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Synthesis of New N-(Trifluoroacetyl) Doxorubicin Analogues

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SYNTHETIC COMMUNICATIONS, 21(7), 931-944 (1991)

SYNTHESIS OF NEW N-(TRIFLUOROACETYL) DOXORUBICIN ANALOGUES.

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Abstract

Four new 14-O-acyl derivatives of N-(trifluoroacetyl) doxorubicin possessing a terminal primary amino group have been synthesized in a two steps process under mild reaction conditions.

The anthracycline antibiotics have attracted considerable interest because of their great therapeutic value in treating a number of human cancers[1,2]. Unfortunately, their use has been limitated by a number of side effects, particularly a dose-related and irreversible cardiotoxicity[3] and by the emergence of multidrug resistance[4]. Therefore, the search for new analogues with reduced toxicity and a broader spectrum of antitumor activity has been of great importance[5]. This kind of research has produced one of the most potent doxorubicin (1a) analogues: the cyanomorpholino derivative[6-10] (2).

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The latter showed 100- to 1000-fold increases in antitumor potency, absence of cardiotoxicity, and activity against tumors that are resistant to doxorubicin. These biological properties have made the cyanomorpholino doxorubicin a candidate of choice for drug-targeting with antibodies[11,12].

We have been trying to incorporate a useful functional group, such an amine, to the cyanomorpholino doxorubicin nucleus. An amino group would help us to covalently link the drug to antibody by standard coupling procedures[13,14] such as those using carbodiimides. In this communication we wish to present our preliminary results on the selective functionalisation of 14-OH of N-(trifluoroacetyl) doxorubicin (1b) (scheme 1).

Preparation of the amino acids

Four commercially available amino acids have been used: beta-alanine (3a), 6-aminohexanoic acid (4a), 11-aminoundecanoic acid (5a), and 4-aminomethylbenzoic acid (6a). Initially these amino acids were protected as their N-p-anisyldiphenylmethyl derivatives[15] (3b-6b). The trityl appeared attractive as a base-stable protecting group that can be cleaved under mild acidic conditions. The result of the methoxytritylation is shown on scheme 2. The low yield of the reaction with 11-aminoundecanoic acid (5a) was due to its poor solubility under the reaction conditions.

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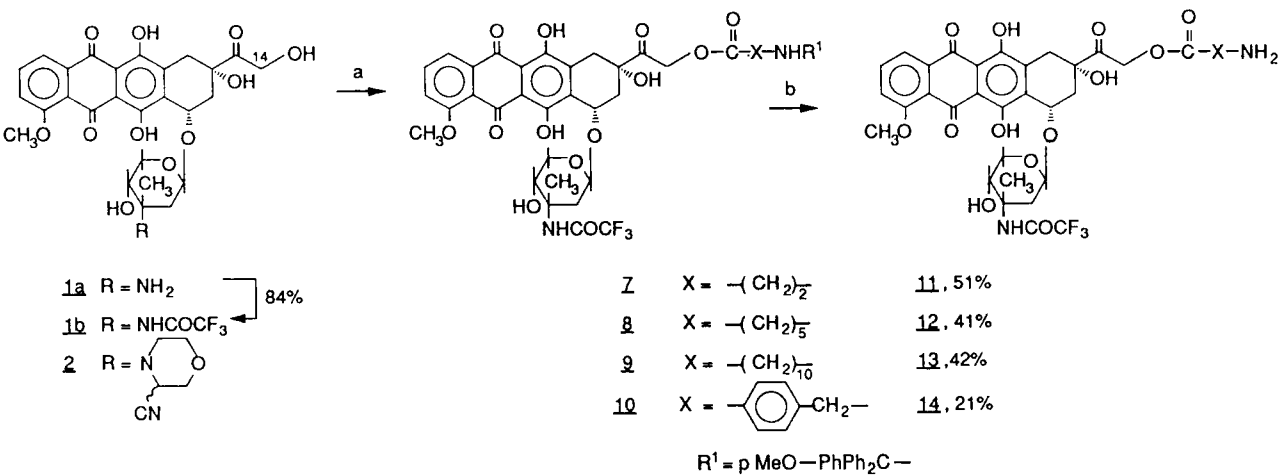
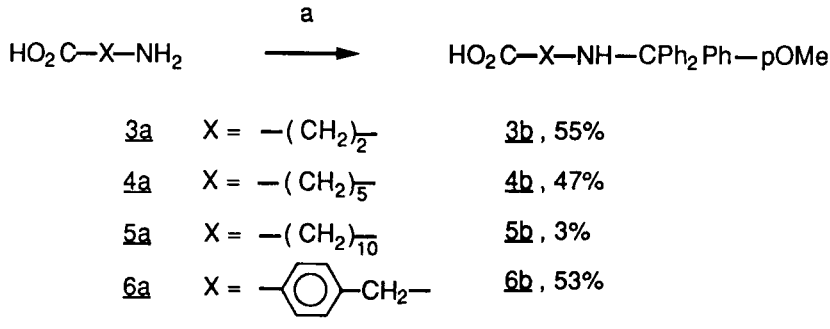


a. AMINO ACID, DCC, 4 - PPY, CH_2Cl_2, 25°C

b. TFA, CH_2Cl_2, 25°C

SCHEME 1



a. p MeO—$PhPh_2CCl$, Et_2NH, H_2O: ^iP_r OH

SCHEME 2

Synthesis of the new analogues.

The N-(trifluoroacetyl) doxorubicin was obtained from doxorubicin upon treatment with S-ethyltrifluoroacetate as described previously[16]. Selective esterification of 14-OH with the various N-protected amino acid was achieved under carefully controlled reaction conditions using 1,3-dicyclohexylcarbodiimide in the presence of a catalytic amount of 4-pyrrolidinopyridine in dichloromethane[17]. The 14-O-acyl derivatives (7-10) were obtained in an average of 38% yield. Thin layer chromatography analysis showed the presence of two minor products presumably the 3'-O-acyl and the 3'-14-O-diacyl derivatives which were not isolated. Subsequent deprotection of the amino group with trifluoroacetic acid gave quantitatively the final products: DOX-ALA-NH_2 (11), DOX-HEX-NH_2 (12), DOX-UND-NH_2 (13), and DOX-ARO-NH_2 (14)

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(scheme 1)[18]. This two-steps process has been successfully applied in this laboratory to functionalised 11-desoxycortisol and hydrocortisone in 84% and 80% yield respectively.

EXPERIMENTAL

Proton NMR spectra were recorded on a Bruker WP-80 (80 MHz) or a General Electric GN 300 (300 MHz) instrument. The following abbreviations have been used: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; dt, doublet of triplets; q, quadruplet; and m, multiplet. Chemical shifts are reported in δ values relative to tetramethysilane (TMS) or chloroform as internal standard. Thin-layer chromatography (TLC) was performed on 0.25 mm Silica Gel plates (Sigma, T 6770). Preparative TLC was performed on 1 mm Silica Gel 60A, 20 x 20 cm plates (Whatman, 4881 840). For flash chromatography, Merck-Kiesel gel 60(230-400 mesh A.S.T.M.) was used. All solvents used in chromatography had been distilled. Unless otherwise noted, starting material and reactant were obtained commercially and were used as such or purified by standard means. Dichloromethane was distilled over phosphorus pentoxide. Anhydrous reactions were performed under an inert atmosphere, the set-up assembled and cooled under nitrogen. Organic solutions

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were dried over sodium sulfate, evaporated on a rotatory evaporator and under reduced pressure.

General Procedure for the Protection of Amino Acids:

N-(p-Anisyldiphenylmethyl) ß-alanine

p-Anisyldiphenylmethyl chloride (1.23 g, 4 mmol) was added in portions with continuous mixing to a solution of ß-alanine (300 mg, 3.36 mmol) in a mixture of water (1.3 ml), diethylamine (1 ml) and isopropanol (2.7 ml). The addition was accomplished within 2 h. The reaction mixture was stirred at 25°C for 15 h. Then, 20 ml of water was added to the reaction mixture. The resulting solution was cooled at 0°C for 30 min and acidified with a cold solution of acetic acid in water (3.3 mmol). Then, the aqueous phase was diluted with more water (30 ml) and extracted with ether (1 x 50 ml and 3 x 20 ml). The ethereal phase was finally washed with water (6 x 20 ml), dried, filtrated and concentrated to a viscous liquid. The crude material was purified by flash chromatography (hexanes:ether, 1:1 and hexanes:acetone, 1:1) to give 670 mg, 55% of N-(p-anisyldiphenylmethyl) ß-alanine. ^{1}H-NMR (δ ppm): 7.3 (12H, m, aromatic protons), 6.73 (2H, d, J = 9.3 Hz, aromatic protons), 6.08 (2H, massive, -N$\underline{H}$CH$_2$CH$_2$CO$_2\underline{H}$), 3.77 (3H, s, -OC$\underline{H}_3$), 2.52 (4H, t, J = 3.2 Hz, -C$\underline{H}_2$C$\underline{H}_2$-).

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6-[N-(p-Anisyldiphenylmethyl) amino] hexanoic acid

^{1}H-NMR (δ ppm): 7.1-7.5 (10H, m, aromatic protons), 7.36 and 6.8 (4H, two d, J = 8.9 Hz, para substituted phenyl group), 5.5 (2H, massive, -N<u>H</u>-$(CH_2)_5$-CO_2<u>H</u>), 3.76 (3H, s, -O<u>CH$_3$</u>), 2.29 (2H, d, J = 7.4 Hz, -NHC<u>H$_2$</u>-), 2.1 (2H, d, J = 7.0 Hz, -C<u>H$_2$</u>CO_2H), 1.56, 1.49 and 1.33 (6H, three m, -NH-CH_2C<u>H$_2$</u>C<u>H$_2$</u>C<u>H$_2$</u>CH_2-CO_2H).

11-[N-(p-Anisyldiphenylmethyl)amino]undecanoic acid

^{1}H-NMR (δ ppm): 7.1-7.5 (10H, m, aromatic protons), 7.36 and 6.8 (4H, two d, J = 8.9 Hz, para substituted phenyl group), 6.65 (2H, massive, -N<u>H</u>-$(CH_2)_{10}$-CO_2<u>H</u>), 3.76 (3H, s, -O<u>CH$_3$</u>), 2.3 (2H, d, J = 7.4 Hz, -NHC<u>H$_2$</u>-), 2.15 (2H, d, J = 7.0 Hz, -C<u>H$_2$</u>CO_2H), 1.6 (2H, m, -NHCH_2C<u>H$_2$</u>-), 1.47 (2H, m, -C<u>H$_2$</u>CH_2-CO_2H), 1.26 and 1.22 (12H, two broad s, -NHCH_2CH_2-(C<u>H$_2$</u>)$_6$-CH_2CH_2-CO_2H).

4-[N-p-Anisyldiphenylmethyl)aminomethyl] benzoic acid

^{1}H-NMR (δ ppm): 7.1-7.6 (10H, m, aromatic protons), 7.1 - 7.6 (2H, massive, -N<u>H</u>- and -COO<u>H</u>), 8.07 and 7.53 (4H, two d, J = 8.8 Hz, para substituted benzoic acid), 7.45 and 6.84 (4H, two d, J = 8.8 Hz, para substituted anisyl group), 3.79 (3H, s, -O<u>CH$_3$</u>), 3.42 (2H, s, -NHC<u>H$_2$</u>-).

<u>General Procedure for Esterification of 14-OH of N-(trifluoroacetyl) Doxorubicin</u>:

N-(Trifluoroacetyl) doxorubicin

14-O-[3'(N-(p-anisyldiphenylmethyl) amino)] propanoate

A solution of N-(trifluoroacetyl) doxorubicin (44.7 mg, 6.99 x 10^{-5} mol), N-(p-anisyldiphenylmethyl) ß-alanine (31 mg, 8.58 x 10^{-5} mol), DCC (17.3 mg, 8.38 X 10^{-5} mol) and PPy (0.5 mg, 3.3 x 10^{-6} mol) in dry dichloromethane (10 ml) was stirred at 25°C for 24 h. Afterwards, the reaction mixture was diluted with dichloromethane (10 ml) and ether (30 ml). The resulting solution washed successively with sodium bicarbonate (2 x 15 ml, 5% aqueous) and water (3 x 20 ml). The organic phase was dried, filtrated and concentrated to a crude material. The residue was purified by preparative TLC (dichloromethane:methanol, 95:5, Rf 0.22 = starting material, Rf 0.46 = final product) to give 18 mg, 40% of starting material along with 21 mg, 30.5% of the desired ester. Corrected yield for N-(trifluoroacetyl) doxorubicin 15-[3'-(N-(p-anisyldiphenylmethyl)amino)] propanoate =51%.

N.B. other secondary materials were pesent but not isolated.

^{1}H-NMR (δ ppm): 13.99 (1H, s, 6-OH), 13.2 (1H, s, 11-OH), 8.02 (1H, d, J = 7.6 Hz, 1-H), 7.78 (1H, t apparent, J = 8.1 Hz, 2-H), 7.1-7.5 (10H, m, aromatic protons), 7.35 and 6.81 (4H, two d, J = 8.8 Hz, para substituted phenyl group), 6.43 (1H, d, J = 8.4 Hz, -N<u>H</u>COCF$_3$), 5.50

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(1H, d, J = 2.8 Hz, 1'-H), 5.26 (1H, s, 7-H), 5.16 (1H, s, 9-OH), 4.76 (2H, s, 14-H), 4.22 (1H, q apparent, J = 6.2 Hz, 5'-H), 4.08 (3H, s, 4-OCH_3), 3.78 (3H, s, -$OC\underline{H}_3$), 3.26 and 2.96 (2H, two d, parts A and B of an AB system, J_{AB} = 18.9 Hz, 10-H), 2.67 (2H, t, J = 6.9 Hz, -$NHC\underline{H}_2$-), 2.44 (2H, t, J = 6.9 Hz, -$C\underline{H}_2CO_2$-), 2.32 and 2.19 (2H, d apparent, J = 14.7 Hz and dd apparent, J = 14.7 and J = 3.9 Hz, 8-H), 1.9 (2H, m, 2'-H), 1.17 (3H, d, J = 6.5 Hz, 6'-H).

N-(Trifluoroacetyl) doxorubicin 14-O-[6'-(N-(p-anisyldiphenylmethyl) amino)] hexanoate

^{1}H-NMR (δ ppm): 13.99 (1H, s, 6-OH), 13.2 (1H, s, 11-OH), 8.03 (1H, d, J = 7.6 Hz, 1-H), 7.78 (1H, t apparent, J = 8.1 Hz, 2-H), 7.1 - 7.5 (10H, m, aromatic protons), 7.36 and 6.81 (4H, two d, J = 8.8 Hz, para substituted phenyl group), 6.34 (1H, d, J = 6.8 Hz, -$N\underline{H}COCF_3$), 5.58 (1H, d, J = 2.8 Hz, 1'-H), 5.29 (1H, s, 7-H), 5.15 (1H, s, 9-OH), 4.76 (2H, s, 14-H), 4.22 (1H, q apparent, J = 6.8 Hz, 5'-H), 4.08 (3H, s, 4-OCH_3), 3.78 (3H, s, -$OC\underline{H}_3$), 3.28 and 2.99 (2H, two d, parts A and B of an AB system, J_{AB} = 18.9 Hz, 10-H), 2.43 (2H, dt, J = 7.4 and J = 1.5 Hz, -$NHC\underline{H}_2$-), 2.33 and 2.2 (2H, d apparent, J = 14.7 Hz and dd apparent, J = 14.7 and 4.0 Hz, 8-H), 2.12 (2H, t, J = 6.8 Hz, -$C\underline{H}_2CO_2$-), 1.93 (2H, m, 2'-H), 1.64, 1.52 and 1.36 (6H, three m, -NH-$CH_2C\underline{H}_2C\underline{H}_2C\underline{H}_2CH_2$-$CO_2$-), 1.18 (3H, d, J = 6.5 Hz, 6'-H).

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N-(Trifluoroacetyl) doxorubicin 14-O-[11'-(p-anisyldiphenylmethyl) amino)] undecanoate

^{1}H-NMR (δ ppm): 14.0 (1H, s, 6-OH), 13.22 (1H, broad s, 11-OH), 8.02 (1H, d, J = 7.6 Hz, 1-H), 7.78 (1H, t apparent, J = 8.2 Hz, 2-H), 7.1 - 7.5 (10H, m, aromatic protons), 7.36 and 6.8 (4H, two d, J = 8.6 Hz, para substituted phenyl group), 6.4 (1H, d, J = 7.2 Hz, -N<u>H</u>COCF$_3$), 5.58 (1H, s, 1'-H), 5.29 (1H, s, 7-H), 5.16 (1H, s, 9-OH), 4.77 (2H, s, 14-H), 4.24 (1H, q apparent, J = 6.2 Hz, 5'-H), 4.08 (3H, s, 4-OCH$_3$), 3.78 (3H, s, -OC<u>H</u>$_3$), 3.28 and 2.99 (2H, two d, parts A and B of an AB system, J_{AB} = 18.9 Hz, 10-H), 2.44 (2H, t, J = 7.3 Hz, -NHC<u>H</u>$_2$-), 2.32 and 2.2 (2H, d apparent, J = 14.7 Hz, and dd apparent, J = 14.7 and J = 3.9 Hz, 8-H), 2.1 (2H, t, J = 6.8 Hz, -C<u>H</u>$_2$CO$_2$-), 1.9 (2H, m, 2'-H), 1.66, 1.46, 1.29 and 1.24 (16H, four m, -NH-CH$_2$-(C<u>H</u>$_2$)$_8$CH$_2$-CO$_2$-), 1.19 (3H, d, J = 6.4 Hz, 6'-H).

N-(Trifluoroacetyl) doxorubicin 14-O-[4'-(N-(p-anisyldiphenylmethyl) aminomethyl)] benzoate

^{1}H-NMR (δ ppm): 14.04 (1H, s, 6-OH), 13.26 (1H, broad s, 11-OH), 8.07 and 7.55 (4H, two d, J = 7.0 Hz, para substituted benzoate ester), 8.03 (1H, partly hidden d, J = 7.6 Hz, 1-H), 7.79 (1H, t apparent, J = 8.2 Hz, 2-H), 7.1 - 7.5 (10H, m, aromatic protons), 7.36 and 6.84 (4H,

two d, J = 7.7 Hz, para substituted anisyl group), 6.56 (1H, d, J = 7.2 Hz, -N<u>H</u>COCF$_3$), 5.68 (1H, s, 1'-H), 5.38 (1H, s, 7-H), 5.35 (1H, s, 9-OH), 4.79 (2H, s, 14-H), 4.35 (1H, q apparent, J = 7.0 Hz, 5'-H), 4.09 (3H, s, 4-OCH$_3$), 3.79 (3H, s, -OC<u>H$_3$</u>), 3.43 (2H, s, -C<u>H$_2$</u>NH-), 3.32 and 3.03 (2H, two d, parts A and B of an AB system, J_{AB} = 18.9 Hz, 10-H), 2.36 and 2.2 (2H, two d apparent, J = 15.5 Hz, 8-H), 1.26 (3H, d, J = 5.2 Hz, 6'-H).

DOX-ALA-NH$_2$, DOX-HEX-NH$_2$, DOX-UND-NH$_2$ and DOX-ARO-NH$_2$

Typical procedure

A solution of protected amine (2 x 10^{-6} mol) in dry dichloromethane (2 ml) was treated with an excess of TFA (2 x 10^{-5} mol) at 25°C, under nitrogen. The reaction mixture was stirred 10 min. Then, the solvent was evaporated. The crude residue was used as such for conjugation or for microcytotoxicity assay. DOX-ALA-NH$_2$: ^{1}H-NMR (DMSO-d$_6$, δ ppm): 14.04 (1H, s, 6-OH), 13.26 (1H, s, 11-OH), 9.4 (1H, d, J = 7.6 Hz, -N<u>H</u>COCF$_3$), 7.92 (4H, m, 1-H, 3-H and -NH$_2$), 7.66 (1H, t apparent, J = 4.7 Hz, 2-H), 5.55 (1H, s, 1'-H), 5.37 (1H, s, 7-H), 5.02 (1H, s, 9-OH), 4.6 (2H, s, 14-H), 4.48 (1H, q apparent, J = 6.7 Hz, 5'-H), 3.98 (3H, s, 4-OCH$_3$), 3.06 (2H, m, -C<u>H$_2$</u>-NH$_2$), 1.06 (3H, d, J = 6.5 Hz, 6'-H).

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ABBREVIATIONS

DCC = N,N-Dicyclohexylcarbodiimide

DOX-ALA-NH_2 = N-(Trifluoroacetyl) doxorubicin 14-O-(3'-amino) propanoate

DOX-HEX-NH_2 = N-(Trifluoroacetyl) doxorubicin 14-O-(6'-amino) hexanoate

DOX-UND-NH_2 = N-(Trifluoroacetyl) doxorubicin 14-O-(11'-amino) undecanoate

DOX-ARO-NH_2 = N-(Trifluoroacetyl) doxorubicin 14-O-(4'-aminomethyl) benzoate

PPy = 4-Pyrrolidinopyridine

TFA = Trifluoroacetic acid

TLC = Thin layer chromatography

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