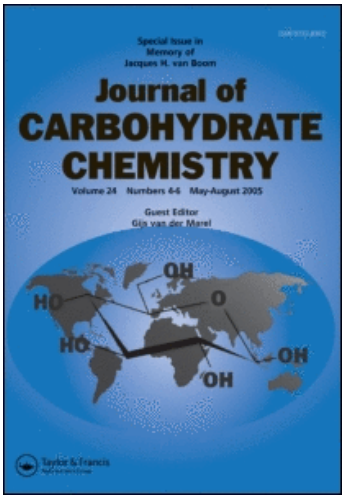

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New Carbohydrate Derivatives of Norfloxacin

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New classes of sugar derivatives of the antibacterial drug norfloxacin (**1**) were synthesized by substituting the N-4′ of the piperazinyl moiety of the molecule. Direct coupling with gluco- and galactopyranosyl units afforded glycosylamines **2**–**5**. Introduction of urea or thiourea linkage between glycosyl and norfloxacin units was produced with the corresponding glycosyl isocyanates or isothiocyanates. For the synthesis of unprotected sugar urea compounds, a new approach was applied by using 1,2-*N,O*-carbonyl-β-D-glycopyranoses. Hydrazinocarbonyl-methyl- and -propyl spacers also were found appropriate for linking norfloxacin with sugar units.

Keywords Norfloxacin, Glycosylamine, Glycosylurea, Glycosylthiourea, Glycosylthiosemicarbazide

INTRODUCTION

Examination of various natural products provided evidence for the important role of carbohydrate units in biomolecules. Recognition of this fact induced

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Dedicated to Professor A. Messmer on the occasion of his 80th anniversary.
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the approach toward combination of pharmacologic active molecules with carbohydrates to improve their properties. Many examples have supported that such combination resulted in favorable changes of pharmacologic effect.[1]

Norfloxacin [1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid] (**1**) is known as an antibacterial drug of widespread efficiency.[2] Due to some unfavorable physical properties of **1** (e.g., weak solubility), hundreds of derivatives with different modifications of the structure were synthesized and tested to produce norfloxacin analogues of better physical and biologic characteristics.[2c,3] Recently, fluoroquinolone carboxylic acid derivatives substituted with glucosyl and galactosyl units were synthesized.[4] Now, we describe new derivatives of norfloxacin coupled with monosaccharide units through various linkages.

RESULTS AND DISCUSSION

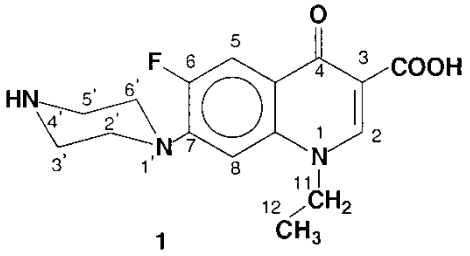
In the norfloxacin molecule (**1**), different functions provide a possibility for chemical modification (Fig. 1).

Among them, the free secondary amine group of the piperazinyl moiety offers easy ways for diverse substitutions with carbohydrate units. By successful realization of some of these approaches we synthesized a large number of new carbohydrate compounds for testing antibacterial activity. In the following sections, we present representative examples from the variety of such combinations.

Glycosylamines

In a publication by Jung et al.,[4] glycosides directly linked to the distal nitrogen atom of the piperazinyl moiety of fluoroquinolones were considered as simplest derivatives. Problems of the synthesis of the corresponding glucopyranosyl- and galactopyranosylamine compounds of norfloxacin (**1**) were also mentioned; however, no details of these two experiments were given.

Repeating the reaction of **1** with D-glucose and D-galactose, we met similar difficulties. Hence, sugar components were applied in protected form.



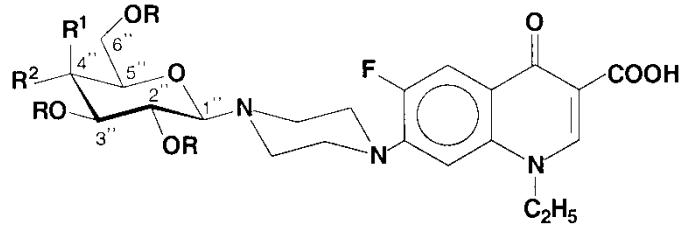
1

Figure 1: Numbering of atoms of norfloxacin (**1**).

Conventional methods,[5] however, using a large excess of amine component could not be applied due to the high price of norfloxacin. We performed a slightly modified Koenigs-Knorr method by using acetylated glycopyranosyl bromides in excess. Thus, *N*-(tetra-*O*-acetyl-β-D-glucopyranosyl)- (**2**) and *N*-(tetra-*O*-acetyl-β-D-galactopyranosyl)-norfloxacin (**3**) were formed in reasonable yield (52% to 63%) from acetobromoglucose and acetobromogalactose, respectively. Deacetylation of **2** and **3** was carried out with ammonia in aqueous ethanol to give the corresponding free glucopyranosyl and galactopyranosyl derivatives **4** and **5**, in the form of ammonium salt crystallizing with 1 molecule of water. The composition of both compounds was supported by microanalysis and corroborated by potentiometric titration indicating ammonium counterion at pK 8.5.

Park et al.,[6] recently stated the formation of stable complexes of several fluoroquinolones with water and protic solvents. Microanalyses found for the new norfloxacin derivatives are in good agreement with this statement. IR spectra of all the new compounds revealed the characteristic bands of the norfloxacin moiety at 1716–1730 (C=O) and 1630 cm^{-1} (C=C). In addition, carbonyl bands of the acetyl groups of pyranosyl units were assigned at 1750 cm^{-1} in the spectra of **2** and **3**. These bands were absent in the spectra of **4** and **5** where broad HO bands appeared between 3600–2400 cm^{-1}, indicating complete deacetylation (Fig. 2).

In both ^{1}H- and ^{13}C-NMR spectra of the new compounds **2** and **3**, characteristic signals of the fluoroquinolone and piperazinyl moieties of norfloxacin as well as patterns of the pyranosyl units were unambiguously assigned. Generally, a small solvent shift was observed for the chemical shifts of the methyl hydrogens in the 1-ethyl group and the C-2 and C-3 atoms, respectively. The β-anomeric structure of the carbohydrate moiety was deduced from the high value of the (*diaxial*-type) vicinal coupling constant $^{3}J_{1,2} = 8.8$ Hz for **2** and $^{3}J_{1,2} = 9.0$ Hz for **3**.



2 : R = Ac, R^1 = H, R^2 = AcO
3 : R = Ac, R^1 = AcO, R^2 = H
4 : R = H, R^1 = H, R^2 = HO
5 : R = H, R^1 = HO, R^2 = H

Figure 2: N–4′–glycosylamines of norfloxacin (**2–5**).

In contrast, ^{1}H-NMR spectra of the deacetylated derivatives **4** and **5** could not be clearly analyzed due to the strong overlapping of the signals of pyranoid ring protons in $(CD_3)_2SO$ solution. However, multiplet of H-6″ was assigned at 3.80–3.67 ppm for **4** beside the characteristic signals of the norfloxacin moiety as in the case of acetylated derivative. Proton signals in ^{1}H-NMR spectrum of **5** were so overlapped that signals for the pyranoid ring could not be assigned. At the same time, optical rotation and molecular ion ($[M + H]^+$ 482) of its mass spectrum unambiguously proved the sugar moiety in the molecule.

The chemical stability of these *N*-glycosides is moderate; in aqueous ethanol the pyranosyl substituent split off, partially, within 48 hr at room temperature.

N-Carbamoyl- and -thiocarbamoyl derivatives

Due to the high stability of the urea system, the instability of *N*-glycosides could be eliminated by binding the sugar moiety with carbamoyl or thiocarbamoyl linkage to the secondary amine N-4′ of the norfloxacin molecule.

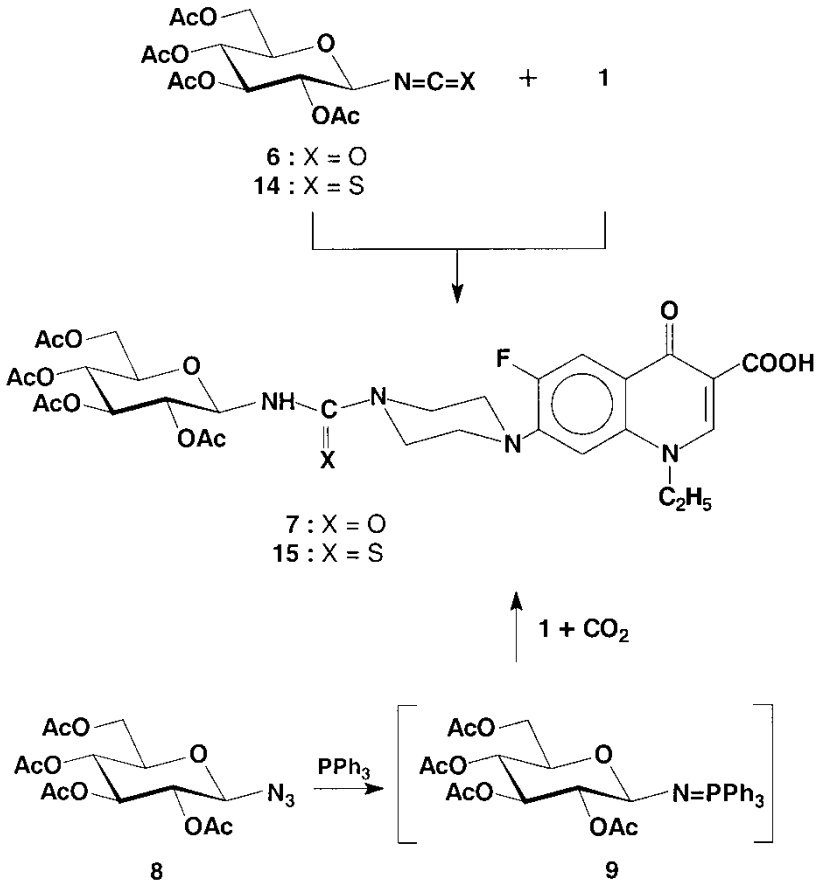
The synthesis of carbamoyl derivatives was performed from **1** by two methods: either with the corresponding isocyanate in dry dichloromethane (method A)[7] or with the phosphinimine reaction of the corresponding azide (method B).[8] The advantage of method B is that azido sugars are more easily available than the corresponding isocyanates (Sch. 1).

Reaction of norfloxacin (**1**) with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isocyanate (**6**)[9] afforded the urea derivative **7** in excellent yield. The structure of the new compound was unambiguously proved by ^{1}H- and ^{13}C-NMR spectra in which all signals of protons and carbon atoms were clearly assigned. Besides the signals of norfloxacin and sugar moieties, the urea group was characterized with the doublet of N—H at δ 6.07 ppm in the ^{1}H-NMR spectrum as well as by the signal of the carbonyl at δ 155.5 ppm in the ^{13}C-NMR spectrum.

The same compound **7** was obtained in similar yield from the reaction of norfloxacin (**1**) with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl azide (**8**).[10] The reaction by method B might be performed either with isolated phosphinimine (**9**)[8] or with *in situ* generated intermediate **9**[11] (Sch. 1).

Several derivatives were synthesized from various protected azido sugars, and structures of those were supported by perfectly analogous IR spectra. Deacetylation of **7** by the Zemplén method was unsuccessful; therefore, norfloxacin (**1**) was treated with 1-*N*,2-*O*-carbonyl-β-D-glucopyranosylamine (**10**) produced from β-D-glucopyranosyl azide with triphenyl phosphine-carbon dioxide reagent,[12] affording the new glucopyranosylaminocarbonyl-norfloxacin **12**.

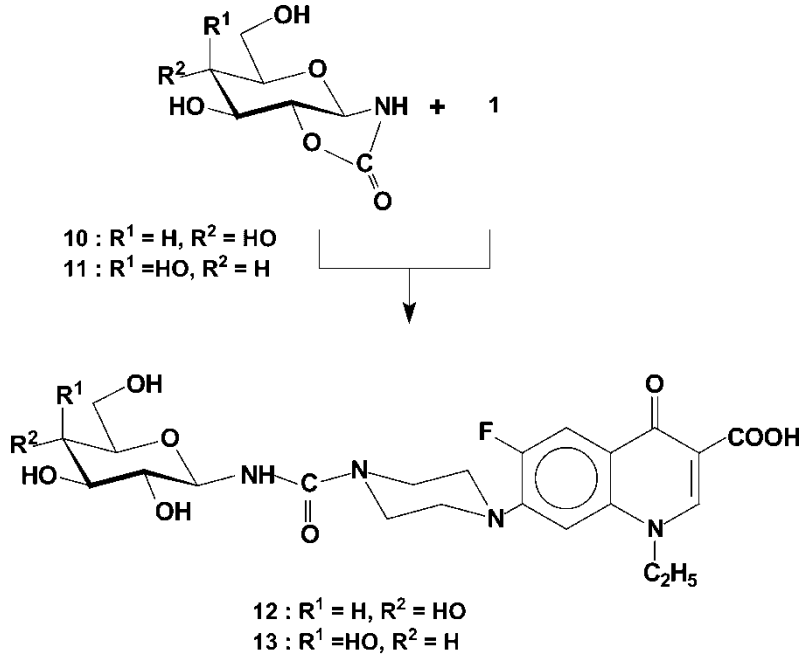
Similarly, the analogous galactopyranosyl compound **13** was formed from the reaction of **1** with 1-*N*,2-*O*-carbonyl-β-D-galactopyranosylamine (**11**)[12] produced from β-D-galactopyranosyl azide (Sch. 2).



Scheme 1: Dual way of a synthesis of N–4′–(glycosylcarbamoyl) derivatives of norfloxacin.

The structure of the new crystalline derivatives **12** and **13** obtained in excellent yield was proved by IR, ^{1}H-, and ^{13}C-NMR spectra. The structure of **12** was corroborated by acetylation with pyridine-acetic anhydride, affording the tetra-*O*-acetate **7** in good yield.

The thiocarbamide analogue of **7** was synthesized with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (**14**)[13] in dry dichloromethane, according to the conventional method[14] (Sch. 1). IR, ^{1}H-, and ^{13}C-NMR spectra of the new glucopyranosylamino-thiocarbonyl derivative **15** exhibited patterns analogous to those of **7**. Signals of norfloxacin and sugar moieties were clearly assigned also in this case. Furthermore, in the ^{1}H-NMR spectrum the characteristic doublet of NH was found at δ 6.90 ppm, while the signal of thiocarbonyl appeared at δ 182.4 ppm in the ^{13}C-NMR spectrum.



Scheme 2: Simplified pathway to free N–4′–(glycosylcarbamoyl) derivatives of norfloxacin (**12** and **13**).

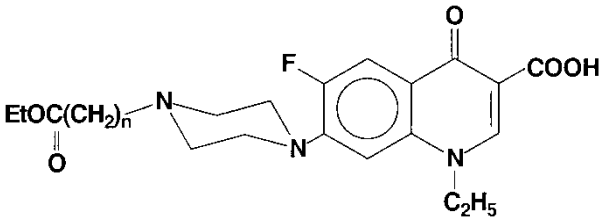
As was expected, the chemical stability of these urea and thiourea derivatives was higher than that of the *N*-glycosides **2**–**5**; in aqueous ethanol, no decomposition was detected by TLC after some days.

N-Hydrazinocarbonylalkyl derivatives

To link the sugar substituent to the piperazinyl group of norfloxacin (**1**), an appropriate spacer was found in the hydrazinocarbonyl-alkyl group. The spacer was built up from the corresponding ethoxycarbonyl-methyl[15] and the new ethoxycarbonyl-propyl derivatives **16** and **17**, respectively. The method described for the synthesis of **16**[15] was successfully modified to give a better yield for both products.

IR spectra of **16** and **17** indicated near analogy in the structure of the two compounds. The structure of the new derivative **17** was clearly proved by the ^{1}H-NMR spectrum exhibiting signals of protons of the three methylene groups at δ 2.49, 2.39, and 1.87 ppm, respectively. In accordance, signals of the corresponding carbon atoms were found at δ 57.2, 52.4, and 31.8 ppm, respectively, in the ^{13}C-NMR spectrum.

The reaction of both **16** and **17** with hydrazine hydrate in boiling ethanol afforded the new acylhydrazine derivatives **18** and **19**, respectively, in good yield (Figs. 3 and 4).



16 : n = 1
17 : n = 3

Figure 3: N–4′–(ethoxycarbonylalkyl) derivatives of norfloxacin (**16** and **17**).

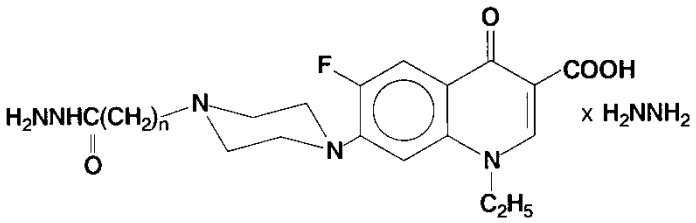
Absence of the ester carbonyl band and appearance of the new amide I band at 1686 and 1670 cm^{-1}, respectively, in the IR spectra of both products supported the expected structures of the two molecules.

Analysis of the ^{1}H-NMR spectra revealed a high value of the integral of NH protons at δ 4.6 ppm in both cases (8H and 4H, respectively), suggesting formation of hydrazinium salts. This assumption was supported, on one hand, by microanalysis indicating hydrates of hydrazinium salts for both **18** and **19**. On the other hand, the hydrazinium counterion was measured by potentiometric titration at pK 8.0 in both cases.

The signals of methylene carbons vicinal to the piperazine-N bearing the side chain (β-CH_2) are separated in the ^{13}C-NMR spectra of **18** and **19** probably due to local molecular asymmetry, which originates from restricted rotation of the amide group in the side chain.

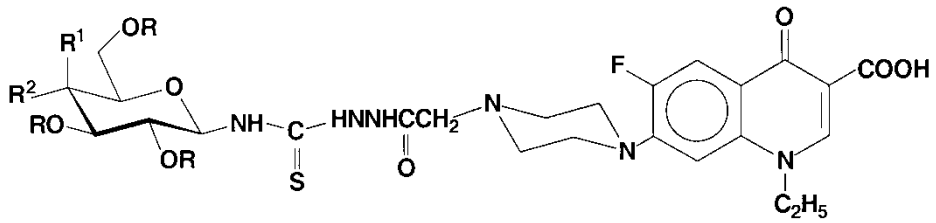
For comparison, norfloxacin (**1**) was similarly treated with hydrazine hydrate, when hydrate of **1** was obtained instead of the expected hydrazinium salt **20**. This composition was corroborated by microanalytical and spectral data.

When the acylhydrazine **18** was treated with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl or 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl isothiocyanate in aprotic solvents, the new crystalline sugar thiosemicarbazide derivatives of norfloxacin **21** and **22** were obtained in good yield (Fig. 5).



18 : n = 1, x = 1
19 : n = 3, x = 1/2

Figure 4: N–4′–(alkoylhidrazine) derivatives of norfloxacin (**18** and **19**).



21 : R = Ac, R^1 = H, R^2 = AcO
22 : R = Ac, R^1 = AcO, R^2 = H
23 : R = H, R^1 = H, R^2 = HO
24 : R = H, R^1 = HO, R^2 = H

Figure 5: N-4′-(glycosylaminothiocarbonyl-hydrazinocarbonylalkyl) derivatives of norfloxacin (**21**–**24**).

Their IR spectra showed intensive NH bands of the thiosemicarbazide group at about 3280 cm^{-1} and ester CO bands of the acetylated sugar at about 1750 cm^{-1}, besides the band of the keton CO of norfloxacin at 1710 cm^{-1}. Structures of **21** and **22** were corroborated by their ^{1}H-NMR spectra exhibiting characteristic signals of norfloxacin and the glycopyranosyl units as well as those of the thiosemicarbazidocarbonylmethyl moiety.

Deacetylation of **21** and **22** was performed with ammonia in aqueous ethanol to give the corresponding free glucopyranosyl and galactopyranosyl derivatives **23** and **24**, respectively. Structures were supported by their IR spectra indicating intensive HO bands between 3600–3400 cm^{-1} and no ester CO band at 1750 cm^{-1}. In accordance, in the ^{1}H-NMR spectra, besides the characteristic signals of the norfloxacin moiety, overlapping signals of the pyranosyl units were identified.

The new acylhydrazines **18** and **19**, as well as acylthiosemicarbazides **21**–**24**, were sufficiently stable for pharmacologic tests.

According to the results of the tests, the antimicrobial activities of these compounds are lower than those of pefloxacin.

EXPERIMENTAL

General Methods

TLC was performed on Silica Gel 60 F_{254} (E. Merck) plates developed with eluents as indicated. Spots were detected by exposure to UV light and by charring with conc. sulfuric acid. Column chromatography was made on silica gel (E. Merck, 0.063–0.2 mesh). IR spectra (KBr) were recorded with a Nicolet 205 FT spectrometer. Optical rotations were measured on a Zeiss-Polamat A polarimeter. The ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ or $(CD_3)_2SO$ solution in 5 mm tubes at rt, either on a Bruker WM-250

spectrometer at 250 (^{1}H) and 62.86 (^{13}C) MHz, or on a Bruker DRX-500 spectrometer at 500 (^{1}H) and 125.7 (^{13}C) MHz with the deuterium signal of the solvent as the lock and TMS as the internal standard. The standard Bruker microprogram NOEMULT to generate NOE[16] and to get DIFFNOE spectra[17,18] was used with a selective preirradiation time. DEPT spectra[19] were run in a standard manner[20] using only a $\Theta = 135^\circ$ pulse to separate the CH/CH_3 and CH_2 lines phased "up" and "down," respectively. The 2D-HSC[21,22] spectra were obtained using the standard Bruker pulse. Potentiometric titrations were carried out in 71 w/w% dimethyl sulfoxide/water mixed solvent and 0.05 M KOH was delivered by a Titrino 716 DMS autoburette, connected to a PC for data collection. A Metrohm combination glass electrode designed for hydroorganic media was calibrated at $23 \pm 1^\circ$C in terms of hydrogen ion concentration.[23] The autoprotolysis constant of the solvent mixture ($pK_w = 16.9$) was found in the literature.[24] Samples for analysis were dissolved in $1 \div 10$ mmol concentration in a standard solution of HCl and titrated alkalimetrically. From the equivalence point of the titration curve, the purity of each ligand could be assessed. Dissociation constants falling into the $3 < pK_a < 12$ range also were determined. Identity of counter ions was confirmed by measuring pK_a values of samples of potassium acetate ($pK_1 = 6.8$), ammonium chloride ($pK_1 = 8.6$), and hydrazinium chloride ($pK_1 = 8.0$), respectively. Mass spectra were measured on a ZAB-SEQ instrument. For FAB measurements, glycerine was used as a matrix with the exception of **4** and **5**, when diethanolamine was used.

*1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-[2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl]-1-piperazinyl)-3-quinolinecarboxylic Acid (**2**)*

Norfloxacin (**1**, 2.4 g, 7.52 mmol) in dichloromethane (70 mL) was mixed with solid potassium hydrogencarbonate (0.825 g, 8.25 mmol), and then a solution of acetobromoglucose (3.45 g, 8.4 mmol) in dichloromethane (30 mL) was added at room temperature under stirring. The mixture was stirred overnight, and then potassium hydrogencarbonate (0.0825 g, 0.825 mmol) and acetobromoglucose (0.345 g, 0.84 mmol) in dichloromethane (5 mL) were added again. Stirring was continued at room temperature for 96 hr; meanwhile, the addition of the same portion of the reagents was repeated twice. After standing for 50 hr, TLC (EtOAc-MeOH-H_2O 30:10:1 or $CHCl_3$-MeOH 6:1) revealed a slight spot of **1**, which did not disappear by further addition of the reagents. The mixture was diluted with dichloromethane (80 mL), and the solid was filtered and washed with dichloromethane (3×40 mL) to give a mixture (1.47 g) of unchanged **1** and potassium salts. The filtrate was evaporated, and the syrupy residue was stirred with diethyl ether (150 mL) overnight at room temperature. Filtration of the mixture afforded a yellowish solid (2.43 g), which was triturated with chloroform (120 mL) to separate a second

crop of **1** (0.38 g). The crude product was purified by column chromatography with acetone affording pure **2** (1.56 g, 32%), mp: 185–189°C; $[\alpha]_D$ −116.0 (*c* 1, $CHCl_3$).

Collected crops of unchanged **1** (total: 1.85 g, 5.79 mmol) were transformed by the same procedure with acetobromoglucose (total: 4.14 g, 10.08 mmol) and potassium hydrogencarbonate (total: 0.99 g, 9.9 mmol) in dichloromethane (30 mL). Thus, an additional crop of pure **2** (0.96 g, 20%), was isolated, mp: 185–189°C; $[\alpha]_D$ −114.0 (*c* 1, $CHCl_3$). IR (KBr): 1755 (AcO), 1730 (shoulder, C=O), 1630 (C=C, C=O), 1228, 1035 cm^{-1} (C–O–C). ^{1}H-NMR (500 MHz, $CDCl_3$): δ ppm 12.50 (s, 1H, COOH), 8.65 (s, 1H, H-2), 7.99 (d, 1H, H-5), 6.81 (d, 1H, H-8), 5.28 (t, $J_{2,3}$ 9.3 Hz, 1H, H-3″) 5.18 (t, $J_{2,3}$ 9.2 Hz, 1H, H-2″), 5.03 (t, $J_{3,4}$ 9.6 Hz, 1H, H-4″), 4.37 (q, 2H, H-11), 4.26 (m, 1H, H-6a″), 4.12 (m, 1H, H-6b″), 4.13 (d, $J_{1,2}$ 8.8 Hz, 1H, H-1″), 3.70 (m, 1H, H-5″), 3.25 (m, 4H, H-2′, and H-6′), 2.85 (m, 4H, H-3′, and H-5′), 2.08 (s, 6H, C*H*$_3$COO), 2.05 (s, 3H, C*H*$_3$COO) 2.04 (s, 3H, C*H*$_3$COO), 1.60 (t, 3H, H-12). ^{13}C-NMR (125.7 MHz, $CDCl_3$): δ ppm 176.5 (*C*OOH), 170.3, 169.8, 169.5, 169.3 (Me*C*OO), 166.8 (C-4), 153.1 (C-6), 146.8 (C-2), 145.7 (C-7), 136.9 (C-8a), 119.9 (C-4a), 112.1 (C-5), 107.8 (C-3), 103.6 (C-8), 92.9 (C-1″), 73.2, 72.9, 68.5, and 67.1 (C-2″-C-5″), 62.0 (C-6″), 49.8 and 47.2 (C-2′,3′,5′,6′), 49.4 (C-11), 20.44, 20.35, and 20.3 (*C*H$_3$COO), 14.2 (C-12). MS (FAB) m/z 650 ($[M+H]^+$).

Anal. Calcd for $C_{30}H_{36}FN_3O_{12}$ (649.64) C, 55.47; H, 5.59; N, 6.47. Found: C, 56.50; H, 5.64; N, 6.44.

*1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-[2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl]-1-piperazinyl)-3-quinolinecarboxylic Acid (**3**)*

A mixture of **1** (3.36 g, 10.5 mmol), acetobromogalactose (4.83 g, 11.76 mmol), and potassium hydrogencarbonate (1.155 g, 11.55 mmol) in dichloromethane (105 mL) was reacted and worked up as described for the preparation of **2** to produce pure **3** (4.32 g, 63%) after column chromatography; mp: 178–181°C; $[\alpha]_D$ −91.0 (*c* 1, $CHCl_3$). IR (KBr): 1751 (AcO), 1727 (shoulder, C=O), 1630 (C=C, C=O), 1227, 1015 cm^{-1} (C–O–C). ^{1}H-NMR (500 MHz, $CDCl_3$): δ ppm 8.89 (s, 1H, H-2), 7.92 (d, 1H, H-5), 7.00 (d, 1H, H-8), 5.46 (d, $J_{3,4}$ 3 Hz, 1H, H-4″), 5.40 (t, $J_{2,3}$ ∼8 Hz, 1H, H-2″), 5.22 (dd, $J_{2,3}$ ∼8 Hz, 1H, H-3″), 4.50 (q, 2H, H-11), 4.26 (d, $J_{1,2}$ 9.0 Hz, 1H, H-1″), 4.18 (m, 2H, H-6″), 4.05 (t, $J_{4,5}$ ∼3 Hz, 1H, H-5″), 3.33 (m, 4H, H-2′, and H-6′), 2.95 (m, 4H, H-3′, and H-5′), 2.19 (s, 3H, C*H*$_3$COO), 2.11 (s, 3H, C*H*$_3$COO), 2.06 (s, 3H, C*H*$_3$COO) 2.01 (s, 3H, C*H*$_3$COO), 1.57 (t, 3H, H-12). ^{13}C-NMR (125.7 MHz, $CDCl_3$): δ ppm 176.7 (*C*OOH), 170.3, 170.1, 169.9 (CH_3*C*OO), 167.0 (C-4), 154.3 (C-6), 146.9 (C-2), 146.0 (C-7), 137.0 (C-8a), 120.1 (C-4a), 112.3 (C-5), 108.0 (C-3), 103.7 (C-8), 93.4 (C-1″), 71.6, 67.2, and 65.0 (C-2″-C-5″), 61.2 (C-6″), 50.0 and 47.0 (C-2′,3′,5′,6′), 49.4 (C-11), 20.8, and 20.5 (*C*H$_3$COO), 14.3 (C-12). MS (FAB) m/z 650 ($[M+H]^+$).

Anal. Calcd for $C_{30}H_{36}FN_3O_{12}$ (649.64) C, 55.47; H, 5.59; N, 6.47. Found: C, 55.52; H, 5.55; N, 6.40.

*1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-[β-D-glucopyranosyl]-1-piperazinyl)-3-quinolinecarboxylic Acid Ammonium Salt (**4**)*

Compound **2** (0.82 g, 1.26 mmol) was dissolved in a mixture of conc. ammonium hydroxide (4.5 mL) and ethanol (9 mL) under stirring at room temperature within some minutes. After standing for 24 hr the almost complete deacetylation was indicated by TLC (aceton-H_2O 9:1 or $CHCl_3$-MeOH-H_2O 3:2:1). The mixture was diluted with acetone to precipitate crude **4**. After filtration the yellowish solid was triturated with a little ethanol and acetone, and then it was stirred overnight with diethyl ether to give pure **4** (0.53 g, 87%), mp: 140–143°C; $[\alpha]_D$ +6.0 (*c* 1, DMF). IR (KBr): 3600–2400 (OH), 1716 (C=O), 1629 cm^{-1} (C=C, C=O). ^{1}H-NMR (250 MHz, $(CD_3)_2SO$): δ ppm 8.88 (s, 1H, H-2), 7.10 (d, 1H, H-8), 4.56 (q, 2H, H-11), 3.67–3.80 (m, 2H, H-6″), 3.33 (m, 4H, H-2′, and H-6′), 2.80 (m, 4H, H-3′, and H-5′), 1.43 (t, 3H, H-12). MS (FAB) m/z 482 ($[M+H]^+$). Potentiometry: $pK_1 = 7.0$, $pK_2 = 8.4$, $pK(NH_4^+) = 8.5$.

Anal. Calcd for $C_{22}H_{28}FN_3O_8.NH_3.H_2O$ (516.50) C, 51.16; H, 6.44; N, 10.85. Found: C, 51.76; H, 6.36; N, 11.70.

*1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-[β-D-galactopyranosyl]-1-piperazinyl)-3-quinolinecarboxylic Acid Ammonium Salt (**5**)*

Compound **3** (1.23 g, 1.89 mmol) was dissolved in a mixture of conc. ammonium hydroxide (7 mL) and ethanol (14 mL) under stirring at room temperature within some minutes. After standing for 24 hr the mixture was worked up as described for the preparation of **4** to give pure **5** (0.55 g, 60%), mp: 226–230°C; $[\alpha]_D$ +7.0 (*c* 1, DMF). IR (KBr): 3600–2400 (OH), 1718 (C=O), 1630, 1580 cm^{-1} (C=C, C=O). MS (FAB) m/z 482 ($[M+H]^+$). Potentiometry: $pK_1 = 7.0$, $pK_2 = 8.5$, $pK(NH_4^+) = 8.5$.

Anal. Calcd for $C_{22}H_{28}FN_3O_8.NH_3.H_2O$ (516.50) C, 51.16; H, 6.44; N, 10.85. Found: C, 51.70; H, 6.36; N, 11.26.

*1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-[2,3,4,6-tetra-O-acetyl-β-D-glucopyranosylaminocarbonyl]-1-piperazinyl)-3-quinolinecarboxylic Acid (**7**)*

Method A

To a mixture of **1** (0.32 g, 1 mmol) in dry dichloromethane (15 mL) was added dropwise a solution of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isocyanate (**6**, 0.37 g, 1 mmol) in dry dichloromethane (5 mL) under stirring at room

temperature. The solid was dissolved, and the reaction was complete within 6 hr as indicated by TLC (BuOAc-AcOH-EtOH-H_2O 3:2:1:1). After evaporation *in vacuo*, the residue was treated with diethyl ether to give a white microcrystalline solid. Purification by precipitation from a dichloromethane solution with diethyl ether afforded pure **7** (0.62 g, 91%), mp: 148–150°C; $[\alpha]_D$ −29.6 (*c* 1, $CHCl_3$). IR (KBr): 1745 (AcO), 1665 (NHCON) 1625 cm^{-1} (C═O). ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 8.63 (s, 1H, H-2), 7.91 (d, 1H, H-5), 6.86 (d, 1H, H-8), 6.07 (d, 1H $J_{1,NH} = 8.8$ Hz, NH), 5.36 (t, 1H, H-3″), 5.24 (t, 1H, H-1″), 5.09 (t, 1H, $J_{3,4} = 9.5$ Hz, $J_{4,5} = 10.0$ Hz, H-4″), 5.00 (t, 1H, $J_{1,2} = 9.5$ Hz, $J_{2,3} = 9.5$ Hz, H-2″), 4.38 (q, 2H, H-11), 4.33 (dd, 1H, $J_{5,6a} = 1.9$ Hz, $J_{6a,6b} = 12.3$ Hz, H-6$_a''$), 4.11 (dd, 1H, $J_{5,6b} = 3.9$ Hz, $J_{6a,6b} = 12.3$ Hz, H-6$_b''$), 3.89 (ddd, 1H, $J_{5,6a} = 1.9$ Hz, $J_{5,6b} = 3.9$ Hz, H-5″), 3.65 (m, 4H, H-2′, and H-6′), 3.37 (m, 4H, H-3′, and H-5′), 2.10, 2.09, 2.05, 2.04 (4s, 12H, C*H*$_3$COO), 1.60 (t, 3H, H-12). ^{13}C-NMR (100 MHz, $CDCl_3$): δ ppm 176.4 (C-4), 171.3 (*C*OOH), 170.5, 169.7, 169.5, and 166.8 (CH_3*C*OO), 155.5 (N*C*ON), 153.0 (C-6), 147.0 (C-2), 145.3 (C-7), 136.9 (C-8a), 120.1 (C-4a), 112.2 (C-5), 107.8 (C-3), 103.9 (C-8), 80.5 (C-1″), 72.9 (C-5″), 72.6 (C-3″), 70.7 (C-2″), 68.1 (C-4″), 61.6 (C-6″), 49.6, 49.1, and 43.1 (2C) (C-11 and C-2′,3′,5′,6′), 20.7, 20.5, and 20.4 (2C) (*C*H_3COO), 14.2 (C-12). MS (FAB) m/z 693 ($[M + H]^+$).

Anal. Calcd for $C_{31}H_{37}FN_4O_{13}.H_2O$ (710.70) C, 52.39; H, 5.53; N, 7.88. Found C, 52.70; H, 5.45; N, 7.60.

Method B

Under continuous bubbling of dry carbon dioxide into a solution of **1** (0.64 g, 2 mmol) and 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl azide (**8**, 0.75 g, 2 mmol) in dimethylformamide (12 mL) a solution of triphenyl phosphine (0.55 g, 2.1 mmol) in dimethylformamide (3 mL) was added dropwise at room temperature. After stirring for 24 hr, TLC (BuOAc-AcOH-EtOH-H_2O 3:2:1:1) indicated complete transformation. The solvent was removed *in vacuo* (1 mmHg, bath temperature: 50°C), and the residue was extracted several times with boiling cyclohexane (5 mL) and then twice with hot carbon tetrachloride (5 mL) to give a solid. After filtration and washing with diethyl ether, pure **7** was obtained in 89% yield. Physical constants and spectra were identical with those of the product obtained by Method A.

Method C

Compound **12** (0.105 g, 0.2 mmol) was added to a mixture of dry pyridine (1.5 mL) and acetic anhydride (0.7 mL) under cooling. After standing overnight at room temperature, the mixture was poured into iced water, and the precipitate was filtered and dried to give a crude product (0.11 g). By precipitation from dichloromethane solution with diethyl ether, pure **7** (0.092 g, 66%) was obtained. Physical constants and spectra were identical with those of an authentic sample.

*1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-[β-D-glucopyranosylaminocarbonyl]-1-piperazinyl)-3-quinolinecarboxylic Acid (**12**)*

To a solution of **1** (0.96 g, 3 mmol) in dimethylformamide (20 mL) was added 1-*N*,2-*O*-carbonyl-β-D-glucopyranosylamine (**10**, 0.62 g, 3 mmol) under stirring at room temperature. The solid dissolved, and after 3 hr a solid began to precipitate. The mixture was left to stand overnight and filtered. The solid was washed with acetone to give a crop of the crude product (0.9 g). By diluting the filtrate with acetone, a second crop (0.53 g) was obtained. Crystallization from aqueous methanol (1:1) furnished colorless needles of pure **12** (1.18 g, 75%); mp: 216–218°C; $[\alpha]_D$ +10.5 (*c* 1, DMSO). IR (KBr): 1716 (COOH), 1644 (NHCON) 1632 (C═O), 1537 cm^{-1} (amide II). ^{1}H-NMR (250 MHz, $(CD_3)_2SO$): δ ppm 8.97 (s, 1H, H-2), 7.97 (d, 1H, H-5), 7.22 (d, 1H, H-8), 7.11 (d, 1H, NH), 4.79 (d, 1H, $J_{1,2} = 8.6$ Hz, H-1″), 4.62 (q, 2H, H-11), 3.8–3.1 (m, 6H, H-2″-6″), 3.66 (m, 4H, H-2′, and H-6′), 3.35 (m, 4H, H-3′, and H-5′), 1.47 (t, 3H, H-12). ^{13}C-NMR (62.9 MHz, $(CD_3)_2SO$): δ ppm 176.5 (C-4), 166.5 (*C*OOH), 156.7 (N*C*ON), 153.0 (C-6), 148.8 (C-2), 145.7 (C-7), 137.4 (C-8a), 119.0 (C-4a), 111.4 (C-5), 107.3 (C-3), 106.3 (C-8), 82.3 (C-1″), 78.5, 77.9, 72.3, and 70.3 (C-2″-5″), 61.2 (C-6″), 49.5 and 49.2 (C-2′,3′,5′,6′), 43.1 (C-11), 14.2 (C-12).

Anal. Calcd for $C_{23}H_{29}FN_4O_9$ (524.51) C, 52.67; H, 5.57; N, 10.68. Found: C, 52.11; H, 6.01; N, 10.48.

*1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-[β-D-galactopyranosylaminocarbonyl]-1-piperazinyl)-3-quinolinecarboxylic Acid (**13**)*

A solution of **1** (0.96 g, 3 mmol) in DMF (20 mL) was treated with 1-*N*,2-*O*-carbonyl-β-D-galactopyranosylamine (**11**, 0.62 g, 3 mmol), and the mixture was worked up as described for **12**. Crystallization of the crude product from aqueous methanol (1:1) gave pure **13** (0.96 g, 61%); mp: 190–192°C; $[\alpha]_D$ +28.3 (*c* 0.7, DMF). IR (KBr): 1719 (COOH), 1640–1620 (NHCON and C═O), 1536 cm^{-1} (amide II). ^{13}C-NMR (62.9 MHz, $(CD_3)_2SO$): δ ppm 176.5 (C-4), 166.5 (*C*OOH), 156.9 (N*C*ON), 151.2 (C-6), 148.8 (C-2), 145.7 (C-7), 137.5 (C-8a), 119.6 (C-4a), 111.4 (C-5), 107.3 (C-3), 106.2 (C-8), 82.8 (C-1″), 76.6, 74.5, 69.7, and 68.5 (C-2″–5″), 60.7 (C-6″), 49.5 and 49.3 (C-2′,3′,5′,6′), 43.2 (C-11), 14.4 (C-12).

Anal. Calcd for $C_{23}H_{29}FN_4O_9$ (524.51) C, 52.67; H, 5.57; N, 10.68. Found: C, 52.23; H, 5.91; N, 10.31.

*1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-[2,3,4,6-tetra-O-acetyl-β-D-glucopyranosylamino-thiocarbonyl]-1-piperazinyl)-3-quinolinecarboxylic Acid (**15**)*

To a mixture of **1** (0.32 g, 1 mmol) in dry dichloromethane (15 mL) was added dropwise a solution of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl

isothiocyanate (**14**, 0.39 g, 1 mmol) in dry dichloromethane (5 mL) under stirring at room temperature. The mixture was treated and worked up as in the case of **7** (method A) to give pure **15** in 90% yield; mp: 153–154°C; $[\alpha]_D$ −25.6 (*c* 1, $CHCl_3$). IR (KBr): 1745 (AcO), 1660 (NHCON) 1625 cm^{-1} (C═O). ^{1}H-NMR (250 MHz, $CDCl_3$): δ ppm 8.60 (s, 1H, H-2), 7.92 (d, 1H, H-5), 6.90 (d, 1H, NH), 6.81 (d, 1H, H-8), 5.85 (dd, 1H, $J_{1,2} = 9.1$ Hz H-1″), 5.41 (t, 1H, H-3″), 5.10 (t, 1H, $J_{3,4} = 9.3$ Hz, $J_{4,5} = 10.2$ Hz, H-4″), 5.06 (t, 1H, $J_{2,3} = 9.7$ Hz, H-2″), 4.33 (m, 2H, H-11), 4.25 and 4.15 (m, 2H, H-6″), 4.15 (m, 4H, H-2′, and H-6′), 3.83 (m, 1H, H-5″), 3.41 (m, 4H, H-3′, and H-5′), 2.08, 2.07, 2.06, and 2.05 (4s, 12H, C*H*$_3$COO), 1.60 (t, 3H, H-12). ^{13}C-NMR (62.9 MHz, $CDCl_3$): δ ppm 182.4 (C = S), 176.6 (C-4), 172.2 (*C*OOH), 170.7, 169.9, 169.7, and 167.1 (Me*C*OO), 152.8 (C-6), 147.1 (C-2), 144.9 (C-7), 137.1 (C-8a), 120.0 (C-4a), 112.5 (d, $^2J_{C,F} = 23$ Hz, C-5), 107.9 (C-3), 103.4 (C-8), 83.8 (C-1″), 73.3 (C-5″), 72.7 (C-3″), 71.3 (C-2″), 68.5 (C-4″), 61.8 (C-6″), 49.8, 48.5, and 47.1 (2C) (C-11 and C-2′,3′,5′,6′), 20.9, 20.8, and 20.6 (2C) (*C*H$_3$COO), 14.4 (C-12).

Anal. Calcd for $C_{31}H_{37}FN_4O_{12}S$ (708.73) C, 52.53; H, 5.26; N, 7.90. Found; C, 51.40; H, 5.24; N, 7.70.

*1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-[ethoxycarbonylmethyl]-1-piperazinyl)-3-quinolinecarboxylic Acid (**16**)*

To a solution of ethyl bromoacetate (5.0 g, 30 mmol) in dry DMF (100 mL), potassium hydrogencarbonate (2.66 g, 26.6 mmol) and norfloxacin (**1**, 8.0 g, 25 mmol) were added under stirring at room temperature. Stirring was continued overnight, and then a mixture of ethyl bromoacetate (0.5 g, 3 mmol) and potassium hydrogencarbonate (0.26 g, 2.6 mmol) in DMF (5 mL) was added. After 24 hr, addition of ethyl bromoacetate (0.5 g, 3 mmol) and potassium hydrogencarbonate (0.26 g, 2.6 mmol) was repeated under continuous stirring. When TLC (BuOAc-AcOH-EtOH-H_2O 3:2:1:1) revealed complete transformation of **1**, the solid was filtered and washed with diethyl ether to give a white solid (10.58 g) containing the product and potassium salts. After trituration with chloroform (2 L) and filtration, the chloroform layer was washed with water (260 mL), and then the aqueous layer was extracted twice with chloroform (100 mL). The organic phases were collected, dried with Na_2SO_4, and concentrated *in vacuo* to dryness to give the crude product. Crystallization from the chloroform-ethanol (1:1) mixture afforded pure **16** (7.52 g, 74%); mp: 231–233°C; lit.[15] mp: 229–231°C. IR (KBr): 1740 (CO ester), 1625, 1610 cm^{-1} (COOH, C═O). MS (EI) *m*/*z* 405.1705 ([M]).

Anal. Calcd for $C_{20}H_{24}FN_3O_5$ (405.43) C, 59.25; H, 5.97; N, 10.36. Found: C, 59.19; H, 5.87; N, 10.30.

*1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-[ethoxycarbonylpropyl]-1-piperazinyl)-3-quinolinecarboxylic Acid (**17**)*

To a solution of ethyl 4-bromobutyrate (9.0 g, 46 mmol) in dry DMF (120 mL), potassium hydrogencarbonate (3.78 g, 37.8 mmol) and norfloxacin (**1**, 12.0 g, 37.6 mmol) were added under stirring at room temperature. Stirring was continued overnight, and then a mixture of ethyl 4-bromobutyrate (0.90 g, 4.6 mmol) and potassium hydrogencarbonate (0.38 g, 3.8 mmol) in DMF (5 mL) was added. After 24 hr, addition of ethyl 4-bromobutyrate (0.90 g, 4.6 mmol) and potassium hydrogencarbonate (0.38 g, 3.8 mmol) was repeated under continuous stirring. When TLC (BuOAc-AcOH-EtOH-H_2O 3:2:1:1) revealed complete transformation of **1**, the mixture was worked up, as in the case of **16**, to give pure **17** (11.30 g, 69%); mp: 171–172°C. IR (KBr): 1740 (CO ester), 1625, 1610 cm^{-1} (COOH and C=O). ^{1}H-NMR (500 MHz, $CDCl_3$): δ ppm 8.65 (s, 1H, H-2), 7.95 (d, 1H, H-5), 6.84 (d, 1H, H-8), 4.35 (q, 2H, H-11), 4.15 (q, $COOCH_2CH_3$), 3.35 (m, 4H, H-2′, and H-6′), 2.70 (m, 4H, H-3′, and H-5′), 2.49, 2.39, and 1.87 (q, 3 × 2H, $(CH_2)_3$), 1.59 (t, 3H, H-12), 1.28 (t, $J = 7.1$ Hz, $COOCH_2CH_3$). ^{13}C-NMR (125.7 MHz, $CDCl_3$): δ ppm 176.8 (*C*OOH), 167.1 (C-4), 153.4 (C-6), 146.9 (C-2), 146.0 (C-7), 137.0 (C-8a), 120.0 (C-4a), 112.2 (C-5), 107.9 (C-3), 103.6 (C-8), 57.2 (NCH_2), 52.4 and 49.6 (C-2′,3′,5′,6′), 49.5 (C-11), 31.8 (CH_2CO), 21.7 (CH_2), 14.1 (C-12), 13.9 $COOCH_2CH_3$). MS (EI) m/z 433.2005 ([M]). Potentiometry: $pK_1 = 5.7$, $pK_2 = 8.5$.

Anal. Calcd for $C_{22}H_{28}FN_3O_5$ (433.49) C, 60.96; H, 6.51; N, 9.69. Found: C, 60.97; H, 6.41; N, 9.67.

*1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-[hydrazinocarbonylmethyl]-1-piperazinyl)-3-quinolinecarboxylic Acid Hydrazinium Salt (**18**)*

Compound **16** (2.0 g, 4.93 mmol) was slowly mixed with 50% aqueous hydrazine hydrate (60 mL) at room temperature. The solid gradually dissolved, and then the product precipitated. The mixture was stirred for 2 hr at room temperature and left to stand overnight in a refrigerator. Then the solid was filtered, washed, and triturated with ethanol to give pure **18** (1.39 g, 70%); mp: 253–256°C. IR (KBr): 3600–2000 (NH^+), 1686 (amide I), 1621, 1488 cm^{-1} (COOH, C=O). ^{1}H-NMR (500 MHz, $(CD_3)_2SO$): δ ppm 8.88 (s, 1H, H-2), 7.84 (d, 1H, H-5), 7.12 (d, 1H, H-8), 4.6 (broad, 8H, NH), 4.55 (q, 2H, H-11), 3.35 (m, 4H, H-2′, and H-6′), 3.05 (m, 2H, CH_2CO) 2.68 (m, 4H, H-3′, and H-5′), 1.43 (t, 3H, H-12). ^{13}C-NMR (125.7 MHz, $(CD_3)_2SO$): δ ppm 176.4 (*C*OOH), 168.5 (CH_2CO), 166.5 (C-4), 153.2 (C-6), 148.7 (C-2), 145.7 (C-7), 137.5 (C-8a), 119.4 (C-4a), 111.6 (C-5), 107.5 (C-3), 105.9 (C-8), 59.7 (CH_2CO), 52.5, 49.6, and 49.1 (C-2′,3′,5′,6′), 49.5 (C-11), 14.4 (C-12). MS (EI) m/z 391.1668 ([M]). Potentiometry: $pK_1 = 4.1$, $pK_2 = 8.5$, $pK(NH_2NH_3^+) = 8.0$.

Anal. Calcd for $C_{18}H_{22}FN_5O_4.N_2H_4.H_2O$ (441.47) C, 48.97; H, 6.39; N, 22.20. Found: C, 49.69; H, 6.25; N, 21.27.

*1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-[hydrazinocarbonylpropyl]-1-piperazinyl)-3-quinolinecarboxylic Acid Hydrazinium Salt (**19**)*

Compound **17** (4.0 g, 9.3 mmol) was slowly mixed with 50% aqueous hydrazine hydrate (70 mL) at room temperature. The mixture was treated and worked up as in the case of **18** to give pure **19** (3.28 g, 82%); mp: 222–224°C. IR (KBr): 3600–2400 (NH^+), 1720 (C═O), 1629 cm^{-1} (amide I, COOH). ^{1}H-NMR (500 MHz, $(CD_3)_2SO$): δ ppm 8.94 (s, 1H, H-2), 7.89 (d, 1H, H-5), 7.15 (d, 1H, H-8), 4.6 (broad, 4H, NH), 4.58 (q, 2H, H-11), 3.31 (m, 4H, H-2′, and H-6′), 2.55 (m, 4H, H-3′, and H-5′), 2.33, 2.07, and 1.70 (q, 3 × 2H, $(CH_2)_3$), 1.42 (t, 3H, H-12). ^{13}C-NMR (125.7 MHz, $(CD_3)_2SO$): δ ppm 176.2 (*C*OOH), 171.6 (CH_2*C*O), 166.0 (C-4), 152.8 (C-6), 148.2 (C-2), 145.4 (C-7), 137.3 (C-8a), 119.1 (C-4a), 111.0 (C-5), 107.3 (C-3), 105.4 (C-8), 56.9 (NCH_2), 52.5, 49.3, and 48.8 (C-2′,3′,5′,6′), 49.5 (C-11), 31.2 (*C*H_2CO), 22.1 (CH_2), 13.9 (C-12). MS (EI) m/z 419.1953 ([M]). Potentiometry: $pK_1 = 5.6$, $pK_2 = 8.5$, $pK(NH_2NH_3^+) = 8.0$.

Anal. Calcd for $C_{20}H_{26}FN_5O_4.1/2N_2H_4.H_2O$ (453,50) C, 52.97; H, 6.84; N, 18.53. Found: C, 52.44; H, 6.70; N, 19.39.

Reaction of 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic Acid with Hydrazine Hydrate

A mixture of norfloxacin (**1**, 2.0 g, 6.3 mmol) with 50% aqueous hydrazine hydrate (60 mL) was stirred for 24 hr at room temperature. The white precipitate was filtered and triturated with ethanol to afford white crystals of **1. H_2O** (1.35 g, 64%); mp: 212–214°C. IR (KBr): 3600–2200 (NH, OH), 1742, 1730, 1618 cm^{-1} (COOH and C═O). ^{1}H-NMR (250 MHz, $(CD_3)_2SO$): δ ppm 8.87 (s, 1H, H-2), 7.80 (d, 1H, H-5), 7.08 (d, 1H, H-8), 4.54 (q, 2H, H-11), 3.22 (m, 4H, H-2′, and H-6′), 2.89 (m, 4H, H-3′, and H-5′), 1.42 (t, 3H, H-12). ^{13}C-NMR (62.9 MHz, $(CD_3)_2SO$): δ ppm 175.9, 165.7 (C-4), 152.6 (C-6), 147.8 (C-2), 145.8 (d, C-7), 137.1 (C-8a), 118.7 (d, C-4a), 110.9 (d, C-5), 107.0 (C-3), 105.0 (C-8), 50.7, 50.6 and 45.2 (C-2′,3′,5′,6′), 48.7 (C-11), 13.9 (C-12). MS (EI) m/z 319.1324 ([M]). Potentiometry: $pK_1 = 5.7$, $pK_2 = 8.6$.

Anal. Calcd for $C_{16}H_{18}FN_3O_3.H_2O$ (337.40) C, 56.96; H, 5.98; N, 12.46. Found: C, 57.20; H, 5.85; N, 12.97.

*1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-[2,3,4,6-tetra-O-acetyl-β-D-glucopyranosylaminothiocarbonyl-hydrazinocarbonylmethyl]-1-piperazinyl)-3-quinolinecarboxylic Acid (**21**)*

Slow addition of a solution of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl isothiocyanate (**14**, 3.6 g, 9.25 mmol) in dry dichloromethane (30 mL) to a mixture

of **18** (1.6 g, 3.9 mmol) in dry dichloromethane (30 mL) resulted in solubilization of the solid. Since after 24 hr at room temperature TLC (acetone-water 95:5) revealed unreacted **18**, a solution of **14** (0.36 g, 0.92 mmol) in dry dichloromethane (3 mL) was added again to complete the reaction. After 24 hr, the solvent was evaporated *in vacuo*, and the syrupy residue was triturated with diethyl ether (100 mL) to give a solid (5.3 g). After filtration it was dissolved in acetone (220 mL), and then a white crystalline solid was slowly precipitated with diethyl ether (1 L). Filtration of the crystals and washing with ether afforded pure **21** (2.73 g, 89%); mp: 155–157°C, $[\alpha]_D + 8.0$ (*c* 1, DMF). IR (KBr): 3286 (NH), 1754 (AcO), 1710 (C═O), 1629 cm^{-1} (amide I). ^{1}H-NMR (250 MHz, $(CD_3)_2SO$): δ ppm 8.94 (s, 1H, H-2), 8.25 (d, 1H, NH), 7.90 (d, 1H, H-5), 7.16 (d, 1H, H-8), 5.85 (t, 1H, H-1″), 5.3, 5.10, 4.95, and 4.25 (d + 3t, 4H, H-2″-5″), 4.60 (q, 2H, H-11), 4.00 (m, 2H, H-6″), 3.40 (m, 6H, CH_2, H-2′, and H-6′), 2.75 (m, 4H, H-3′, and H-5′), 2.00 and 1.95 (2s, 12H, C*H*$_3$COO), 1.42 (t, 3H, H-12). MS (FAB) *m*/*z* 781 ($[M + H]^+$).

Anal. Calcd for $C_{33}H_{41}FN_6O_{13}S.H_2O$ (798.82) C, 49.61; H, 5.43; N, 10.52. Found: C, 49.15; H, 5.32; N, 10.18.

*1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-[2,3,4,6-tetra-O-acetyl-β-D-galactopyranosylaminothiocarbonyl-hydrazinocarbonylmethyl]-1-piperazinyl)-3-quinolinecarboxylic Acid (**22**)*

A solution of 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl isothiocyanate (2.9 g, 7.46 mmol) in dry dichloromethane (30 mL) was slowly added to a mixture of **18** (1.3 g, 3.2 mmol) in dry dichloromethane (30 mL). After 24 hr at room temperature, TLC (acetone-water 95:5) indicated unreacted **18**. Then a solution of isothiocyanate (0.3 g, 0.75 mmol) in dry dichloromethane (3 mL) was added again, and the mixture was kept overnight. This procedure was repeated after an additional 24 hr when TLC revealed complete reaction. The solid was filtered and washed with dichloromethane (2 × 5 mL) to give a solid (2.06 g). Concentration of the dichloromethane solution afforded a second crop (0.12 g) of the crude product. The combined crude products were dissolved in acetone (90 mL), and then a white crystalline solid was slowly precipitated with diethyl ether (550 mL). Filtration and washing with ether afforded crystals of pure **22** (1.99 g, 80%); mp: 192–195°C, $[\alpha]_D + 23.0$ (*c* 1, DMF). IR (KBr): 3275 (NH), 1751 (AcO), 1710 (C═O), 1629 cm^{-1} (amide I). ^{1}H-NMR (250 MHz, $(CD_3)_2SO$): δ ppm 8.94 (s, 1H, H-2), 8.26 (d, 1H, NH), 7.89 (d, 1H, H-5), 7.16 (d, 1H, H-8), 5.87 (t, 1H, H-1″), 5.30 and 5.20 (m, 3H, H-2″-4″), 4.28 (m, 1H, H-5″), 4.60 (q, 2H, H-11), 4.05 (m, 2H, H-6″), 3.35 (m, 6H, CH_2, H-2′, and H-6′), 2.75 (m, 4H, H-3′, and H-5′), 2.12, 2.00, 1.97, and 1.93 (4s, 12H, C*H*$_3$COO), 1.43 (t, 3H, H-12). MS (FAB) *m*/*z* 781 ($[M + H]^+$).

Anal. Calcd for $C_{33}H_{41}FN_6O_{13}S.H_2O$ (798.82) C, 49.61; H, 5.43; N, 10.52. Found: C, 48.92; H, 5.35; N, 10.39.

*1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-[β-D-glucopyranosylaminothiocarbonyl-hydrazinocarbonylmethyl]-1-piperazinyl)-3-quinolinecarboxylic Acid (**23**)*

Compound **21** (1.3 g, 1.66 mmol) was slowly added to a mixture of ethanol and 25% aqueous ammonia (2:1, 11 mL), and the resulting solution was kept at room temperature for 2 d when TLC ($CHCl_3$-MeOH 6:1) revealed complete deacetylation. Addition of acetone (100 mL) to the mixture precipitated a semi-solid material, which was triturated with acetone (200 mL) and kept overnight to give a solid (0.82 g). Trituration of the crude product with diethyl ether (100 mL), filtration, and washing with ether afforded purified **23** (0.78 g, 76%); mp: 170–174°C, $[\alpha]_D$ +26.0 (*c* 1, DMF). IR (KBr): 3600–2400 (OH), 1702 (C═O), 1629 cm^{-1} (amide I). ^{1}H-NMR (250 MHz, $(CD_3)_2SO$): δ ppm 8.94 (s, 1H, H-2), 7.80 (d, 1H, H-5), 7.20 (d, 1H, H-8), 5.40–4.20 (m, 5H, H-1″-5″), 4.60 (q, 2H, H-11), 3.68 (m, 2H, H-6″), 3.38 (m, 6H, CH_2, H-2′, and H-6′), 2.75 (m, 4H, H-3′, and H-5′), 1.43 (t, 3H, H-12). MS (FAB) m/z 613 ($[M + H]^+$).

Anal. Calcd for $C_{25}H_{33}FN_6O_9S.2H_2O$ (648.67) C, 46.29; H, 5.75; N, 12.96. Found: C, 46.31; H, 5.66; N, 13.02.

*1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(4-[β-D-galactopyranosylaminothiocarbonyl-hydrazinocarbonylmethyl]-1-piperazinyl)-3-quinolinecarboxylic Acid (**24**)*

Compound **22** (1.7 g, 2.18 mmol) was slowly added to a mixture of ethanol and 25% aqueous ammonia (2:1, 11 mL), and the resulting solution was kept at room temperature for 2 d when TLC ($CHCl_3$-MeOH 6:1) revealed complete deacetylation. Working up as in the case of **23** afforded purified **24** (1.14 g, 85%); mp: 162–166°C, $[\alpha]_D + 28.0$ (*c* 1, DMF). IR (KBr): 3600–2400 (OH), 1700 (C═O), 1630 cm^{-1} (amide I). MS (FAB) m/z 613 ($[M + H]^+$).

Anal. Calcd for $C_{25}H_{33}FN_6O_9S.H_2O$ (630.70) C, 47.61; H, 5.59; N, 13.33. Found: C, 47.00; H, 5.64; N, 13.55.

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