Asymmetric Synthesis and DNA Intercalation of (−)-6-[[(Aminoalkyl)oxy]methyl]-4-demethoxy-6,7-dideoxydaunomycinones[1,†]

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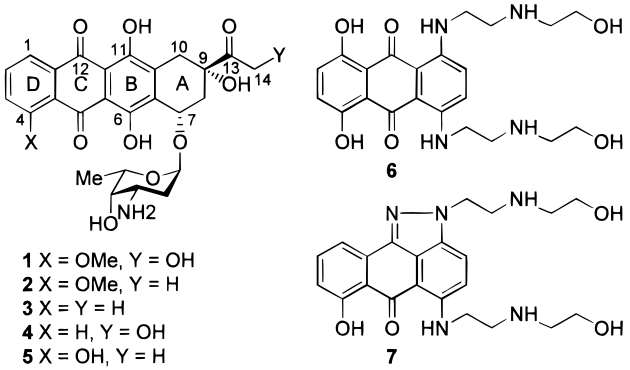
The BF_3·Et_2O-promoted Diels−Alder addition of 1-acetylvinyl RADO(Et)-ate (RADO(Et)-ate = 3-ethyl-2-oxo-6,8-dioxa-3-azabicyclo[3.2.1]octane-7-*exo*-carboxylate) to 1-(dimethoxymethyl)-2,3,5,6-tetramethylidene-7-oxabicyclo[2.2.1]heptane led to one major monoadduct that added to 1,2-didehydrobenzene and was converted into (−)-4-demethoxy-7-deoxydaunomycinone and (2*R*)-12-acetoxy-2-acetyl-5-(bromomethyl)-1,2,3,4-tetrahydronaphthacen-2-yl RADO(Et)-ate. The latter compound was used to construct (8*R*)-8-acetyl-6,8-dihydroxy-11-[[(3′-[(aminopropyl)oxy]-, -4′-[(aminobutyl)oxy], and -5′-[(aminopentyl)oxy]methyl]-7,8,9,10-tetrahydronaphthacene-5,12-dione hydrochloride (−)-**8**, (−)-**9**, (−)-**10**, respectively, as well as (8*R*)-8-acetyl-6,8-dihydroxy-11- [[[2′-[(3″-aminopropyl)amino]ethyl]oxy]- ((−)-**11**) and -[[3′-[(3″-aminopropyl)amino]propyl]oxy]methyl]-7,8,9,10-tetrahydronaphthacene-5,12-dione hydrochloride ((−)-**12**). (8*R*)-8-Acetyl-6,8-dihydroxy-11-[[(α-L-daunosaminyl)oxy]methyl]-7,8,9,10-tetrahydronaphthacene-5,12-dione hydrochloride ((−)-**13**), a mimic of idarubicin, was also prepared. Absorbance and fluorescence titration experiments showed (−)-**8**, (−)-**9**, and (−)-**10** to intercalate calf thymus DNA whereas (−)-**11**, (−)-**12**, and (−)-**13** did not. The best intercalator was (−)-**9** ($K_b = (1.1 \pm 0.1) \times 10^5$ M^{-1}) with the [(4′-aminobutyl)oxy]-methyl chain. Inhibition of topoisomerase II-induced DNA strand religation was observed for (−)-**8** at a concentration of 50 μM.

The clinical utility of the anthracycline antitumor antibiotics such as adriamycin (**1**) and daunomycin (**2**) is well demonstrated.[3] Their effectiveness, however, is restricted due to acute bone marrow toxicity, cardiotoxicity, and drug resistance development.[4] More than 2000 analogues have been synthesized and tested during the last 30 years. Among them, idarubicin (**3**), 4-demethoxyadriamycin (**4**),[5,6] and carminomycine (**5**)[7] have comparable or better *in vivo* activity than **1** and **2** at lower dose.

Simple 1,4-bis[(aminoalkyl)amino]-9,10-anthraquinones[8] such as mitoxantrone (**6**)[9] and isosoxantrone (**7**)[10] also have useful anticancer properties. These observations together with the fact that polyamines are able to inhibit cancer cell growth[11] led us to conceive a new series of anthracycline analogues (−)-**8** - (−)-**12** bearing aminoalkyl chains through a benzyl ether link at C(6). We have also prepared the α-L-daunosaminyl derivative (−)-**13** and have evaluated the ability of these new analogues to bind calf thymus DNA through interacation[12] and, for some of them, their ability to inhibit topoisomerisase



† Dedicated to Prof. Horst Prinzbach on the occasion of his 65th birthday, with thanks and admiration.

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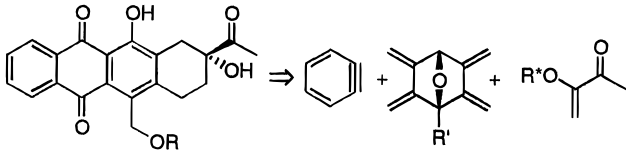
I-induced relaxation of circular plasmids and topoisomerase II-induced DNA strand religation. The newly developed synthetic approach in this work has led us to propose a new synthesis of (−)-(*R*)-4-demethoxy-7-deoxydaunomycinone,[1] a known precursor[13] of idarubicin (**3**). While (−)-**11** − (−)-**13** do not intercalate calf thymus DNA, (−)-**8** − (−)-**10** do bind with DNA.

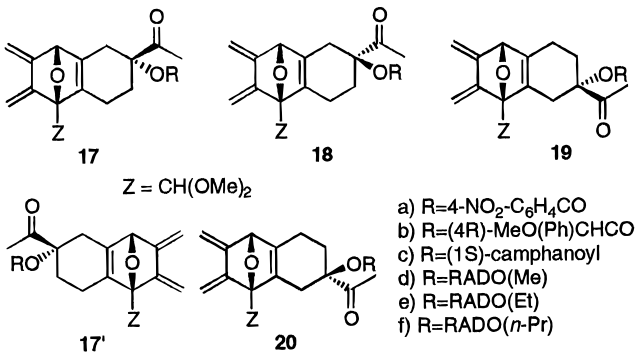


(-)-**8** R = $(CH_2)_3NH_2$·HCl
(-)-**9** R = $(CH_2)_4NH_2$·HCl
(-)-**10** R = $(CH_2)_5NH_2$·HCl
(-)-**11** R = $(CH_2)_2NH(CH_2)_3NH_2$·$(HCl)_2$
(-)-**12** R = $(CH_2)_3NH(CH_2)_3NH_2$·$(HCl)_2$
(-)-**13** R = α-daunosaminyl·HCl

14 R' = H **16** (a-f)
15 R' = $(MeO)_2CH$

In 1979, our group[14] demonstrated that the readily available tetraene **14** can be converted through two successive Diels−Alder additions with two different dienophiles into a variety of linearly condensed polycyclic systems including racemic anthracyclinone derivatives. The method has been applied to the synthesis of (±)-4-demethoxydaunomycinone,[15] (±)-daunomycinone,[16] and (±)-11-deoxydaunomycinone.[17] 1-(Dimethoxymethyl)-2,3,5,6-tetramethylidene-7-oxabicyclo[2.2.1]heptane (**15**), a tetraene obtained readily from furfural,[18] was envisaged as a precursor of anthracyclinone analogues bearing a carbon substituent at C(6), as required for (−)-**8**−(−)-**13**.

Desymmetrization through Diels−Alder Additions. The cycloadditions of tetraene **15** to 1-acetylvinyl esters of type **16** are expected to generate eight diastereomeric monoadducts of type **17**−**20**. There are 16 modes of addition corresponding to the "*para*" (→ **17** + **18**) and "*meta*" (→ **19** + **20**) orientations, to the "Alder" or "anti-Alder" rule and whether the *exo* or *endo* face of the bicyclic exocyclic diene is involved. Under thermal conditions the Diels−Alder addition of methyl vinyl ketone to **15** is not stereoselective and gives a mixture of all possible monoadducts.[18] However, when methyl vinyl ketone was coordinated to a strong Lewis acid some selectivity could be observed, the best results being obtained for $EtAlCl_2$. It was found also that the regioselectivity (product ratio) depends on the solvent.[18] A single monoadduct (**17a**) was obtained for the cycloaddition of **15** to 1-acetylvinyl *p*-nitrobenzoate[19] precomplexed with BF_3·Et_2O.[20] For all these Diels−Alder additions the formation of the bis-adduct of **15** were at least 100 times slower than the additions of the first equivalent of the dienophile.[21]



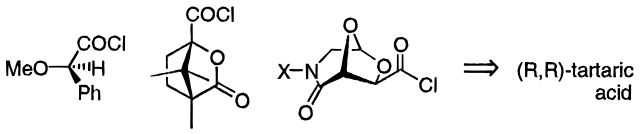
17 **18** **19**

Z = $CH(OMe)_2$

17' **20**

a) R=4-NO_2-C_6H_4CO
b) R=(4R)-MeO(Ph)CHCO
c) R=(1S)-camphanoyl
d) R=RADO(Me)
e) R=RADO(Et)
f) R=RADO(*n*-Pr)

Since preliminary experiments with optically pure Lewis acids such as (−)-BINAP-$TiCl_2$ and (+)-$Eu(tfc)_3$ failed to induce useful asymmetry in the Diels−Alder additions of **15** to 1-acetylvinyl *p*-nitrobenzoate, we explored the possibility to apply optically pure 1-acetylvinyl esters as dienophiles to generate optically active monoadducts.



21b **21c**

21d X = Me RADO(Me)-Cl
21e X = Et RADO(Et)-Cl
21f X = *n*-Pr RADO(*n*-Pr)-Cl

(R,R)-tartaric acid

Condensation of diacetyl with the optically pure acyl chlorides **21b**−**f** in toluene (pyr., 0 °C) gave the corresponding dienophiles **16b**−**f**. Compound **16e** added slowly and with low stereoselectivity to tetraene **15** on heating. Mixture of eight monoadducts were obtained in all cases. When mixed with Me_3Al, Et_2AlCl, $EtAlCl_2$, $TiCl_4$, Ti(O-*i*-Pr)Cl_3, BF_3·Me_2O, BBr_3, $BBr(OMe)_2$, or Et_2-BBr, polymerization of **15** and **16** occurred between −78 and −50 °C. For the reaction of **15** with **16c**[22] coordinated to Ti(O-*i*-Pr)$_2Cl_2$, a 19:10:5:3 mixture of four diastereomeric monoadducts (64% yield) was obtained. Lewis acids such as (*t*-Bu)$Me_2SiOSO_2CF_3$ (−30 °C), $B(OMe)_3$ (25 °C), or $Eu(tfc)_3$ (25 °C) did not promote the cycloadditions of **15** to **16e**. However, in the presence of a large excess of BF_3·Et_2O, **15** added to **16b**−**f** giving the corresponding monoadducts **17b**−**f** and **17′b**−**f** (see Table 1) with less than 10% (by 360 MHz ^{1}H NMR) of the other stereomeric adducts, thus confirming the high regio- and stereoselectivity of the Lewis-acid-promoted Diels−Alder additions. The best diastereoselectivity was observed with **16e**[22] (product ratio **17e**/**17′e** 87:13) in CH_2Cl_2. Changing the solvent from pure CH_2Cl_2 to CH_2Cl_2/$MeNO_2$ 6:1 (−78 °C) led to a higher yield of **17e** + **17′e** but with lower diastereoselectivity (83:17). In CH_2Cl_2/hexane 5:1 (−55 °C) and CH_2Cl_2/CF_2Cl_2 2:1 (−78 °C) the yields and diastereoselectivity were not improved (51% (81:19) and 20% (82:18), respectively). Surprisingly, in pure propionitrile the yield climbed to 90% but with lower and reversed diastereoselectivity (43:57). The structure and absolute configuration of the major adduct **17e** have been established as shown in Table 1.

The 87:13 mixture of **17e** and **17e′** ($CHCl_3$, drying with 3 Å molecular sieves) reacted with didehydrobenzene (generated through nitrosation of anthranilic acid with

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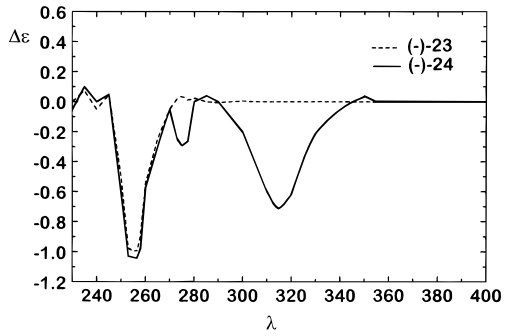


Figure 1. Superimposed CD spectra ($CHCl_3$) of (−)-**23** and (−)-**24**.

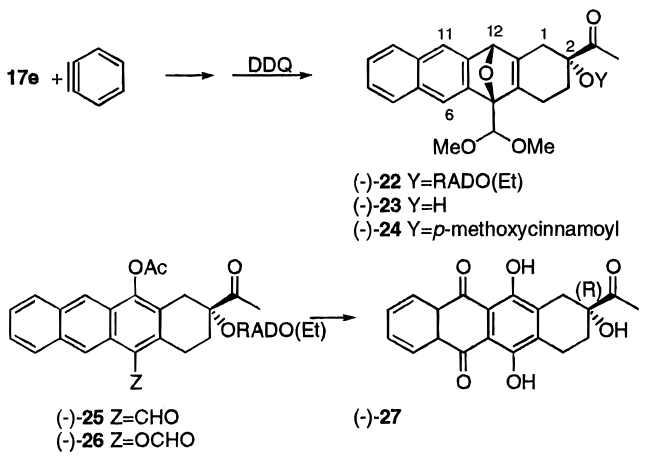
Table 1. Yields and Diastereoselectivity (Product Ratio 17/17′) for the BF_3·Et_2O (15 Equiv)-promoted Cycloadditions of 15 to 16 (1.2 Equiv) in CH_2Cl_2 (7 days)

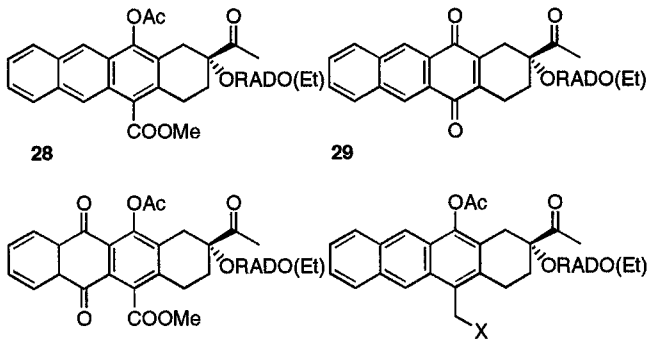
dienophile	**16b**	**16c**	**16d**	**16d**	**16e**	**16f**
T (°C)	−78	−55	−78	−50	−78	−78
yield of **17** + **17′** (%)	53	66	46	50	79	48
[**17**]/[**17′**]	56:44	36:64	85:15	81:19	87:13	84:16

isopentyl nitrite in 1,2-dimethoxyethane (DME)/$CHCl_3$). After treatment with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ in DME, 55 °C) the naphthacenyl methyl ketone (−)-**22** ((2*R*,5*S*,12*R*)-2-acetyl-5-(dimethoxymethyl)-1,2,3,4,5,12-hexahydro-5,12-epoxynaphthacen-2-yl RADO-(Et)-ate) was obtained (60%, purified by medium-pressure liquid chromatography). Saponification of (−)-**22** with 1 M NaOH/EtOH gave (−)-**23**, the esterification of which with *p*-methoxycinnamic anhydride (DMAP, CH_2Cl_2, 25 °C, 50 h) afforded (−)-**24**. The CD spectrum of (−)-**24** showed a typical exciton type of Cotton effect[23] (see Figure 1) consistent with a negative chirality, which implies the (*R*) configuration at C(2). The relative configuration of center C(2) was confirmed by NOE measurements in the ^{1}H NMR spectrum observed between the aromatic protons at C(6) and C(11) and the protons of the RADO(Et) moiety of (−)-**22**.

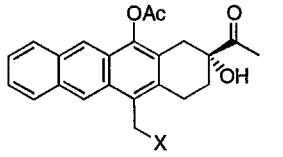
The absolute configuration of C(2) in (−)-**22** was further confirmed by converting it into the known (−)-7-deoxyidarubicinone ((−)-**27**).[24] The treatment of crude (−)-**22** with $Me_3SiOSO_2CF_3$ in CH_2Cl_2 (0 °C), followed by acetylation (Ac_2O/pyridine, 25 °C) and two recrystallization from EtOAc furnished the diastereomerically pure naphthacenyl methyl ketone (−)-**25** (55%). Baeyer−Villiger oxidation with SeO_2 and H_2O_2 led to the formate (−)-**26** (94%), which upon oxidation (4 N Jones-reagent, acetone, 0−20 °C) and saponification (1 M NaOH/THF, 20 °C) afforded (−)-**27** in 80% yield.

Synthesis of 6-[[(Aminoalkyl)oxy]methyl]-4-demethoxy-6,7-dideoxydaunomycinones. Oxidation of the dimethyl acetal of naphthacenecarbaldehyde (−)-**25** with PDC (pyridium dichromate[25]) led to a mediocre yield (33%) of the corresponding methyl ester **28**. The reaction was accompanied with the formation of the naphthacene-5,12-quinone **29**. Oxidation of (−)-**25** with $RuCl_3$/$NaIO_4$[26] or with $KMnO_4$/18-crown-6 ether/benzene[27] generated a complex mixture containing a small amount of **29**. The direct transformation of (−)-**25** into **30** was accomplished using Bu_4NMnO_4 in pyridine as oxidant,[28] followed by esterification with CH_2N_2. Unfortunately, the yield never surpassed 45%. The reduction of aldehyde (−)-**25** with $NaBH_3CN$ in AcOH/MeOH/$CHCl_3$ (20 °C) was selective (no reduction of the methyl ketone moiety) and afforded the benzyl alcohol (−)-**31** (93%), which was converted into bromide (−)-**32** (97%) on treatment with AcBr in $CHCl_3$.

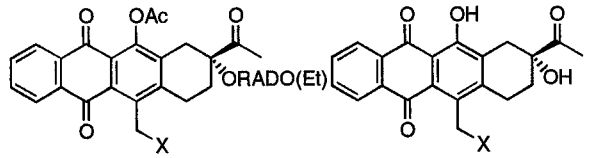




(-)-**31** X=OH
(-)-**32** X=Br
(-)-**33** X=O(CH_2)$_3$NHBoc
(-)-**37** X=O(CH_2)$_3$NHAlloc
(-)-**40** X=O(CH_2)$_3$N(Alloc)(CH_2)$_3$OH
(-)-**46** X=O(CH_2)$_3$N(Alloc)(CH_3)NHAlloc
(-)-**48** X=O(CH_2)$_3$NHCOCF$_3$
(-)-**50** X=O(CH_2)$_4$NHCOCF$_3$
(-)-**51** X=O(CH_2)$_5$NHCOCF$_3$
(-)-**52** X=O(CH_2)$_2$N(COCF$_3$)(CH_2)$_3$NHCOCF$_3$



(-)-**34** X=O$CH_2CH_2CH_2$NHBoc
(-)-**35** X=Cl
(-)-**36** X=OH
(-)-**38** X=O(CH_2)$_3$NHAlloc
(-)-**39** X=O(CH_2)$_3$$NH_2$·HCl
(-)-**41** X=O(CH_2)$_3$N(Alloc)(CH_2)$_3$OH
(-)-**42** X=O(CH_2)$_3$NH(CH_2)$_3$OH·HCl



(-)-**43** X=O(CH_2)$_3$NHAlloc
(-)-**47** X=O(CH_2)$_3$N(Alloc)-(CH_2)$_3$NHAlloc
(-)-**49** X=O(CH_2)$_3$NHCOCF$_3$
(-)-**53** X=O(CH_2)$_4$NHCOCF$_3$
(-)-**54** X=O(CH_2)$_5$NHCOCF$_3$
(-)-**55** X=O(CH_2)$_2$N(COCF$_3$)(CH_2)$_3$NHCOCF$_3$

(-)-**44** X=O(CH_2)$_3$NHAlloc
(-)-**45** X=O(CH_2)$_3$NHCOC$_6$H$_4$NO$_2$
(-)-**56** X=O(CH_2)$_4$NHCOC$_6$H$_4$NO$_2$
(-)-**57** X=O(CH_2)$_5$NHCOC$_6$H$_4$NO$_2$

Alcoholysis of bromide (−)-**32** with $HOCH_2CH_2CH_2$-NHBoc in anhydrous THF and in the presence of $AgOSO_2$-

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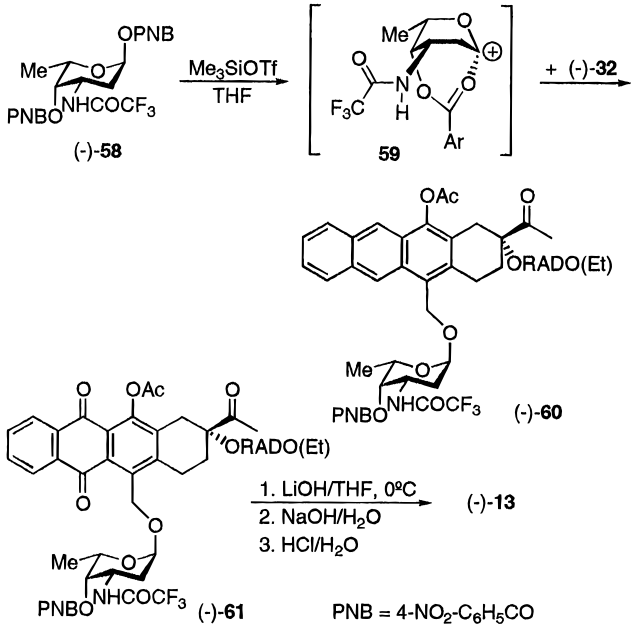
CF_3[29] gave (−)-**33** (71%). Saponification of (−)-**33** with aqueous 1 M NaOH in THF (0 °C) cleaved selectively the RADO(Et) ester group with formation of (−)-**34** (84%). At this stage we failed in our attempts to deprotect the amino moiety.[30] When (−)-**34** was treated with gaseous HCl in CH_2Cl_2 (0 °C)[31] the benzyl chloride (−)-**35** was obtained in 90% yield. Aqueous 3 M HCl in AcOEt (20 °C, 12 h) provided the corresponding alcohol (−)-**36** (74%). These experiments showed that the Boc protective group does not suit our purposes; we thus prepared the Alloc[32] -protected amine (−)-**37** (80%) as above by treatment of bromide (−)-**32** with $HO(CH_2)_3NHCOOCH_2CH{=}CH_2$ and AgOTf. Selective saponification of (−)-**37** with NaOH/H_2O/THF provided (−)-**38** (84%). Palladium-catalyzed ($Pd(Ph_3P)_4$) reduction (Bu_3SnH) of the Alloc group,[33] followed by treatment with aqueous HCl (pH ≥ 3.0 to avoid ether cleavage) furnished (−)-**39** (66%). In a similar way, the analogues (−)-**40** (60%), (−)-**41** (53%), and (−)-**42** (70%) were prepared.

Because the direct oxidation of the anthracene units of (−)-**39** and (−)-**42** led only to decomposition, we turned back to the protected amines (−)-**37** and (−)-**40**. Oxidation of (−)-**37** with aqueous 4 N Jones reagent (CrO_3/H_2SO_4) in acetone (0 °C, 30 min, 20 °C, 1.5 h) led to the corresponding anthraquinone (−)-**43** (91%). Saponification of (−)-**43** with LiOH/H_2O/THF gave (−)-**44**, which was contaminated with about 10% of an unknown compound. Treatment of this crude product with Pd-$(Ph_3P)_2(OAc)_2$ and Bu_3SnH in wet CH_2Cl_2 (20 °C, 20 min) afforded the unprotected amine. After acidification with 0.1 M aqueous HCl (to pH = 2.3−3.0) and purification by flash chromatography on silica gel, the pure hydrochloride (−)-**8** was obtained in 45% yield. The structure of this compound was given by its spectral data (see Experimental Part) and was further characterized as its *p*-nitrobenzamide (−)-**45**. Under similar conditions we prepared also the diamine double hydrochloride (−)-**12**. The reaction of bromide (−)-**32** with *N,N*-bis(allyloxy)-carbamate of 3-[(3′-aminopropyl)amino]propanol[34] and AgOTf (THF, molecular sieves) gave (−)-**46** (84%). Oxidation (Jones) led to anthraquinone (−)-**47** (94%), the saponification of which with aqueous 1 M LiOH in THF (0 °C), followed by palladium-catalyzed reduction provided (−)-**12** (16%) after acidification with aqueous HCl.

Another route toward the anthracycline analogue (−)-**8** was opened that relies on the nucleophilic displacement of bromide (−)-**32** with 3-(trifluoroacetamido)propanol (AgOTf, THF, 20 °C). The benzyl ether (−)-**48** (87%) so-obtained was oxidized (Jones) into (−)-**49** (85%). Treatment of (−)-**49** with LiOH (THF, H_2O, 0 °C) cleaved the RADO(Et) and acetate esters. Without isolating the corresponding alcohol, the mixture was then treated with aqueous 0.02 M NaOH (25 °C) and finally with aqueous HCl to furnish (−)-**8** (62%). The same method was applied to the synthesis of analogues (−)-**9**, (−)-**10**, and (−)-**11**. The reactions of (−)-**32** with $HO(CH_2)_4NHCOCF_3$, $HO(CH_2)_5NHCOCF_3$, and $HO(CH_2)_2N(COCF_3)(CH_2)_3NHCOCF_3$ (AgOTf, THF) gave (−)-**50** (79%), (−)-**51** (63%), and (−)-**52** (75%), respectively. Oxidation (Jones) provided (−)-**53** (82%), (−)-**54** (77%), and (−)-**55** (76%), respectively. These compounds were hydrolyzed as above into (−)-**9** (26%), (−)-**10** (30%), and (−)-**11** (60%), respectively. Amines (−)-**9** and (−)-**10** were characterized as their *p*-nitrobenzamide (−)-**56** (81%) and (−)-**57** (81%), respectively.



Glycosidation of the benzyl alcohol (−)-**31** with the appropriate protected L-daunosamine derivative (−)-**58**[35] in the presence of Me_3SiOTf in THF (−78 to −25 °C) provided a 3:1 mixture of α- and *β*-pyranosides **60** in 25% yield only. Yields were lower under Terashima's conditions[36] (CH_2Cl_2/ether 2:1, −40 °C), probably because of solubility problems. Adding CH_3CN led to a loss of selectivity with formation of 1:1 mixture of α- and *β*-anomers, probably because of the nitrilium ion effect.[37] Finally, we found that the slow addition of (−)-**31** in $CHCl_3$ at −25 °C to a solution of (−)-**58** pretreated with Me_3SiOTf in THF at 0 °C (formation of intermediate **59**) led to a 2:1 mixture of the α- and *β*-anomers with 75% yield. The α/*β* selectivity could be raised to 7:1 on carrying out the glycosidation at 0 °C. Unfortunately, the yield dropped to 15% under these conditions. The desired glycoside (−)-**60** was separated from its *β*-anomer by high-pressure liquid chromatography and was isolated in 50% yield. Oxidation of (−)-**60** (Jones) gave (−)-**61** (71%). Ester cleavage with LiOH/H_2O/THF and hydrolysis of the trifluoroacetamido moiety with aqueous 0.02 M NaOH, followed by acidification with aqueous 0.1 M HCl, provided (−)-**13** (66%; 23% overall yield based on (−)-**31**).

DNA Intercalation. The molecular recognition of DNA by small molecules is an important macromolecular

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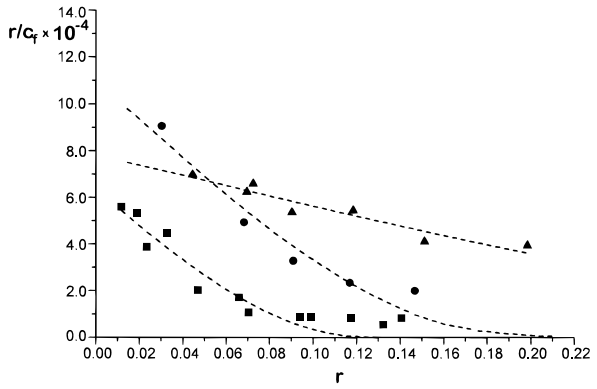

Figure 2. Equilibrium binding isotherms of (−)-**8** (■), (−)-**9** (●) and (−)-**10** (▲) (*r*: concentration ratio of intercalator/DNA in base pairs; free intercalator concentration).

receptor−drug interaction in the field of chemotherapy. Intercalation is one of the ways that a foreign molecule can interact with DNA.[12,38] It involves compounds possessing a planar aromatic chromophore stacked between the adjacent base pairs of DNA and stabilized by van der Waals forces. Hydrogen bonding or ionic interaction involving substituents attached to the chromophore frequently impart further stabilization and even DNA sequence selectivity to the binding process.[39,40] The relationship between structural alterations of DNA caused by intercalators and biological activity such as frameshift mutagenicity, B-DNA-transition, and antitumor activity is now clearly established. Changing the structure of RNA and DNA causes inhibition of some very important enzymes such as DNA, RNA polymerases, and DNA topoisomerases.[41]

Binding affinities of naphthacenequinones (−)-**8**−(−)-**13** and of naphthacene derivatives (−)-**39** and (−)-**42** to calf thymus DNA Type XV were evaluated by absorbance titration[42] and by fluorescence titration.[43] The data were analyzed by Scatchard-plots.[44] The dashed lines shown in Figure 2 for (−)-**8**, (−)-**9**, and (−)-**10** were calculated according to the neighbor exclusion model[45] and were obtained with n = 7.0 ± 0.1, 4.6 ± 0.4, and 2.0 ± 0.2, respectively, as exclusion parameters in base pairs. In the cases of (−)-**8** and (−)-**9** (data were not accurate enough for (−)-**10**) we calculated $K_b = (6.1 \pm 0.1) \times 10^4$ M^{-1} and $(1.1 \pm 0.1) \times 10^5$ M^{-1}, respectively, as intrinsic binding constants. These results suggests that (−)-**8** and (−)-**9** are better intercalators than daunomycinone (K_b = (1−3) × 10^4 M^{-1})[46] but weaker intercalators than adriamycin (**1**, $K_b = (2-4) \times 10^6$ M^{-1}) and daunomycin (**2**, $K_b = (4-6) \times 10^6$ M^{-1} or 1.2×10^6 M^{-1} under our conditions[47]).

Compounds (−)-**39** and (−)-**42** did not intercalate calf thymus DNA. This shows that the quinonic moiety is a requirement for intercalation of these 2-napthacenyl methyl ketones. Among the monoamine derivatives, (−)-**9** with a butyl chain seems to be the best adapted to calf thymus DNA structure under the studied conditions. It probably realizes a suitable arrangement for interaction between its ammonium unit and the phosphate backbone of DNA on one hand and simultaneous intercalation of its naphthacenequinone moiety on the other hand. The fact that the analogues (−)-**11** and (−)-**12** with diamino chains do not intercalate DNA suggests that these systems have geometries that do not allow their naphthacenequinone moieties to intercalate because their bis-ammonium systems are held by the phosphate anions away from the suitable base sequence. The inability of the α-daunosaminyl analogue (−)-**13** to intercalate shows the severe steric demand of DNA for the 6-substituted 2-naphthacenyl methyl ketone system to enter between the suitable base pairs.

Effect on Topoisomerases. Intercalators induce supercoils in circular plasmids. Topoisomerase I enzyme catalyzes the relaxation of supercoils, a phenomenon that depends on the concentration and affinity of the intercalator and that can be monitored by electrophoresis (unwinding assay).[48] pGEM plasmid was taken in entirely supercoiled form and was incubated (37 °C, 30 min, in the dark) with yeast topoisomerase I and different concentrations (50, 100, 200, 500 μM) of compounds (−)-**8**, (−)-**13**, (−)-**39**, and (−)-**42**.[49] After protein separation, samples were extracted (phenol/$CHCl_3$) and subjected to agarose gel electrophoresis using daunomycin (**2**) as standard. The topoisomerase I-induced relaxation was inhibited only with (−)-**8** at 200 μM. Under the same conditions, daunomycin inhibited the DNA unwinding at 20 μM. DNA intercalators also inhibit topoisomerase II, an important enzyme that catalyzes many topological interconversions of DNA loops during replication and transcription.[50] Anthracyclines are known to inhibit both DNA relaxation and DNA strand religation by topoisomerase II.[51] Inhibition of these two activities was tested for (−)-**8**, (−)-**11**, (−)-**12**, and (−)-**13**[49] using human topoisomerase II enzyme and pGEM DNA. Samples of different concentrations (50−500 μM) were incubated at 37 °C in the dark. After protein digestion (proteinase K) and extraction (phenol/$CHCl_3$), aliquots were subjected to agarose gel electrophoresis. In order to assay the topoisomerase II-induced relaxation inhibition, agarose without ethidium bromide was used, whereas agarose with ethidium bromide was employed in the topoisomerase II-induced DNA cleavage assays (distinction between linear DNA band and relaxed DNA bands).[52] Topoisomerase II-induced relaxation was inhibited at 150, 50, 150, 400, and 5 μM for (−)-**8**, (−)-**11**, (−)-**12**, (−)-**13**, and **2**, respectively. Inhibition of the topoisomerase II-induced DNA strand religation was observed only for (−)-**8** at a concentration of 50 μM (2 μM for **2** under the same conditions).

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The nonquinonic analogues (−)-**39** and (−)-**42** were also assayed as above. They did not show any inhibiting activity (up to 500 μM concentration) toward topoisomerase II.

Our results demonstrate that intercalation is a necessary condition for our anthracycline analogues to act as inhibitors of topoisomerase II-mediated DNA strand religation. The weak inhibiting activities observed for the nonintercalating systems (−)-**11**, (−)-**12**, and (−)-**13** toward topoisomerase II-induced DNA relaxation might be assigned to the association of the ammonium ions of these compounds with the phosphate DNA backbone, a nonspecific effect.

Conclusion

Optically pure (−)-6-[[(aminoalkyl)oxy]methyl]-4-demethoxy-6,7-dideoxydaunomycinone derivatives have been obtained through asymmetric Diels−Alder additions of 1-(dimethoxymethyl)-2,3,5,6-tetramethylidene-7-oxabicyclo[2.2.1]heptane. Those bearing (3′-aminopropyl)oxy ((−)-**8**), (4′-aminobutyl)oxy ((−)-**9**), and (5′-aminopentyl)oxy ((−)-**10**) chains are calf thymus DNA intercalators, whereas analogues with two amine units such as [*N*-(3″-aminopropyl)-2′-aminoethyl]oxy ((−)-**11**) or [*N*-(3″-aminopropyl)-3′-aminopropyl]oxy ((−)-**12**) chain attached at the 6-methyl moiety do not intercalate DNA. This was also the case for the 6-[(α-daunosaminyloxy)methyl]-4-demethoxy-6,7-dideoxydaunomycinone ((−)-**13**). Only compounds that were calf thymus DNA intercalators were inhibitors of human topoisomerase II-induced DNA strand religation. The best results were obtained with the (4′-aminobutyl)oxy derivative (−)-**9** that showed an intrinsic binding constant with calf thymus DNA $K_b = (1.1 \pm 0.1) \times 10^5\ M^{-1}$ ($n = 4.6 \pm 0.4$); this is about 10 times smaller than the binding constant measured for daunomycin.

Experimental Section

General Remarks. See ref 53. None of the procedures were optimized. Flash column chromatography (FC) was performed on Merck silica gel (230−400 mesh). Thin layer chromatography (TLC) was carried out on silica gel (Merck aluminum foils). ^{1}H NMR signal assignments were confirmed by double irradiation experiments and, when required, by 2-D-NOESY and COSY spectra. *J* values are given in Hz.

(8*R*)-8-Acetyl-6,8-dihydroxy-11-[(3′-aminopropyloxy)-methyl]-7,8,9,10-tetrahydronaphthacene-5,12-dione Hydrochloride ((−)-8). Method A. A mixture of (−)-**44** (12 mg, 24 μmol), $Pd(Ph_3P)_2(OAc)_2$ (catalytic amount), and Bu_3SnH (13 μL, 2 equiv) was stirred in wet CH_2Cl_2 (2 mL) in the dark at 20 °C for 20 min. After solvent evaporation, the residue was dissolved in H_2O (3 mL) and acidified to pH 2.5−3.0 with aqueous 0.1 M HCl. It was then washed with Et_2O (10 mL, seven times) and evaporated to give a yellow solid that can be purified by FC (silica gel, 5 g, CH_2Cl_2/MeOH 4/1, R_f 0.19 (alizarine)) to yield 5 mg of (−)-**8** (45%). The product is very sensitive to light.

Method B. (−)-**49** (16 mg, 21 μmol) was dissolved in degasssed THF (1 mL). After the solution was cooled to 0 °C aqueous 1 M LiOH (0.1 mL) was added and the mixture was stirred in the dark for 20 min. After addition of saturated aqueous solution of NH_4Cl (5 mL) the product was extracted ($CHCl_3$, 15 mL, three times). The organic extracts were dried ($MgSO_4$). Solvent evaporation yielded a yellow solid that was immediately dissolved in THF (0.2 mL) and H_2O (2 mL). The THF was evaporated, and the suspension was treated with aqueous 0.02 M NaOH (0.2 mL, changed to red) at 25 °C for 20 min. It was then neutralized (aqueous 0.1 M HCl) and washed with Et_2O (10 mL, five times). The aqueous layer was evaporated, and the yellow residue was taken up with EtOH (1 mL) to allow filtration of the insoluble NaCl. After filtration (Celite), Et_2O was added to precipitate 6 mg (62%), yellow powder: $[\alpha]^{25}_D = -53$ ($c = 0.25$, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 8.23, 7.89 (2m), 4.98 (2d, $^2J = 10$), 3.91 (t, $^2J = 5$), 3.30−2.90 (m), 2.41 (s), 2.15−1.85 (m).

(8*R*)-8-Acetyl-6,8-dihydroxy-11-[(4′-aminobutyloxy)-methyl]-7,8,9,10-tetrahydronaphthacene-5,12-dione Hydrochloride ((−)-9). (−)-**53** (10 mg, 13 μmol) was dissolved in degassed THF/H_2O 3/1 (2 mL). After the solution was cooled to 0 °C, $LiOH{\cdot}H_2O$ (4 mg, 95 μmol) was added and the red mixture was stirred in the dark for 20 min. After addition of saturated aqueous solution of NH_4Cl (5 mL), the product was extracted ($CHCl_3$, 15 mL, three times). The organic extracts were dried ($MgSO_4$), and solvent evaporation yielded a yellow solid that was immediately dissolved in THF (0.2 mL) and H_2O (2 mL). The THF was evaporated and the suspension was treated with aqueous 0.02 M NaOH (0.1 mL, changed to red) at 25 °C for 30 min. It was then neutralized (aqueous 0.1 M HCl) and washed with Et_2O (10 mL, five times). The aqueous layer was evaporated, the yellow residue was taken up with EtOH (99.8%, 1 mL), and the insoluble NaCl was filtered off (cotton−wool). The filtrate was concentrated, and Et_2O was added to precipitate about 2 mg (30%) of (−)-**9** as a yellow powder. The quantity was measured more precisely (1.92 mg) by ^{1}H NMR using tris−HCl as internal standard. The product can be purified in low yield by FC in the dark (CH_2Cl_2/MeOH 4/1, $R_f = 0.4$, UV, alizarin). Other eluants such as *i*-PrOH/MeCN or EtOH/MeCN with 1% of CF_3COOH led to decomposition. Separation by HPLC on reversed phase (MN, CC 125/4, Nucleosil 100−5, C18 AB) was also attempted. With MeCN/H_2O 1/1 as eluant, no separation was achieved, while with a more polar mixture, no fractions of the desired product could be detected (probably due to low sensibility of Kontron IOTA 2 refractometer). Product degradation can be observed on storing in the solid state under argon at −18 °C: $[\alpha]^{25}_D = -55$ ($c = 0.2$, EtOH 95%); ^{1}H NMR (400 MHz, CD_3OD) δ 8.29, 7.91 (2m), 5.07 (d, $^2J = 10$), 3.79 (m), 3.30−2.90 (m), 2.42 (s), 2.10−1.90, 1.81 (2m).

(8*R*)-8-Acetyl-6,8-dihydroxy-11-[[(5′-aminopentyl)oxy]-methyl]-7,8,9,10-tetrahydronaphthacene-5,12-dione Hydrochloride ((−)-10). The same procedure as for the preparation of (−)-**9** was followed, starting with (−)-**54** (12 mg, 16 μmol). FC (−18 °C, in the dark, silica gel, CH_2Cl_2/MeOH 4/1, $R_f = 0.33$, UV, alizarin) gave 2 mg (26%) of a yellow gum: $[\alpha]^{25}_D = -49$ ($c = 0.2$, EtOH 95%); ^{1}H NMR (400 MHz, CD_3OD) δ 8.22, 7.86 (2m), 5.02, 4.96 (2d, $^2J = 10$), 3.71, 3.16 (2m), 3.09, 2.95 (2d, $^2J = 18.5$), 2.39 (s), 2.08−1.90, 1.70, 1.50 (3m).

(8*R*)-8-Acetyl-6,8-dihydroxy-11-[[2′-[(3″-aminopropyl)-amino]ethoxy]methyl]-7,8,9,10-tetrahydronaphthacene-5,12-dione Hydrochloride ((−)-11). The same procedure as for the preparation of (−)-**9** was followed, starting with (−)-**55** (8 mg, 9 μmol): yield 3 mg (60%); $[\alpha]^{25}_D = -6$ ($c = 0.1$, EtOH 95%); ^{1}H NMR (400 MHz, CD_3OD) δ 8.29, 7.92 (2m), 4.92 (d, $^2J = 10$), 4.08 (br s), 3.70, 3.20−2.90 (2m), 2.41 (s), 2.12−1.95, 1.65 (2m).

(8*R*)-8-Acetyl-6,8-dihydroxy-11-[[[3′-[(3″-aminopropyl)-amino)propyl]oxy]methyl]-7,8,9,10-tetrahydronaphthacene-5,12-dione Hydrochloride ((−)-12). (−)-**46** (19 mg, 22 μmol) was dissolved in degassed THF (2 mL). After the solution was cooled to 0 °C, aqueous 1 M LiOH (0.1 mL) was added and the mixture was stirred in the dark for 10 min. After addition of a saturated aqueous solution of NH_4Cl (5 mL) the product was extracted ($CHCl_3$, 15 mL, three times) and the combined organic extracts were dried ($MgSO_4$). Solvent evaporation yielded a yellow solid with about 10% impurity that could not be separated by chromatography or crystallization. The product obtained above, $Pd(Ph_3P)_2(OAc)_2$ (catalytic amount) and Bu_3SnH (20 μL, 74 μmol), was stirred in *wet* CH_2Cl_2 (2 mL) in the dark at 20 °C for 40 min. After solvent evaporation the residue was dissolved in H_2O (3 mL) and acidified to pH 2.5−3.0 with aqueous 0.1 M HCl. It was then washed with Et_2O (10 mL, seven times) and evaporated to give

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2 mg (16%) of a yellow solid: mp 130−135 °C (Et_2O/EtOH); $[\alpha]^{25}_D = -2$ ($c = 0.1$, EtOH 95%); ^{1}H NMR (400 MHz, CD_3OD) δ 8.29, 7.92 (2m), 4.92 (d), 3.91 (t, $^2J = 2.5$), 3.35−2.90 (m), 2.41 (s), 2.12−1.95 (m).

(8*R*)-8-Acetyl-6,8-dihydroxy-11-[[(2′,3′,6′-trideoxy-4′-hydroxy-3′-amino-α-L-*lyxo*-hexopyranosyl)oxy]methyl]-7,8,9,10-tetrahydronaphthacene-5,12-dione Hydrochloride ((−)-13). (−)-**61** (11 mg, 11 μmol) was dissolved in degassed THF (0.1 mL). After the solution was cooled to 0 °C, aqueous 1 M LiOH (0.1 mL) was added and the mixture was stirred in the dark for 20 min. After addition of a saturated aqueous solution of NH_4Cl (5 mL) the product was extracted ($CHCl_3$, 15 mL, three times), and the combined organic extracts were dried ($MgSO_4$). Solvent evaporation yielded a yellow solid that was immediately dissolved in THF (2 mL) and H_2O (2 mL). THF was then evaporated, and the suspension was treated with aqueous 0.02 M NaOH (0.2 mL, changed to red) at 25 °C for 20 min. It was then neutralized (aqueous 0.1 M HCl) and washed with Et_2O (10 mL, five times). The aqueous layer was evaporated, and the yellow residue was taken up with EtOH (1 mL) to allow filtration of the insoluble NaCl. After filtration (Celite), Et_2O was added to precipitate 4 mg (66%) of a yellow powder: mp 172−174 °C; $[\alpha]^{25}_D = -22$ ($c = 0.1$, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 8.23, 7.90 (2m), 5.48 (d, $^3J = 11$), 5.30 (m), 5.22 (d, $^3J = 1$), 5.00−4.80 (m), 4.26, 3.20−1.95 (2m), 2.42 (s), 2.20−1.95 (m), 1.28 (d, $^3J = 6$).

3′-Oxobut-1′-en-2′-yl (2*R*)-2-Methoxy-2-phenylacetate ((+)-16b). A mixture of (+)-2-methoxy-2-phenylacetic acid (100 mg, 0.6 mmol), DMF (0.047 mL, 0.6 mmol), and oxalyl chloride (0.25 mL, 0.3 mmol) was stirred for 1 h in hexane at 20 °C. It was then decanted from the white precipitate and evaporated to give 90 mg of a colorless oil that was dissolved without further purification in anhydrous toluene (5 mL) at 0 °C, and diacetyl (0.053 mL, 0.6 mmol) with Et_3N (0.18 mL, 1.2 mmol) were added. The mixture was stirred at 20 °C for 24 h. It was then poured into EtOAc (10 mL) and washed with aqueous 0.1 M HCl (10 mL, three times), with H_2O (10 mL, three times), and with brine (10 mL, three times). After drying ($MgSO_4$), evaporation, FC (silica gel, 10 g, light petroleum/EtOAc 5/1 ($KMnO_4$)) gave 49 mg (35%) of a colorless oil: $[\alpha]^{25}_D = +4$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.56−7.30 (m), 5.97, 5.58 (2d, $^2J = 3$), 4.99, 3.52, 2.22 (3s).

3′-Oxobut-1′-en-2′-yl (1*S*)-3-Oxo-4,7,7-trimethyl-2-oxabicyclo[2.2.1]heptane-1-carboxylate ((−)-16c). A mixture of diacetyl (0.1 mL, 1.12 mmol), Et_3N (0.3 mL, 2 mmol), and camphanic acid chloride (217 mg, 1 mmol) was stirred at 20 °C in anhydrous toluene (3 mL) for 24 h. It was then poured into EtOAc (20 mL) and washed with aqueous 0.1 M HCl (15 mL, three times), with H_2O (15 mL, three times), and finally with brine (15 mL, three times). After drying ($MgSO_4$) and solvent evaporation, FC (silica gel, 10 g, EtOAc/light petroleum 1/1 ($KMnO_4$)) gave 157 mg (60%) of colorless crystals: mp 61−64 °C (Et_2O); $[\alpha]^{25}_D = -2$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.02, 5.75 (2d, $^2J = 2.8$), 2.48 (ddd, $^2J = 13.5$, $^3J = 11$, 4.5), 2.09 (ddd, $^2J = 13.5$, $^3J = 9.5$, 4.5), 1.96 (ddd, $^2J = 13.5$, $^3J = 11$, 4.5), 1.68 (ddd, $^2J = 13.5$, $^3J = 9.5$, 4.5), 2.4, 1.12, 1.10, 1.08 (3s).

3′-Oxobut-1′-en-2′-yl (1*S*,5*R*,7*S*)-3-Methyl-2-oxo-6,8-dioxa-3-azabicyclo[3.2.1]octane-7-*exo*-carboxylate (3-Oxobut-1-en-2-yl RADO(Me)-ate, (−)-16d). RADO(Me)-OH[41] (187 mg, 1 mmol) was boiled under reflux in $SOCl_2$ (15 mL) for 1 h. The excess reagent was distilled off, and the residue was dissolved in EtOAc and precipitated with Et_2O to yield a pale yellow solid that was used without further purification. A mixture of diacetyl (0.1 mL, 1.12 mmol), Et_3N (0.3 mL, 2 mmol), and the acid chloride obtained above was stirred for 36 h at 20 °C in anhydrous toluene (3 mL). It was then poured into EtOAc (20 mL) and washed with aqueous 0.1 M HCl (15 mL, three times), with H_2O (15 mL, three times), and finally with brine (15 mL, three times). After drying ($MgSO_4$) and solvent evaporation, FC (silica gel, 10 g, EtOAc/light petroleum 3/1 ($KMnO_4$)) gave 153 mg (60%) of colorless crystals: mp 113−114 °C (Et_2O); $[\alpha]^{25}_D = -77$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.00, 5.76 (2d, $^2J = 3.0$), 5.97 (d, $^3J = 2.5$), 5.18, 4.90 (2s), 3.55 (dd, $^2J = 12.5$, $^3J = 2.5$), 3.22 (d, $^3J = 12.5$), 2.95, 2.39 (2s).

3′-Oxobut-1′-en-2′-yl (1*S*,5R,7*S*)-6,8-Dioxa-2-oxo-3-propyl-3-azabicyclo[3.2.1]octane-7-*exo*-carboxylate (3-oxobut-1-en-2-yl RADO(Pr)-ate, (−)-16f). RADO(Pr)-OH[41] (215 mg, 1 mmol) was boiled under reflux in $SOCl_2$ (15 mL) for 1 h. The excess of reagent was distilled off to yield a yellowish oil that was used without further purification. A mixture of diacetyl (0.1 mL, 1.12 mmol), Et_3N (0.3 mL, 2 mmol), and the acid chloride obtained above was stirred for 24 h at 20 °C in anhydrous toluene (3 mL). It was then poured into EtOAc (20 mL) and washed with aqueous 0.1 M HCl (15 mL, three times), with H_2O (15 mL, three times), and with brine (15 mL, three times). After drying ($MgSO_4$) and solvent evaporation, FC (silica gel, 10 g, EtOAc/light petroleum 1/1 ($KMnO_4$)) gave 119 mg (42%) of a colorless oil: $[\alpha]^{25}_D = -50$ ($c = 1.0$, $CHCl_3$).

(1′*R*,4′*R*,8′*S*)-4′-Acetyl-8′-(dimethoxymethyl)-9′,10′-dimethylidene-11′-oxatricyclo[6.2.1.0$^{2',7'}$]undec-2′(7′)-en-4′-yl (1*R*,5*S*,7*R*)-3-ethyl-2-oxo-6,8-dioxa-3-azabicyclo[3.2.1]-octane-7-*exo*-carboxylate ((−)-17e). (−)-**16e**[41] (500 mg, 1.86 mmol) was dissolved in anhydrous CH_2Cl_2 (9 mL) under Ar. The solution was cooled to −78 °C, and $BF_3{\cdot}Et_2O$ (3.5 mL, 28 mmol, 15 equiv) was added under vigorous stirring. After 30 min, a solution of **15**[40] (490 mg, 2.23 mmol, 1.2 equiv) in anhydrous CH_2Cl_2 (9 mL) was introduced slowly. The mixture was stirred for 1 week at −78 °C, and then it was poured at once into an ice-cold mixture of EtOAc and saturated aqueous solution of $NaHCO_3$ (100 mL, 1/1). The organic layer was washed with aqueous $NaHCO_3$ (50 mL), with H_2O (50 mL, twice), and finally with brine (50 mL, three times). After drying ($MgSO_4$) and evaporation of the solvent, the residue was purified on Florisil (50 g, 60−100 mesh, EtOAc/light petroleum 1/1) to give 50 mg of **15** (R_f 0.9) and 825 mg of a mixture of isomeric adducts (R_f 0.6). Two successive chromatographies (Lobar, CH_2Cl_2/EtOAc) gave an analytical sample of (−)-**17e** (69%) as an amorphous solid: mp 77−80 °C (hexane/Et_2O); $[\alpha]^{25}_D = -1$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 5.80 (d, $^3J = 2$), 5.37, 5.30, 5.28, 5.01 (4s), 4.95, 4.82 (2s), 4.60, 4.54 (2d, $^3J = 0.5$), 3.59, 3.57 (2s), 3.47 (dd, $^2J = 2$, $^3J = 12$), 3.40, 3.38 (2q, $^3J = 7.2$), 3.20 (d, $^3J = 12$), 3.01 (dt, $^2J = 18.5$, $^3J = 2$), 2.60−2.38 (m), 2.20−2.05 (m), 2.08 (s), 1.90−1.70 (m), 1.09 (t, $^3J = 7.2$).

(2′*R*,5′*S*,12′*R*)-2′-Acetyl-5′-(dimethoxymethyl)-1′,2′,3′,4′,5′,12′-hexahydro-5′, 12′-epoxynaphthacen-2′-yl (1*R*,5*S*,7*R*)-3-Ethyl-2-oxo-6,8-dioxa-3-azabicyclo[3.2.1]-octane-7-*exo*-carboxylate ((−)-22). A mixture of crude adduct (−)-**17e** and isomers (650 mg, 1.33 mmol), molecular sieves (3 Å, 10 pieces), isopentyl nitrite (250 μL, 1.86 mmol, 1.4 equiv), and anthranilic acid (430 mg in $MeOCH_2CH_2OMe$ (DME, 2 mL), 3.13 mmol, 2.4 equiv) was stirred for 45 min in $CHCl_3$ (6 mL) at 0 °C. A deep red precipitate was formed. The mixture was heated rapidly under vigourous stirring to 55 °C and allowed to react until the end of the gas evolution (about 25 min). It was then diluted with $CHCl_3$ (15 mL), and 3,6-dichloro-2,3-dicyanobenzoquinone (DDQ, 328 mg in DME (2 mL), 1.45 mmol, 1.1 equiv) was added. After 35 min at 55 °C, it was cooled, diluted ($CHCl_3$, 50 mL), and washed with 5% aqueous $NaHSO_3$ (30 mL, three times), with a saturated aqueous solution of $NaHCO_3$ (30 mL, three times), and finally with brine (30 mL, three times). Drying and solvent evaporation gave an oil that was subjected to chromathography (silica gel, 50 g, EtOAc/light petroleum 1/1, then 3/1, R_f 0.6). A mixture of starting material and different isomers of (−)-**22** was obtained and then separated on a Lobar column (EtOAc/light petroleum 1/1) to yield 180 mg of **17e** (72% conversion), 370 mg of pure (−)-**22**, and 80 mg of isomers of (−)-**22**. Recrystallization of the second fraction ((−)-**22**) gave colorless crystals: mp 98−102 °C (Et_2O); $[\alpha]^{25}_D = -31$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.72, 7.52 (2s), 7.72 (m), 7.38, 7.36 (2d, $^3J = 3.2$), 5.53, 5.05 (2s), 4.82 (d, $^3J = 2$), 4.00, 3.42, 3.75, 3.65 (4s), 3.20−2.90 (m), 2.77 (dd, $^2J = 12$, $^3J = 2$), 2.62 (dm, $^2J = 15$), 2.50 (d, $^2J = 12$), 2.40 (dm, $^2J = 15$), 2.20−2.00 (m), 2.05 (s), 1.90−1.70 (m), 0.95 (t, $^3J = 7$).

(2*R*,5*S*,12*R*)-5-(Dimethoxymethyl)-1,2,3,4,5,12-hexahydro-2-hydroxy-5,12-epoxynaphthacen-2-yl Methyl Ketone ((−)-23). (−)-**22** (28 mg, 0.05 mmol) was dissolved in

EtOH (3 mL, at 25 °C). Aqueous 0.1 M NaOH (0.5 mL) was added, and the mixture was stirred for 10 min. It was then neutralized with aqueous 0.2 M HCl, and EtOH was evaporated. The residue was dissolved in EtOAc (20 mL) and washed with brine (10 mL, three times). Drying ($MgSO_4$) and solvent evaporation yielded 17 mg (89%) of colorless crystals: mp 68−70 °C (Et_2O); $[\alpha]^{25}_D = -19$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.70 (m), 7.66, 7.51 (2s), 7.41 (m), 5.54, 5.08, 3.73, 3.66 (4s), 2.90 (ddd, $^2J = 18$, $^3J = 4$, 3), 2.62 (dm, $^2J = 18$), 2.21 (s), 2.15−2.00 (m), 1.98−1.79 (m), 1.62−1.52 (m).

(2*R*,5*S*,12*R*)-2-Acetyl-5-(dimethoxymethyl)-2-hydroxy-1,2,3,4,5,12-hexahydro-5,12-epoxynaphthacen-2-yl 3-(4′-Methoxyphenyl)prop-2-enoate ((−)-24). (−)-**23** (9 mg, 24 μmol) was dissolved in anhydrous Et_2O (1 mL) at 0 °C. Paramethoxycinnamic acid anhydride (25 mg, 48 μmol) and 4-(dimethylamino)pyridine (2 mg) were added. After being stirred at 40 °C for 50 h, the mixture was poured into ice−water and extracted with EtOAc (10 mL, three times). The combined organic extracts were washed with aqueous 0.1 M HCl (10 mL, twice), a saturated aqueous solution of $NaHCO_3$ (10 mL, twice), and finally with brine (10 mL, twice). Drying ($MgSO_4$), solvent evaporation, FC (silica gel, 1 g, EtOAc/light petroleum 1/1, R_f 0.5 ($KMnO_4$)) afforded 4 mg (31%) of a pale yellow solid: mp 99−102 °C (Et_2O); ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.68, 7.50 (2s), 7.62 (m), 7.22 (m), 6.86 (d, $^3J = 15$), 6.62 (m), 5.58 (s), 5.05 (s), 5.04 (d, $^3J = 15$), 3.82, 3.72, 3.68 (3s), 2.93 (dm, $^2J = 18$), 2.62 (m), 2.42 (d, $^2J = 18$), 2.18 (m), 2.15 (s), 2.10−1.97 (m).

(2′*R*)-12′-Acetoxy-2′-acetyl-5′-formyl-1′,2′,3′,4′-tetrahydronaphthacen-2′-yl (1*R*,5*S*,7*R*)-3-Ethyl-2-oxo-6,8-dioxa-3-azabicyclo[3.2.1]octane-7-*exo*-carboxylate ((−)-25). Crude adduct **22** (563 mg, 1 mmol) was dissolved in anhydrous CH_2Cl_2 (70 mL) at 0 °C. TMSOTf (1.0 mL, 6 equiv) was added at once, and the red mixture was stirred for 45 min. It was then rapidly poured into a mixture of EtOAc and saturated aqueous solution of $NaHCO_3$ (100 mL, 4:1, 0 °C). The organic layer was washed with a saturated aqueous solution of $NaHCO_3$ (30 mL), with H_2O (30 mL, twice), and with brine (30 mL, three times). After drying ($MgSO_4$), the solvent was evaporated. The brown solid was immediately treated with pyridine (5 mL) and Ac_2O (5 mL) under Ar at 0 °C. After 3 h of stirring at 20 °C, the solution was poured into ice−water and extracted with EtOAc (30 mL, three times). The combined organic extracts were washed with aqueous 1 M H_2SO_4 (30 mL, three times), with saturated aqueous solution of $NaHCO_3$ (30 mL, three times), and finally with brine (30 mL, three times), dried ($MgSO_4$), and evaporated. The residue was purified by two successive crystallizations (EtOAc) to yield 250 mg of pure (de > 95% by ^{1}H NMR: ^{13}C−^{1}H satellites) (−)-**25**. The filtrate was purified by FC (silica gel, 15 g, CH_2Cl_2/EtOAc 3/1, R_f 0.38 (yellow spot)) to give 120 mg of a yellow solid from which 60 mg of pure (−)-**25** can be obtained by two recrystallizations from EtOAc. Yield: 310 mg (55%) of optically pure (−)-**25** and 60 mg (11%) of a 1/1 mixture of diastereoisomers. Compound (−)-**25** was a yellow powder: mp 216 °C (EtOAc); $[\alpha]^{25}_D = -22$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 11.1, 9.50, 8.32 (3s), 8.05, 7.50 (2m), 5.70 (d, $^2J = 2$), 4.81, 4.72 (2s), 3.60−3.30 (m), 3.42 (dd, $^2J = 12$, $^3J = 2.5$), 3.31, 3.28 (2q, $^3J = 7$), 3.12 (d, $^2J = 12$), 2.58 (s), 2.60−2.50 (m), 2.26 (s), 2.30−2.10 (m), 1.05 (t, $^3J = 7$).

(2′*R*)-12′-Acetoxy-2′-acetyl-5′-(formyloxy)-1′,2′,3′,4′-tetrahydronaphthacen-2′-yl (1*R*,5*S*,7*R*)-3-Ethyl-2-oxo-6,8-dioxa-3-azabicyclo[3.2.1]octane-7-*exo*-carboxylate ((−)-26). A mixture of (−)-**25** (30 mg, 54 μmol), SeO_2 (1 mg, 10 μmol), and 30% aqueous H_2O_2 (10 μL, 100 μmol) was stirred vigorously for 24 h at 20 °C in CH_2Cl_2 (3 mL). It was then diluted with EtOAc (20 mL) and washed with 5% aqueous $NaHSO_3$ (10 mL, twice), with 10% aqueous Na_2CO_3 (10 mL, twice), and finally with brine (10 mL, twice). After drying ($MgSO_4$) and solvent evaporation, the residue was recrystallized from Et_2O, giving 29 mg (94%) of colorless crystals: mp 240−241 °C (Et_2O); $[\alpha]^{25}_D = -13$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.60, 8.38, 8.30 (3s), 8.00, 7.51 (2m), 5.78 (d, $^2J = 2$), 4.72, 4.68 (2s), 3.60−2.90 (m), 3.35 (dd, $^2J = 12$, $^3J = 2.5$), 3.08 (d, $^2J = 12$), 2.58 (s), 2.60−2.50 (m), 2.27 (s), 2.20−2.05 (m), 1.03 (t, $^3J = 7$).

(8*R*)-8-Acetyl-7,8,9,10-tetrahydro-6,8,11-trihydroxynaphthacene-5,12-dione ((−)-27). (−)-**25** (16 mg, 28 μmol) was dissolved in acetone (4 mL, 0 °C). Jones reagent (4 N, 0.12 mL, 0.17 mmol) was added dropwise, and the reaction mixture was stirred in the dark for 30 min at 0 °C and for 3 h at 20 °C. The excess of the oxidant was destroyed with *i*-PrOH (1 mL, changed to green). After solvent evaporation, the residue was taken up with EtOAc (20 mL) and washed with 10% aqueous $NaHCO_3$ (10 mL) and finally with brine (10 mL, four times). After drying ($MgSO_4$) and solvent evaporation, the yellow residue was immediately treated with 0.2 mL of aqueous 1 M NaOH in THF/H_2O (2 mL, 1/1) at 0 °C. After being stirred for 4 h at 20 °C, the solution was neutralized with 0.1 M aqueous HCl and extracted with $CHCl_3$ (10 mL, three times). The combined extracts were washed with brine (15 mL, three times). Drying ($MgSO_4$), solvent evaporation, and FC (silica gel, 1 g, CH_2Cl_2/EtOAc 6/1, R_f 0.45 (red spot)) gave 8 mg (80%) of red crystals, mp 216 °C (benzene) (lit.[24] mp 218-219 °C (benzene)). Spectral data were in agreement with those reported for this compound.[24]

(2′*R*)-12′-Acetoxy-2′-acetyl-5′-(methoxycarbonyl)-1′,2′,3′,4′-tetrahydronaphthacen-2′-yl (1*R*,5*S*,7*R*)-3-Ethyl-2-oxo-6,8-dioxa-3-azabicyclo[3.2.1]octane-7-*exo*-carboxylate (28). (−)-**25** (20 mg, 36 mmol) was dissolved in DMF (2 mL) at 20 °C. Activated molecular sieves (3 Å), MeOH (10 mL), and PDC (80 mg, 6 equiv) was added, and the mixture was stirred for 24 h. It was then diluted with CH_2Cl_2 and filtered (Celite), and the solvent was evaporated. The residue was directly subjected to FC (silica gel, 10 g, CH_2Cl_2/EtOAc 3/1) to give 7 mg (33%) of **28** (R_f 0.5) and 4 mg of **29** (R_f 0.85). Data for **28**: white solid; ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.34, 8.28 (2s), 7.98, 7.48 (2m), 5.80 (d, $^3J = 2$), 4.85, 4.70, 4.12 (3s), 3.41 (dd, $^2J = 12$, $^3J = 2$), 3.40−3.10 (m), 3.12 (d, $^2J = 12$), 2.60 (s), 2.60−2.50 (m), 2.26 (s), 2.25−2.20 (m), 1.08 (t, $^3J = 7$).

(2′*R*)-2′-Acetyl-5′,12′-dioxo-1′,2′,3′,4′-tetrahydronaphthacen-2′-yl (1*R*,5*S*,7*R*)-3-Ethyl-2-oxo-6,8-dioxa-3-azabicyclo[3.2.1]octane-7-*exo*-carboxylate (29): yellow solid; ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.65, 8.64 (2s), 8.07, 7.68 (2m), 5.88 (d, $^3J = 2$), 4.91, 4.72 (2s), 3.48 (dd, $^2J = 12$, $^3J = 2$), 3.40−3.30 (m), 3.19 (d, $^2J = 12$), 3.15−2.88 (m), 2.62−2.50 (m), 2.24 (s), 2.10−2.00 (m), 1.10 (t, $^3J = 7$).

(2′*R*)-12′-Acetoxy-2′-acetyl-5′-(methoxycarbonyl)-6′,11′-dioxo-1′,2′,3′,4′-tetrahydronaphthacen-2′-yl (1*R*,5*S*,7*R*)-3-Ethyl-2-oxo-6,8-dioxa-3-azabicyclo[3.2.1]octane-7-*exo*-carboxylate (30). (−)-**25** (10 mg, 18 μmol) in pyridine (1 mL) was treated with 19 mg of *n*-Bu_4MnO_4 (3 equiv) at 20 °C. After 1 h of stirring, it was diluted with $CHCl_3$ (10 mL) and washed with aqueous 1 M H_2SO_4 (10 mL, three times), with 5% aqueous $NaHSO_3$ (10 mL, three times), and finally with brine (10 mL, three times). After drying ($MgSO_4$) and solvent evaporation, the residue was dissolved in THF (3 mL). CH_2N_2 (1 mL, 0.6 M in Et_2O) was added, and 15 min later, the excess of the reagent was destroyed with AcOH (0.5 mL). After solvent evaporation, FC (silica gel, 5 g, CH_2Cl_2/EtOAc, R_f 0.55) gave 5 mg (45%) of a yellow solid: ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.21, 7.80 (2m), 5.82 (d, $^3J = 2$), 5.77, 4.85, 4.70, 4.09 (4s), 3.50−3.30 (m), 3.16 (d, $^2J = 12$), 2.56 (s), 2.60−2.50 (m), 2.26 (s), 2.20−2.00 (m), 1.10 (t, $^3J = 7$).

(2′*R*)-12′-Acetoxy-2′-acetyl-5′-(hydroxymethyl)-1′,2′,3′,4′-tetrahydronaphthacen-2′-yl (1*R*,5*S*,7*R*)-3-Ethyl-2-oxo-6,8-dioxa-3-azabicyclo[3.2.1]octane-7-*exo*-carboxylate ((−)-31). $Na(CN)BH_3$ (20 mg, 0.27 mmol) in MeOH (1 mL) was added to (−)-**25** (150 mg, 0.268 mmol) in $CHCl_3$ (10 mL) at 20 °C, and the mixture was acidified to pH = 6 with AcOH (20% in MeOH). The reaction was followed by TLC (R_f 0.5, EtOAc); more AcOH must be added if the reaction stops. After ca. 4 h, the mixture was diluted with CH_2Cl_2 (100 mL) and washed with brine (20 mL, four times). The combined organic extracts were dried ($MgSO_4$), and the solvent was evaporated. The residue was recrystallized from EtOAc (10 mL) to give 140 mg (93%) of colorless needles: mp 194−196 °C (EtOAc); $[\alpha]^{25}_D = -45$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.89, 8.33 (2s), 8.03, 7.48 (2m), 5.70 (d, $^3J = 2$), 5.38, 5.24 (2d, $^2J = 12$),

4.60 (s), 3.65−3.05 (m), 3.02 (d, ^2J = 12), 2.50−2.40 (m), 2.58, 2.28 (2s), 2.12−1.98 (m), 0.98 (t, ^3J = 7).

(2′*R*)-12′-Acetoxy-2′-acetyl-5′-(bromomethyl)-1′,2′,3′,4′-tetrahydronaphthacen-2′-yl (1*R*,5*S*,7*R*)-3-Ethyl-2-oxo-6,8-dioxa-3-azabicyclo[3.2.1]octane-7-*exo*-carboxylate ((−)-32). Freshly distilled AcBr (0.5 mL) was added to a solution of (−)-**31** (56 mg, 0.1 mmol) in $CHCl_3$ (10 mL) at 20 °C. After being stirred for 15 min, the mixture was poured into brine (30 mL). The aqueous layer was washed with $CHCl_3$ (10 mL, four times). The combined organic extracts were dried ($MgSO_4$), and the solvent was evaporated to give a yellow solid that was recrystallized from EtOAc, yielding 60 mg (97%) (TLC, EtOAc/light petroleum 1/1, R_f 0.63) of yellow needles: mp 173−174 °C; $[\alpha]^{25}_D = -65$ (c = 1.0, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.64, 8.33 (2s), 8.05, 7.51 (2m), 5.78 (d, ^3J = 2), 5.15, 5.08 (2d, ^2J = 12), 4.67, 4.64 (2s), 3.47 (dd, ^2J = 12, ^3J = 2), 3.40−3.10 (m), 3.10 (d, ^2J = 12), 2.65−2.55 (m), 2.58, 2.27 (2s), 2.12−1.98 (m), 1.04 (t, ^3J = 7).

(2′*R*)-12′-Acetoxy-2′-acetyl-5′-[[[3″-[[(*tert*-butyloxy)carbonyl]amino]propyl]oxy]methyl]-1′,2′,3′,4′-tetrahydronaphthacen-2′-yl (1*R*,5*S*,7*R*)-3-Ethyl-2-oxo-6,8-dioxa-3-azabicyclo[3.2.1]octane-7-*exo*-carboxylate ((−)-33). To a mixture of (−)-**32** (40 mg, 64 μmol), the *N*-(*tert*-butyloxy)-carbamate of 3-aminopropanol (56 mg, 0.32 mmol), activated molecular sieves (4 Å), and CaH_2 (20 mg) in anhydrous THF (1 mL) at 20 °C was added a solution of AgOTf (16 mg, 64 μmol) in THF (0.5 mL) under vigorous stirring. After 5 min, the white precipitate was filtered off (rinsing with EtOAc), and the filtrate was washed with brine (20 mL). After drying ($MgSO_4$) and solvent evaporation, FC (silica gel 10 g, washing off the excess of alcohol with light petroleum/EtOAc 3/1, then 1/1, R_f 0.11) gave 32 mg (71%) of colorless crystals: mp 182−183 °C (Et_2O); $[\alpha]^{25}_D = -38$ (c = 1.0, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.72, 8.29 (2s), 8.01, 7.49 (2m), 5.78 (d, ^3J = 2), 5.11, 4.68, 4.66 (3s), 3.74 (t, ^3J = 5.7), 3.49 (dd, ^2J = 12, ^3J = 2), 3.45−3.20 (m), 3.10 (d, ^2J = 12), 2.55−2.50 (m), 2.58, 2.26 (2s), 2.22−2.15 (m), 1.84 (m), 1.35 (s), 1.05 (t, ^3J = 7).

(2*R*)-2-Acetyl-2-hydroxy-5-[[[3′-[[(*tert*-butyloxy)carbonyl]amino]propyl]oxy]methyl]-1,2,3,4-tetrahydronaphthacen-12-yl Acetate ((−)-34). (−)-**33** (32 mg, 45 μmol) was dissolved in THF (4 mL) and cooled to 0 °C. Aqueous 1 M NaOH (0.5 mL) was added, and the mixture was stirred at 20 °C for 3 h. It was then neutralized with aqueous 1 M HCl. After evaporation of the THF, the mixture was extracted with $CHCl_3$ (10 mL, three times) and washed with brine (10 mL, three times). Drying ($MgSO_4$), solvent evaporation, and FC (silica gel, flash, EtOAc/light petroleum 1/1, R_f 0.75) gave 20 mg (84%) of a yellowish solid: mp 89−91 °C (Et_2O); $[\alpha]^{25}_D$ = −18 (c = 1.0, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.72, 8.28 (2s), 8.04, 7.47 (2m), 5.12 (s), 3.75 (t, ^3J = 5.7), 3.45−2.85 (m), 2.57, 2.36 (2s), 2.20 (m), 2.05 (m), 1.83 (m), 1.45 (s).

(2*R*)-2-Acetyl-2-hydroxy-5-(hydroxymethyl)-1,2,3,4-tetrahydronaphthacen-12-yl Acetate ((−)-36). Method A. (−)-**34** (6 mg, 11 mmol) was dissolved in a mixture of EtOAc (1 mL) and aqueous 3 M HCl (1 mL) and stirred at 20 °C for 12 h. The organic phase was diluted, separated, and washed with brine (10 mL, 5 times). Drying and solvent evaporation gave 3 mg (74%) of a yellowish solid.

Method B. (−)-**31** (15 mg, 0.027 mmol) was dissolved in THF (5 mL) and cooled to 0 °C. Aqueous 1 M NaOH (0.5 mL) was added, and the mixture was stirred at 20 °C for 2 h. It was then neutralized with aqueous 1 M HCl. After evaporation of the THF, the mixture was extracted with $CHCl_3$ (10 mL, three times) and washed with brine (10 mL, three times). After drying ($MgSO_4$) and solvent evaporation, FC (silica gel, 5 g, EtOAc/light petroleum 3/1, R_f 0.53) yielded 8 mg (79%) of a yellowish solid: mp 84−87 °C (Et_2O); $[\alpha]^{25}_D = -32$ (c = 0.25, $CHCl_3$).

(2′*R*)-12′-Acetoxy-2′-acetyl-5′-[[[3″-[[(allyloxy)carbonyl]-amino]propyl]oxy]methyl]-1′,2′,3′,4′-tetrahydronaphthacen-2′-yl (1*R*,5*S*,7*R*)-3-Ethyl-2-oxo-6,8-dioxa-3-azabicyclo[3.2.1]octane-7-*exo*-carboxylate ((−)-37). To a mixture of (−)-**32** (40 mg, 64 μmol), the *N*-(allyloxy)carbamate of 3-aminopropanol (48 mg, 0.32 mmol), and activated molecular sieves (4 Å) in anhydrous THF (1 mL) at 20 °C was added a solution of AgOTf (16 mg, 64 μmol) in THF (0.5 mL) under vigorous stirring. After 5 min, the white precipitate was filtered off (rinsing with EtOAc), and the filtrate was washed with brine (20 mL). The solution was dried ($MgSO_4$), and the solvent was evaporated. FC (silica gel 10 g, washing off the excess of alcohol with EtOAc/light petroleum 1/1, then 3/1) gave a main fraction (R_f 0.58), 36 mg (80%) as colorless crystals: mp 175 °C (Et_2O); $[\alpha]^{25}_D = -23$ (c = 1.0, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.70, 8.29 (2s), 7.98, 7.48 (2m), 5.80 (d, ^3J = 2), 5.82 (m), 5.30−5.02 (m), 5.11, 4.70, 4.62 (3s), 4.45 (dm, ^3J = 5.5), 3.73 (t, ^3J = 6.0), 3.40 (dd, ^2J = 12, ^3J = 2), 3.45−3.20 (m), 3.10 (d, ^2J = 12), 2.59 (s), 2.55−2.50 (m), 2.27 (s), 2.22−2.15 (m), 1.85 (m), 1.04 (t, ^3J = 7).

(2*R*)-2-Acetyl-5-[[[3′-[(allyloxy)carbonyl]amino]propyl]oxy]methyl]-1,2,3,4-tetrahydronaphthacen-12-yl Acetate ((−)-38). (−)-**37** (28 mg, 0.040 mmol) was dissolved in THF (4 mL) and cooled to 0 °C. Aqueous 1 M NaOH (0.5 mL) was added, and the mixture was stirred at 20 °C for 3 h. It was then neutralized with aqueous 1 M HCl, and the THF was evaporated. After dilution with H_2O, the mixture was extracted with $CHCl_3$ (10 mL, three times), and the combined extracts were washed with brine (10 mL, three times). Drying ($MgSO_4$), solvent evaporation, and FC (silica gel, 5 g, light petroleum/EtOAc 3/1, R_f 0.81) gave 17 mg (84%) of yellowish crystals: mp 79−80 °C (CH_2Cl_2/hexane); $[\alpha]^{25}_D = -17$ (c = 1.0, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.70, 8.29 (2s), 8.00, 7.46 (2m), 5.82 (m), 5.30−5.02 (m), 5.10 (s), 4.45 (dm, ^3J = 5.5), 3.74 (t, ^3J = 5.5), 3.45−2.85 (m), 2.57, 2.35 (2s), 2.20, 2.05, 1.84 (3m).

(2*R*)-2-Acetyl-2-hydroxy-5-[[(3′-aminopropyl)oxy]methyl]-1,2,3,4-tetrahydronaphthacen-12-yl Acetate Hydrochloride ((−)-39). A mixture of (−)-**38** (10 mg, 19 μmol), $Pd(Ph_3P)_4$ (catalytic amount), and Bu_3SnH (4 μL, 2 equiv) was stirred in the dark at 20 °C in wet CH_2Cl_2 (2 mL) for 20 min. After solvent evaporation, the residue was dissolved in H_2O/MeOH 9/1 (3 mL) and acidified to pH 2.5−3.0 with aqueous 0.1 M HCl. The MeOH was evaporated and the suspension washed with Et_2O (10 mL, seven times) and evaporated to give a pale yellow solid that was purified by FC (silica gel 5 g, CH_2Cl_2/MeOH 4/1, R_f 0.19 (alizarin)) to yield 6 mg (66%) as a yellowish solid: mp 152−154 °C (EtOH/Et_2O); $[\alpha]^{25}_D = -18$ (c = 1.0, EtOH 95%); ^{1}H NMR (250 MHz, CD_3OD) δ 8.79, 8.39 (2s), 8.08, 7.50 (2m), 5.20 (s), 3.82 (t, ^3J = 5.5), 3.45−2.85 (m), 3.03 (t, ^3J = 5.5), 2.61, 2.40 (2s), 2.20−1.90 (m).

(2′*R*)-12′-Acetoxy-2′-acetyl-5′-[[[3″-[*N*-[(allyloxy)carbonyl]-*N*-(hydroxypropyl)amino]propyl]oxy]methyl]-1′,2′,3′,4′-tetrahydronaphthacen-2′-yl (1*R*,5*S*,7*R*)-3-Ethyl-2-oxo-6,8-dioxa-3-azabicyclo[3.2.1]octane-7-*exo*-carboxylate ((−)-40). To a mixture of (−)-**32** (30 mg, 53 μmol), the *N*-allyloxycarbamate of 1-dipropanolamine (53 mg, 0.32 mmol), and activated molecular sieves (4 Å) in anhydrous THF (1 mL) at 20 °C was added a solution of AgOTf (14 mg, 0.055 mmol) in THF (0.5 mL) under vigorous stirring. After 5 min, the white precipitate was filtered off (rinsing with EtOAc), and the filtrate was washed with brine (20 mL). Drying ($MgSO_4$), solvent evaporation, and FC (silica gel 10 g, washing off the excess of alcohol with EtOAc/light petroleum 3/1, then EtOAc, R_f 0.19) gave a main fraction, which provided 26 mg (about 60%) of a 1:1 mixture of protected dipropanolamine/(−)-**40**: $[\alpha]^{25}_D = -25$ (as mixture, c = 1.0, $CHCl_3$); ^{1}H NMR of (−)-**40** (250 MHz, $CDCl_3$) δ 8.70, 8.28 (2s), 7.98, 7.48 (2m), 5.80 (d, ^3J = 2), 5.82 (m), 5.30−5.02 (m), 5.10, 4.72, 4.70 (3s), 4.56 (dm), 3.70−3.20 (m), 3.11 (d, ^2J = 12), 2.59 (s), 2.55−2.50 (m), 2.25 (s), 2.22−2.15 (m), 1.90−1.70 (m), 1.05 (t, ^3J = 7).

(2*R*)-2-Acetyl-2-hydroxy-5-[[[3′-[*N*-[(allyloxy)carbonyl]-*N*-(hydroxypropyl)amino]propyl]oxy]methyl]-1,2,3,4-tetrahydronaphthacen-12-yl Acetate ((−)-41). (−)-**40** with some alcohol impurity (26 mg, about 32 μmol) was dissolved in THF (4 mL) and cooled to 0 °C. Aqueous 1 M NaOH (0.5 mL) was added, and the mixture was stirred at 20 °C for 3 h. It was then neutralized with aqueous 1 M HCl, and after evaporation of the THF, it was extracted with $CHCl_3$ (10 mL, three times) and washed with brine (10 mL, three times). After drying ($MgSO_4$) and solvent evaporation, FC (silica gel, 10 g, EtOAc, R_f 0.50) yielded 9 mg (32% for two steps) of a yellowish solid: mp 47−50 °C (Et_2O/hexane); $[\alpha]^{25}_D = -14$ (c = 1.0, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.70, 8.28 (2s), 7.98, 7.48,

5.85, 5.30−5.02 (4m), 5.11 (s), 4.56 (dm, ^3J = 5.5), 3.68, 3.42 (2t, ^3J = 5.5), 3.45−2.85 (m), 2.58, 2.36 (2s), 2.20, 2.05, 1.90, 1.52 (4m).

(2*R*)-2-Acetyl-2-hydroxy-5-[[[3′-[(3″-hydroxypropyl)-amino]propyl]oxy]methyl]-1,2,3,4-tetrahydronaphthacen-12-yl Acetate Hydrochloride((−)-42). A mixture of (−)-**41** (10 mg, 19 μmol), $Pd(Ph_3P)_4$ (catalytic amount), and Bu_3SnH (4 μL, 2 equiv) was stirred in the dark at 20 °C in wet CH_2Cl_2 (3 mL) for 20 min. After solvent evaporation, the residue was dissolved in H_2O/MeOH 9/1 (3 mL) and acidified to pH 2.5−3.0 with aqueous 0.1 M HCl. The MeOH was evaporated, and the suspension was washed with Et_2O (10 mL, seven times) and evaporated to give a pale yellow solid that was purified by FC (silica gel, 5 g, CH_2Cl_2/MeOH 4/1, R_f 0.55 (alizarin)) to yield 6 mg (70%) of a yellowish solid: mp 140−144 °C (EtOH/Et_2O); $[\alpha]^{25}_D$ = −21 (*c* = 0.1, EtOH 95%); ^{1}H NMR (400 MHz, CD_3OD) δ 8.77, 8.39 (2s), 8.10, 7.51 (2m), 5.18 (s), 3.85 (t, ^3J = 5.5), 3.48, 3.45−2.85, 3.10, 2.95 (4m), 2.62, 2.40 (2s), 2.20−1.65 (m).

(2′*R*)-12′-Acetoxy-2′-acetyl-6′,11′-dioxo-5′-[[[3″-[[(allyloxy)carbonyl]amino]propyl]oxy]methyl]-1′,2′,3′,4′-tetrahydronaphthacen-2′-yl (1*R*,5*S*,7*R*)-3-Ethyl-2-oxo-6,8-dioxa-3-azabicyclo[3.2.1]octane-7-*exo*-carboxylate ((−)-43). (−)-**37** (40 mg, 57 μmol) was dissolved in acetone (10 mL) and cooled to 0 °C. Jones reagent (4 N, 0.25 mL, 0.34 mmol) was added dropwise, and the reaction mixture was stirred in the dark at 0 °C for 30 min and at 20 °C for 1 h. It was cooled again to 0 °C, and 40 μL of Jones reagent was added. After the mixture was stirred at 20 °C for 1 h, the excess of the oxidant was destroyed with *i*-PrOH (1 mL, changed to green). Solvent evaporation afforded a residue that was taken up with EtOAc (20 mL) and washed with 10% aqueous $NaHCO_3$ (10 mL) and finally with brine (10 mL, four times). After drying, solvent evaporation gave 36 mg (91%) of a yellowish gum: $[\alpha]^{25}_D$ = −13 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.13, 7.73, 5.90 (3m), 5.82 (d, ^3J = 2), 5.20 (m), 4.98 (br s), 4.75, 4.70 (2s), 4.51 (d, ^3J = 5.5), 3.75 (t, ^3J = 5.5), 3.42 (dd, ^2J = 12, ^3J = 2), 3.40−3.00 (m), 3.15 (d, ^2J = 12), 2.55, 2.26 (2s), 2.15−2.00, 1.85 (2m), 1.10 (t, ^3J = 7).

(8*R*)-8-Acetyl-6,8-dihydroxy-11-[[[3′-[[(allyloxy)carbonyl]amino]propyl]oxy]methyl]-7,8,9,10-tetrahydronaphthacene-5,12-dione ((−)-44). Method A. A mixture of (−)-**37** (20 mg, 32 μmol) and $HO(CH_2)_3NHAlloc$ (27 mg, 6 equiv) in THF at −30 °C was treated with NaH (85% in oil, 3 mg, 3 equiv). The temperature was allowed to rise to 20 °C. After being stirred for 6 h, the red solution was neutralized with aqueous 1 M HCl and evaporated. The residue was dissolved in EtOAc (2 mL) and treated with aqueous 1 M HCl (2 mL). After being stirred overnight, the organic phase was separated, washed with brine (10 mL, three times), and dried ($MgSO_4$), and the solvent was evaporated. The residue was purified by FC (silica gel, 5 g, light petroleum/EtOAc 1/1, R_f 0.53 (UV, green with vanillin)) to yield 6 mg (37%) of (−)-**44** with some impurity. **Method B.** (−)-**43** (36 mg, 49 μmol) was dissolved in degassed THF (2 mL). After the solution was cooled to 0 °C, aqueous 1 M LiOH (0.1 mL) was added, and the mixture was stirred in the dark for 10 min. After addition of a saturated aqueous solution of NH_4Cl (5 mL), the product was extracted with $CHCl_3$ (15 mL, three times) and the combined extracts were dried ($MgSO_4$). Solvent evaporation yielded 24 mg of a yellow solid with about 10% impurity, which could not be separated by chromatography or crystallization: $[\alpha]^{25}_D$ = −7 (*c* = 0.5, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 13.81 (s), 8.24, 7.80, 5.83 (3m), 5.49 (br. t), 5.28−5.10 (m), 5.10, 4.83 (2d, ^2J = 10), 4.48 (dm, ^3J = 5.5), 3.92 (s), 3.75, 3.40−3.11 (2m), 3.11, 2.98 (2d, ^2J = 18), 2.40 (s), 2.12−1.90, 1.84 (2m).

(8*R*)-8-Acetyl-6,8-dihydroxy-11-[[[3′-(*p*-nitrobenzamido)-propyl]oxy]methyl]-7,8,9,10-tetrahydronaphthacene-5,12-dione ((−)-45). (−)-**8** (5 mg, 13 μmol) was dissolved in anhydrous CH_2Cl_2 (1 mL) at 0 °C. Et_3N (2 μL, 13 μmol) and *p*-nitrobenzoyl chloride (3 mg, 16 μmol) were added. After being stirred for 30 min, the mixture was diluted with CH_2Cl_2 and washed with aqueous 0.1 M HCl (10 mL, three times) and brine (10 mL, three times). Drying ($MgSO_4$) and solvent evaporation gave 5 mg (67%) of yellow prisms: mp 86−88 °C (hexane/$CHCl_3$ 1/1); $[\alpha]^{25}_D$ = −36 (*c* = 0.25, $CHCl_3$).

(2′*R*)-12′-Acetoxy-2′-acetyl-5′-[[[3″-[*N*-[(allyloxy)carbonyl]-*N*-[3‴-[(allyloxy)carbonyl]amino]propyl]amino]-propyl]oxy]methyl]-1′,2′,3′,4′-tetrahydronaphthacen-2′-yl (1*R*,5*S*,7*R*)-3-Ethyl-2-oxo-6,8-dioxa-3-azabicyclo[3.2.1]-octane-7-*exo*-carboxylate ((−)-46). A solution of AgOTf (16 mg, 64 μmol) in 0.5 mL of THF was added to a stirred mixture of (−)-**32** (40 mg, 64 μmol), the *N*,*N*-bis(allyloxy)carbamate of 3-[(3′-aminopropyl)amino]propanol (100 mg, 0.33 mmol), and activated molecular sieves (4 Å) in anhydrous THF (1 mL) at 20 °C. After 5 min, the white precipitate was filtered off (rinsing with EtOAc), and the filtrate was washed with brine (20 mL). Drying ($MgSO_4$) and solvent evaporation gave a residue that was purified by FC (silica gel 10 g, washing off the excess of alcohol with acetone/light petroleum 1/1, then 3/1, (R_f 0.32)) to give 40 mg of a mixture of (−)-**46** and the starting alcohol (yield: about 84%). An analytical sample was obtained by recrystallization from EtOAc/hexane as an amorphous solid: mp ca. 27 °C; $[\alpha]^{25}_D$ = −33 (*c* = 1.0, CH_3CN); ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.71, 8.29 (2s), 7.98, 7.48, 5.90 (3m), 5.78 (d, ^3J = 2), 5.30−5.10 (m), 5.11, 5.08 (2d, ^2J = 7), 4.68, 4.67 (2s), 4.55, 4.52 (2d, ^3J = 5.5), 3.68 (m), 3.38 (dd, ^2J = 12, ^3J = 2), 3.45−3.00 (m), 3.10 (d, ^2J = 12), 2.58 (s), 2.55−2.50 (m), 2.26 (s), 2.22−2.15, 1.90−1.60 (2m), 1.04 (t, ^3J = 7).

(2′*R*)-12′-Acetoxy-2′-acetyl-6′,11′-dioxo-5′-[[[3″-[*N*-[(allyloxy)carbonyl]-*N*-[3‴-[(allyloxycarbonyl]amino]propyl]amino]propyl]oxy]methyl]-1′,2′,3′,4′-tetrahydronaphthacen-2′-yl (1*R*,5*S*,7*R*)-3-Ethyl-2-oxo-6,8-dioxa-3-azabicyclo[3.2.1]octane-7-*exo*-carboxylate ((−)-47). (−)-**46** (40 mg, 47 μmol) was dissolved in acetone (10 mL) and then cooled to 0 °C. Jones reagent (4 N, 0.21 mL, 0.28 mmol) was added dropwise, and the reaction mixture was stirred in the dark at 0 °C for 30 min and at 20 °C for 1 h. It was cooled again to 0 °C, and 40 μL of Jones reagent was added. After the mixture was stirred at 20 °C for 1 h, the excess of the oxidant was destroyed with *i*-PrOH (1 mL, changed to green). After solvent evaporation, the residue was dissolved in EtOAc (20 mL) and washed with 10% aqueous $NaHCO_3$ (10 mL) and finally with brine (10 mL, four times). After drying, solvent evaporation gave 39 mg (94%) of an amorphous yellow solid: mp ∼27 °C (Et_2O); $[\alpha]^{25}_D$ = −14 (*c* = 1.0, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.13, 7.72, 5.90 (3m), 5.83 (d, ^3J = 2), 5.40−5.10 (m), 5.05, 4.88 (2d, ^2J = 10), 4.76, 4.68 (2s), 4.60 (dt, ^2J = 5.5, ^3J = 1.2), 4.51 (d, ^3J = 5.5), 3.67 (t, ^3J = 5.5), 3.42 (dd, ^2J = 12, ^3J = 2), 3.40−3.00 (m), 3.15 (d, ^2J = 12), 2.52, 2.24 (2s), 2.15−2.00 (m), 1.90, 1.70 (2m), 1.10 (t, ^3J = 7).

(2′*R*)-12′-Acetoxy-2′-acetyl-5′-[[[3″-(trifluoroacetamido)-propyl]oxy]methyl]-1′,2′,3′,4′-tetrahydronaphthacen-2′-yl (1*R*,5*S*,7*R*)-3-Ethyl-2-oxo-6,8-dioxa-3-azabicyclo[3.2.1]-octane-7-*exo*-carboxylate ((−)-48). A solution of AgOTf (16 mg, 64 μmol) in THF (0.5 mL) was added to a stirred mixture of (−)-**32** (40 mg, 64 μmol), 3-(trifluoroacetamido)propanol (55 mg, 0.32 mmol), and activated molecular sieves (4 Å) in anhydrous THF (1 mL) at 20 °C. After 5 min, the white precipitate was filtered off (rinsing with EtOAc), and the filtrate was washed with brine (20 mL). After drying ($MgSO_4$) and solvent evaporation, FC (silica gel 10 g, washing off the excess of alcohol with light petroleum/EtOAc 2/1, then 1/1) gave 44 mg (87%) of colorless crystals: mp 181 °C (Et_2O/hexane 5/1); $[\alpha]^{25}_D$ = −12 (*c* = 0.5, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.68, 8.30 (2s), 7.98, 7.46 (2m), 5.77 (d, ^3J = 2), 5.20, 5.06 (2d, ^2J = 12), 4.65, 4.40 (2s), 3.76 (t, ^3J = 6.0), 3.50−3.10 (m), 3.08 (d, ^2J = 12), 2.58 (s), 2.55−2.40 (m), 2.26 (s), 2.18−2.05 (m), 1.89 (m), 1.01 (t, ^3J = 7).

(2′*R*)-12′-Acetoxy-2′-acetyl-6′,11′-dioxo-5′-[[[3″-(trifluoroacetamido)propyl]oxy]methyl]-1′,2′,3′,4′-tetrahydronaphthacen-2′-yl (1*R*,5*S*,7*R*)-3-Ethyl-2-oxo-6,8-dioxa-3-azabicyclo[3.2.1]octane-7-*exo*-carboxylate ((−)-49). (−)-**48** (22 mg, 31 μmol) was dissolved in acetone (5 mL) and then cooled to 0 °C. Jones reagent (4 N, 0.12 mL, 0.17 mmol) was added dropwise, and the reaction mixture was stirred in the dark at 0 °C for 30 min and at 20 °C for 1 h. It was cooled again to 0 °C, and Jones reagent (20 μL) was added. After being stirred at 20 °C for 1 h, the excess of the oxidant was destroyed with *i*-PrOH (1 mL, changed to green). Solvent evaporation afforded a residue that was taken up with EtOAc (10 mL) and washed with brine (10 mL, four times). Drying

and solvent evaporation gave 18 mg (85%) of an amorphous pale yellow solid: mp 35−40 °C (CH_2Cl_2/hexane); $[\alpha]^{25}_D = -14$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.13 (m), 8.00 (br s), 7.76 (m), 5.82 (d, $^3J = 2$), 4.99, 4.82 (2 br. d, $^2J = 10$), 4.68, 4.62 (2s), 3.80 (t, $^3J = 5.5$), 3.42 (dd, $^2J = 12$, $^3J = 2$), 3.60−3.10 (m), 3.15 (d, $^2J = 12$), 2.55, 2.26 (2s), 2.15−1.90 (m), 1.10 (t, $^3J = 7$).

(2′*R*)-12′-Acetoxy-2′-acetyl-5′-[[[4″-(trifluoroacetamido)-butyl]oxy]methyl]-1′,2′,3′,4′-tetrahydronaphthacen-2′-yl (1*R*,5*S*,7*R*)-3-Ethyl-2-oxo-6,8-dioxa-3-azabicyclo[3.2.1]-octane-7-*exo*-carboxylate ((−)-50). A solution of AgOTf (16 mg, 64 μmol) in THF (0.5 mL) was added to a stirred mixture of (−)-**32** (40 mg, 64 μmol), 4-(trifluoroacetamido)butanol (80 mg, 0.43 mmol), and activated molecular sieves (4 Å) in anhydrous THF (1.5 mL) at 20 °C. After 5 min, the white precipitate was filtered off (rinsing with EtOAc), and the filtrate was washed with brine (20 mL, three times). The combined aqueous fractions were reextracted with EtOAc (as many times as needed, checked by TLC). After drying ($MgSO_4$) and solvent evaporation, FC (silica gel 10 g, washing off the excess of alcohol with light petroleum/EtOAc 2/1, then 1/1, then 1/3) afforded the main fraction (R_f 0.54, UV), which gave 37 mg (79%) of colorless crystals: mp 158−160 °C (EtOAc/hexane); $[\alpha]^{25}_D = -15$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.68, 8.30 (2s), 7.98, 7.48, 7.25 (3m), 5.78 (d, $^3J = 2$), 5.19, 5.05 (2d, $^2J = 12$), 4.68, 4.55 (2s), 3.68 (m), 3.50−3.10 (m), 3.10 (d, $^2J = 12$), 2.58 (s), 2.55−2.40 (m), 2.26 (s), 2.18−2.05, 1.66 (2m), 1.03 (t, $^3J = 7$).

(2′*R*)-12′-Acetoxy-2′-acetyl-5′-[[[5″-(trifluoroacetamido)pentyl]oxy]methyl]-1′,2′,3′,4′-tetrahydronaphthacen-2′-yl (1*R*,5*S*,7*R*)-3-Ethyl-2-oxo-6,8-dioxa-3-azabicyclo[3.2.1]-octane-7-*exo*-carboxylate ((−)-51). A solution of AgOTf (16 mg, 64 μmol) in THF (0.5 mL) was added to a stirred mixture of (−)-**32** (40 mg, 64 μmol), 5-(trifluoroacetamido)pentanol (90 mg, 0.45 mmol), and activated molecular sieves (4 Å) in anhydrous THF (1.5 mL) at 20 °C. After 5 min, the white precipitate was filtered off (rinsing with EtOAc), and the filtrate was washed with brine (20 mL, three times). The combined aqueous fractions were reextracted with EtOAc (as many times as needed, checked by TLC). After drying ($MgSO_4$) and solvent evaporation, FC (silica gel 20 g, washing off the excess of alcohol with light petroleum/EtOAc 2/1, the 1/2, then 1/3) afforded the main fraction (R_f 0.52, UV), which gave 30 mg (63%) of colorless crystals: mp 151 °C (EtOAc/hexane); $[\alpha]^{25}_D = -18$ ($c = 1.0$, $CDCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.72, 8.28 (2s), 7.98, 7.46 (2m), 5.70 (d, $^3J = 2$), 5.10 (br s), 4.67 (s), 3.62 (dt, $^2J = 2.2$, $^3J = 6.0$), 3.40−3.00 (m), 2.58 (s), 2.55−2.40 (m), 2.27 (s), 2.25−2.10, 1.63, 1.35 (3m), 1.02 (t, $^3J = 7$).

(2′*R*)-12′-Acetoxy-2′-acetyl-5′-[[[2″-[*N*-(trifluoroacetyl)-*N*-[3‴-(trifluoroacetamido)propyl]amino]ethyl]oxy]-methyl]-1′,2′,3′,4′-tetrahydronaphthacen-2′-yl (1*R*,5*S*,7*R*)-3-Ethyl-2-oxo-6,8-dioxa-3-azabicyclo[3.2.1]octane-7-*exo*-carboxylate ((−)-52). A solution of AgOTf (16 mg, 0.064 mmol) in THF (0.5 mL) was added to a stirred mixture of (−)-**32** (20 mg, 32 μmol), the *N*,*N*-bis(trifluoroacetamide) of 2-[(3′-aminopropyl)amino]propanol (100 mg, 0.33 mmol), and activated molecular sieves (4 Å) in anhydrous THF (1 mL) at 20 °C. After being stirred for 5 min, the white precipitate was filtered off (rinsing with EtOAc) and the filtrate was washed with brine (20 mL). After drying ($MgSO_4$) and solvent evaporation, FC (silica gel 10 g, washing off the excess of alcohol with EtOAc/light petroleum 1/1, then 3/1, (R_f 0.32)) afforded 20 mg (75%) of an amorphous solid: mp ca. 30 °C (from CH_2Cl_2/hexane); $[\alpha]^{25}_D = -12$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.64, 8.30 (2s), 7.98, 7.48 (2m), 5.81 (d, $^3J = 2$), 5.15, 5.13 (2d, $^2J = 7$), 4.65, 4.53 (2s), 3.88, 3.79, 3.61 (3t, $^3J = 5$), 3.45−2.95 (m), 2.58 (s), 2.55−2.50 (m), 2.26 (s), 2.22−2.15, 1.60 (2m), 1.04 (t, $^3J = 7$).

(2′*R*)-12′-Acetoxy-2′-acetyl-6′,11′-dioxo-5′-[[[4″-(trifluoroacetamido)butyl]oxy]methyl]-1′,2′,3′,4′-tetrahydronaphthacen-2′-yl (1*R*,5*S*,7*R*)-3-Ethyl-2-oxo-6,8-dioxa-3-azabicyclo[3.2.1]octane-7-*exo*-carboxylate ((−)-53). (−)-**50** (35 mg, 48 μmol) was dissolved in acetone (5 mL) and then cooled to 0 °C. Jones reagent (4 N, 0.22 mL, 0.31 mmol) was added dropwise, and the reaction mixture was stirred in the dark at 0 °C for 30 min and at 20 °C for 2 h. The excess of the oxidant was destroyed with *i*-PrOH (1 mL). Solvent evaporation afforded a residue that was taken up with EtOAc (10 mL) and washed with brine (10 mL, four times). After drying and solvent evaporation the product still contained nonoxidized starting (−)-**50** (about 15% by ^{1}H NMR), so it was redissolved in acetone (5 mL) and treated in the same manner with 110 μL of 4 N Jones reagent. The usual workup gave 30 mg (82%) of an amorphous pale yellow solid: mp 74−77 °C (CH_2Cl_2/Et_2O/hexane); $[\alpha]^{25}_D = -10$ ($c = 1.0$, $CHCl_3$).

(2′*R*)-12′-Acetoxy-2′-acetyl-6′,11′-dioxo-5′-[[[5″-(trifluoroacetamido)pentyl]oxy]methyl]-1′,2′,3′,4′-tetrahydronaphthacen-2′-yl (1*R*,5*S*,7*R*)-3-ethyl-2-oxo-6,8-dioxa-3-azabicyclo[3.2.1]octane-7-*exo*-carboxylate ((−)-54) was prepared by the same procedure as for the preparation of (−)-**53**, starting with (−)-**51** (20 mg, 27 μmol): yield 16 mg (77%) of an amorphous, pale yellow solid; mp 73−75 °C (CH_2Cl_2/Et_2O/hexane); $[\alpha]^{25}_D = -11$ ($c = 1.0$, $CHCl_3$).

(2′*R*)-12′-Acetoxy-2′-acetyl-6′,11′-dioxo-5′-[[[2″-[*N*-(trifluoroacetyl)-*N*-[3‴-(trifluoroacetamido)propyl]amino]-ethyl]oxy]methyl]-1′,2′,3′,4′-tetrahydronaphthacen-2′-yl (1*R*,5*S*,7*R*)-3-ethyl-2-oxo-6,8-dioxa-3-azabicyclo[3.2.1]-octane-7-*exo*-carboxylate ((−)-55) was prepared by the same procedure as for the preparation of (−)-**53**, starting with (−)-**52** (15 mg, 17 μmol): yield 12 mg (76%) of an amorphous yellow solid; mp ca. 29 °C (Et_2O); $[\alpha]^{25}_D = -10$ ($c = 1.0$, $CHCl_3$).

(8*R*)-8-Acetyl-6,8-dihydroxy-11-[[[4′-(*p*-nitrobenzamido)-butyl]oxy]methyl]-7,8, 9,10-tetrahydronaphthacene-5,12-dione ((−)-56) was prepared by the same procedure as for the preparation of (−)-**45**, starting with (−)-**9** (2 mg, 4 μmol): yield 2 mg (81%), recrystallization from CH_2Cl_2/hexane; mp 89−92 °C; $[\alpha]^{25}_D = -23$ ($c = 0.2$, $CHCl_3$).

(8*R*)-8-Acetyl-6,8-dihydroxy-11-[[[5′-(*p*-nitrobenzamido)-pentyl]oxy]methyl]-7,8, 9,10-tetrahydronaphthacene-5, 12-dione ((−)-57) was prepared by the same procedure as for the preparation of (−)-**45**, starting with (−)-**10** (2 mg, 4 μmol): yield 2 mg (81%) of a yellow gum; $[\alpha]^{25}_D = -40$ ($c = 0.2$, $CHCl_3$).

(2′*R*)-12′-Acetoxy-2′-acetyl-5′-[(2″,3″,6″-trideoxy-4″-*O*-(*p*-nitrobenzoyl)-3″-(trifluoroacetamido)-α-L-*lyxo*-hexopyranosyl]oxy]methyl]-1′,2′,3′,4′-tetrahydronaphthacen-2′-yl (1*R*,5*S*,7*R*)-3-Ethyl-2-oxo-6,8-dioxa-3-azabicyclo[3.2.1]-octane-7-*exo*-carboxylate ((−)-60). Fifty mg (93 μmol) of (−)-**58** (2,3,6-trideoxy-4-*O*-(*p*-nitrobenzoyl)-3-(trifluoroacetamido)-α-L-*lyxo*-hexopyranosyl *p*-nitrobenzoate) was dissolved under Ar in anhydrous THF (3 mL). After the mixture was cooled to −78 °C, $Me_3SiOSO_2CF_3$ (42 μL, 0.2 mmol) was added and the temperature was allowed to rise to −25 °C. After the mixture was stirred at −25 °C for 30 min, (−)-**31** (42 mg in $CHCl_3$ (1 mL), 74 μmol) was introduced slowly and the mixture was stirred at −25 °C for 3 h. It was then poured into a saturated aqueous solution of $NaHCO_3$ (20 mL, cooled to 0 °C) and extracted with EtOAc (15 mL, three times). The combined organic extracts were washed with brine (15 mL, three times) and dried ($MgSO_4$). After solvent evaporation, FC (silica gel, 10 g, EtOAc/light petroleum 1/1, R_f 0.66) gave two anomers (52 mg, 75%, $\alpha/\beta = 2/1$) that could only be separated by HPLC (Nucleosil 100−3, EtOAc/light petroleum 3/2) to give 34 mg (50%) of (−)-**60** and 16 mg of its β-anomer.

Data for (−)-**60**: colorless crystals; mp 260 °C dec (Et_2O); $[\alpha]^{25}_D = -45$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.79, 8.32 (2s), 8.40−8.20, 8.02, 7.47 (3m), 6.50 (d, $^3J = 7$), 5.80 (d, $^3J = 2$), 5.47 (br s), 5.30 (s), 5.07 (d, $^3J = 1$), 4.85−4.65 (m), 4.66, 4.58 (2s), 4.37 (q, $J = 6$), 3.40−3.10 (m), 2.59 (s), 2.55−2.45 (m), 2.27 (s), 2.15−1.80 (m), 1.26 (d, $^3J = 6$), 1.01 (t, $^3J = 7$).

(2′*R*)-12′-Acetoxy-2′-acetyl-6′,11′-dioxo-5′-[[[2″,3″,6″-trideoxy-4″-O-(*p*-nitrobenzoyl)-3″-(trifluoroacetamido)-α-L-*lyxo*-hexopyranosyl]oxy]methyl]-1′,2′,3′,4′-tetrahydronaphthacen-2′-yl (1*R*,5*S*,7*R*)-3-Ethyl-2-oxo-6,8-dioxa-3-azabicyclo[3.2.1]octane-7-*exo*-carboxylate ((−)-61). (−)-**60** (15 mg, 16 μmol) was dissolved in acetone (4 mL) and then cooled to 0 °C. Jones reagent (4 N, 0.07 mL, 0.1 mmol) was added dropwise, and the reaction mixture was stirred in the dark at 0 °C for 20 min and at 20 °C for 30 min. The excess of the oxidant was destroyed with *i*-PrOH (0.5 mL). After solvent evaporation, the residue was taken up with EtOAc (20

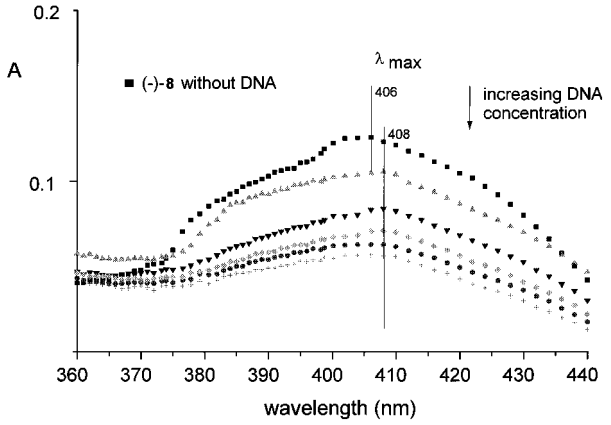

Figure 3. Normalized UV spectra of (−)-**8** with increasing amounts of CT-DNA. At 406 nm wavelength $\epsilon_f = 3740 \pm 180$ and $\epsilon_b = 2740 \pm 240$.

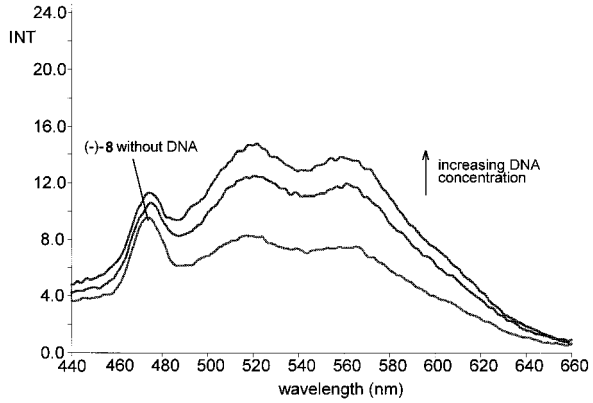

Figure 4. Normalized fluorescence spectra of (−)-**8** with increasing amounts of CT-DNA. Excitation wavelength $\lambda_{ex} =$ 408 nm.

mL) and washed with 10% aqueous $NaHCO_3$ (10 mL) and then with brine (10 mL, four times). After drying, solvent evaporation gave 11 mg (71%) of a yellow powder: mp 130−134 °C (Et_2O); $[\alpha]^{25}_D = -73$ ($c = 1.0$, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 8.40−8.20, 8.15, 7.75 (3m), 6.54 (d, $^3J = 7$), 5.85 (d, $^3J = 2$), 5.50 (br s), 5.35, 5.18 (2d, $^2J = 12$), 5.28 (d, $^3J = 1$), 4.72, 4.65 (2s), 4.60−4.35, 3.42−3.14 (2m), 2.55 (s), 2.55−2.40 (m), 2.25 (s), 2.25−1.90 (m), 1.27 (d, $^3J = 6$), 1.05 (t, $^3J = 7$).

Example of Intercalation Assay by Absorbance Titration. The electronic absorption spectrum of (−)-**8** in the presence of increasing amounts of CT-DNA shows strong decrease of the peak intensity (Figure 3). This hypochromicity attains 60% with only 2 mm red shift of the absorbance maximum.

Example of Intercalation Assay by Fluorescence Titration. An interesting property of the fluorescence emission spectrum was the increase of intensity ($P = 2.38$) with increasing amount of DNA without any change in the shape of the spectrum (Figure 4).

Intercalation Assays. Calf thymus DNA type XV was purchased from Sigma. Purity was checked by measuring the ratio of the absorbance at 260−280 nm (1.9). Extinction coefficient at 260 nm: 12824 M^{-1} in base pairs. All samples were dissolved in 10 mM Tris−HCl buffer, 1 mM Na_2EDTA, 100 mM NaCl pH 7.4. Yeast topoisomerase I and pGEM plasmid (Promega) were a generous gift of Dr. Y. Vassetzky and D. Bragulia (ISREC, Lausanne), while human topoisomerase II and other reagents for inhibition assays were received in a Topoisomerase II drug screening kit (TopoGEN, Inc., Columbus, OH).

(A) Absorbance Titration. Absorbance titration experiments were performed on a Hewlett-Packard 8450A diode array spectrometer maintained at 25 °C. Every 15 min,

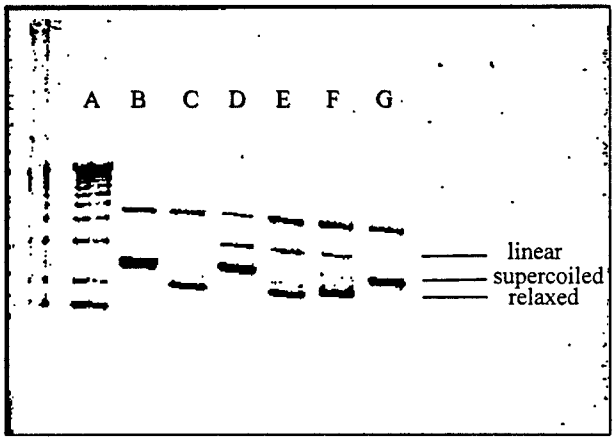

Figure 5. Effect of DNA intercalators on topoisomerase II strand passing activity: (A) 1 kd ladder; (B) pGEM supercoiled DNA without enzyme; (C) with enzyme; (D) daunomycin in 2 μM, (E) (−)-8 in 50 μM, (F) in 150 μM, and (G) in 500 μM concentration.

aliquots of potential intercalator solution (about 100 μM in Tris−HCl buffer) were added to a solution of CT-DNA (about 200 μM in Tris−HCl buffer) under continous stirring. The base-line absorbance was recorded and subtracted from all subsequent readings. All the spectrum was transferred into Microcal Origin software. For the calculation of free and bound intercalator concentration, only absorbance at the λ_{max} wavelength, the exact concentration of the intercalator, and the DNA in base pairs are necessary. Three readings were recorded at each addition and averaged. ϵ_b was determined by extrapolation to high DNA concentration. The titration was repeated by addition of aliquots of DNA solution to intercalator solution (see Figure 3 for an example).

(B) Fluorescence Titration. Titration experiments were performed on a Perkin-Elmer LS-50B spectrofluorometer maintained at 25 °C. A slit width of 4 nm was used with $\lambda_{ex} = \lambda_{max}$ of the UV spectra. Excitation and emission spectra of potential intercalator alone were recorded and used to determine the wavelength of maximum intensity in fluorescence (λ_{em}, I_{max}). Every 15 min aliquots of intercalator solution (about 10 μM in Tris−HCl buffer) were added to a solution of CT-DNA (about 20 μM in Tris−HCl buffer) under continous stirring. The base-line fluorescence was recorded and substracted from all subsequent readings. For the calculation of free and bound intercalator concentration, fluorescence intensity at the I_{max} wavelength, the exact concentration of the intercalator and the DNA in base pairs are only necessary. Three readings were recorded at each addition and averaged. k_b was determined by extrapolation to high DNA concentration. The titration was repeated by addition of aliquots of DNA solution to intercalator solution. Data obtained from absorbance and fluorescence titrations were used to calculate the free intercalator concentration (see Figure 4 for an example).

Topoisomerase Inhibition. (A) Unwinding Assay. Reactions were carried out in 20 μL assay buffer (10 mM Tris−HCl, pH 7.9, 50 mM NaCl, 50 mM KCl, 5 mM $MgCl_2$, 0.1 mM EDTA, and 2.5 wt % glycerol) that contained 270 ng of pGEM plasmid, increasing quantities of the potential inhibitor (50, 100, 200, 500 μM final concentration), and 10 units of topoisomerase I. Following a 30 min incubation at 37 °C (in the dark), samples were extracted with phenol/chloroform and subjected to electrophoresis (1% agarose, TRIS−borate, without ethidium bromide, 10 V/cm).

(B) Topoisomerase II Assay. Reactions were carried out in 20 μL cleavage buffer (10× to detect enzyme-mediated

clevage, 1× contained 30 mM Tris−HCl, pH 7.6, 3 mM ATP, 60 mM NaCl, 8 mM $MgCl_2$, and 15 mM mercaptoethanol) or in assay buffer (10× to detect relaxation, 1× contained 50 mM Tris−HCl, pH 8.0, 0.5 mM ATP, 120 mM KCl, 10 mM $MgCl_2$, and 0.5 mM dithiothreitol) that contained about 300 ng of pGEM plasmid, increasing quantity of potential inhibitor (2−500 μM final concentration), and four units of topoisomerase II. Following 40 min incubation at 37 °C (in the dark), the reaction was stopped by addition of 2 μL of SDS (10%), and proteins were digested on adding proteinase K (50 μg/mL) in 30 min at 37 °C. Samples were then extracted with phenol/chloroform and subjected to electrophoresis (1% agarose, TRIS−borate, with or without ethidium bromide (0.5 μg/mL), 10 V/cm). After running, agarose gels were examined under a UV lamp (when no ethidium bromide was added before the electrophoresis, it was added afterwards for visualization) and photographed (see Figure 5 for an example).

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Supporting Information Available: Spectral data and elemental analysis for various compounds (30 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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