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Synthesis of Carbon-14 Labelled (3-{[(*Z*)-5-Chloro-2,3-dihydro-3-(hydroxy-2-thienylmethylene)-2-oxo-1*H*-indol-1-yle]carbonylamino}propyl)trimethylammonium Chloride, a Potential Cartilage-Targeted Antirheumatic Drug

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SUMMARY

A [^{14}C]-labelled form of (3-{[(*Z*)-5-chloro-2,3-dihydro-3-(hydroxy-2-thienylmethylene)-2-oxo-1*H*-indol-1-yle]carbonylamino}propyl)trimethylammonium chloride, a potential cartilage-targeted antirheumatic drug, was required for pharmacokinetic studies. This compound, labelled with [^{14}C] located in the C-3 methylene position, was prepared in four steps starting from *N*-[3-(dimethylamino)propyl]-5-chloro-2,3-dihydro-2-oxo-1*H*-indole-1-carboxamide and 2-thiophene-[^{14}C]carbonyl chloride, previously synthesized by a two-step sequence from barium [^{14}C]-carbonate and 2-thienyllithium. The desired product was obtained with a specific activity of 359 MBq/mmol (9.7 mCi/mmol). The overall radiochemical yield was 50% based on barium [^{14}C]-carbonate.

Key words: carbon-14, Tenidap, quaternary ammonium, cartilage targeting, antirheumatic drug.

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INTRODUCTION

Antirheumatic oxindoles are a new class of drugs for the treatment of arthritis (1,2). Tenidap (Figure 1), the lead compound of this family, has demonstrated excellent activity in clinical trials with patients suffering from rheumatoid arthritis or osteoarthritis (3,4). Tenidap produces the same effect as classical non-steroidal anti-inflammatory drugs through cyclo-oxygenase inhibition but also exhibits unique properties by decreasing the release and activity of the proinflammatory cytokines interleukine 1 (IL1), interleukine 6 (IL6) and tumor necrosis factor alpha (TNFα) in both *in vitro* and *in vivo* settings (1-6). However, the further development of Tenidap may be compromized by its renal toxicity and to a lesser extent by its gastro-intestinal toxicity (7).

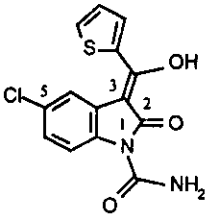


Figure 1. Structure of Tenidap, (*Z*)-5-chloro-2,3-dihydro-3-(hydroxy-2-thienyl-methylene)-2-oxo-1*H*-indole-1-carboxamide.

The side effects of Tenidap prompted us to integrate this oxindole derivative into a cartilage targeting program developed in our laboratory. The objective of the project is to increase the specificity of antirheumatic agents for cartilage, in order to minimize their efficient dose and adverse reactions. As we discovered in a previous pharmacokinetic work that the presence of the quaternary ammonium entity in some structures was responsible for their affinity for cartilaginous tissues (8-10), our strategy is based on the conjugation of the antirheumatic drug with the quaternary ammonium moiety (11).

The demonstration of the targeting of cartilage for the Tenidap-quaternary ammonium conjugate (Figure 2) required a pharmacokinetic study in animal models;

consequently, a [^{14}C]-labelled analog must be synthesized. The preparation of this radiolabelled form is described in this paper.

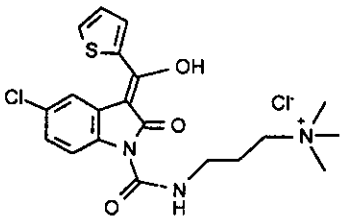


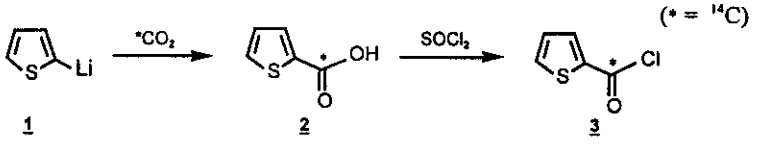
Figure 2. Structure of the quaternary ammonium derivative of Tenidap, (3-{[(*Z*)-5-chloro-2,3-dihydro-3-(hydroxy-2-thienylmethylene)-2-oxo-1*H*-indol-1-yle]carbonyl-amino}propyl)trimethylammonium chloride.

RESULTS AND DISCUSSION

Radiosynthesis of Tenidap has been previously described with [^{14}C] located in the benzo portion or in the methylene of the C-3 substituent of the indole ring (12). Since earlier metabolic studies in rats of [^{14}C]-Tenidap did not show a loss of the C-3 substituent, the ammonium derivative was labelled with [^{14}C] located in the C-3 methylene (13). In this case, the radioactivity was introduced late in the synthetic sequence (see Scheme 3). The [^{14}C]-precursor, 2-thiophene-[^{14}C]carbonyl chloride, was easily obtained from barium [^{14}C]-carbonate.

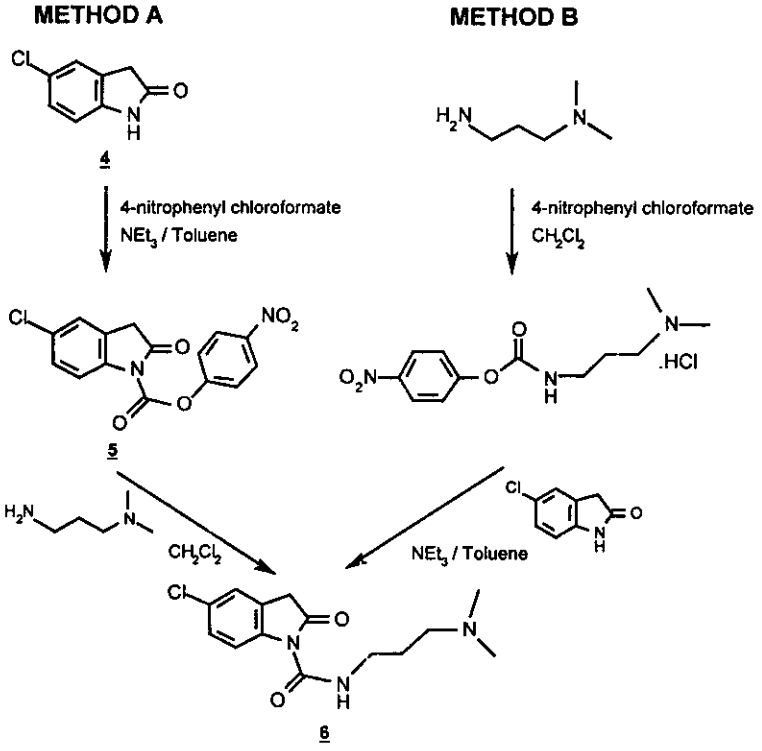
2-Thiophene-[^{14}C]carbonyl chloride was prepared according to the route outlined in Scheme 1. The initial step was the carbonation of commercially available 2-thienyllithium (**1**) with [^{14}C]-carbon dioxide, generated from barium [^{14}C]-carbonate and sulfuric acid, leading to 2-thiophene-[^{14}C]carboxylic acid (**2**) with a radiochemical yield of 97%. This carboxylic acid could have been also obtained with a similar yield by the carbonation of the corresponding magnesium salt (14). Compound **2** was then treated with thionyl chloride to quantitatively give the [^{14}C]-acid chloride **3**.

Scheme 1.



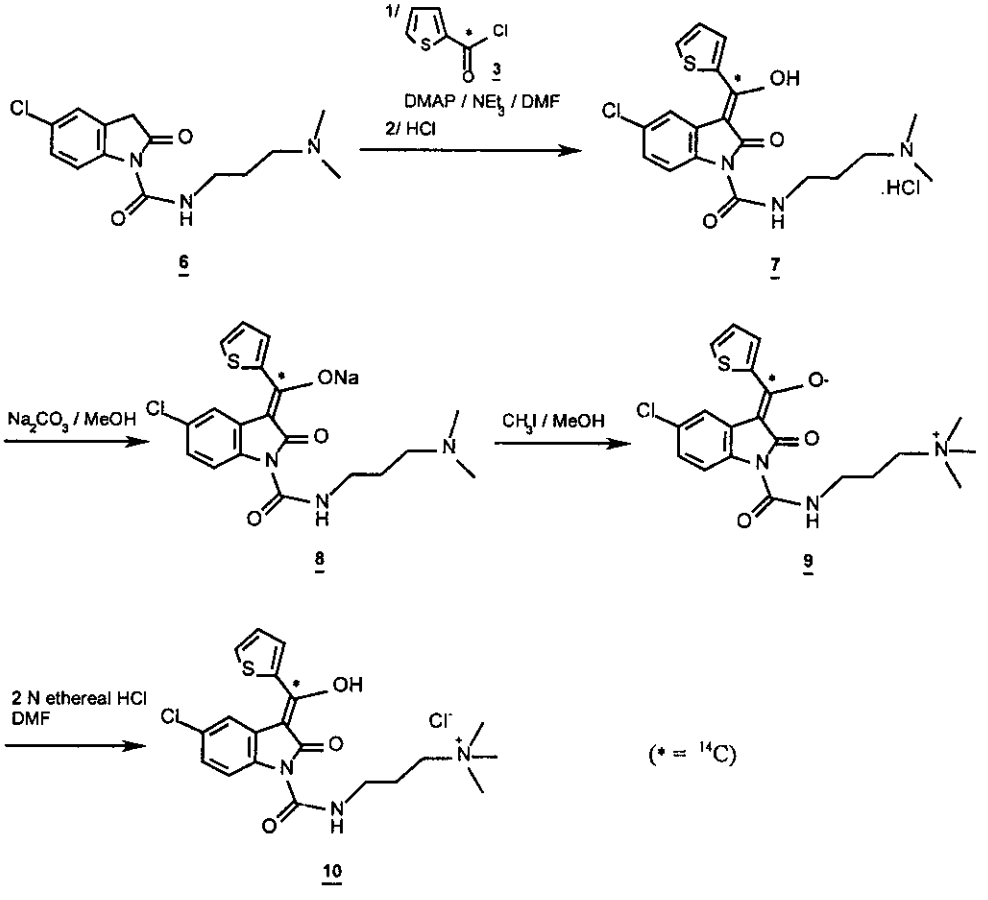
Compound **3** was immediately condensed with the *N*-substituted oxindole-1-carboxamide **6**, previously synthesized by a two-step sequence from the commercial oxindole **4** as displayed in Scheme 2. In fact two reversed synthetic routes were explored for the preparation of **6** (methods A and B). Method A was based on the initial condensation of the oxindole **4** with 4-nitrophenyl chloroformate to give the derivative **5** in 73% yield; this was further converted by treatment with 3-(dimethylamino)propylamine into the desired *N*-substituted oxindole-1-carboxamide **6** in 86% yield (63% overall yield). In method B, 4-nitrophenyl chloroformate was first treated with 3-(dimethylamino)propylamine to provide a carbamate derivative in 95% yield, which was subsequently condensed with **4** to give **6** in 35% yield (33% overall yield). The better overall yield for method A was expected. The limiting step in the two proposed sequences was the condensation involving the oxindole **4**. As 4-nitrophenyl chloroformate is a more activated product than the intermediate carbamate involved in method B, its condensation with **4** was easier.

Scheme 2.



The reaction of compound **6** with the [^{14}C]-acid chloride **3** was carried out in *N,N*-dimethylformamide, using 4-*N,N*-dimethylaminopyridine as catalyst (Scheme 3). After acidification of the mixture with hydrochloric acid, the hydrochloride **7** was isolated with a radiochemical yield of 63%. Compound **7** was then converted into its sodium salt **8** in high yield (91%) by treatment with sodium carbonate in a methanolic solution. Quaternarization of the free tertiary amine of **8** by methyl iodide in methanol gave the ammonium derivative **9** in 90% yield. Finally, the reaction of **9** with ethereal hydrochloric acid in *N,N*-dimethylformamide provided the target enol-quaternary ammonium **10** in quantitative yield.

Scheme 3.



The quaternary ammonium derivative of Tenidap (**10**) was isolated with a specific activity of 359 MBq/mmol (9.7 mCi/mmol) and a radiochemical purity determined to be better than 99% by thin layer chromatography analysis. The overall radiochemical yield of the labelling sequence was 50% based on barium [^{14}C]-carbonate.

EXPERIMENTAL

General comments. 2-Thienyllithium and 5-chloro-2,3-dihydro-2*H*-indole-2-one were purchased from Aldrich and barium [^{14}C]-carbonate from NEN. All solvents and reagents obtained from commercial sources were used without further purification. Proton and carbon nuclear magnetic resonance spectra (^{1}H-NMR and ^{13}C-NMR) were performed at respectively 200.133 MHz and 50.323 MHz on a Brüker AM 200 (4.5 T) spectrometer. Chemical shifts (δ) are reported in parts per million relative to the internal tetramethylsilane standard for ^{1}H-NMR and the solvents for ^{13}C-NMR ($CDCl_3$: δ = 77.0 ppm, DMSO-d_6: δ = 39.5 ppm). Coupling constants (J values) are given in hertz. Infrared (IR) spectra were recorded on a Brüker Vector 22 instrument. Electrospray ionization mass spectrometry (MS) was carried out on a Hewlett Packard MS Enging 5989 spectrometer. Elemental analyses for carbon, hydrogen and nitrogen obtained from CNRS Service Central d'Analyse at Vernaison (France) were within $\pm$ 0.4% of theory for the formulae given. Melting points (mp) were determined on a Electrothermal digital apparatus and are uncorrected. Analytical thin layer chromatography (TLC) was conducted on precoated silica gel plates (SDS, 60 F254, 0.25 mm thick) with both detection by ultraviolet light at 254 nm and visualisation with iodine. Silica gel 60 (Chromagel, 35-60 μm, SDS) was used for medium pressure chromatography using the indicated solvent mixture expressed as volume/volume ratios. Radiochemical purity was determined by scanning the TLC plates with an Ambis 4000 detector. Specific activity of compounds was measured in a Wallac Winspectral 1414 liquid scintillation counter. All labelled compounds were compared with the unlabelled reference compounds by TLC and by NMR.

2-Thiophene-[^{14}C]carboxylic acid (2). 2-Thienyllithium (1) (13 mmol, 13 mL of a 1 M solution in tetrahydrofuran) was carbonated by [^{14}C]-CO_2, generated from barium [^{14}C]-carbonate (2.24 g, 11.35 mmol, 4070 MBq (110 mCi)) and concentrated sulfuric acid (40 mL) in a vacuum manifold. The mixture was stirred for 1 h at -40°C, then for 2 h at room temperature. After hydrolysis with 1 N hydrochloric acid, the organic phase was extracted with 1 N sodium hydroxide. The aqueous fraction was then made acidic and re-extracted with ether. The ether phase was dried over magnesium sulfate, filtered and evaporated under reduced pressure to give 2 as a white solid (1.41 g, 3951 MBq (106.8 mCi), 97%) with a specific activity of 359 MBq/mmol (9.7 mCi/mmol). The radiochemical purity was 98.2% as determined by TLC analysis (*Rf* = 0.40, cyclohexane/ethyl acetate 1/1).

2-Thiophene-[^{14}C]carbonyl chloride (3). Compound 2 (1.39 g, 10.85 mmol, 3892 MBq (105.2 mCi)) was treated with thionyl chloride (5 mL) with a few drops of *N,N*-dimethylformamide under a nitrogen atmosphere for 2 h at 70°C. The resulting solution was evaporated under reduced pressure to afford 3 (1,16 mL, quantitative yield) which was immediately used in the next step.

4-Nitrophenyl (5-chloro-2,3-dihydro-2-oxo-1*H*-indole-1-carboxylate) (5). To a stirred suspension of 5-chloro-2,3-dihydro-2*H*-indole-2-one (4) (3.92 g, 23.39 mmol) and triethylamine (6.52 mL, 46.78 mmol) in toluene (40 mL) was added, at 0°C, under nitrogen atmosphere, a solution of 4-nitrophenyl chloroformate (9.43 g, 46.78 mmol) in the same solvent (50 mL). The mixture was then heated at 110°C for 6 h. The suspension was filtered and the precipitated solid washed with toluene. After concentration of the combined filtrates under reduced pressure, the residue was purified by chromatography on silica gel eluting with dichloromethane to provide 5 as a clear solid (5.68 g, 73%). TLC (*Rf* = 0.48, dichloromethane); mp 194-195°C (dec); ^{1}H-NMR (DMSO-d_6) δ: 3.92 (s, 2H, H-3), 7.43 (d, *J* = 8.6, 1H, H-6), 7.48 (s, 1H, H-4), 7.63 (d, *J* = 9, 2H, H $ArNO_2$), 7.79 (d, *J* = 8.6, 1H, H-7), 8.37 (d, *J* = 9, 2H, H $ArNO_2$); ^{13}C-NMR (DMSO-d_6) δ: 36.00 (C-3), 116.31 (C-7), 123.07 (C

$ArNO_2$), 124.54 (C-4), 125.44 (C $ArNO_2$), 126.95 (C-3a), 127.52 (C-6), 128.77 (C-5), 138.68 (C-7a), 145.38 147.97 (C $ArNO_2$), 154.49 (CO-NH), 171.57 (C-2); IR (KBr) ν cm^{-1}: 1771 1740 (C=O).

***N*-[3-(Dimethylamino)propyl]-5-chloro-2,3-dihydro-2-oxo-1*H*-indole-1-carboxamide (6).** To a stirred solution of 5 (4.02 g, 12.08 mmol) in dichloromethane (70 mL) was added, at room temperature, 3-(dimethylamino)propylamine (1.52 mL, 12.08 mmol). The reaction was immediate. The solution was then extracted with 0.05 N sodium hydroxide. The organic phase was dried over magnesium sulfate, filtered and evaporated to dryness under reduced pressure to give 6 as a brown solid (3.07 g, 86%). TLC (*Rf* = 0.30, dichloromethane/methanol 1/1); mp 84-85°C; ^{1}H-NMR (DMSO-d_6) δ: 1.66 (qt, *J* = 6.9, 2H, $(CH_3)_2N$-CH_2-CH_2-CH_2), 2.16 (s, 6H, $N(CH_3)_2$), 2.30 (t, *J* = 6.9, 2H, $(CH_3)_2N$-CH_2-CH_2-CH_2), 3.32 (q, *J* = 6.9, 2H, $(CH_3)_2N$-CH_2-CH_2-CH_2), 3.88 (s, 2H, H-3), 7.33 (d, *J* = 8.6, 1H, H-6), 7.37 (s, 1H, H-4), 8.02 (d, *J* = 8.6, 1H, H-7), 8.71 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ: 27.03 ($(CH_3)_2N$-CH_2-CH_2-CH_2), 36.52 (C-3), 38.30 ($(CH_3)_2N$-CH_2-CH_2-CH_2), 45.32 ($N(CH_3)_2$), 57.15 ($(CH_3)_2N$-CH_2-CH_2-CH_2), 117.35 (C-7), 123.91 (C-4), 124.52 (C-3a), 128.11 (C-6), 129.50 (C-5), 140.26 (C-7a), 151.67 (CO-NH), 176.18 (C-2); IR (KBr) ν cm^{-1}: 1753 1686 (C=O).

(*Z*)-*N*-[3-(Dimethylamino)propyl]-5-chloro-2,3-dihydro-3-(hydroxy-2-thienyl-[^{14}C]methylene)-2-oxo-1*H*-indole-1-carboxamide hydrochloride (7). To a solution of 6 (2.20 g, 7.44 mmol) and 4-*N*,*N*-dimethylaminopyridine (186 mg) in *N*,*N*-dimethylformamide (5 mL), was added, under a nitrogen atmosphere, at 0°C, triethylamine (2.09 mL, 15.02 mmol) and 3 (800 µL, 7.44 mmol, 2671 MBq (72.2 mCi)). The mixture was stirred at 25°C for 3 h and then treated with methanol (4 mL) and 12 N hydrochloric acid (4 mL). The resulting precipitate was filtered, washed with cold water (2 mL) and dried *in vacuo* to give 7 as a yellow solid (2.10 g, 1684 MBq (45.5 mCi), 63%) with a specific activity of 359 MBq/mmol (9.7 mCi/mmol). The radiochemical purity was 99.8% as determined by TLC analysis (*Rf* = 0.60, dichloromethane/methanol/ammonium hydroxide 50/48/2).

Analytical data for the unlabelled compound 7: mp 197-198°C (dec); ^{1}H-NMR (DMSO-d_6) δ: 1.84-1.98 (m, 2H, $(CH_3)_2HN^+$-CH_2-CH_2-CH_2), 2.84 (d, *J* = 4.8, 6H,

$^{+}NH(CH_3)_2$), 3.02-3.12 (m, 2H, $(CH_3)_2HN^{+}$-CH_2-CH_2-CH_2), 3.35 (q, J = 6.3, 2H, $(CH_3)_2HN^{+}$-CH_2-CH_2-CH_2), 7.01 (d, J = 8.6, 1H, H-6), 7.15 (dd, J = 4.5, J = 3.6, 1H, H-4'), 7.45 (s, 1H, OH enol), 7.76 (d, J = 4.5, 1H, H-3'), 8.00 (s, 1H, H-4), 8.05 (d, J = 8.6, 1H, H-7), 8.24 (d, J = 3.6, 1H, H-5'), 9.54 (s, 1H, CO-NH), 10.04 (s, 1H, $^{+}NH(CH_3)_2$); ^{13}C-NMR ($CDCl_3$) δ: 25.08 ($(CH_3)_2HN^{+}$-CH_2-CH_2-CH_2), 37.22 ($(CH_3)_2HN^{+}$-CH_2-CH_2-CH_2), 43.04 ($^{+}NH(CH_3)_2$), 55.74 ($(CH_3)_2HN^{+}$-CH_2-CH_2-CH_2), 99.96 (C-3), 117.06 (C-7), 118.98 (C-4), 122.79 (C-3a), 126.54 (C-4'), 128.03 (C-6), 129.59 (C-5), 132.14 (C-3'), 132.85 (C-5'), 133.86 (C-2'), 134.83 (C-7a), 152.14 (CO-NH), 167.51 (C=C(OH)), 172.71 (C-2); IR (KBr) ν cm^{-1}: 1716 1644 (C=O); MS (electrospray) *m/z*: 406.0 and 408.1 $[M(^{35}Cl,^{37}Cl)]^{+}$; Anal. ($C_{19}H_{20}ClN_3O_3S.HCl \cdot 0.3H_2O$) C, H, N.

(*Z*)-*N*-[3-(Dimethylamino)propyl]-5-chloro-2,3-dihydro-3-(hydroxy-2-thienyl-[^{14}C]methylene)-2-oxo-1*H*-indole-1-carboxamide sodium salt (8). A suspension of 7 (1.11 g, 2.49 mmol) and sodium carbonate (131 mg, 1.25 mmol) in methanol (70 mL) was stirred at 25°C for 5 h. The mixture was then concentrated under reduced pressure and filtered. After washing with cold water and drying, the resulting solid was treated again with sodium carbonate (122 mg, 1.15 mmol) in methanol (70 mL) at 25°C for 30 min. Evaporation to dryness of the mixture provided 8 as a yellow solid (969 mg, 814 MBq (22.0 mCi), 91%) with a specific activity of 359 MBq/mmol (9.7 mCi/mmol). The radiochemical purity was 97.4% as determined by TLC analysis (*Rf* = 0.60, dichloromethane/methanol/ammonium hydroxide 50/48/2).

Analytical data for the unlabelled compound 8: mp 211-212°C (dec); ^{1}H-NMR (DMSO-d_6) δ: 1.68 (qt, J = 7.1, 2H, $(CH_3)_2N$-CH_2-CH_2-CH_2), 2.28 (s, 6H, $N(CH_3)_2$), 2.35 (t, J = 7.1, 2H, $(CH_3)_2N$-CH_2-CH_2-CH_2), 3.30 (q, J = 6.6, 2H, $(CH_3)_2N$-CH_2-CH_2-CH_2), 6.80 (dd, J = 8.5, J = 2.3, 1H, H-6), 7.07 (dd, J = 4.9, J = 3.8, 1H, H-4'), 7.58 (dd, J = 4.9, J = 1.1, 1H, H-3'), 8.02 (d, J = 8.5, 1H, H-7), 8.16 (d, J = 2.3, 1H, H-4), 8.59 (dd, J = 3.8, J = 1.1, 1H, H-5'), 9.99 (t, J = 5.5, 1H, CO-NH); ^{13}C-NMR (DMSO-d_6) δ: 27.19 ($(CH_3)_2N$-CH_2-CH_2-CH_2), 36.84 ($(CH_3)_2N$-CH_2-CH_2-CH_2), 44.88 ($N(CH_3)_2$), 56.44 ($(CH_3)_2N$-CH_2-CH_2-CH_2), 94.31 (C-3), 113.85 (C-7), 117.40

(C-4), 118.44 (C-6), 126.08 (C-3a), 127.02 (C-4'), 129.23 (C-3'), 129.65 (C-5'), 130.14 (C-5), 131.93 (C-2'), 149.10 (C-7a), 154.31 (CO-NH), 165.88 (C=*C*(OH)), 176.93 (C-2); IR (KBr) ν cm^{-1}: 1691 1632 (C=O).

(*Z*)-(5-Chloro-1,2-dihydro-2-oxo-1-{[3-(trimethylammonio)propyl]aminocarbonyl}-3*H*-indol-3-ylidene)-2-thienyl-[^{14}C]methanolate (9). To a solution of **8** (950 mg, 2.22 mmol) in methanol (20 mL) was added, under a nitrogen atmosphere, methyl iodide (0.21 mL, 3.33 mmol). The stirring of the mixture for 3 h at 25°C led to the progressive precipitation of the quaternary ammonium derivative. The precipitate was filtered, washed with methanol and ether and dried *in vacuo* to give **9** as a yellow solid (839 mg, 718 MBq (19,4 mCi), 90%) with a specific activity of 359 MBq/mmol (9.7 mCi/mmol). The radiochemical purity was 99.8% as determined by TLC analysis (*Rf* = 0.14, dichloromethane/methanol/ammonium hydroxide 50/48/2). Analytical data for the unlabelled compound **9**: mp 260-261°C (dec); ^{1}H-NMR (DMSO-d_6) δ: 1.98 (qt, *J* = 8,0, 2H, $(CH_3)_3N^+$-CH_2-CH_2-CH_2), 3.04 (s, 9H, $N^+(CH_3)_3$), 3.31-3.39 (m, 4H, $(CH_3)_3N^+$-CH_2-CH_2-CH_2), 6.80 (dd, *J* = 8.5, *J* = 2.4, 1H, H-6), 7.06 (dd, *J* = 4,9, *J* = 3.8, 1H, H-4'), 7.59 (dd, *J* = 4,9, *J* = 1.1, 1H, H-3'), 8.02 (d, *J* = 8.5, 1H, H-7), 8.16 (d, *J* = 2.4, 1H, H-4), 8.59 (dd, *J* = 3.8, *J* = 1.1, 1H, H-5'), 10.11 (t, *J* = 5.6, 1H, CO-NH); ^{13}C-NMR (DMSO-d_6) δ: 23.46 ($(CH_3)_3N^+$-CH_2-CH_2-CH_2), 36.01 ($(CH_3)_3N^+$-CH_2-CH_2-CH_2), 52.26 ($^+N(CH_3)_3$), 63.61 ($(CH_3)_3N^+$-CH_2-CH_2-CH_2), 94.32 (C-3), 113.91 (C-7), 117.47 (C-4), 118.51 (C-6), 126.23 (C-3a), 126.98 (C-4'), 129.30 (C-3'), 129.68 (C-5'), 130.06 (C-5), 132.02 (C-2'), 149.02 (C-7a), 154.54 (CO-NH), 165.81 (C=*C*(OH)), 177.02 (C-2); IR (KBr) ν cm^{-1}: 1690 1634 (C=O); MS (electrospray) *m/z*: 420.0 and 422.0 [M(^{35}Cl,^{37}Cl+H]$^+$; Anal. ($C_{20}H_{22}ClN_3O_3S$) C, H, N.

(3-{[(*Z*)-5-chloro-2,3-dihydro-3-(hydroxy-2-thienyl-[^{14}C]methylene)-2-oxo-1*H*-indol-1-yle]carbonylamino}propyl)trimethylammonium chloride (10). To a solution of **9** (400 mg, 0.95 mmol) in *N,N*-dimethylformamide (7 mL) was added 2 N ethereal hydrochloric acid (2.5 mL). The reaction mixture was stirred for 10 minutes at 25°C and then poured into ether (100 mL). The resulting precipitate was filtered, washed with ether and dried *in vacuo* to afford **10** as a yellow solid (452

mg, 340 MBq (9.2 mCi), quantitative yield) with a specific activity of 359 MBq/mmol (9.7 mCi/mmol). The radiochemical purity was 99.5% as determined by TLC analysis (*Rf* = 0.14, dichloromethane/methanol/ammonium hydroxide 50/48/2). Analytical data for the unlabelled compound **10**: mp 209-211°C (dec); ^{1}H-NMR (DMSO-d_6) δ: 1.98 (qt, *J* = 8.0, 2H, $(CH_3)_3N^+$-CH_2-CH_2-CH_2), 3.04 (s, 9H, $N^+(CH_3)_3$), 3.31-3.39 (m, 4H, $(CH_3)_3N^+$-CH_2-CH_2-CH_2), 6.85 (s, 1H, OH enol), 6.94 (d, *J* = 8.6, 1H, H-6), 7.12 (dd, *J* = 4.7, *J* = 3.5, 1H, H-4'), 7.70 (d, *J* = 4.7, 1H, H-3'), 8.02-8.07 (m, 2H, H-4, H-7), 8.34 (d, *J* = 3.5, 1H, H-5'), 9.75 (s, 1H, CO-NH); IR (KBr) ν cm^{-1}: 1711 1646 (C=O); MS (electrospray) *m/z*: 420.0 and 422.0 $[M(^{35}Cl,^{37}Cl)]^+$; Anal. ($C_{20}H_{23}Cl_2N_3O_3S \cdot 1.1H_2O$) C, H, N.

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