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Synthesis of Actinomycin D Analogs: XXIII.[1] Actinocin Derivatives Containing Azacrown Fragments

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Abstract—A number of actinocin amides containing residues of aza-15-crown-5 and aza-18-crown-6 where crown fragments were separated from the heterocyclic chromophore by the residues of ω-amino acids were obtained as actinomycin D models.

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Earlier [2] we showed that actinomycin D was able to complexation with Na^+ cations, similarly to crown compounds. We ascribed this ability of actinomycin D to the structure of its pentapeptidelactone rings consisting of alkylaminoacids. Thereupon as model structures for actinomycin D we designed and synthesized actinocin derivatives, containing radicals of benzo-15-crown-5 and benzo-18-crown-6, connected either directly to phenoxazinee chromophore [3], or via spacers of various length [1]. Studying a way of binding of such compounds with DNA [4, 5] and their antineoplastic properties [6, 7] has shown that they depend both on structure of crown fragment and on its position in a drug molecule. Among such compounds there can be potential anticancer drugs, while studying of their interaction with DNA can clarify the mode of binding of such compounds to DNA [8–11].

The key intermediates in the synthesis of substituted actinocin amides are the corresponding amides of 2-amino-3-hydroxy-4-X-benzoic acids. In our case they are {[aza-15(18)-crown-5(6)]-ylaminocarbamoyl}-2-amino-3-hydroxy-4-methylbenzamids (**Ia–If)**. The most convenient approach to such compounds is catalytic reduction of the corresponding 2-nitro-3-benzyloxy-4X-benzamides. In the synthesis of *N*-{[benzo-18(15)-crown-6(5)]-4'-ylaminocarbamoyl}alkinyl-2-nitro-3-benzyloxy-4-methylbenz-amides [1] we started from 2-nitro-3-benzyloxy-4-methylbenzoyl chloride [12] and hydrochloride of an appropriate amino acid ester. Obtained *N*-alkoxycarbonylalkyl-2-nitro-3-benzyloxy-4-methylbenzamides were converted into the corresponding acyl azides and coupled with aminobenzocrowns.

At such approach in a stage of azide synthesis (in case of amino-benzocrowns) the yields seldom exceeded 60–70%, and due to inaccessibility of intermediate compounds and complexity of removal of nonreacted starting material the use of considerable excess of one of the components to achieve higher yields was not possible. To avoid these obstacles in this work we obtained compounds **IIa–IIf** from 2-nitro-3-benzyloxy-4-methyl-benzamides **Ia–Ie**, which, in turn, were prepared using Schotten–Baumann procedure from the corresponding amino acids and 2-nitro-3-benzyloxy-4-methylbenzoyl chloride.

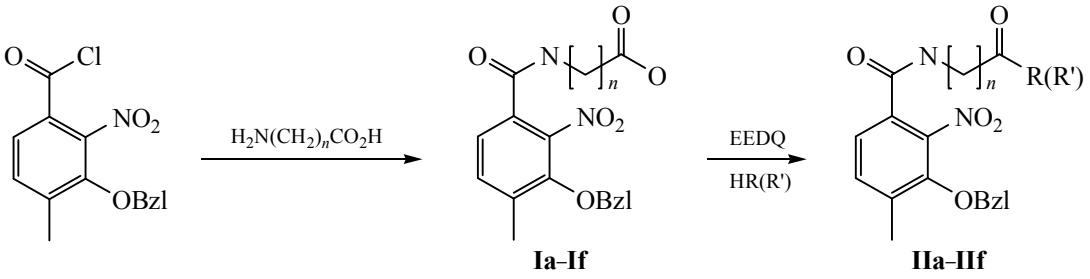
Compounds **Ia–Ie** were subjected to condensation with the corresponding aza-crown ethers using *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) [12] (Scheme 1). Structures of the obtained compounds are shown in Scheme 3.

Compounds **IIa–IIf** have been catalytically reduced to the corresponding 2-amino-3-hydroxy benzamides **IIIa–IIIf** which upon oxidation with *p*-quinone led to the target compounds **IVa–IVf** (Scheme 2).

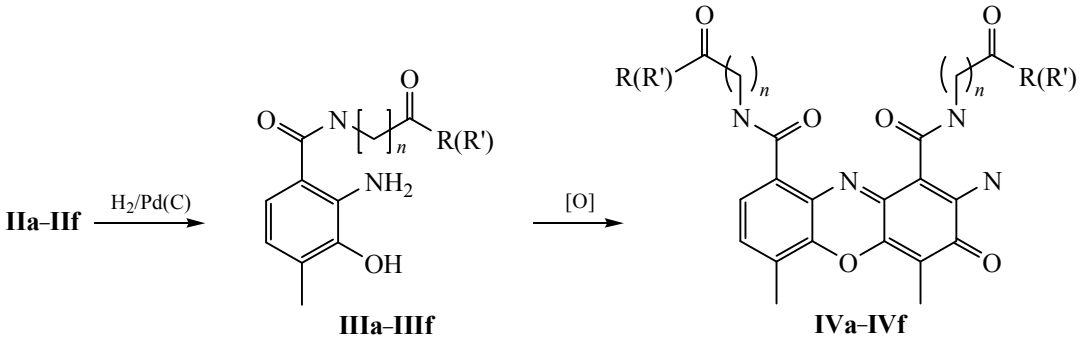
[1] For communication XXII, see [1].

† Deceased.

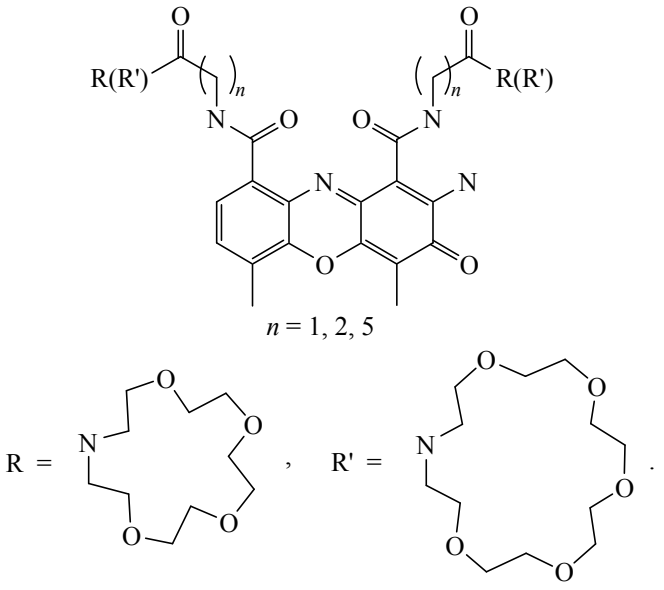
Scheme 1.



Scheme 2.



Scheme 3.



EXPERIMENTAL

^{1}H NMR spectra of compounds **IIa–IIf** and **IVa–IVf** were recorded on a Bruker WM400 (400 MHz) instrument in DMSO–d_6 or $CDCl_3$. Melting points were determined on a Koefler model HMK apparatus and were published without correction. TLC analyses were carried out on Merck silicagel 60 F_{254} plates using the following systems: methanol–chloroform, 1:4 (A), methanol–chloroform–3% aqueous ammonia solution (B), ethyl acetate–hexane, 1:1 (C). Commercial reagents were used as obtained, while solvents were additionally purified by known techniques and stored over molecular sieves 4 Å in the darkness.

***N*-[(1-Aza-15-crown-5)-yl-carbamoyl]methyl-2-nitro-3-benzyloxy-4-methylbenzamide (IIa).** To a

stirred suspension of 0.172 g (0.5mmol) of *N*-glycyl-(4-methyl-3-benzyloxy-2-nitro)benzamide in 3ml of anhydrous dioxane under argon at ambient temperature 0.252 g (0.55 mmol) of EEDQ was added in one portion, followed by a solution of 0.110 g (0.5 mmol) of 1-aza-15-crown-5 in 2ml of anhydrous dioxane. The reaction mixture was stirred till complete disappearance of **Ia** (monitored by TLC in system A). After removing volatiles *in vacuo* on a rotary evaporator the residue was crystallized from an ethyl acetate-hexane mixture to give **IIa** as a white solid chromatographically homogenous according to TLC (system A). Yield 0.234 g (86 %), mp, 154°C. ^{1}H NMR spectrum, δ, ppm: 2.31 s (3H, Ar–CH_3), 3.52 s (2H, CO–CH_2–NH), 3.54–4.32 br.m (16H, OCH_2CH_2O), 4.45–4.65 br.m (4H, NCH_2CH$_2$O), 5.21 s (2H, O–CH_2–Ph), 7.25–7.42 br.m (5H, Ar–H, 2H, Ar–H). Found, %: C 59.63, 59.78; H 6.65, 6.72; N 7.57, 7.64. $C_{27}H_{35}N_3O_9$. Calculated, %: C 59.44; H 6.47; N 7.70.

***N*-[(1-Aza-15-crown-5)-yl-carbamoyl]ethyl-2-nitro-3-benzyloxy-4-methylbenzamide (IIb)** was obtained similarly to **IIa** from 0.179 g (0.5 mmol) of *N*-β-alanyl-(4-methyl-3-benzyloxy-2-nitro)benzamide and 0.110 g (0.5mmol) of 1-aza-15-crown-5. Yield 0.258 g (92%), chromatographically homogenous according to TLC (systems A, B), mp 162°C. ^{1}H NMR spectrum, δ, ppm: 2.33 s (3H, Ar–CH_3); 2.71 t (2H, COCH$_2$CH_2N); 3.08 t (2H, COCH_2CH$_2$N); 3.48–4.30 br.m (16H, OCH_2CH_2O); 4.43–4.64 br.m (4H, NCH_2CH$_2$O); 5.23 s (2H, O–CH_2–Ph) 7.26–7.38 m (5H, Ar–H); 7.41, 7.43 d (2H, Ar–H) Found, %: C 60.23, 60.41; H 6.94, 7.12; N 7.28, 7.35. $C_{28}H_{37}N_3O_9$. Calculated, %: C 60.10; H 6.66; N 7.51.

***N*-[(1-Aza-15-crown-5)-yl-carbamoyl]penthyl-2-nitro-3-benzyloxy-4-methylbenzamide (IIc)** was obtained similarly to **IIa** from 0.2 g (0.5 mmol) of *N*-ε-caproyl-(4-methyl-3-benzyloxy-2-nitro)benzamide and 0.110 g (0.5 mmol) of 1-aza-15-crown-5. Yield 0.264 g (88%), chromatographically homogenous according to TLC (systems A, B), mp 158°C. ^{1}H NMR spectrum, δ, ppm: 2.15 m (2H, CH_2N); 2.31 s (3H, Ar–CH_3), 3.12 m (2H, CH$_2$ CH_2CH$_2$); 3.25 m (2H, CH$_2$CH_2CH$_2$); 3.34 m (2H, CH$_2$CH_2CH$_2$); 3.47 m (2H, COCH_2); 3.5–4.32 br.m (16H, OCH_2CH_2O); 4.42–4,61 br.m (4H, NCH_2CH$_2$O); 5.24 s (2H, O–CH_2–Ph) 7.27–7.39 m (5H, Ar–H); 7.4, 7.43 d (2H, Ar–H). Found, %: C 61.63, 61.79; H 7.24, 7.32; N 7.18, 7.35. $C_{31}H_{43}N_3O_9$. Calulated, %: C 61.88; H 7.20; N 6.98.

***N*-[(1-Aza-18-crown-6)-yl-carbamoyl]methyl-2-nitro-3-benzyloxy-4-methylbenzamide (IId)** was obtained similarly to **IIa** from 0.172 g (0.5 mmol) of *N*-glycyl-(4-methyl-3-benzyloxy-2-nitro)benzamide and 0.132 g (0.5 mmol) of 1-aza-18-crown-6. Yield 0.251 g (85%), chromatographically homogenous according to TLC (systems A, B), mp 161°C. ^{1}H NMR spectrum, δ, ppm: 2.34 s (3H, Ar–CH_3); 3.5 br.s (2H, CO–CH_2–NH); 3.54–4.56 br.m (20H, OCH_2CH_2O); 4.60–4.72 br.m (4H, NCH_2CH$_2$O); 5.25 s (2H, O–CH_2–Ph); 7.25–7.38 m (5H, Ar–H); 7.41, 7.43 d (2H, Ar–H). Found, %: C 59.71, 59.84; H 7.44, 7.53; N 7.16, 7.21. $C_{29}H_{39}N_3O_{10}$. Calculated, %: C 60.08; H 7.32; N 6.78.

***N*-[(1-Aza-18-crown-6)-yl-carbamoyl]ethyl-2-nitro-3-benzyloxy-4-methylbenzamide (IIe)** was obtained similarly to **IIa** from 0.129 g (0.5 mmol) of *N*-β-alanyl-(4-methyl-3-benzyloxy-2-nitro)benzamide and 0.132 g (0.5 mmol) of 1-aza-18-crown-6. Yield 0.253 g (84%), chromatographically homogenous according to TLC (systems A, B), mp 171°C, ^{1}H NMR spectrum, δ, ppm: 2.32 s (3H, Ar–CH_3); 2.69 t (2H, COCH$_2$CH_2N); 3.04 t (2H, COCH_2CH$_2$N); 3.44–4.51 br.m (20H, OCH_2CH_2O); 4.56–4.59 br.m (4H, NCH_2CH$_2$O); 5.26 s (2H, O–CH_2–Ph) 7.25–7.36 m (5H, Ar–H); 7.4, 7.42 d (2H, Ar–H). Found, %: C 60.38, 60.51; H 7.72, 7.81; N 6.30, 6.46. $C_{30}H_{41}N_3O_{10}$. Calculated, %: C 60.65; H 7.48; N 6.63.

***N*-[(1-Aza-18-crown-6)-yl-carbamoyl]penthyl-2-nitro-3-benzyloxy-4-methylbenzamide (IIf)** was obtained similarly to **IIa** from 0.2 g (0.5 mmol) of *N*-ε-caproyl-(4-methyl-3-benzyloxy-2-nitro)benzamide and 0.132 g (0.5 mmol) of 1-aza-18-crown-6. Yield 0.297 g (92%), chromatographically homogenous according to TLC (systems A, B), mp 169°C, ^{1}H NMR spectrum, δ, ppm: 2.18 m (2H, CH_2N); 2.35 s (3H, Ar–CH_3), 3.11 m (2H, CH$_2$CH_2CH$_2$); 3.24 m (2H, CH$_2$CH_2CH$_2$); 3.31 m (2H, CH$_2$CH_2CH$_2$); 3.45 m (2H, COCH_2); 3.52–4.57 br.m (20H, OCH_2CH_2O); 4.58–4.71 br.m (4H, NCH_2CH$_2$O); 5.22 s (2H, O–CH_2–Ph) 7.26–7.40 m (5H, Ar–H); 7.39, 7.42 d (2H, Ar–H). Found, %: C 61.82, 61.95; H 8.21, 8.33; N 6.02, 6.15. $C_{33}H_{47}N_3O_{10}$. Calculated, %: C 62.20; H 7.90; N 6.22.

2-Amino-*N*,*N*'-bis-[(1-aza-15-crown-5)-yl-carbamoyl]methyl-4,6-dimethyl-3*H*-3-oxophenoxazine-1,9-dicarboxyamide (IVa). A solution of 0.055 g (0.1 mmol) of **IIa** in 5 ml of methanol was hydrogenated over Pd catalyst (5% Pd/C) at room temperature and 1 bar pressure till complete reduction to the corresponding 2-amino-3-hydroxy-4-metyl

benzoic acid **IIIa** derivative (TLC monitoring, system A). The catalyst was filtered off, washed with a little portion of methanol and to thus obtained clear filtrate a solution of 0.016 g (0.15 mmol) of *p*-quinone in 2 ml of methanol was added in one portion. After keeping in darkness for 15 h, the volatiles were removed *in vacuo* on a rotary evaporator and the residue was redissolved in 2 ml of acetonitrile and applied on a chromatographic column (2×4 cm, Merck Kieselgel 60, 40–63 μm). After washing with acetonitrile to remove colored impurities, the eluent was changed for a chloroform–methanol mixture (4 : 1) to elute the product. Fractions containing **IVa** (TLC control, system A) were collected, volatiles were removed *in vacuo* on a rotary evaporator, and the residue was crystallized from an ethyl acetate–hexane mixture to afford **IVa**. Yield 0.037 g (87%), chromatographically homogenous according to TLC (systems A, B), mp 184°C, ^{1}H NMR spectrum, δ, ppm: 1.66 s (3H, Ar–CH_3); 2.58 s (3H, Ar–CH_3); 3.26 s (4H, COCH_2N); 3.45–3.91 br.m (16H, OCH_2CH_2O); 4.43–4.62 br.m (4H, NCH_2CH$_2$O); 7.24, 7.33 d (2H, Ar–H). Found, %: C 56.52, 56.63; H 6.84, 6.91; N 9.64, 9.72. $C_{40}H_{56}N_6O_{14}$, Calculated, %: C 56.86; H 6.68; N 9.95.

2-Amino-*N,N′*-bis-[(1-aza-15-crown-5)-yl-carbamoyl]ethyl-4,6-dimethyl-3*H*-3-oxophenoxazine-1,9-dicarboxyamid (IVb) was obtained similarly to **IVa** from 0.056 g (0.1 mmol) of **IIb**, Yield 0.034 g (79%), chromatographically homogenous according to TLC (systems A, B), mp 191°C, ^{1}H NMR spectrum, δ, ppm: 1.64 s (3H, Ar–CH_3); 2.53 s (3H, Ar–CH_3); 2.76 t (2H, COCH$_2$CH_2N); 3.11 t (2H, COCH_2CH$_2$N); 3.44–3.93 br.m (16H, OCH_2CH_2O); 4.46–4.67 br.m (4H, NCH_2CH$_2$O); 7.22, 7.32 d (2H, Ar–H). Found, %: C 57.52, 57.59; H 7.03, 7.11; N 9.31, 9.45. $C_{42}H_{60}N_6O_{14}$. Calculated, %: C 57.79; H 6.93; N 9.63.

2-Amino-*N,N′*-bis-[(1-aza-15-crown-5)-yl-carbamoyl]penthyl-4,6-dimethyl-3*H*-3-oxophenoxazine-1,9-dicarboxyamid (IVc) was obtained similarly to **IVa** from 0.060 g (0.1 mmol) of **IIc**. Yield 0.043 g (89%), chromatographically homogenous according to TLC (systems A, B), mp 187°C, ^{1}H NMR spectrum, δ, ppm: 1.68 s (3H, Ar–CH_3); 2.05 m (2H, CH_2N); 2.29 s (3H, Ar–CH_3); 3.11 m (2H, CH$_2$ CH_2CH$_2$); 3.23 m (2H, CH$_2$CH_2CH$_2$); 3.36 m (2H, CH$_2$CH_2CH$_2$); 3.44 m (2H, COCH_2); 3.46–4.25 br.m (16H, OCH_2CH_2O); 4.48–4.71 br.m (4H, NCH_2CH$_2$O); 7.18, 7.34 d (2H, Ar–H). Found, %: C 59.92, 60.03; H 7.83, 7.91; N 8.52, 8.65. $C_{48}H_{72}N_6O_{14}$ Calculated, %: C 60.24; H 7.58; N 8.78.

2-Amino-*N,N′*-bis-[(1-aza-18-crown-6)-yl-carbamoyl]methyl-4,6-dimethyl-3*H*-3-oxophenoxazine-1,9-dicarboxyamid (IVd) was obtained similarly to **IVa** from 0.059 g (0.1 mmol) of **IId**. For chromatographic purification a 1:1 mixture of chloroform–methanol was used. Yield 0.039 g (83%), chromatographically homogenous according to TLC(systems A, B), mp 193°C, ^{1}H NMR spectrum, δ, ppm: 1.62 s (3H, Ar–CH_3); 2.49 s (3H, Ar–CH_3); 3.18 br.s (4H, COCH_2N); 3.38–4.46 br.m (20H, OCH_2CH_2O); 4.61–4.75 br.m (4H, NCH_2CH$_2$O); 7.25, 7.36 d (2H, Ar–H). Found, %: C 56.35, 56.47; H 7.09, 7.18; N 8.79, 8.83. $C_{44}H_{64}N_6O_{16}$. Calculated, %: C 56.64; H 6.91; N 9.01.

2-Amino-*N,N′*-bis-[(1-aza-18-crown-6)-yl-carbamoyl]ethyl-4,6-dimethyl-3*H*-3-oxophenoxazine-1,9-dicarboxyamid (IVe) was obtained similarly to **IVa** from 0.060 g (0.1 mmol) of **IIe**. For chromatographic purification a 1:1 mixture of chloroform–methanol was used. Yield 0.044 g (91%), chromatographically homogenous according to TLC (systems A, B), mp 203°C, ^{1}H NMR spectrum, δ, ppm: 1.65 s (3H, Ar–CH_3); 2.47 s (3H, Ar–CH_3); 2.64 br.t (2H, COCH$_2$CH_2N); 3.07 br.t (2H, COCH_2CH$_2$N); 3.41–4.52 br.m (20H, OCH_2CH_2O); 4.58–4.72 br.m (4H, NCH_2CH$_2$O,); 7.23, 7.35 d (2H, Ar–H). Found, %: C 57.23, 57.31; H 7.17, 7.24; N 8.45, 8.56. $C_{46}H_{68}N_6O_{16}$. Calculated, %: C 57.49; H 7.13; N 8.74.

2-Amino-*N,N′*-bis-[(1-aza-18-crown-6)-yl-carbamoyl]penthyl-4,6-dimethyl-3*H*-3-oxophenoxazine-1,9-dicarboxyamid (IVf) was obtained similarly to **IVa** from 0.065 g (0.1 mmol) of **IIf**. For chromatographic purification a 1:1 mixture of chloroform–methanol was used. Yield 0.042 g (81%), chromatographically homogenous according to TLC (systems A, B), mp 198°C, ^{1}H NMR spectrum, δ, ppm: 1.66 s (3H, Ar–CH_3); 2.01 m (2H, CH_2N); 2.45 s (3H, Ar–CH_3); 3.08 m (2H, CH$_2$CH_2CH$_2$); 3.18 m (2H, CH$_2$CH_2CH$_2$); 3.34 m (2H, CH$_2$CH_2CH$_2$); 3.43 m (2H, COCH_2); 3.40–4.56 br.m (20H, OCH_2CH_2O); 4.61–4.75 br.m (4H, NCH_2CH$_2$O); 7.21, 7.34 d (2H, Ar–H). Found, %: C 59.53, 59.62; H 7.85, 7.98; N 7.81, 7.86. $C_{52}H_{80}N_6O_{16}$. Calculated, %: C 59.75; H 7.71; N 8.04.

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