a), 34.7 (C-9), 33.3 (C-15), 26.4 (9-Me), 25.7 (9-Me), 23.6 (C-8), 14.5 (Me). *m/z*: (EI) 390 (M^+, 7%), 345 (M^+ − OC_2H_5, 3%), 260 (M^+ − 130, 3%),

158 ($C_{11}H_{12}N^+$, 2%), 146 ($C_{10}H_{12}N^+$, 100%), 130 ($C_9H_8N^+$, 4%), 104($C_8H_8^+$, 9%).

Crystal structure data for 7b. $C_{25}H_{30}N_2O_2$, $M = 390.51$, crystal size 0.7 × 0.4 × 0.3 mm, monoclinic, space group $C2$, $a = 24.525(5)$, $b = 8.188(2)$, $c = 26.609(4)$ Å, $\alpha = 90°$, $\beta = 116.853(14)°$, $\gamma = 90°$, $V = 4767.1(18)$ Å^3, $D_c = 1.088$ Mg m^{-1}, $T = 293(2)$ K, $Z = 8$, $\mu = 0.069$ mm^{-1}, $\lambda = 0.71073$ Å, reflections collected/unique: 5982/5841 [R(int) = 0.0383], final R indices [$I > 2\sigma(I)$]: $R1 = 0.0806$, $wR2 = 0.2046$, R indices (all data): $R1 = 0.1373$, $wR2 = 0.2376$. CCDC reference number 243553. See http://dx.doi.org/10.1039/b503281d for crystallographic data in CIF or other electronic format.

(8a*R*,14a*S*,14b*S*)-9,9-Dimethyl-7,8,8a,9,14,14a,14b,15 octahydro-5*H*-benzo[*b*]isoquino[2,3-*h*]-1,7-naphthyridine-11-carboxylic acid (8)

KOH (141 mg, 2.51 mmol) was added to a solution of **7a** (280 mg, 0.71 mmol) in ethanol (10 mL) and water (2 mL), and the reaction mixture was heated at reflux for 4 h. After being concentrated, the residual layer was adjusted to pH 8–9 with 6 N HCl (ice-cooling) and extracted four times with CH_2Cl_2–methanol (10 : 1). The combined extracts were dried ($MgSO_4$), and the solvent was removed to give **8** (230 mg, 0.64 mmol, 89%) as an amorphous solid, $[\alpha]_D^{22}$ +183.15 (*c* 1.65, MeOH). $\tilde{\nu}_{max}$ (KBr)/cm^{-1} 3416 (br), 3067, 3028, 2964 (m), 2906, 2873, 2806, 2771 (m), 1703 (s), 1606, 1512, 1497 (s), 1303, 1251 (s), 776, 744 (m). δ_H (400 MHz, DMSO-d_6) 11.9 (s, br, COOH), 7.68 (d, *J* 1.8, 10-H), 7.48 (dd, *J* 8.5, 1.8, 12-H), 7.20–7.11 (m, 1,2,3-H), 7.09–7.03 (m, br, 4-H), 6.76 (d, *J* 8.5, 13-H), 6.30 (s, br, NH), 3.89 (d, *J* 15.5, 5-Heq), 3.66 (s, br, 14a-H), 3.45 (dd, *J* 16.5 and 11.7, 15-Hax), 3.26 (d, *J* 15.5, 5-Hax), 3.03 (m, 7-Heq), 2.70 (dd, *J* 16.5 and 4.1, 15-Heq), 2.47 (ddd, *J* 11.7, 4.1 and 1.0, 14b-H), 2.08 (m, 7-Hax), 1.57–1.46 (m, 8-Heq, 8a-H), 1.32 (s, 9-Me), 1.19 (s, 9-Me), 1.14–1.10 (m, 8-Hax). δ_C (100 MHz, $CDCl_3$) 167.7 (C=O), 147.7 (C-13a), 133.6 (C-15a), 133.58 (C-4a), 128.3 (C-9a), 128.0 (C-12), 127.6, 126.2, 125.6 (C-10, C-1,2,3), 126.0 (C-4), 116.5 (C-11), 112.8 (C-13), 60.5(C-14b), 57.5 (C-5), 55.6 (C-7), 49.1 (C-14a), 43.4 (C-8a), 34.8 (C-9), 32.9 (9-Me), 30.0 (C-15), 25.2 (9-Me), 21.8 (C-8). *m*/*z* (EI): 362 (M^+, 3%), 318 (M^+ − COOH, 2%), 158 ($C_{11}H_{12}N^+$, 16%), 146 ($C_{10}H_{12}N^+$, 100%), 130 ($C_9H_8N^+$, 31%), 104 ($C_8H_8^+$, 18%). HRMS $C_{23}H_{26}N_2O$: calcd.362.1994, found 362.1992.

2-(Dimethylamino)ethyl(8a*R*,14a*S*,14b*S*)-9,9-dimethyl-7,8,8a,9,14,14a,14b,15-octahydro-5*H*-benzo[*b*]isoquino[2,3-*h*]-1,7-naphthyridine-11-carboxylate (9)

DCC (69.8 mg, 0.336 mmol) was added to a suspension of **8** (110 mg, 0.28 mmol) in dry CH_2Cl_2 (15 mL) at 0 °C. After stirring for 10 min, DMAP (7 mg, 0.06 mmol) and *N*,*N*-dimethylaminoethanol (0.034 mL, 0.336 mmol) in CH_2Cl_2 (2 mL) were added. The reaction mixture was stirred at room temperature for 16 h and then filtered through Celite. The filtrate was washed with aqueous NH_4Cl solution (2 × 10 mL), dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography (R_f 0.22, SiO_2, pentane–CH_2Cl_2–NEt_3, 10 : 5 : 1) to give **9** (98 mg, 0.23 mmol, 81%) as colourless needles, $[\alpha]_D^{22}$ +139.9 (*c* 0.67, $CHCl_3$). Found: C, 74.49; H, 8.23; N, 9.50. $C_{27}H_{35}N_3O_2$ (433.6) requires C, 74.79; H, 8.14; N, 9.69. $\tilde{\nu}_{max}$ (KBr)/cm^{-1} 3390 (s), 3067, 3028 (m), 2946, 2904, 2872, 2818, 2768 (m), 1695 (s), 1604, 1512, 1456 (s), 1242 (s), 769, 741 (m). δ_H (600 MHz, $CDCl_3$) 7.87 (d, *J* 2.0, 10-H), 7.64 (dd, *J* 8.4 and *J* 2.0, 12-H), 7.17–7.12 (m, 1,2,3-H), 7.03 (dd, 4-H), 6.47 (d, *J* 8.4, 13-H), 4.49 (br dd, NH), 4.37 (m, OCH_2), 3.95 (d, *J* 15.3, 5-Heq), 3.70 (br m, 14a-H), 3.38 (dd, *J* 15.3 and ^5J 1.1, 5-Hax), 3.16 (br dd, *J* 16.7, 11.3 and ^5J 1.1, 15-Hax), 3.13 (ddd, *J* 11.5, 4.1 and 2.7, 7-Heq), 2.84 (dd, *J* 16.7 and 4.8, 15-Heq), 2.70 (m, NCH_2), 2.56 (ddd, *J* 11.3, 4.8 and 2.3, 14b-H), 2.34 (s, NMe_2), 2.17 (ddd, *J* 12.8, 11.6 and 3.3, 7-Hax), 1.60 (dqd, *J* 13.3, 3.3, 3.4, 2.7 and ^4J(8eq,14a) 1.3, 8-Heq), 1.50 (dtd, *J* 12.3, 3.4, 3.1 and ^4J(8a,NH) 1.5, 8a-H), 1.40 (dddd, *J* 13.3, 12.8, 12.3 and 4.1, 8-Hax), 1.39 (s, 9-Me), 1.27 (s, 9-Me). δ_C (100 MHz, $CDCl_3$) 167.0 (C=O), 146.8 (C-13a), 133.7 (C-15a), 132.6 (C-4a), 128.8 (C-12), 128.6 (C-10), 128.2, 126.4, 125.9, 125.9 (C-1,2,3,4), 127.6 (C-9a), 118.3 (C-11), 113.8 (C-13), 62.3 (OCH_2), 60.6 (C-14b), 58.1 (C-5), 57.9 (NCH_2), 56.3 (C-7), 49.6 (C-14a), 45.8 (NMe_2), 44.7 (C-8a), 35.4 (C-9), 33.1 (9-Me), 30.9 (C-15), 25.5 (9-Me), 22.1 (C-8). *m*/*z* (EI) 433 (M^+, 1%), 362 ($M^+ - O(CH_2)_2N(CH_3)_2$, 7%), 158 ($C_{11}H_{12}N^+$, 12%), 146 ($C_{10}H_{12}N^+$, 100%), 104 ($C_8H_8^+$, 10%).

(8a*R*,14a*S*,14b*S*)-*N*-(2-Hydroxyethyl)-9,9-dimethyl-7,8,8a,9,14,14a,14b,15-octahydro-5*H*-benzo[*b*]isoquino-[2,3-*h*]-1,7-naphthyridine-11-carboxamide (10a)

Under an inert gas atmosphere, aminoethanol (0.31 mL, 5.12 mmol) was added to Na (59 mg, 2.56 mmol) in dry toluene (10 mL). After the Na had dissolved, a solution of **7a** (100 mg, 0.256 mmol) in dry toluene (2 mL) was added dropwise. The reaction mixture was heated at 80 °C for 16 h, cooled to room temperature and was then hydrolyzed with satd. NH_4Cl solution. The aqueous layer was washed with CH_2Cl_2 (3 × 5 mL). The combined organic layers were dried ($MgSO_4$), and the solvent was removed. The residue was purified by flash chromatography (SiO_2, CH_2Cl_2–MeOH, 10 : 1 with 5% NEt_3) to give **10a** (65 mg, 0.16 mmol, 62%) as yellow crystals, $[\alpha]_D^{22}$ +127.8 (*c* 1.05, $CHCl_3$). $\tilde{\nu}_{max}$ (ATR (attenuated total reflection))/cm^{-1} 3315 (s), 3066, 3028 (m), 2965, 2927, 2904, 2871, 2854, 2769 (m), 1603, 1542 (s), 767, 738 (m). δ_H (600 MHz, $CDCl_3$) 7.70 (d, *J* 2.2, 10-H), 7.32 (dd, *J* 8.4 and 2.2, 12-H), 7.18–7.12 (m, 1,2,3-H), 7.03 (dd, 4-H), 6.43 (br t, CONH), 6.48 (d, *J* 8.4, 13-H), 4.45 (br m, NH), 3.95 (d, *J* 15.3, 5-Heq), 3.74 (m, OCH_2), 3.69 (br m, 14a-H), 3.58 (m, NCH_2), 3.40 (d, *J* 15.3 and ^5J 1.1, 5-Hax), 3.24 (ddd, *J* 16.7, 11.3 and ^5J 1.1, 15-Hax), 3.14 (ddd, *J* 11.6, 4.1 and 2.9, 7-Heq), 2.83 (dd, *J* 16.7 and 4.8, 15-Heq), 2.57 (ddd, *J* 11.3, 4.8 and 2.0, 14b-H), 2.17 (ddd, *J* 12.8, 11.6 and 2.4, 7-Hax), 1.60 (dqd, *J* 13.3, 3.3, 3.2, 2.9 and ^4J(8,14a) 1.3, 8-Heq), 1.50 (dtd, *J* 12.4, 3.2, 3.2 and ^4J(8a,NH) 1.2, 8a-H), 1.38 (s, 9-Me), 1.40 (dddd, *J* 13.3, 12.8, 12.4 and 4.1, 8-Hax) 1.38 (s, 9-Me), 1.26 (s, 9-Me). δ_C (100 MHz, $CDCl_3$) 169.1 (C=O), 145.7 (C-13a), 132.6 (C-15a, C-4a), 128.3, 126.6, 126.3 (C-1,2,3), 128.1 (C-9a), 126.1 (C-10), 126.0 (C-4), 125.5 (C-12), 122.1 (C-11), 114.0 (C-13), 62.8 (OCH_2), 60.8 (C-14b), 57.9 (C-5), 56.2 (C-7), 49.5 (C-14a), 44.6 (C-8a), 43.1 (NCH_2), 35.5 (C-9), 33.2 (C-16), 30.8 (9-Me), 25.6 (9-Me), 22.0 (C-8). *m*/*z* (EI): 387 ($M^+ - H_2O$, 1%), 229 ($C_{16}H_{23}N^+$, 5%), 213 ($C_{15}H_{19}N^+$, 5%), 158 ($C_{11}H_{12}N^+$, 15%), 146 ($C_{10}H_{12}N^+$, 100%), 104 ($C_8H_8^+$, 12%).

(8a*R*,14a*R*,14b*S*)-*N*-(2-Hydroxyethyl)-9,9-dimethyl-7,8,8a,9,14,14a,14b,15-octahydro-5*H*-benzo[*b*]isoquino-[2,3-*h*]-1,7-naphthyridine-11-carboxamide (10b)

A solution of **7b** (100 mg, 0.256 mmol) in abs. THF (2 mL) was added to a solution of Na (59 mg, 2.56 mmol) in abs. THF (10 mL), and the reaction mixture heated at 80 °C for 16 h. The reaction mixture was then cooled to room temperature and hydrolyzed with satd. NH_4Cl solution (10 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 5 mL), and the combined organic layers were dried ($MgSO_4$). After removal of the solvent, the residue was purified by flash chromatography (R_f 0.11, SiO_2, CH_2Cl_2–MeOH, 20 : 1) to give **10b** (73 mg, 0.18 mmol, 70%) as a yellowish amorphous solid, $[\alpha]_D^{22}$ −222.1 (c 1.21, $CHCl_3$). Found: C, 73.81; H, 7.81; N, 10.25. $C_{25}H_{31}N_3O_2$ (405.5) requires C, 74.04; H, 7.70; N, 10.36. δ_H (600 MHz, $CDCl_3$) 7.70 (d, *J* 2.2, 10-H), 7.32 (dd, *J* 8.4 and 2.2, 12-H), 7.17–7.12 (m, 1,2,3-H), 7.04 (br m, 4-H), 6.50 (d, *J* 8.4, 13-H), 6.48 (t, CONH), 4.35 (br s, NH), 3.92 (d, *J* 15.1, 5-Heq), 3.81 (br t, OCH_2), 3.60 (br q, NCH_2), 3.45 (br dd, *J* 15.1 and ^5J 1.3, 5-Hax), 3.22 (dt, *J* 11.5, 3.8 and 3.0, 7-Heq), 3.18 (dd, *J* 15.9 and 4.3, 15-Heq), 3.09 (dd, *J* 10.7 and 8.5, 14a-H), 2.88 (ddd, *J* 15.9, 10.5 and ^5J 1.1, 15-Hax), 2.29 (ddd, *J* 11.5, 12.2 and 2.5, 7-Hax), 2.28

(ddd, *J* 10.5, 4.3 and 8.5, 14b-H), 1.92 (dq, *J* 13.1, 3.0 and 2.5, 8-Heq), 1.65 (qd, *J* 13.1, 12.3, 12.2 and 3.8, 8-Hax), 1.54 (ddd, *J* 10.7, 12.3 and 3.3, 8a-H), 1.48 (s, 9-Me), 1.22 (s, 9-Me). δ_C (100 MHz, $CDCl_3$) 168.9 (C=O), 145.7 (C-13a), 133.5 (C-15a), 132.07 (C-4a), 130.1 (C-9a), 128.1, 126.4, 125.9 (C-1,2,3), 126.0 (C-10), 125.8 (C-4), 125.7 (C-12), 122.1 (C-11), 113.6 (C-13), 62.8 (C-19), 62.4 (C-14b), 57.6 (C-5), 55.8 (C-14a), 55.7 (C-7), 44.7 (C-8a), 42.9 (C-18), 34.6 (C-9), 33.2 (C-15), 26.4 (C-16), 25.7 (C-17), 23.5 (C-8). *m/z* (EI): 387 ($M^+ - H_2O$, 12%) 229 ($C_{16}H_{23}N^+$, 5%), 213 ($C_{15}H_{19}N^+$, 5%), 158 ($C_{11}H_{12}N^+$, 12%), 146 ($C_{10}H_{12}N^+$, 100%), 104 ($C_8H_8{}^+$, 100%).

Preparation of (8a*R*,15a*S*,15b*S*) (12a) and (8a*R*,15a*R*,15b*S*)-9,9-dimethyl-7,8,8a,9,15,15a,15b,15-octahydro-5*H*[1,3]benzodioxolo[5,6-*b*]isoquino[2,3-*h*]-1,7-naphthyridine (12b)

As described above for **7a**, using **4** (1.5 g, 6.20 mmol), 3,4-(methylenedioxy)aniline (850 mg, 6.20 mmol), 4 Å molecular sieves (10 g), and a 1 M solution of $SnCl_4$ (8 mL, 8 mmol), with purification by flash chromatography (SiO_2, pentane–CH_2Cl_2–NEt_3, 15 : 3 : 1), to give **12b** in the first fraction as a brown amorphous solid (R_f 0.41, 800 mg, 2.21 mmol, 37%), and **12a** in the second fraction as a brown amorphous solid (R_f 0.29, 500 mg, 1.38 mmol, 22%). **12a**: Found: C, 75.82; H, 7.14, N, 7.78. $C_{23}H_{26}N_2O_2$ (362.5) requires C, 76.21; H, 7.23; N, 7.73. $\tilde{\nu}_{max}$ (ATR)/ cm^{-1} 3389 (s), 2963, 2932 (m), 1620, 1510, 1479 (s), 1030 (s), 746 (m). δ_H (400 MHz, $CDCl_3$) 7.18–7.13 (m, 2,3-H), 7.11–7.08 (m, 1-H), 7.06–7.01 (m, 4-H), 6.68 (s, 10-H), 6.20 (s, 13-H), 5.81 and 5.79 (d, *J* 1.4, OCH_2O), 4.19 (d, *J* 16.8, 5-Heq), 3.67 (br s, NH), 3.65 (d, *J* 16.8, 5-Hax), 3.47 (br s, 14a-H), 3.25 (ddd, *J* 12.3, 5.0 and 4.9, 14b-H), 3.04 (dd, *J* 16.9 and 12.3, 15-Hax), 2.63 (dt, *J* 11.8, 11.8 and 3.2, 7-Hax), 2.52 (ddd, *J* 11.8, 4.9 and 2.8, 7-Heq), 2.45 (dd, *J* 16.9 and 4.9, 15-Heq), 1.62–1.52 (m, 8a-H, 8-Heq), 1.44–1.37 (m, 8-Hax), 1.27 (s, 9-Me), 1.25 (s, 9-Me). δ_C(100 MHz, $CDCl_3$) 146.0 (C-13a), 139.9 (C-12), 137.4 (C-11), 133.9 (C-4a), 133.3 (C-15a), 128.9 (C-1), 126.7 (C-4), 126.2, 126.1 (C-2,3), 121.0 (C-9a), 105.9 (C-10), 100.2 (OCH_2O), 97.3 (C-13), 57.5 (C-14b), 56.6 (C-5), 50.3 (C-14a), 46.3 (C-7), 38.1 (C-8a), 35.1 (C-9), 33.9 (9-Me), 26.5 (9-Me), 24.6 (C-15), 23.3 (C-8). *m/z* (EI): 362 (M^+, 7%), 347 ($M^+ - OC_2H_5$, 3%), 216 ($M^+ - 130$, 3%), 158 ($C_{11}H_{12}N^+$, 2%), 146 ($C_{10}H_{12}N^+$, 100%), 130 ($C_9H_8N^+$, 4%), 104 ($C_8H_8{}^+$, 9%). **12b**: Found: C, 76.40; H, 7.19; N, 7.80. $C_{23}H_{26}N_2O_2$ (362.5) requires C, 76.21; H, 7.23; N, 7.73. $\tilde{\nu}_{max}$ (ATR)/cm^{-1} 3394(s), 2961, 2928, 2872, 2816 (m), 1631, 1500, 1479 (s), 1033 (s), 740 (m). δ_H (400 MHz, $CDCl_3$) 7.16–7.10 (m, 1,2,3-H), 7.04–7.03 (m, 4-H), 6.75 (s, 10-H), 6.14 (s, 13-H), 5.81 (s, OCH_2O), 3.92 (d, *J* 15.1, 5-Heq), 3.69 (br s, NH), 3.43 (d, *J* 15.1, 5-Hax), 3.21–3.16 (m, 15-Heq, 7-Heq), 2.98 (dd, *J* 10.4 and 8.4, 14a-H), 2.84 (dd, *J* 15.7 and 10.4, 15-Hax), 2.30–2.22 (m, 7-Hax, 14b-H), 1.89 (dd, *J* 12.4, 3.4, 2.9 and 2.9, 8-Heq), 1.64 (dq, *J* 12.4, 12.4, 12.4 and 3.6, 8-Hax), 1.55 (dt, *J* 12.4, 10.4 and 3.4, 8a-H), 1.30 (s, 9-Me), 1.12 (s, 9-Me). δ_C (100 MHz, $CDCl_3$) 146.1 (C-13a), 140.2 (C-12), 137.1 (C-11), 133.7 (C-4a), 132.5 (C-15a), 128.2, 126.4, 125.9 (C-1,2,3), 125.8 (C-4), 123.2 (C-9a), 106.3 (C-10), 100.4 (OCH_2O), 96.5 (C-13), 63.2 (C-14b), 57.8 (C-5), 56.0 (C-14a), 55.8 (C-7), 45.4 (C-8a), 34.8 (C-9), 33.5 (C-15), 27.2 (9-Me), 27.0 (9-Me), 23.8 (C-8). *m/z* (EI): 362 (M^+, 7%), 347 ($M^+ - OC_2H_5$, 3%), 216 ($M^+ - 130$, 3%) 158 ($C_{11}H_{12}N^+$, 2%), 146 ($C_{10}H_{12}N^+$, 100%), 130 ($C_9H_8N^+$, 4%), 104 ($C_8H_8{}^+$, 9%).

(3*S*)-3-(Hydroxymethyl)-*N*-(3-pentynyl)-1,2,3,4-tetrahydroisoquinoline (14)

Bromopentyne (2 g, 13.60 mmol) was added with ice-cooling to a suspension of K_2CO_3 (1.44 g, 11.43 mmol) and **13** (1.85 g, 11.31 mmol) in abs. toluene (20 mL), and the reaction mixture was heated at reflux for 72 h. After cooling to room temperature, the reaction mixture was extracted with 2 N HCl (4 × 25 mL). The aqueous layer was washed with Et_2O (3 × 20 mL), adjusted to pH 8–9 with conc. NH_3 solution and extracted with CH_2Cl_2 (4 × 20 mL). The combined extracts were dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography (R_f 0.17, SiO_2, pentane–EtOAc, 1 : 1), to give **14** (1.97 g, 8.59 mmol, 75%) as a yellowish oil, $[\alpha]_D^{22}$ −8.9° (*c* 1.02, $CHCl_3$). Found: C, 78.56; H, 8.30; N, 6.14. $C_{15}H_{19}NO$ (227.32) requires C, 78.56; H, 8.35; N, 6.11. $\tilde{\nu}_{max}$ (ATR)/cm^{-1} 3393 (br), 3064, 3022 (m), 2916, 2851 (m), 1586, 1495, 1452 (s), 741 (m). δ_H (400 MHz, $CDCl_3$) 7.18–7.12 (m, 6,7-H), 7.10–7.05 (m, 5-H), 7.04–7.02 (m, 8-H), 3.94 (d, *J* 16.4, 1-H_a), 3.77 (d, *J* 16.4, 1-H_b), 3.58 (dd, *J* 11.0 and 5.6, 1″-H_a), 3.54 (dd, *J* 11.0 and 8.7, 1″-H_b), 3.19 (dddd, *J* 8.7, 7.3, 5.8 and 5.6, 3-H), 2.79 (dd, *J* 16.7 and 5.8, 4-H_a), 2.73 (ddd, *J* 12.7, 7.3 and 7.3, 1′-H_a), 2.58 (dt, *J* 12.7, 6.4 and 6.4, 1′-H_b), 2.50 (dd, *J* 16.7 and 7.3, 4-H_b), 2.37–2.31 (m, 2′-CH_2), 1.78 (t, *J* 2.5, 4′-Me). δ_C (100 MHz, $CDCl_3$) 134.2 (C-4a), 133.9 (C-8a), 128.8 (C-5), 126.9 (C-8), 126.5, 126.0 (C-6,7), 77.6 (C-4′), 77.4 (C-3′), 62.3 (C-1″), 58.8 (C-3), 50.6 (C-1), 50.1 (C-1′), 26.5 (C-4), 18.8 (C-2′), 3.4 (4′-Me). *m/z* (EI): 198 ($M^+ - CH_2OH$, 100%), 196 ($M^+ - CH_2OH - 2$, 21%), 176 ($C_{11}H_{14}NO^+$, 80%), 130 ($C_9H_8N^+$, 17%), 117 (25%), 115 (29%).

(3*S*)-*N*-(3-Pentynyl)-1,2,3,4-tetrahydroisoquinoline-3-carbaldehyde (15)

DMSO (0.75 mL, 10.95 mmol) in abs. CH_2Cl_2 (4 mL) was added dropwise over 30 min to a solution of $(COCl)_2$ (0.47 mL, 5.60 mmol) in abs. CH_2Cl_2 (15 mL) at −50 °C. After stirring for 15 min, a solution of **14** (500 mg, 2.18 mmol) in abs. CH_2Cl_2 (10 mL) was added dropwise over 45 min, and the reaction mixture was stirred at −50 °C for 16 h. NEt_3 (1.8 mL) was added dropwise over 30 min, the reaction mixture stirred for a further 15 min and then allowed to warm up to room temperature. The organic layer was washed with water (3 × 50 mL) and dried ($MgSO_4$). The solvent was removed under vacuum to give **15** (495 mg, quant.) as a yellow oil. Found: C, 79.38; H, 7.55; N, 6.11. $C_{15}H_{17}NO$ (227.30) requires C, 79.26; H, 7.54; N, 6.16. ν_{max} (neat)/cm^{-1} 3064 (m), 3047 (m), 3023 (m), 2980 (s), 2925 (s), 2914 (s), 1730 (s), 1454 (s), 745 (s). δ_H (200 MHz, $CDCl_3$) 9.77 (d, *J* 1.1, CHO), 7.18–7.12 (m, 5,6,7-H), 7.05–7.03 (m, 8-H), 4.05 and 3.94 (d, *J* 16.0, 1-CH_2), 3.54 (td, *J* 6.4 and 1.3, 3-H), 3.07–2.73 (m, 4-CH_2, 1′-CH_2), 2.45–2.38 (m, 2′-CH_2), 1.79 (t, *J* 2.5, 3′-Me). δ_C (50 MHz, $CDCl_3$) 203.3 (C=O), 133.7 (C-4a), 132.0 (C-8a), 128.7 (C-5), 126.7 (C-8), 126.6 (C-6), 126.3 (C-7), 77.0 (C-3′), 76.8 (C-4′), 66.4 (C-3), 53.6 (C-1), 52.1 (C-1′), 26.0 (C-4), 18.6 (C-2′), 3.4 (4′-Me).

Ethyl (14b*S*)-9-methyl-7,8,14b,15-tetrahydro-5*H*-benzo[*b*]isoquino[2,3-*h*]-1,7-naphthyridine-11-carboxylate (17)

As described above for **7a**, using **15** (488 mg, 2.15 mmol), **5** (355 mg, 2.15 mmol), 4 Å molecular sieves (5 g), and $BF_3{\cdot}OEt_2$ (0.56 mL, 4.30 mmol), with purification by flash chromatography (R_f 0.30, SiO_2, pentane–CH_2Cl_2–NEt_3, 20 : 2 : 1, to give **17** (300 mg, 0.81 mmol, 38%) as a yellow amorphous solid, $[\alpha]_D^{22}$ −267.9 (*c* 1.06, $CHCl_3$). Found: C, 77.40; H, 6.45; N, 7.54. $C_{24}H_{24}N_2O_2$ (372.46) requires C, 77.39; H, 6.49; N, 7.52. $\tilde{\nu}_{max}$ (ATR)/cm^{-1} 3064, 3027 (m), 2976, 2958, 2925, 2903, 2837, 2754 (m), 1709 (s), 1615, 1581, 1496 (s), 1239 (s), 789, 733 (m). δ_H (400 MHz, $CDCl_3$) 8.77 (d, *J* 1.6, 10-H), 8.16 (dd, *J* 8.8 and 1.6, 12-H), 7.97 (d, *J* 8.8, 13-H), 7.26–7.15 (m, 1,2,3-H), 7.12–7.10 (m, 4-H), 4.37 (q, *J* 7.1, OCH_2), 4.04 (d, *J* 14.9, 5-Heq), 3.83 (dd, *J* 16.4 and 4.2, 15-Heq), 3.78 (dd, *J* 10.8 and 4.2, 14b-H), 3.73 (d, *J* 14.9, 5-Hax), 3.25 (ddd, *J* 11.6, 5.7 and 2.1, 7-Heq), 3.13 (ddd, *J* 11.6, 11.6 and 5.7, 8-Hax), 3.05–3.01 (m, 8-Heq), 2.83 (dd, *J* 16.4 and 10.8, 15-Hax), 2.61 (ddd, *J* 11.6, 11.6 and 3.9, 7-Hax), 2.68 (s, 9-Me), 1.47 (t, *J* 7.1, CH_2CH_3). δ_C (100 MHz, $CDCl_3$) 166.6 (C=O), 160.4 (C-14a), 148.2 (C-13a), 142.9 (C-9), 134.6 (C-15a), 133.8 (C-4a), 127.5 (C-11), 126.8 (C-9a), 129.7 (C-10), 128.9 (C-1), 127.8 (C-12), 126.7 (C-13), 126.4, 126.0, 125.7 (C-2,3,4), 63.6 (C-14b), 61.2 (OCH_2), 58.6 (C-5), 50.9 (C-7), 35.1 (C-15), 26.9 (C-8), 14.4 (CH_2CH_3), 13.7 (9-Me). *m/z* (EI): 372

(M^+, 100%), 357 ($M^+ - CH_3$, 7%), 343 ($M^+ - C_2H_5$, 8%), 327 ($M^+ - OC_2H_5$, 8%), 104 (60%).

(11a*S*)-1-*N*-(4-Carboxyethylphenyl)-2-(1-chloroethylidene)-1,2,3,4,6,11-hexahydrobenzo[*g*]quinolidine (18)

As described above for **7a**, using **15** (100 mg, 0.44 mmol), **5** (72 mg, 0.44 mmol), 4 Å molecular sieves (1 g), and a 1 M solution of $EtAlCl_2$ (0.88 mL, 0.88 mmol), with purification by chromatography (R_f 0.11, SiO_2, pentane–CH_2Cl_2–NEt_3, 20 : 2 : 1), to give **18** (67 mg, 0.16 mmol, 37%) as a yellow amorphous solid, $[a]_D^{22}$ −76.8 (*c* 1.07, $CHCl_3$). Found: C, 70.15; H, 6.60; Cl, 8.67; N, 6.83. $C_{24}H_{27}ClN_2O_2$ (410.94) requires C, 70.15; H, 6.6.62; Cl, 8.63; N, 6.82. $\tilde{\nu}_{max}$ (ATR)/cm^{-1} 3365 (br), 3062, 3020 (m), 2926, 2917, 2841 (m), 1697 (s), 1601, 1520, 1476 (s), 1269, 1170, 1100 (s), 768, 747 (m). δ_H (400 MHz, $CDCl_3$) 7.89–7.86 (m, 3′,5′-H), 7.19–7.13 (m, 8,9-H), 7.10–7.06 (m, 10-H), 7.05–7.01 (m, 7-H), 6.57–6.54 (m, 2′,6′-H), 5.48 (d, *J* 8.5, NH), 4.31 (q, *J* 7.1, OCH_2), 4.31–4.28 (m, 1-H), 4.26 (d, *J* 16.8, 6-H_a), 3.71 (d, *J* 16.8, 6-H_b), 3.49 (ddd, *J* 12.4, 4.9 and 2.5, 11a-H), 2.91 (dd, *J* 16.8 and 12.4, 11-Hax), 2.82–2.77 (m, 3-H_a), 2.74–2.61 (m, 4-CH_2), 2.48 (dd, *J* 16.8 and 4.9, 11-Heq), 2.39–2.31 (m, 3-H_b), 2.37 (d, *J* 2.5, 1″-Me), 1.35 (t, *J* 7.1, CH_2CH_3). δ_C (100 MHz, $CDCl_3$) 166.7 (C=O), 150.4 (C-1′), 133.4 (C-10a), 132.5 (C-6a), 131.5 (C-3′,5′), 129.1 (C-7), 129.0 (C-2), 126.9 (C-1″), 126.8 (C-10), 126.4 (C-8,9), 119.0 (C-4′), 112.3 (C-2′,6′), 60.2 (OCH_2), 58.3 (C-11a), 56.1 (C-6), 53.6 (C-1), 46.2 (C-4), 26.9 (C-3), 23.9 (C-11), 22.3 (1″-Me), 14.4 (CH_2CH_3). *m*/*z* (EI): 410 (M^+, 1%), 374 (M^+ − HCl, 40%), 244 ($C_{15}H_{15}ClN^+$, 39%), 146 ($C_{10}H_{12}N^+$, 100%), 130 ($C_9H_8N^+$, 29%).

UV experiments

The UV spectra were recorded at 25 °C on a Perkin–Elmer Lambda 40 UV-VIS spectrophotometer, and for the melting experiments on a JASCO W-550 spectrophotometer equipped with a thermostated cell. Melting of the oligonucleotides was followed by UV absorbance. Dilution experiments were performed starting from a concentrated solution (10^{-4} M) of the drug in water, which was diluted to 10^{-7} M, in the presence of NaCl (0.02 M). For the aggregation process a dimerization model was considered to be a sufficient approximation,[26] because we have just used the solutions (in the range 10^{-5}–10^{-7} M) which gave spectra with the isosbestic point (385, 380, 395, 398 and 402 nm for **7a**, **7b**, **9**, **10a** and **10b** respectively). The dimerization constants K_D were calculated by using standard equations. The binding constants *K* for the nucleotide–drug interaction were obtained by titration experiments, performed by adding increasing amounts of a 5.0×10^{-6} M solution in water of the drug to a 1.0×10^{-5} M solution of the oligonucleotide, until R = [drug]/[DNA] = 5. Then the binding constants *K* were calculated by including the dimerization equilibrium. The system of non-linear equations[8] was solved by using MATLAB software (v. 5.1). The calculation of thermodynamic data and *K* values from equilibrium melting curves was performed following well-established procedures.[27]

NMR experiments

^1H and ^{31}P NMR spectra of the complexes were recorded on a Bruker AMX-600 NMR spectrometer, operating at a frequency of 600.13 MHz for the ^1H nucleus and at 242.94 MHz for the ^{31}P nucleus, equipped with a z-gradient 5 mm reverse probe. ^1H and ^{13}C spectra of the free molecules were recorded on Bruker AMX-600 and AMX-400 spectrometers. D_2O and H_2O–D_2O (90 : 10) were used as solvents for the complexes with the oligonucleotides; $CDCl_3$ and DMSO-d_6 were used for proton and ^{13}C assignments in the free molecules, and for the measurements of the H,H coupling constants. ^1H chemical shifts (δ) were measured in ppm, using as internal references tetramethylsilane (TMS) for $CDCl_3$ and DMSO-d_6, and the residual water signal, set at $\delta = 4.80$ ppm, for aqueous solutions, respectively. Estimated accuracy is ±0.005 ppm for ^1H spectra, and ± 0.1 ppm for ^{13}C spectra. The coupling constants were obtained from one-dimensional spectra and measured in Hz, with an accuracy of ±0.05 Hz, unless specified in Table 1. The titration experiments were performed by adding increasing amounts of the drug to the solution (1–3 mM) of the oligonucleotide (DNA), until R = [drug]/[DNA] = 2. As the ligands are not completely soluble in water, the NMR experiments were performed by adding a 12 mM solution in DMSO-d_6 to the oligonucleotide.

NOESY and ROESY spectra were acquired in the phase-sensitive TPPI mode, with 2K × 512 complex fids, a spectral width of 6666.667 Hz, recycling delay of 1.3 s, 72 scans, at temperature of +20 °C, with a mixing time of 300 ms. TOCSY and ROESY spectra were acquired with the use of MLEV-17 spin-lock pulse[28] (field strength 7576 Hz, 60 ms total duration). All spectra were transformed and weighted with a 90° shifted sine-bell squared function to 1K × 1K real data points. Water suppression was achieved by the pre-saturation technique, placing the carrier frequency on the H_2O resonance. The ^1H assignments for free and bound molecules were performed by using the coupling constant values, and NOESY, ROESY, COSY and TOCSY experiments. In particular, the assignment of 4-H *vs.* 1-H in water was established by ROESY, using the NOE interactions with 5-H and 15-H, respectively, then 2-H *vs.* 3-H by the interaction with 1-H and 4-H, respectively. The assignment of 5-Heq *vs.* 5-Hax was made by the five-bond coupling between 15-Hax and 5-Hax: the homoallylic coupling is maximum when the orientation of the interacting protons is parallel to the plane of the π-bonds. The assignments for the free oligonucleotides $d(AAGAATTCTT)_2$, $d(CGACGTCG)_2$ and $d(GCGATCGC)_2$ have been reported previously.[6,8] The sequential assignment of nucleotide units in the free nucleotide and in the complexes was performed by applying well established procedures[11] for the analysis of double-stranded oligomers in the B-DNA form. The ^{13}C assignments were performed by DEPT and HSQC experiments.

Molecular modeling

Molecular models were built using a Silicon Graphics 4D35GT workstation running the INSIGHT II & DISCOVER software. MM and MD simulations of oligomers were carried out starting from the B-DNA conformation generated by using standard bond lengths and angles contained in the INSIGHT library. For the models generated by DISCOVER we used the CVFF force field supported by the Biosym program. The system was surrounded by a sphere of water molecules with a radius of 25 Å. The models of the complexes were developed by docking the interacting molecules into the DNA in an orientation suggested by the experimental NOEs. In the first step, we performed a minimization by DISCOVER, applying 100 steps of steepest-descendent algorithm followed by conjugate gradient minimization, until the energy difference between successive minimization steps was less than 0.1 kcal mol^{-1}. Then, a 10 ps simulation was run at a constant temperature of 300 K. No restraints were applied, except for the NOE contacts and the hydrogen bonds between the base pairs (1.7–1.9 Å), including those of the terminal base pairs. The models of AATT/**9**, AATT/**10a** and ACGT/**10b** yielded structures with total energy equal to −19588, −19888 and −21698 kcal mol^{-1}, respectively. An average structure for each complex was created in order to calculate the RMS deviation over all heavy atoms, relative to each single frame of the MD calculation; the RMS value converged to 0.9 ± 0.3 Å for AATT models, and 1.0 ± 0.3 Å for ACGT model. The convergence of the energy was ensured and the stability of the system was checked: a plot of the total energy of the drug/nucleotide complexes *vs.* the time of MD simulation showed that after only 2 ps the systems reached equilibrium.

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References

1 A. Monsees, S. Laschat, M. Hotfilder, J. Wolff, K. Bergander, L. Terfloth and R. Fröhlich, *Bioorg. Med. Chem. Lett.*, 1997, **7**, 2945.
2 A. Monsees, S. Laschat, M. Hotfilder and P. G. Jones, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 2881.
3 M. Demeunynck, C. Bailly and W. D. Wilson, *DNA and RNA Binders*, Wiley, Weinheim, 2003.
4 L. H. Hurley, *Nat. Rev. Cancer*, 2002, **2**, 188.
5 B. H. Long and B. N. Balasubramanian, *Expert Opin. Ther. Pat.*, 2000, **10**, 635.
6 S. Mazzini, M. C. Bellucci, S. Dallavalle, F. Fraternali and R. Mondelli, *Org. Biomol. Chem.*, 2004, **2**, 505.
7 T.-K. Li, E. Bathory, E. J. LaVoie, A. R. Srinivasan, W. K. Olson, R. R. Sauers, L. F. Liu and D. S. Pilch, *Biochemistry*, 2000, **39**, 7107.
8 S. Mazzini, M. C. Bellucci and R. Mondelli, *Bioorg. Med. Chem.*, 2003, **11**, 505.
9 (*a*) P. Krishnan and K. F. Bastow, *Anti-Cancer Drug Des.*, 2000, **15**, 255; (*b*) N. Iizuka, K. Miyamoto, K. Okita, A. Tangoku, H. Hayashi, S. Yosino, T. Abe, T. Morioka, S. Hazama and M. Oka, *Cancer Lett.*, 2000, **148**, 19; (*c*) K. Fukuda, Y. Hibiya, M. Mutoh, M. Koshiji, S. Akao and H. Fujiwara, *J. Ethnopharmacol.*, 1999, **66**, 227; (*d*) J. G. Lin, J. G. Chung, L. T. Wu, G. W. Chen, H. L. Chang and T. F. Wang, *Am. J. Chin. Med.*, 1999, **27**, 265.
10 (*a*) S. Laschat, *Liebigs Ann./Recl.*, 1997, 1; (*b*) O. Temme, S. Laschat, R. Fröhlich, B. Wibbeling, J. Heinze and P. Hauser, *J. Chem. Soc., Perkin Trans. 2*, 1997, 2083; (*c*) S. Laschat and J. Lauterwein, *J. Org. Chem.*, 1993, **58**, 2856.
11 D. Neuhaus, M. Williamson, *The Nuclear Overhauser Effect in Structural and Conformational Analysis*, VCH, New York, 1998.
12 A. Monsees, S. Laschat, S. Kotila, T. Fox and E.-U. Würthwein, *Liebigs Ann./Recl.*, 1997, 533.
13 F. Linkert, S. Laschat, S. Kotila and T. Fox, *Tetrahedron*, 1996, **52**, 955.
14 F. Linkert, S. Laschat and M. Knickmeier, *Liebigs Ann. Chem.*, 1995, 985.
15 CCDC-243553 contains the crystallographic data for this paper. See http://dx.doi.org/10.1039/b503281d for crystallographic data in CIF or other electronic format.
16 (*a*) U. Omura and D. Swern, *Tetrahedron*, 1978, **34**, 1651; (*b*) A. J. Mancuso, S. L. Huang and D. Swern, *J. Org. Chem.*, 1978, **43**, 2480.
17 H. Günther, *Modern NMR Spectroscopy*, John Wiley & Sons, New York, 1995.
18 S. Mazzini, G. Musco, E. Ragg and S. Penco, *Magn. Reson. Chem.*, 1994, **32**, 139.
19 H. Loskotová and V. Brabec, *Eur. J. Biochem.*, 1999, **266**, 392.
20 R. Bortolini, S. Mazzini, R. Mondelli, E. Ragg, C. Ulbricht, S. Vioglio and S. Penco, *Appl. Magn. Reson.*, 1994, **7**, 71.
21 S. Mazzini, R. Mondelli and E. Ragg, *J. Chem. Soc, Perkin Trans. 2*, 1998, 1983, and references quoted therein.
22 A. Pulmann and B. Pulmann, *Q. Rev. Biophys.*, 1981, **14**, 289.
23 S. Bommarito, N. Peyret and J. SantaLucia, Jr., *Nucleic Acids Res.*, 2000, **28**, 1929.
24 J. Tuma, R. Paulini, J. A. Rojas Stütz and C. Richert, *Biochemistry*, 2004, **43**, 15680.
25 M. B. Hansen, S. E. Nielsen and K. Berg, *J. Immunol. Methods*, 1989, **119**, 203.
26 (*a*) A. N. Gough, R. L. Jones and W. D. Wilson, *J. Med. Chem.*, 1979, **22**, 1551; (*b*) J. B. Chairs, N. Dattagupta and D. M. Crothers, *Biochemistry*, 1982, **21**, 3927.
27 L. A. Marky and K. J. Breslauer, *Biopolymers*, 1987, **26**, 1601.
28 A. Bax and D. G. Davis, *Magn. Reson.*, 1985, **65**, 35.