DOI: 10.1002/ejoc.200500981

N-Carboxymethylated 6,7-Dimethoxy-4-trifluoromethylcarbostyrils as Fluorescence Markers for Amino Acids, Peptides, Amino Carbohydrates and Amino Polysaccharides

Naresh S. Badgujar,[a] Marek Pazicky,[a] Pedro Traar,[a] Anamaria Terec,[a] Georg Uray,[a] and Wolfgang Stadlbauer*[a]

Keywords: Fluorescent probes / Photoaffinity labeling / Amino acids / Peptides / Carbohydrates

The highly fluorescent 6,7-dimethoxy-3-trifluoromethylcarbostyril **2** was regioselectively carboxymethylated with bromoacetates **4** at N-1 to give esters of type **5** in good yield. After saponification to **8**, succinimidoyl (OSu) esters **9** were prepared. Dyestuffs **9** were reacted in slightly basic aqueous media under mild conditions with free amino acids, esters and peptides to give the fluorescently labeled amino acid derivatives **11** and **13** in good yields. Similarly, aminoglucose and chitosane reacted to form the fluorescently labeled carbohydrates **15** and **17**. Fluorescence quantum yields of 0.3–0.4, their photostability and a pH-independence between 3 and 10 make this class of compounds useful for linking to biological samples in aqueous media.

Introduction

Coumarins ([1]benzopyran-2-ones) are used in a great number of fluorescence applications.[1] Less attention has been paid to the aza-analogue carbostyrils [quinolin-2(1*H*)-ones], which in general seem to have the disadvantage of shorter absorption and emission wavelengths.[2] One reason for this is the significantly smaller red-shift on changing from 4-methylcarbostyril (absorption λ_{max} 331 nm and emission at 368 nm) to the 7-amino derivative (absorption λ_{max} 342 nm and fluorescence λ_{max} 393 nm) compared with the nonfluorescent 4-methylcoumarin[1o] and its 7-amino derivative (absorption shifted from 313 nm to 357 nm and strong emission at 427 nm).[2g] Recently we reported systematic studies about the fluorescence properties of differently substituted carbostyrils.[3] The important structural elements of these carbostyrils, which shift the absorption up to 440 nm and the emission maxima to 540 nm, are electron-donating substituents such as amino or methoxy groups in positions 6 and 7 and an electron-deficient substituent in position 4.[3c] These properties also make them interesting for use in sensor devices utilizing the new blue laser diodes. In contrast to coumarins[1m,1n] and to many other fluorescent dyestuffs, such push-pull-substituted carbostyrils have the important advantage that they are highly stable to further chemical reaction (e.g. compared with fluorescein type dyes), thermal and photochemical stress (e.g. compared with azodyes) and are not sensitive to oxygen quenching (compared with, e.g., 1,10-phenanthroline complexes).

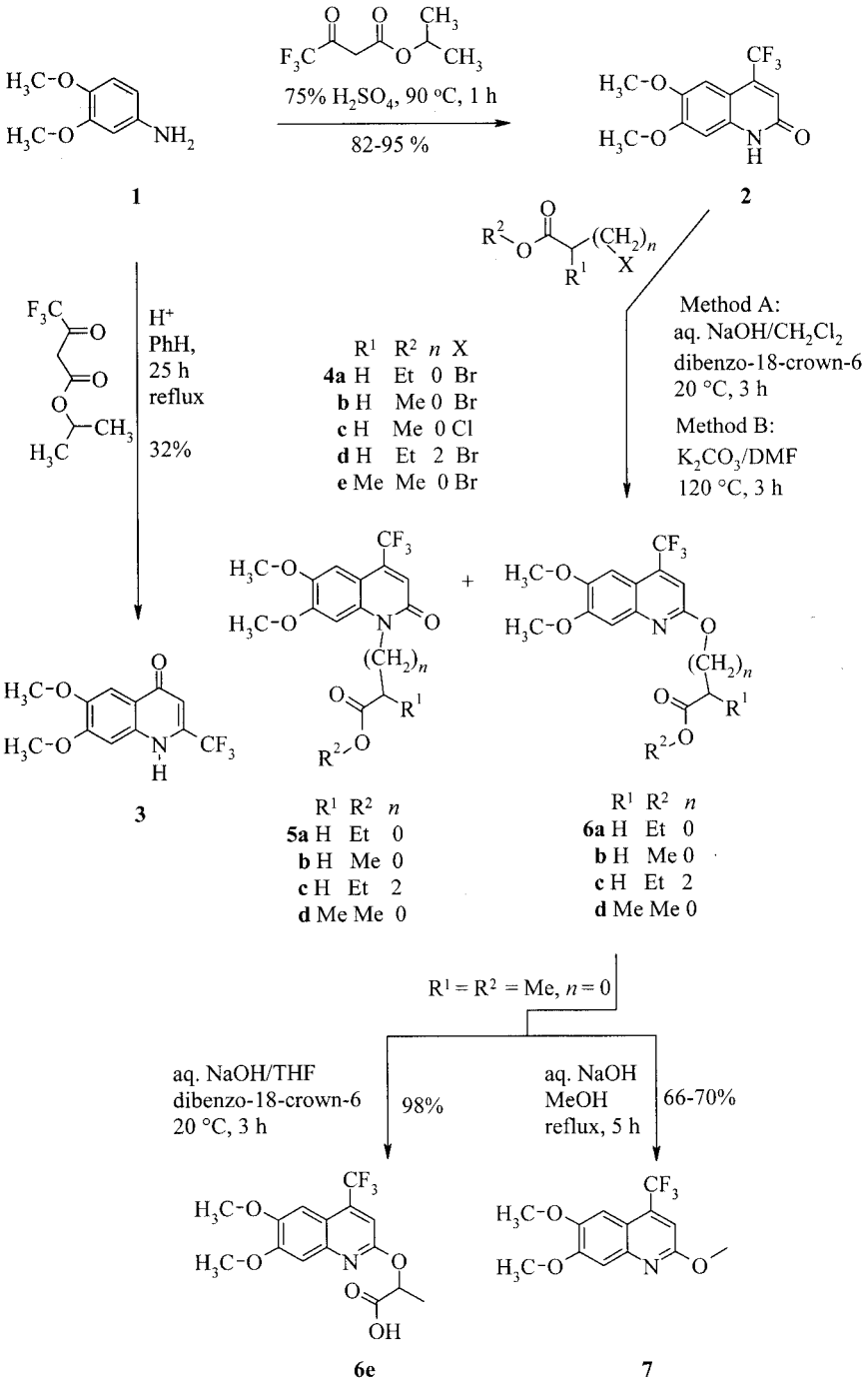
[a] Department of Chemistry, Karl Franzens University of Graz
Heinrichstrasse 28, 8010 Graz, Austria

The aforementioned dyes are frequently used as fluorescent labels for natural polymers.[4] In this contribution investigations into the introduction of reactive linker groups at the N1 position of 6,7-dimethoxycarbostyrils are described. This allows labeling of natural substrates such as amino acids, proteins, amino-carbohydrates or amino-polysaccharides without causing a blue-shift as seen with amide binding in 7-aminocoumarins and 7-aminocarbostyrils. 6,7-Dimethoxycarbostyrils suffer disadvantages in terms of slightly shorter absorption and emission wavelengths at $\lambda \geq 370$ nm and 440 nm (in contrast to aminocarbostyrils with $\lambda \approx 440$ nm absorption and 540 nm emission maxima[3c]). However, other advantages such as largely pH-independent photophysical properties and high chemical stability, e.g. against oxidation processes,[1p] directed our choice to the 6,7-dimethoxycarbostyrils. The advantage of 3,4-dimethoxycarbostyril derivatives was shown recently in their use as fluorescence resonance energy transfer (FRET) systems.[2h]

Results and Discussion

Carbostyril **2** possessing a trifluoromethyl group in position 4 and methoxy groups at positions 6 and 7 shows excellent photophysical properties.[3] It has a broad absorption maximum close to the visible light region ($\lambda \geq 370$ nm), a sufficiently large Stokes shift (80 nm), a useful extinction coefficient of about 10^4 and a solvent dependent fluorescence quantum yield of up to 0.5. The synthesis of carbostyril **2** was performed by adopting the published procedure for 3,4-dimethoxyaniline **1** and isopropyl trifluoroace-

toacetate.[3,5] The intermediate trifluoroacetoacetanilide was cyclized without isolation with sulfuric acid as the catalyst to give mainly 6,7-dimethoxy-4-trifluoromethyl-2-quinolone **2** together with a small amount of the isomer 6,7-dimethoxy-2-trifluoromethyl-4-quinolone **3** in a 10:1 ratio. Pure 2-quinolone **2** was obtained after recrystallization in an overall yield of about 70%. The isomer 4-quinolone **3** was prepared for comparison by adopting a literature known method:[6] the key intermediate, imino-acetoacetate, was obtained from aniline **1** and trifluoroacetoacetate using montmorillonite K10 as the acid catalyst and then cyclized thermally without isolation to give 4-quinolone **3** in 32% yield. Recently,[7] a new preparative method for 4-methyl-2-quinolones of type **2** from anilines, such as **1** with acetoacetates, was described which uses the continued addition of small amounts of water to direct the first reaction step regioselectively to form the acetanilides and prevents the formation of the isomeric imino-acetoacetates. We tried to transpose this method to our problem, however the results were much worse than with our method. Obviously electron-donating groups on the aniline part are not preferred using the "watering protocol".

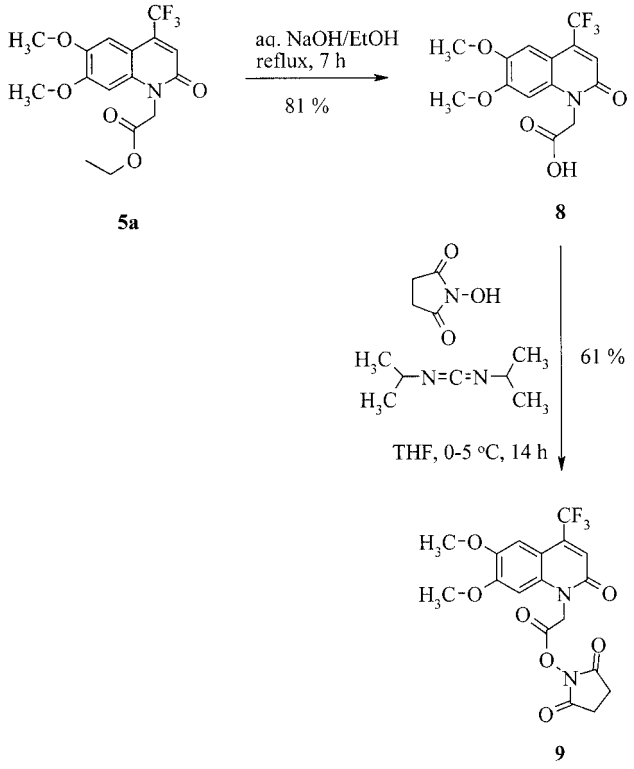


Scheme 1.

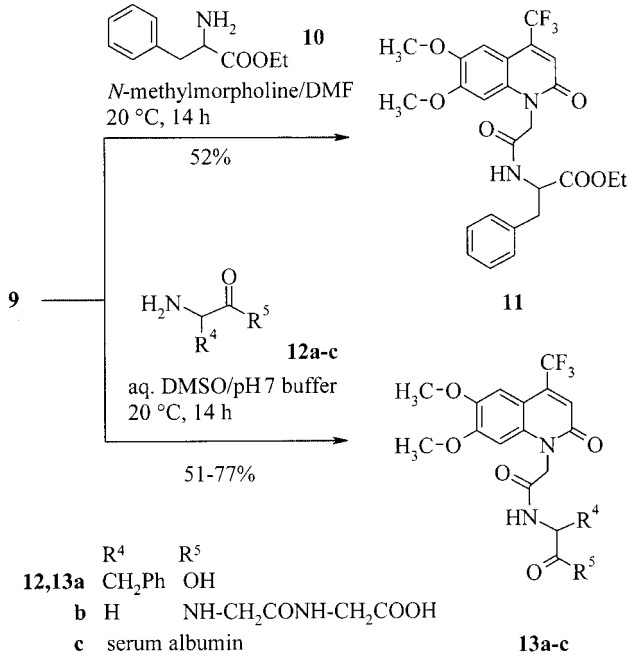
To attach a reactive linker group to the fluorescent carbostyril **2** at the N1 we followed the strategy of alkylating the carbostyril **2** at N1 with alpha halogen-substituted esters. After conversion to the reactive reagents, one should be able to label natural substrates such as amino acids, proteins, amino-carbohydrates or amino-polysaccharides without loss of the fluorescence properties. In contrast to N7-acyl, the N1-alkyl substituent has almost no influence on the fluorescence properties which can be shown with the *N*-methyl derivative of **2**.[9] For N1 functionalization a phase transfer method described for the *N*-benzylation of quinolones assisted by dibenzo-18-crown-6[8] was adopted. When the alkylation of **2** was performed with bromoacetates **4a**,**b** or chloroacetate **4c** in dimethylformamide and potassium carbonate as the base (method B), the ratio of carbostyrils **5** to 2-alkoxquinolines **6** was about 4:5. Using a binary mixture of sodium hydroxide/water and dichloromethane (method A), alkylation of **2** with 2-bromoacetates **4a**,**b** gave the desired *N*-alkylated carbostyrils **5a**,**b** in excellent yields. Only about 12–15% of *O*-alkylated side product **6a**,**b** was formed. Using method A, 4-bromobutanoate **4d** gave similar results. However, with method B the ratio was 1:4 for **5c** to **6c**, but the yield was only about 32%. With 2-bromopropanoate **4e**, 2-alkoxyquinoline **6d** was obtained in 52% yield, which gave the carboxylic acid **6e** upon hydrolysis with ethanolic sodium hydroxide. However, the use of methanolic sodium hydroxide resulted in the exclusive formation of 2-methoxyquinoline **7** (Scheme 1).

Hydrolysis of quinolinyl-1-acetates **5a**,**b** in aqueous ethanolic sodium hydroxide gave quinolinyl-1-acetic acid **8** in excellent yields. In the next step we chose the preparation of the reactive succinimidoyl active ester **9** (OSu ester). This class of compounds is reported to have the advantage of both stability and high reactivity with amino groups in aqueous solution.[10] The preparation of **9** was performed using the acid **8** and *N*-hydroxysuccinimide in dry tetrahydrofuran with diisopropylcarbodiimide as the water scavenger. Experiments using chloroform as the solvent resulted in the partial transesterification because of the ethanol content used as a stabilizer in chloroform.

The linkage of fluorescent dyes with biopolymers such as proteins or polysaccharides is a method used for many purposes; e.g. refs.[4a,4c,10c,11] The reaction of OSu ester **9** with the amino group of peptides or proteins should allow the labeling of such biocompounds under gentle conditions, whereas our attempts to conjugate the methyl or ethyl esters **5a**,**b** to amino acids, proteins or sugars at temperatures between 20 and 40 °C were not successful. The reaction of OSu ester **9** with the phenylalanine ester **10** as an example procedure was performed at room temperature in dimethylformamide as the solvent and *N*-methylmorpholine as basic catalyst. It afforded the carbostyril **11** linked with the amino group of phenylalanine ester in about 50% yield. With phenylalanine (**12a**), the reaction of OSu ester **9** (Scheme 2) was performed under biochemical conditions using aqueous dimethylsulfoxide as the solvent and with aq. pH 7 buffer; this reaction afforded the amino-linked carbostyril **13a** in nearly 80% yield. In the same manner, the tripeptide glycyl-glycyl-glycine (**12b**) reacted in about 50% yield with the amino-linked carbostyril **13b**. The reaction of OSu ester **9** with bovine serum albumine (**12c**) in aqueous dimethylformamide/hydrogencarbonate buffer and subsequent purification gave the labeled protein **13c** (Scheme 3). Labeling was found to be efficient with about 20% based on a fluorescence comparison with the OSu ester **9**.

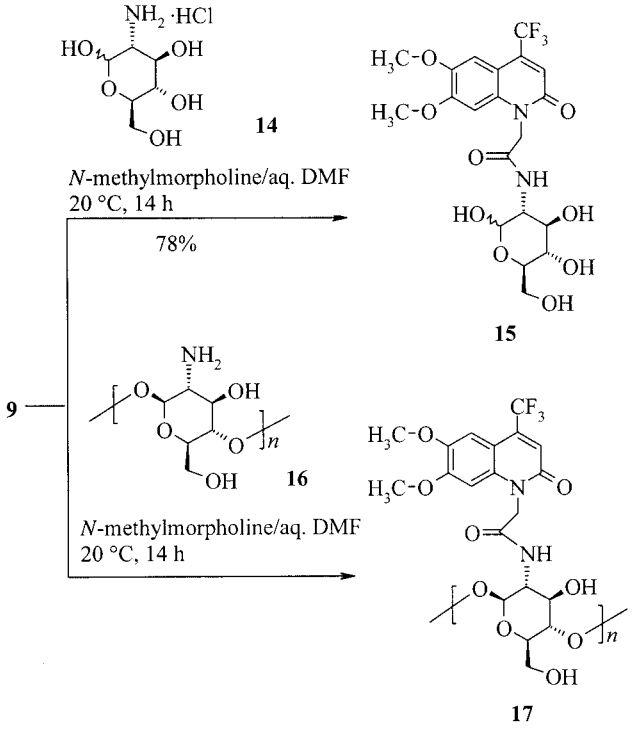


Scheme 2.



Scheme 3.

Amino groups of sugars or oligo- and polysaccharides are a further target for the linkage of the OSu ester **9**. The reaction of glucosamine **14** in aqueous dimethylformamide with *N*-methylmorpholine as basic catalyst gave in about 80% yield the amino sugar-linked carbostyril **15**. Chitosan oligosaccharide **16** gave, under the same conditions, the aminooligosaccharide-linked product **17** (Scheme 4), which showed, after purification, a labeled aminochitosan with a linking grade of 15–20%, again based on a fluorescence comparison with the OSu ester **9**.



Scheme 4.

Conclusions

Absorption spectra of all *N*-substituted carbostyrils (including those already linked to biopolymers) are all very similar with λ_{max} = 367–370 nm in DMSO and 359–361 nm in water. The extinction coefficients are sufficiently high with values between ε = 9000 and 10500 and do not differ from the *N*-unsubstituted carbostyril **2** (λ_{max} = 360 and 367 in water and DMSO, ε = 9300 and 10400). This means that alkylation and further reactions do not influence the electronic structure of the carbostyril. The emission spectra give values ranging between 440 nm in DMSO and 430 nm in water; the Stokes shifts are λ_F = 69–73 nm and the quantum yields are sufficiently high with values between Φ_F = 0.22–0.41 in DMSO and 0.11–0.34 in water. An example absorption and emission spectrum of the labeled phenylalanine **13a** can be found in Figure 1, all spectroscopic data are compiled in Table 1. The absorption and emission spectra of the labeled bovine serum albumine **13c** and the oligosaccharide chitosan **17** give absorptions at 371 and 370 nm, and emission peaks at 437 and 430 nm with linking grades of about 20% based on comparison with OSu-ester **9**, respectively. All these findings reveal that OSu-esters of 6,7-dimethoxy-4-trifluoromethylcarbostyrils are very useful because of their stability, their high reactivity in aqueous solvents at room temperature and their photophysical properties.

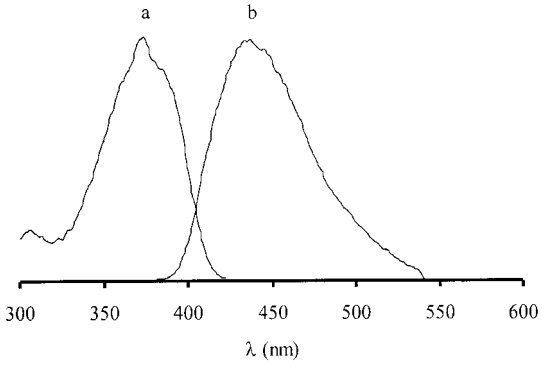


Figure 1. Absorption (a) and emission (b) spectra of **13a** in DMSO.

Experimental Section

General Remarks: Melting points were determined using a Gallenkamp Melting Point Apparatus, Mod. MFB-595 in open capillary tubes. ^{1}H NMR and ^{13}C NMR spectra were recorded using a Bruker AMX 360 instrument (360 or 90 MHz) or a Bruker Avance DRX 500 instrument (500 or 125 MHz). Chemical shifts are given in ppm (δ) from the internal TMS standard. IR spectra were recorded using a Mattson Galaxy Series FTIR 7020 instrument with potassium bromide discs. Elemental analyses were performed using a Fisons elemental analyzer Mod. EA 1108. Mass spectra were

Table 1. Photophysical data for the electronic absorption (abs) and fluorescence (flu) of carbostyrils **2**–**15**; solvent temperatures: 25 °C; λ in nm. λ_{flu}-values in DMSO were found with 440 nm, in water with 430 nm.

No.	λ_{abs} (DMSO)	ε (DMSO)	λ_{abs} (water)	ε (water)	$\Delta\lambda$ (DMSO)	Φ_F (DMSO)	$\Delta\lambda$ (water)	Φ_F (water)
2	367	9400	360	10440	73	0.41	70	0.34
5a	367	9000	359	8780	73	0.39	71	0.21
8	368	10500	360	9520	72	0.32	70	0.27
9	368	9500	359	8710	72	0.40	71	0.25
11	369	9400	363	8960	71	0.33	67	0.25
13a	370	9900	361	10600	70	0.36	69	0.22
13b	369	9700	360	8960	71	0.33	70	0.26
15	370	9000	360	8980	70	0.22	70	0.11

obtained from a HP 1100 LC/MSD mass spectral instrument (positive or negative ACPI ion source, 50–200 V, nitrogen). UV/Vis spectra were recorded using a Shimadzu UV/Vis scanning spectrophotometer UV-2101 PC; concentration: 0.01 mg/mL. Excitation and emission spectra were recorded using on a Shimadzu RF-5001 PC spectrofluorometer (150-W Xe lamp, 6 selectable slits: 1.5, 3, 5, 10, 15, 20 nm, R452-01 photomultiplier; monochromator: ion-blazed holographic concave grating F/2.5); concentration: 0.001 mg/mL. Determination of quantum yields: emission signals were set in relation to the known signal of quinine sulfate at pH 1. Analytical HPLC was performed on a Shimadzu LC 20 system equipped with a diode array detector (215 and 254 nm) on a Pathfinder AS reversed phase (4.6 × 150 mm, 5 μm) column, running a acetonitrile/water gradient (30–100% acetonitrile).

All reactions were monitored by thin layer chromatography on 0.2 mm silica gel F-254 (Merck) plates using uv light (254 and 366 nm) for detection. Common reagent-grade chemicals were either commercially available and were used without further purification or prepared by standard literature procedures. All optical measurements were performed using analytical grade solvents.

3,4-Dimethoxyaniline (1): This compound is commercially available.

6,7-Dimethoxy-4-(trifluoromethyl)quinolin-2(1*H*)-one (2): This compound was prepared according to ref.[3a]

6,7-Dimethoxy-2-(trifluoromethyl)-1*H*-quinolin-4-one (3): To a solution of 3,4-dimethoxyaniline (**1**) (1.53 g, 10 mmol) and isopropyl 4,4,4-trifluoroacetoacetate (1.98 g, 10 mmol) in benzene (10 mL), montmorillonite K10 (0.1 g) was added and the mixture heated at reflux for 24 h using a Dean–Stark apparatus. The catalyst was filtered, benzene was removed under reduced pressure and the residue was dissolved in diphenylether (20 mL) and heated for 15 min under reflux. The mixture was diluted with cyclohexane (20 mL) and cooled to 20 °C. A solid separated, which was filtered by suction, washed with cyclohexane (50 mL) and dried at 40 °C under reduced pressure to afford 0.87 g (32% yield) of colorless needles, m.p. 302–304 °C (ethanol/water). ^{1}H NMR ($CDCl_3$): δ = 3.92 and 3.95 (2 s, 6 H, 6-OMe and 7-OMe), 7.01 (s, 1 H, 3-H), 7.38 (s, 1 H, 8-H), 7.43 (s, 1 H, 5-H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 56.1 and 56.2 (6-OMe and 7-OMe), 100.5 (ArC), 106.9 (ArC), 116.9 (ArC), 121.9 (q, J = 270 Hz, 2-CF_3), 123.5 (ArC), 143.1 (q, J = 27 Hz, 2-C), 150.0 (6-C), 153.9 (7-C), 195.0 (4-C=O) ppm. IR (KBr): $\tilde{\nu}$ = 3260 w, 3180 sh, 3090 m, 2980 s, 2120 w, 1625 s cm^{-1}. $C_{12}H_{10}F_3NO_3$ (273.21): calcd. C 52.75, H 3.69, N 5.13; found C 53.04, H 3.68, N 5.08.

Ethyl [6,7-Dimethoxy-2-oxo-4-(trifluoromethyl)-1,2-dihydroquinolin-1-yl]acetate (5a). Method A: To a solution of **2** (1.10 g, 4 mmol) in dichloromethane (120 mL) 50% aq. NaOH (3.5 mL) and dibenzo-18-crown-6 (0.12 g, 0.2 mmol) was added whilst stirring. Then **4a** (0.67 g, 4 mmol) was added and stirred for about 3–4 h until the starting material had disappeared (checked by TLC monitoring). The mixture was filtered and the solvent removed under reduced pressure. The combined solids were rinsed with hexane (25 mL), filtered, washed with hexane and dried at 75 °C. TLC analysis showed two products (*N*-alkylated **5a** and *O*-alkylated **6a**), (1.40 g; 97% yield), which were separated by dry flash column chromatography[12] (Merck silica gel 60 H, 5–40 μm). Toluene as eluent afforded **6a**, toluene/acetone (9:1) as eluent afforded **5a** (1.15 g, 80% yield) as light green prisms, m.p. 150–151 °C (ethanol).

Method B: To a solution of **2** (10.1 g, 37 mmol) in dry DMF (600 mL) K_2CO_3 (5.2 g, 37 mmol) and **4a** (6.2 g, 37 mmol) were added and the temperature was raised slowly to 80 °C. The mixture was kept at this temperature for about 3 h until TLC showed no more starting material. The temperature was raised to 110 °C for 2 h, then the mixture was filtered and the solvent removed under reduced pressure. A thick brown oil was obtained, which crystallized after standing overnight and afforded 11.9 g (93% yield) of a 1:1 mixture of **5a** and **6a**, which were separated by dry flash column chromatography as described in method A. Toluene/acetone (9:1) as eluent afforded **5a** (5.5 g, 42% yield), light green prisms, m.p. 150–151 °C (ethanol). ^{1}H NMR ($CDCl_3$): δ = 1.27 (t, J = 7.2 Hz, 3 H, ester CH_3), 3.95 and 3.97 (2 s, 2 × 3 H, 6-OMe, 7-OMe), 4.27 (q, J = 7.1 Hz, 2 H, ester OCH_2), 5.13 (s, 2 H, NCH_2), 6.59 (s, 1 H, 3-H), 7.02 (s, 1 H, 8-H), 7.23 (s, 1 H, 5-H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 14.4 (ester CH_3), 44.9 (NCH_2), 56.2 and 56.6 (6-OMe, 7-OMe), 62.0 (ester OCH_2), 99.0 (ArC), 105.8 (ArC), 107.6 (ArC), 117.1 (ArC), 123.0 (q, J = 270 Hz, 4-CF_3), 136.1 (q, J = 27 Hz, 4-C), 136.5 (ArC), 145.8 (6-C), 153.4 (7-C), 160.3 (N-C=O), 168.4 (ester CO) ppm. IR (KBr): $\tilde{\nu}$ = 1733 s, 1667 s, 1625 w, 1602 m, 1558 m cm^{-1}. UV (DMSO): λ_{max} (nm) = 359, 293; (water): 359, 293. $C_{16}H_{16}F_3NO_5$ (359.30): calcd. C 53.49, H 4.49, N 3.90; found C 53.58, H 4.39. N 3.83.

Methyl [6,7-Dimethoxy-2-oxo-4-(trifluoromethyl)-1,2-dihydroquinolin-1-yl]acetate (5b). Method A: A mixture of **2** (0.49 g, 1.8 mmol) and **4b** (0.35 g, 2.3 mmol) was reacted, worked-up, and separated according to method A as described for **5a**. Toluene/acetone (9:1) as eluent afforded **5b** (0.54 g, 87% yield), colorless prisms, m.p. 171–172 °C (ethanol).

Method B: A mixture of **2** (5.0 g, 18.5 mmol) and **4c** (3.0 g, 27.5 mmol) was reacted, worked-up, and separated according to method B as described for **5a**. Toluene/acetone (9:1) as eluent afforded **5b** (2.87 g, 45% yield). ^{1}H NMR ($CDCl_3$): δ = 3.79 (s, 3 H, ester OMe), 3.95 and 3.97 (2 s, 2 × 3 H, 6-OMe and 7-OMe), 5.14 (s, 2 H, NCH_2), 6.59 (s, 1 H, 3-H), 7.03 (s, 1 H, 8-H), 7.24 (s, 1 H, 5-H) ppm. IR (KBr): $\tilde{\nu}$ = 1755 m, 1665 s, 1624 s, 1599 s, 1559 w, 1528 m cm^{-1}. $C_{15}H_{14}F_3NO_5$ (345.28): calcd. C 52.18, H 4.09, N 4.06; found C 52.36, H 4.15, N 3.95.

Ethyl 4-[6,7-Dimethoxy-2-oxo-4-(trifluoromethyl)quinolin-1(2*H*)-yl]-butanoate (5c). Method A: A mixture of **2** (0.50 g, 1.83 mmol) and **4d** (0.46 g, 2.38 mmol) was reacted for 42 h at 60 °C, worked-up, and separated according to method A as described for **5a**. Toluene/acetone (9:1) as eluent afforded **5c** (0.57 mg, 83% yield), colorless prisms, m.p. 100–101 °C (ethanol).

Method B: A mixture of **2** (2.0 g, 7.3 mmol), ethyl **4d** (2.15 g, 11 mmol) and K_2CO_3 (1.01, 7.3 mmol) was reacted for 6 h at 110 °C, worked-up and separated according to method B as described for **5a**. Toluene/acetone (9:1) as eluent afforded **5c** (0.30 g, 11% yield). ^{1}H NMR ([D$_6$]DMSO): δ = 1.17 (s, J = 7.0 Hz, 3 H, ester CH_3), 1.83–1.88 (m, 2 H, 3-CH_2 of butanoic acid), 2.46 (t, J = 7.1 Hz, 2 H, 2-CH_2 of butanoic acid), 3.81 and 3.99 (2 s, 2 × 3 H, 6-OMe and 7-OMe), 4.05 (q, J = 7.1 Hz, 2 H, ester OCH_2), 4.29 (t, J = 6.9 Hz, 2 H, NCH_2), 6.89 (s, 1 H, 3-H), 7.06 (s, 1 H, 8-H), 7.26 (s, 1 H, 5-H) ppm. IR (KBr): $\tilde{\nu}$ = 3600–3400 m br., 3004–2937 m br., 1730 m, 1660 s, 1624 w, 1601 m, 1560 w, 1528 m cm^{-1}. $C_{18}H_{20}F_3NO_5$ (387.36): calcd. C 55.81, H 5.20, N 3.62; found C 55.45, H 5.56, N 3.98.

Methyl 2-[6,7-Dimethoxy-2-oxo-4-(trifluoromethyl)-1,2-dihydroquinolin-1-yl]propanoate (5d). Method B: A mixture of **2** (1.36 g, 5 mmol) and **4e** (1.09 g, 6 mmol) was reacted, worked-up, and separated according to method B as described for **5a**. Toluene/acetone (9:1) as eluent afforded **5d** (0.06 g, 2% yield), colorless prisms, m.p. 148–150 °C (ethanol). ^{1}H NMR ($CDCl_3$): δ = 1.73 (d, J = 7.2 Hz, 3 H, Me of propanoate), 3.66 (s, 3 H, ester OMe), 3.94 and 3.95 (2 s, 2 × 3 H, 6-OMe and 7-OMe), 6.69 (s, 1 H, 3-H), 6.99 (s, 1 H, 8-H), 7.22 (s, 1 H, 5-H) ppm. ^{13}C NMR ($CDCl_3$): δ = 14.1 (Me of

propanoate), 51.2 (NCH of propanoate), 52.8 (ester OMe), 56.2 (6-OMe and 7-OMe), 97.6 (ArC), 106.9 (ArC), 109.1 (ArC), 117.8 (ArC), 123.1 (q, J = 270 Hz, CF_3), 134.9 (ArC), 136.9 (q, J = 27 Hz, 4-C), 145.5 (6-C), 152.8 (7-C), 160.1 (N-C=O), 171.2 (ester CO) ppm. MS: *m*/*z* (%): 360 (22) [M + 1]. $C_{16}H_{16}F_3NO_5$ (359.30): calcd. C 53.49, H 4.49, N 3.90; found C 53.21, H 4.81, N 4.28.

Ethyl [6,7-Dimethoxy-4-(trifluoromethyl)quinolin-2-yloxy]acetate (6a). Method A: 6a was obtained from the reaction of **2** (1.10 g, 4 mmol), **4a** (0.67 g, 4 mmol) and **5a** according to the method described for **5a** (method A). Toluene as eluent afforded **6a** (0.21 g, 15% yield); light green prisms, m.p. 147–148 °C (ethanol).

Method B: 6a was obtained from the reaction of **2** (10.1 g, 37 mmol), **4a** (6.2 g, 37 mmol) and **5a** according to the method described for **5a** (method B). Toluene as eluent afforded **6a** (6.70 g, 51% yield). ^{1}H NMR ($CDCl_3$): δ = 1.30 (t, J = 6.9 Hz, 3 H, ester Me), 4.01 and 4.03 (2 s, 2×3 H, 6-OMe and 7-OMe), 4.27 (q, J = 6.9 Hz, 2 H, ester OCH_2), 5.03 (s, 2 H, 2-OCH_2), 7.22–7.24 (m, 3 H, 3 ArH) ppm. ^{13}C NMR ([D_6]DMSO): δ = 19.3 (ester Me), 60.9 and 61.1 (6-OMe, 7-OMe), 65.7 (2-OCH_2), 102.1 (ArC), 107.8 (ArC), 108.1 (ArC), 114.2 (ArC), 125.1 (q, J = 270 Hz, 4-CF_3), 135.0 (q, J = 27 Hz, 4-C), 143.8 (ArC), 149.3 (ArC), 153.5 (6-OMe), 158.8 (7-OMe), 172.2 (ester CO) ppm. IR (KBr): $\tilde{\nu}$ = 1751 s, 1613 m, 1517 m cm^{-1}. $C_{16}H_{16}F_3NO_5$ (359.30): calcd. C 53.49, H 4.49, N 3.90; found C 53.51, H 4.52, N 3.77.

Methyl [6,7-Dimethoxy-4-(trifluoromethyl)quinolin-2-yloxy]acetate (6b). Method A: 6b was obtained from the reaction of **2** (0.49 g, 1.8 mmol), **4b** (0.35 g, 2.3 mmol) and **5b** according to the method described for **5a** (method A). Toluene as eluent afforded **6b** (0.07 g, 12% yield), colorless prisms, m.p. 136–137 °C.

Method B: It was obtained from **2** (5.0 g, 18.5 mmol) and **4c** (3.0 g, 27.5 mmol) together with **5b** according to the method described at **5a** (method B). Toluene as eluent afforded **6b** (3.32 g, 52% yield). ^{1}H NMR ([D_6]DMSO): δ = 3.65 (s, 3 H, ester OMe), 3.88 and 3.94 (2 s, 2×3 H, 6-OMe and 7-OMe), 5.12 (s, 2 H, 2-OCH_2), 7.15 (s, 1 H, 3-H), 7.22 (s, 1 H, 8-H), 7.34 (s, 1 H, 5-H) ppm. IR (KBr): $\tilde{\nu}$ = 2960 w, 1722 s, 1620 sh, 1615 s, 1590 w cm^{-1}. $C_{15}H_{14}F_3NO_5$ (345.28): calcd. C 52.18, H 4.09, N 4.06; found C 51.96, H 4.17, N 4.25.

Ethyl 4-[6,7-Dimethoxy-4-(trifluoromethyl)quinolin-2-yloxy]butanoate (6c). Method A: 6c was obtained from the reaction of **2** (0.50 g, 1.83 mmol), **4d** (0.46 g, 2.38 mmol) and **5c** after 42 h at 60 °C according to the method described for **5a** (method A). Toluene as eluent afforded **6c** (10.3 mg, 15% yield), colorless prisms, m.p. 85–86 °C (ethanol).

Method B: 6c was obtained from the reaction of **2** (2.0 g, 7.3 mmol) and **4d** (2.15 g, 11 mmol) after 6 h at 110 °C together with **5c** according to the method described for **5a** (method B). Toluene as eluent afforded **6c** (900 mg, 32% yield). ^{1}H NMR ([D_6]DMSO): δ = 1.15 (t, J = 7.1 Hz, 3 H, ester CH_3), 2.00–2.05 (m, 2 H, 3-CH_2 of butanoic acid), 2.47 (t, 2 H, 2-CH_2 of butanoic acid), 3.86 and 3.93 (2 s, 2×3 H, 6- OMe, 7-OMe), 4.04 (t, J = 7.0 Hz, 2 H, ester OCH_2), 4.41 (t, J = 7.0 Hz, 2 H, 2-OCH_2), 7.12 (s, 1 H, 3-H), 7.17 (s, 1 H, 8-H), 7.27 (s, 1 H, 5-H) ppm. IR (KBr): $\tilde{\nu}$ = 3400 m br., 2972 m, 2933 sh, 1731 s, 1616 s, 1580 w, 1516 s cm^{-1}. $C_{18}H_{20}F_3NO_5$ (387.36): calcd. C 55.81, H 5.20, N 3.62; found C 56.17, H 5.01, N 3.29.

Methyl 2-[6,7-Dimethoxy-4-(trifluoromethyl)quinolin-2-yloxy]propanoate (6d). Method B: 6d was obtained from the reaction of **2** (1.36 g, 5 mmol), **4e** (1.09 g, 6 mmol) and **5d** according to the method described for **5a** (method B). Toluene as eluent afforded **6d** (1.20 g, 52% yield), colorless prisms, m.p. 127–129 °C. ^{1}H NMR ([D_6]DMSO): δ = 1.67 (d, J = 7.2 Hz, 3 H, Me of propanoate), 3.76 (s, 3 H, ester OMe), 4.00 and 4.01 (2 s, 2×3 H, 6-OMe, 7-OMe), 5.54 (m, 1 H, OCH of propanoate), 7.18 (s, 1 H, 3-H), 7.21 (s, 1 H, 8-H), 7.25 (s, 1 H, 5-H) ppm. ^{13}C NMR ([D_6]DMSO): δ = 17.8 (Me of propanoate), 52.5 (ester OMe), 56.1 and 56.3 (6-OMe, 7-OMe), 70.3 (OCH of propanoate), 102.1 (ArC), 107.8 (ArC), 108.2 (ArC), 114.2 (ArC), 125.1 (q, J = 270 Hz, 4-CF_3), 134.9 (q, J = 27 Hz, 4-C), 143.9 (ArC), 149.3 (ArC), 153.5 (6-C), 158.8 (7-C), 172.2 (ester CO) ppm. IR (KBr): $\tilde{\nu}$ = 2974 w, 1754 s, 1615 m, 1513 s cm^{-1}. $C_{16}H_{16}F_3NO_5$ (359.30): calcd. C 53.49, H 4.49, N 3.90; found C 53.39, H 4.39, N 3.84.

2-[6,7-Dimethoxy-4-(trifluoromethyl)quinolin-2-yloxy]propanoic Acid (6e): 0.01 M aq. NaOH (1 mL) was added to a solution of **6d** (89 mg, 0.25 mmol) in ethanol (5 mL) and stirred at 20 °C for 5 h. After cooling the mixture was poured into water (20 mL) and acidified with 2 M HCl (1 mL) to pH 1–2. The resulting solid was filtered by suction and washed with water to afford 50 mg (66% yield) of colorless prisms, m.p. 170–171 °C (ethyl acetate). ^{1}H NMR ($CDCl_3$): δ = 1.71 (t, J = 6.9 Hz, 3 H, Me of propanoate), 3.98 and 3.99 (2 s, 2×3 H, 6-OMe and 7-OMe), 5.55 (q, J = 6.8 Hz, 1 H, OCH of propanoate), 7.18 (s, 1 H, 3-H), 7.22 (s, 2 H, 5-H, 8-H) ppm. ^{13}C NMR ([D_6]DMSO): δ = 17.3 (Me of propanoate), 55.6 and 55.8 (6-OMe, 7-OMe), 69.6 (OCH of propanoate), 101.6 (ArC), 107.3 (ArC), 107.8 (ArC), 113.5 (ArC), 123.3 (q, J = 270 Hz, 4-CF_3), 134.2 (q, J = 27 Hz, 4-C), 143.4 (ArC), 148.6 (ArC), 152.9 (6-C), 158.6 (7-C), 172.6 (acid CO) ppm. $C_{15}H_{14}F_3NO_5$ (345.28): calcd. C 52.18, H 4.09, N 4.06; found C 52.52, H 4.39, N 3.78.

2,6,7-Trimethoxy-4-(trifluoromethyl)quinoline (7): A solution of **6d** (89 mg, 0.25 mmol) in methanol (5 mL) and 0.01 M aq. NaOH (1 mL) was heated at reflux for 5 h. The mixture was cooled to 20 °C and poured into water (20 mL), acidified with 2 M HCl to pH 1–2 and the resulting solid was filtered by suction and washed with water to afford 50 mg (70% yield), colorless prisms, m.p. 132–133 °C (methanol). ^{1}H NMR ($CDCl_3$): δ = 4.01 and 4.05 (2 s, 2×3 H, 6-OMe and 7-OMe), 4.07 (s, 3 H, 2-OMe), 7.10 (s, 1 H, 3-H), 7.26 (s, 1 H, 8-H), 7.30 (s, 1 H, 5-H) ppm. MS: *m*/*z* (%) = 287 (21) [M], 272 (82), 257(61), 229 (21). $C_{13}H_{12}F_3NO_3$ (287.24): calcd. C 54.36, H 4.21, N 4.88; found C 54.68, H 4.52, N 4.57.

[6,7-Dimethoxy-2-oxo-4-(trifluoromethyl)-1,2-dihydroquinolin-1-yl]-acetic Acid (8): A solution of **5a** (0.31 g, 1 mmol) in ethanol (5 mL) and 1 M aq. NaOH (2 mL) was heated at reflux for 7 h, the ethanol was removed under reduced pressure and the residue dissolved in water (5 mL) under cooling. The mixture was acidified with conc. HCl to pH 1–2 and the resulting precipitate filtered by suction and washed with water and cyclohexane which afforded 0.26 g (81% yield), pale yellow prisms, m.p. 284–285 °C (ethyl acetate). ^{1}H NMR ([D_6]DMSO): δ = 3.82 and 3.91 (2 s, 2×3 H, 6-OMe and 7-OMe), 5.13 (s, 2 H, NCH_2), 6.96 (s, 1 H, 3-H), 7.03 (s, 1 H, 8-H), 7.09 (s, 1 H, 5-H) ppm. IR (KBr): $\tilde{\nu}$ = 3450 br., 1737 m, 1644 m, 1624 w, 1554 m cm^{-1}. UV (DMSO): λ_{max} (nm) = 368, 295; (water): 360, 294. $C_{14}H_{12}F_3NO_5$ (331.25): calcd. C 50.76, H 3.65, N 4.23; found C 50.53, H 3.46, N 4.15.

1-{[(6,7-Dimethoxy-2-oxo-4-(trifluoromethyl)-1,2-dihydroquinolin-1-yl)acetyl]oxy}pyrrolidine-2,5-dione (9): *N*-Hydroxysuccinimide (81 mg, 0.7 mmol) was added slowly whilst stirring to a solution of **8** (200 mg, 0.6 mmol) in dry tetrahydrofuran (20 mL) at 0 °C. Then *N*,*N*-diisopropylcarbodiimide (88 mg, 0.7 mmol) was added at 0–5 °C dropwise whilst stirring which formed a yellowish-white precipitate; the mixture was stirred 0–5 °C for 14–15 h and then filtered by suction. The solid was washed well with dry tetrahydrofuran and then stirred in dry ethanol (50 mL) at 20 °C for 30 min to remove *N*,*N*′-diisopropylurea formed during the reac-

tion. Suction filtration afforded 183 mg (61% yield), pale yellow prisms, m.p. 228–229 °C (ethanol). ^{1}H NMR ($CDCl_3$): δ = 2.86 (s, 2 H, 2 CH_2 of succinimide), 3.95 and 4.11 (2 s, 2×3 H, 6-OMe and 7-OMe), 5.53 (s, 2 H, NCH_2), 6.66 (s, 1 H, 3-H), 7.02 (s, 1 H, 8-H), 7.23 (s, 1 H, 5-H) ppm. ^{13}C NMR ([D$_6$]DMSO): δ = 25.5 (2 CH_2 of succinimide), 44.5 (NCH_2), 56.2 and 56.5 (6-OMe, 7-OMe), 99.1 (ArC), 105.8 (ArC), 107.6 (ArC), 117.2 (ArC), 125.9 (q, J = 270 Hz, 4-CF_3), 136.1 (q, J = 27 Hz, 4-C), 146.5 (ArC), 153.2 (6-C), 160.1 (7-C), 169.8 and 169.9 (2 N-C=O of succinimide), 173.7 (N-C=O) ppm. IR (KBr): $\tilde{\nu}$ = 1815 w, 1786 m, 1747 s, 1663 s, 1623 w, 1597 w cm^{-1}. UV (DMSO): λ_{max} (nm) = 368, 295; (water): 359, 292. $C_{18}H_{15}F_3N_2O_7$ (428.32): calcd. C 50.48, H 3.53, N 6.54; found C 50.25, H 3.45, N 6.35.

Ethyl 2-[2-(6,7-Dimethoxy-4-methyl-2-oxo-2*H*-quinolin-1-yl)acetylamino]-3-phenylpropanoate (11): To a solution of ethyl (D,L)-2-amino-3-phenyl propanoate (**10**) (24 mg, 0.125 mmol) and *N*-methylmorpholine (11 mg, 0.125 mmol) in DMF (1 mL), **9** (53 mg, 0.125 mmol) in DMF (1 mL) was added dropwise at 20 °C. Then the mixture was stirred at 20 °C for 14–15 h and poured into water (20 mL). The precipitate was filtered by suction and washed with water to afford 33 mg (52% yield) of colorless prisms, m.p. 173–174 °C (ethyl acetate). ^{1}H NMR ($CDCl_3$): δ = 1.22 (t, J = 7.1 Hz, 3 H, ester Me), 2.91 (dd, J = 8.2 and 8.3 Hz Hz, 1 H, ½Ph-CH_2), 3.13 (dd, J = 5.5 and 5.2 Hz Hz, 1 H, ½Ph-CH_2), 3.98 and 3.99 (s, 2×3 H, 6-OMe, 7-OMe), 4.20 (q, J = 7.0 Hz, 2 H, ester OCH_2), 4.60 (d, J = 15.3 Hz, 1 H, ½N-CH_2), 4.79 (dd, J = 8.4 and 8.0 Hz Hz, 1 H, CH), 5.12 (d, J = 15.1 Hz, 1 H, ½N-CH_2), 6.85–6.87 (m, 2 H, ArH), 6.95–6.98 (m, 4 H, ArH), 7.04 (d, J = 7.14 Hz, 1 H, NH), 7.09 (s, 1 H, ArH), 7.21 (s, 1 H, ArH) ppm. IR (KBr): $\tilde{\nu}$ = 1736 m, 1666 s, 1622 w, 1605 w, 1548 m cm^{-1}. UV (DMSO): λ_{max} (nm) = 369, 296; (water): 363, 295. MS: *m*/*z* (%) = 507 (21, M + 1), 506 (56, M), 314 (16), 313 (100, carbonylmethyl-carbostyril). $C_{25}H_{25}F_3N_2O_6$ (506.48): calcd. C 59.29, H 4.98, N 5.53; found C 59.37, H 4.59, N 5.14.

2-[2-(6,7-Dimethoxy-2-oxo-4-trifluoromethyl-1,2-dihydroquinolin-1-yl)acetylamino]-3-phenylpropionic Acid (13a): To a solution of (D,L)-phenylalanine (**12a**) (11 mg, 0.0625 mmol) in DMSO (90%, 1.5 mL), a solution of succinimide ester **9** (27 mg, 0.062 mmol) in DMSO (90%, 1.5 mL) was added dropwise at 20 °C. Then aq. pH 7 buffer (0.5 mL) was added, the mixture stirred for 14 h at 20 °C, poured into water (20 mL) and then acidified with conc. HCl. A solid was separated, which was filtered by suction and washed with copious amounts of water to afford 23 mg (77% yield) of colorless prisms, m.p. 242–243 °C (acetone); HPLC: byproduct (r_t = 6.912, 3.4%), **13a** (r_t = 7.474, 96.5%). ^{1}H NMR ([D$_6$]DMSO): δ = 2.87–3.09 (m, 2 H, PhCH_2), 3.78 and 3.82 (s, 2×3 H, 6-OMe, 7-OMe), 4.88 (d, J = 15.5 Hz, 1 H, ½NCH_2), 5.13 (d, J = 15.6 Hz, 1 H, ½NCH_2), 6.70 (s, 1 H, ArH), 6.95 (s, 1 H, ArH), 7.06 (s, 1 H, ArH), 7.23 (s, 5 H, PhH), 8.71 (d, J = 6.2 Hz, 1 H, NH) ppm. IR (KBr): $\tilde{\nu}$ = 3437 s, 1656 s, 1624 w, 1584 w, 1530 m cm^{-1}. UV (DMSO): λ_{max} (nm) = 370, 296; (water): 361, 295. MS: *m*/*z* (%) = 479 (20) [M + 1], 478 (89) [M] 314 (15), 313 (100) [carbonylmethyl-carbostyril]. $C_{23}H_{21}F_3N_2O_6$ (478.43): calcd. C 57.74, H 4.42, N 5.86; found C 57.36, H 4.03, N 5.60.

[2-{2-[2-(6,7-Dimethoxy-2-oxo-4-(trifluoromethyl)-1,2-dihydroquinolin-1-yl)acetylamino]acetylamino}acetylamino]acetic Acid (13b): Glycyl-glycyl-glycine (**12b**) (12 mg, 0.0625 mmol) was reacted and worked-up as described for **13a** to yield 16 mg (51% yield) of colorless prisms, m.p. 285–286 °C (ethyl acetate); HPLC: **13b** (r_t = 3.942, 99.3%), byproduct (r_t = 8.233, 0.6%). ^{1}H NMR ([D$_6$]DMSO): δ = 3.70–3.73 (m, 4 H, 2 peptide-CH_2), 3.78–3.80 (m, 2 H, peptide-CH_2), 3.82 and 3.91 (2 s, 2×3 H, 6-OMe, 7-OMe), 5.08 (s, 2 H, NCH_2), 6.90 (s, 1 H, ArH), 6.95 (s, 1 H, ArH), 7.07 (s, 1 H, ArH), 8.16 (t, J = 5.8 Hz, 1 H, NH), 8.26 (t, J = 5.4 Hz, 1 H, NH), 8.62 (t, J = 5.3 Hz, 1 H, NH) ppm. UV (DMSO): λ_{max} (nm) = 369, 296; (water): 360, 295. MS: *m*/*z* (%) = 390 (16) [M – 112] 348 (6), 347 (40), 314 (25), 313 (100) [carbonylmethyl-carbostyril]. $C_{20}H_{21}F_3N_4O_8$ (502.41): calcd. C 47.81, H 4.21, N 11.15; found C 47.47, H 3.85, N 10.82.

[6,7-Dimethoxy-2-oxo-4-(trifluoromethyl)-1,2-dihydroquinolin-1-yl]-acetyl-Labeled Bovine Serum Albumin (13c): To a solution of bovine serum albumin (5 mg) in 0.1 M aq. hydrogencarbonate buffer (1 mL, pH 8.5–9.0), a solution of succinimide ester **9** (1.0 mg) in dry dimethylformamide (50 μL) was added dropwise. The reaction mixture was gently stirred at 20 °C for 1 h. The labeled protein was purified by gel chromatography (Sephadex G25, Pharmacia, as stationary phase) using a phosphate buffer of pH 7.2 as the eluent. The first fluorescent band was identified as the labeled protein. The second fluorescent band was identified as the unreacted succinimide ester **9** which moves much slower.

2-[6,7-Dimethoxy-2-oxo-4-(trifluoromethyl)-2*H*-quinolin-1-yl]-*N*-[(3*R*,4*R*,5*S*,6*R*)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydropyran-3-yl]acetamide (15): To a solution of D-glucosamine hydrochloride (**14**) (27 mg, 0.125 mmol) in DMF/water (9:1, 2.0 mL), **9** (53 mg, 0.125 mmol) in DMF/water (9:1, 2.0 mL) was added dropwise at 20 °C. Then *N*-methylmorpholine (11 mg, 0.125 mol) was added, the mixture stirred for 14 h at 20 °C, poured into water (15 mL) and stirred for 30 min. The precipitate was filtered by suction and washed with water to afford 48 mg (78% yield) of a colorless solid, m.p. 248–250 °C (ethyl acetate). ^{1}H NMR ([D$_6$]DMSO): δ = 3.61 (d, J = 4.7 Hz, 2 H, 7′-CH_2), 3.81 and 3.89 (2 s, 2×3 H, 6-OMe, 7-OMe), 4.45 (t, J = 7.2 Hz, 1 H, 5′-H), 4.76 (d, J = 4.7 Hz, 1 H, 4′-H), 4.96–4.97 (m, 1 H, 3′-H), 5.04 (s, b, 1 H, ½NCH_2), 5.15 (s, b, 1 H, ½N-CH_2), 6.60 (d, J = 3.2 Hz, 1 H, α-H), 6.81 (s, 1 H, ArH), 6.95 (s, 1 H, ArH), 7.06 (s, 1 H, Ar-H), 8.34 (d, J = 7.9 Hz, 1 H, NH) ppm. IR (KBr): $\tilde{\nu}$ = 3524 w, 3432 w, 3289 w, 1662 s, 1598 m, 1561 m, 1529 m cm^{-1}. UV (DMSO): λ_{max} (nm) = 370, 295. MS: *m*/*z* (%) = 491 (10) [M – 1], 473 (10) [M – 19], 417 (100) [M – 75], 380 (21), 371 (34), 297 (20), 295 (34). $C_{20}H_{23}F_3N_2O_9$ (492.41): calcd. C 48.79, H, 4.71, N 5.69; found C 48.43, H 4.73, N 5.30.

***N*-[2-(6,7-Dimethoxy-2-oxo-4-trifluoromethyl-2*H*-quinolin-1-yl)acetyl]chitosan (17):** To a suspension of chitosan oligosaccharide lactate **16** (21 mg) in DMF/water (9:1, 2.0 mL), **9** (27 mg, 0.0625 mmol) in DMF (2.0 mL) was added dropwise at 20 °C. Then *N*-methylmorpholine (11 mg, 0.125 mol) was added, stirred for 14 h at room temperature, poured into water (15 mL), acidified with 1 M HCl acid to pH 1–2 and stirred for 4 h. The precipitate formed was isolated by centrifuge and washed several times with water and identified as quinolone **7**. The filtrate was neutralized with K_2CO_3 and then stirred for 2 h. During this time a solid formed, which was separated by centrifuge to afford 4 mg of **17** as colorless prisms in 15–20% purity (based on uv and fluorescence comparison with **9**). UV (DMSO): λ_{max} (nm) = 370, 294.

Acknowledgments

This research was supported by scholarships from the Austrian Academic Exchange Service (N. S. B.) and the CEEPUS network (M. P. and A. T.).

[1] a) R. Raue, in: *Ullmannns Encyclopedia of Industrial Chemistry*, vol. A15, 5th ed. (Eds.: B. Elvers, S. Hawkins, G. Schulz), VCH Verlagsgesellschaft, Weinheim, **1990**, p. 155–157; b) B. B. Snavely, in: *Organic Molecular Photophysics*, Vol. 1 (Ed.: J. B. Birks);

Wiley, Chichester, **1973**, chapter 5; c) R. P. Haughland, in: *Handbook of Fluorescence Probes and Research Chemicals* (Ed.: K. D. Larison); Molecular Probes Inc., Eugene, USA, **1992**; d) O. S. Wolfbeis, in: *Molecular Spectrosopy: Methods and Applications* (Ed.: S. G. Schulman); John Wiley & Sons, New York, **1985**, chapter 3; e) Gold H, in: *Environmental quality and safety, Supplement Volume IV: The Chemistry of Fluorescent Whitening Agents* (Eds.: R. Anliker, G. Müller, R. Raab, R. Zinkernagel), Georg Thieme, Stuttgart, **1975**, p. 25–46; f) J. Kido, Y. Lizumi, *Appl. Phys. Lett.* **1998**, *73*, 2721–2723; g) A. Niko, S. Tasch, F. Meghdadi, C. Brandstätter, G. Leising, *J. Appl. Phys.* **1997**, *82*, 4177–4182; h) A. E. Siegrist, H. Hefti, H. R. Meyer, E. Schmidt, *Rev. Prog. Coloration* **1987**, *17*, 39–55; i) *Organic Luminescent Materials* (Eds.: B. M. Krasovitskii, B. M. Bolotin), Wiley-VCH, Weinheim, **1988**; j) R. M. Christie, *Rev. Prog. Coloration* **1993**, *23*, 1–18; k) N. A. Kuznetsova, O. L. Kaliya, *Russ. Chem. Rev.* **1992**, *61*, 683–696; l) O. A. Ponmarev, E. R. Vasina, V. G. Mitina, A. A. Sukhorukov, *Russ. J. Phys. Chem.* **1990**, *64*, 518–521; m) R. M. Christie, C.-H. Lui, *Dyes Pigm.* **1999**, *42*, 85–93; n) C. Eggeling, R. R. J. Widengren, C. A. M. Seidel, *Anal. Chem.* **1998**, *70*, 2651–2659; o) J. B. Gallivan, *Molecular Photochem.* **1970**, *2*, 191–211; p) G. Piszczek, B. P. Maliwal, I. Gryczynski, D. Jonathan, J. R. Lakowicz, *J. Fluoresc.* **2001**, *11*, 101–107.

[2] a) R. Nakagaki, N. Kitamura, I. Aoyama, H. Ohtsubo, *J. Photochem. Photobiol.* **1994**, *A80*, 113–119; b) G. Saroja, N. B. Sankaran, A. Samanta, *Chem. Phys. Lett.* **1996**, *249*, 392–398; c) O. A. Ponomarev, Yu. F. Pedash, O. V. Prezhdo, *Zh. Fiz. Khim.* **1991**, *65*, 1846–1850; d) J. Chen, P. R. Selvin, *J. Photochem. Photobiol. A* **2000**, *135*, 27–32; e) M. Li, P. R. Selvin, *J. Am. Chem. Soc.* **1995**, *117*, 8132–8138; f) C. Marzano, A. Chilin, A. Guiotto, F. Baccichetti, F. Carlassare, F. Bordin, *Farmaco* **2000**, *55*, 650–658; g) O. A. Ponomarev, E. R. Vasina, S. N. Yarmolenko, V. G. Mitina, N. S. Pivnenko, *Zh. Obshch. Khim.* **1990**, *60*, 1161–1170, *J. Gen. Chem. USSR, Transl. Plenum Pub. Corp.* **1990**, *60*, 1035–1042; h) E. K. Kainmüller, E. P. Olle, W. Bannwarth, *Chem. Commun.* **2005**, *43*, 5459–5461.

[3] a) G. Uray, K.-H. Niederreiter, F. Belaj, W. M. F. Fabian, *Helv. Chim. Acta* **1999**, *82*, 1408–1417; b) W. M. F. Fabian, K. S. Niederreiter, G. Uray, W. Stadlbauer, *J. Mol. Struct.* **1999**, *477*, 209–220; c) G. A. Strohmeier, W. M. F. Fabian, G. Uray, *Helv. Chim. Acta* **2004**, *87*, 215–226.

[4] a) F. A. Bennett, D. J. Barlow, A. N. O. Dodoo, R. C. Hider, A. B. Lansley, M. J. Lawrence, C. Marriott, S. S. Bansal, *Anal. Biochem.* **1999**, *270*, 15–23; b) C. Charitos, G. Kokotos, C. Tzougraki, *J. Heterocycl. Chem.* **2001**, *38*, 153–158; c) J. E. T. Corrie, V. Munasinghe, N. Ranjit, *J. Heterocycl. Chem.* **2000**, *37*, 1447–1454; d) A. P. Esteves, L. M. Rodrigues, M. E. Silva, S. Gupta, A. M. F. Oliveira-Campos, O. Machalicky, A. J. Mendonca, *Tetrahedron* **2005**, *61*, 8625–8632; e) J. Fernandez-Carneado, M. J. Kogan, S. Castel, E. Giralt, *Angew. Chem. Int. Ed.* **2004**, *43*, 1811–1814; f) X. Luo, X. Naiyun, L. Cheng, D. Huang, *Dyes Pigm.* **2001**, *51*, 153–159; g) M. C. Rideout, A. C. Raymond, A. B. Burgin Jr, *Nucleic Acids Res.* **2004**, *32*, 4657–4664; h) M. S. Schiedel, C. A. Briehn, P. Bäuerle, *Angew. Chem. Int. Ed.* **2001**, *40*, 4677–4680; i) P. D. Edwards, R. C. Mauger, K. M. Cottrell, F. X. Morris, K. K. Pine, M. A. Sylvester, C. W. Scott, S. T. Furlong, *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2291–2294; j) C. A. M. Seidel, A. Schulz, M. H. M. Sauer, *J. Phys. Chem.* **1996**, *100*, 5541–5553; k) A. Adronov, S. L. Gilat, J. M. Frechet, K. Ohta, F. V. R. Neuwahl, G. R. Fleming, *J. Am. Chem. Soc.* **2000**, *122*, 1175–1185; l) K. H. Shaughnessy, P. Kim, J. F. Hartwig, *J. Am. Chem. Soc.* **1999**, *121*, 2123–2132.

[5] L. Limpach, *Ber. Dtsch. Chem. Ges.* **1931**, *64*, 970–971.

[6] W. Werner, *Tetrahedron* **1969**, *25*, 255–261.

[7] a) O. Lefebvre, M. Marull, M. Schlosser, *Eur. J. Org. Chem.* **2003**, 2115–2121; b) M. Marull, O. Lefebvre, M. Schlosser, *Eur. J. Org. Chem.* **2004**, 54–63; c) M. Marull, M. Schlosser, *Eur. J. Org. Chem.* **2003**, 1576–1588; d) D. O. Berbasov, V. A. Soloshonok, *Synthesis* **2003**, 2005–2010; e) H.-K. Lee, H. Cao, T. M. Rana, *J. Comb. Chem.* **2005**, *7*, 279–284.

[8] E. V. Dehmlov, J. Bollhoefer, G. Thye, *J. Chem. Res. Synopsis* **2001**, 113–115.

[9] N. S. Badgujar, S. Kovackova, G. Uray, W. Stadlbauer, paper in preparation.

[10] a) G. W. Anderson, J. E. Zimmerman, F. M. Callahan, *J. Am. Chem. Soc.* **1964**, *86*, 1839–1842; b) M. Adamczyk, J. R. Fishpaugh, K. J. Heuser, *Bioconjug. Chem.* **1997**, *8*, 253–255; c) M. Bodanszky, A. Bodanszky: *The Practice of Peptide Synthesis*, Springer, Berlin, Heidelberg, New York, **1994**, p. 125; d) H.-G. Hahn, K. H. Chang, K. D. Nam, S. Y. Bae, H. Mah, *J. Heterocycl. Chem.* **2000**, *37*, 1003–1008; e) K. Stembera, A. Buchynskyy, S. Vogel, D. Knoll, A. A. Osman, J. A. Ayala, P. Welzel, *ChemBioChem* **2002**, *3*, 332–340.

[11] a) M. V. Reddington, *J. Chem. Soc., Perkin Trans. 1* **1998**, 143–148; b) B. J. Balcolm, N. O. Petersen, *J. Org. Chem.* **1989**, *54*, 1922–1927; c) U. Boas, J. B. Christensen, K. J. Jensen, *J. Comb. Chem.* **2004**, *6*, 497–503; d) J. E. Churchich, *Agric. Biochem.* **1993**, *213*, 229–233.

[12] L. M. Harwood, *Aldrichimica Acta* **1985**, *18*, 25.

Received: December 17, 2005
Published Online: April 10, 2006

