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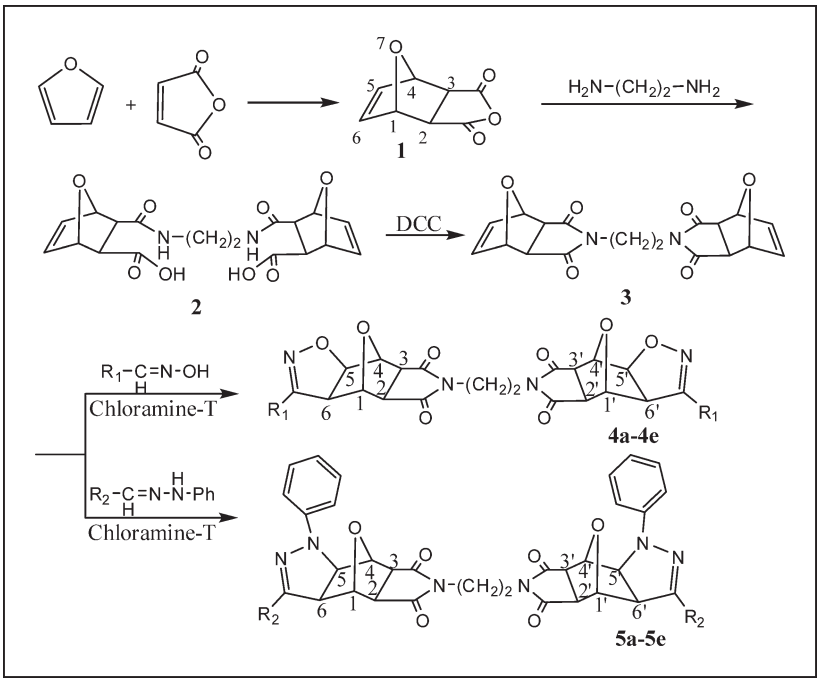

1 2 3 4a-4e 5a-5e

We use one molecule of ethylene diamine as a connecting arm to combine two molecules of 5,6-dehydronorcantharidin. Then, ten novel norcantharidin derivatives were synthesized in a single step by the [3 + 2] 1,3-dipolar cycloaddition reaction with oxime or hydrazone in the presence of chloramine-T, which is simpler than the conventional method.

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INTRODUCTION

Cantharidin (CAN,exo,exo-2,3-dimethyl-7-oxabicyclo [2.2.1] heptane-2,3-dicarboxylic acid anhydride, Fig. 1) is an active ingredient of Chinese blister beetles Mylabris [1], which has been used in China as a medicinal agent for the treatment of cancer, in particular to hepatoma [2]. Recently, cantharidin has been found active in cervical, tongue, ginival, bone, leukaemia, ovarian, and colon cancer cells [3]. However, the renal toxicity of this drug has limited its application [4]. Norcantharidin (NCTD, the demethylated cantharidin derivative, Fig. 1) appeared to improve the awkward side of cantharidin, making the drug safer in application. It was recently found to be capable of inducing apoptosis in human cervical, tongue, ginival, mucoepidermoid carcinoma, adenocystic carcinoma, neuroblastoma, bone, leukaemia, ovarian, and colon cancer cell lines [5]. We have recently referred to all the known Cantharidin SAR data, briefly, no modification of the bicyclo [2.2.1] skeleton is permissible, the 7-oxa bridge are required to maintain activity, the presence of double bond (5,6-ene) has little effect on activity [6–10].

Isoxazoline and pyrazoline derivatives possess a wide range of pharmacological activities [11]. Thus, it seemed of interest to combine isoxazoline or pyrazoline with norcantharidin derivatives in a single molecule. We have successfully synthesized some compounds before [12], but the method is somewhat complex, because we have to synthesize nitrile oxide by the reaction of nitrile oxides with tert-butyl hypochloride. With our sustained interest in the synthesis of norcantharidin derivatives,

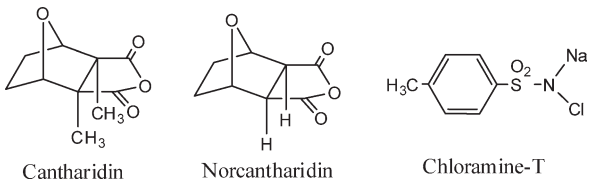

Figure 1. Chemical structures of CAN, NCTD, and chloramine-T.

we have achieved a facile 1,3-dipolar cycloaddition method by using chloramine-T (Fig. 1). Chloramine-T, which is a versatile reagent in organic synthesis [13], was used in this article for the *in situ* oxidation of oximes and hydrazones of aldehydes to generate the nitrile oxides; compared with the conventional method, the synthetic route is more facile, and the reaction rate is enhanced tremendously.

In addition, the dimer may have a character or function, which is not possessed in a single state. Therefore, we use ethylenediamine as a connecting arm to synthesize norcantharidin-dimer derivatives. Cooperating with isoxazole or pyrazole, we look forward to the compounds obtained having a good biological activity.

RESULTS AND DISCUSSION

The synthetic route of the compounds mentioned are outlined in Scheme 1. Such type of compounds (Table 1) with versatile activities may be of interest in chemistry, biochemistry, and pharmacology [14].

EXPERIMENTAL

Melting points were obtained on a B-540 Bűchi melting point apparatus and were uncorrected. ^{1}H NMR spectra were recorded on a Brucker AM-400 M Hz spectrometer with $SiMe_4$ as the internal standard in $CDCl_3$. Element analyses were performed on an EA-1110 instrument.

Nitrile oxides are of great synthetic interest because the product, isoxazolines and pyrazolines, are versatile intermediates for the synthesis of bifunctional compounds. We have carried out the [4 + 2] cycloaddition of furan with maleic anhydride to obtain 5,6-dehydronorcantharin **1**. And then, we use one molecule of ethylene diamine as a connecting arm to combine two molecules of 5,6-dehydronorcantharidin, giving compound **2**. After that, we reacted compound **2** with DCC to get compound **3**. At last, we carried out the [3 + 2] cycloaddition of **3** with oxime or hydrazone in the presence of chloramine-T. Thus, we get compounds **4a–4e** and **5a–5e** [12,15].

General procedure for the preparation of the compound 2. Ethylene diamine (10 mmol) was slowly added to a solution of compound **1** (20 mmol) in acetone (30 mL). The reaction mixture was refluxed in acetone for 8 h, and then leached. The residue was dried, giving the compound **2**.

General procedure for the preparation of the compound 3. Compound **2** (5 mmol) dissolved in DMF (15 mL), being ice-bath. When the solution was down to 0°C, DCC (10 mmol) was added. The reaction mixture was refluxed in DMF for 9 h, and then leached. The extracts were poured into ice water (50 mL), separating out crystal, then leached. The residue was dried then recrystallized from methanol to give the compound **3**.

General procedure for the preparation of the 5,6-dehydronorcantharidin-isoxazoline and 5,6-dehydronorcantharidin-pyrazoline adducts (4a–4e and 5a–5e). Chloramine-T (2.4 mmol) was added to a solution of **2** (1 mmol) and 4-fluorobenzaldehyde oxime (2 mmol) in ethanol (20 mL). The reaction mixture was refluxed in ethanol for 14 h, and then leached. The residue was dried then recrystallized from methanol to give the compound **4a**.

The synthesis of compounds **4b**–**4e** and **5a**–**5e** were performed using the same method.

Data

(1R,2S,3R,4S)-3-(2-((1R,3R,4S)-3-carboxy-7-oxabicyclo [2.2.1]hept-5-enecarboxamido)ethylcarbamoyl)-7-oxabicyclo[2.2.1]hept-5-ene-2-carboxylic acid (2). This compound was obtained as beige crystals, yield 67.5%, m.p. 97°C; 1H NMR(DMSO-d_6) δ: 8.07 (s, 2H, N—H), 5.71 (s, 4H, C_5—H, C_5'—H, C_6—H, C_6'—H), 4.77 (s, 2H, C_1—H, C_1'—H), 4.59 (s, 2H, C_4—H, C_4'—H), 3.47 (s, 4H, (CH2)2), 3.17 (s, 2H, C_3—H, C_3'—H), 3.01 (s, 2H, C_2—H, C_2'—H). Anal. Calcd. for $C_{18}H_{20}N_2O_8$: C, 55.10; H, 5.14; N, 7.14. Found: C, 55.12; H, 5.17; N, 7.13.

2,2′-(Ethane-1,2-diyl)bis[4,7-epoxy-3a,4,7,7a-tetrahydro-1H-isoindole-1,3(2H)-dione] (3). This compound was obtained as beige crystals, yield 25.5%, m.p. 219°C; ^{1}H NMR(DMSO-d_6) δ: 5.65 (s, 4H, C_5—H, C_5'—H, C_6—H, C_6'—H), 4.71 (s, 4H, C_1—H, C_1'—H, C_4—H, C_4'—H), 3.79 (s, 4H, $(CH_2)_2$), 3.11 (s, 4H, C_2—H, C_2'—H, C_3—H, C_3'—H). Anal. Calcd. for

Scheme 1

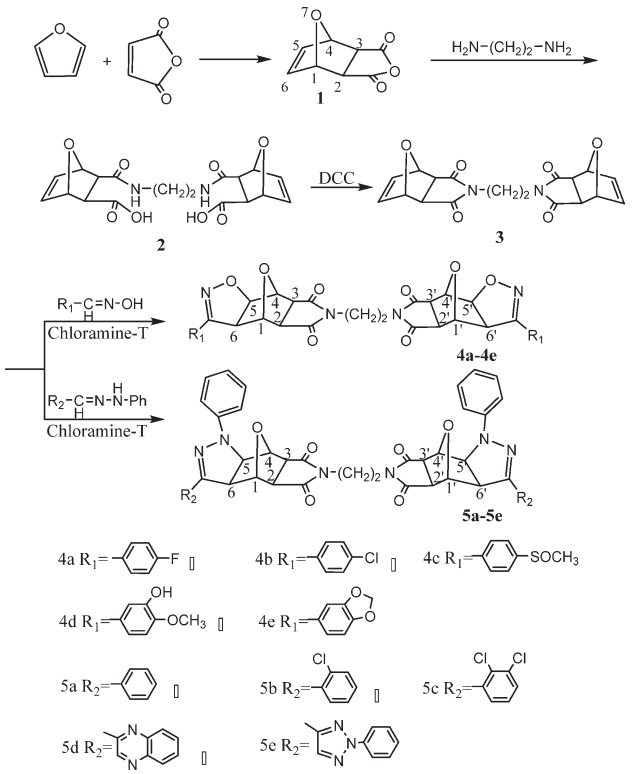

Table 1

Physical data of compounds.

Compound	R_1/R_2	Time (h)	m.p. (°C)	Yield (%)	Molecular formula	Analysis (%) calcd./found C	H	N
2	/	8	97	67.5	$C_{18}H_{20}N_2O_8$	55.10	5.14	7.14
						55.12	5.17	7.13
3	/	9	219	25.5	$C_{18}H_{16}N_2O_6$	60.67	4.53	7.86
						60.65	4.55	7.87
4a	C_6H_4F	22.5	>300	37.4	$C_{32}H_{24}N_4O_8F_2$	60.95	3.84	8.89
						60.94	3.87	8.91
4b	C_6H_4Cl	22.5	>300	51.8	$C_{32}H_{24}N_4O_8Cl_2$	57.93	3.65	8.44
						57.89	3.66	8.42
4c	$C_6H_4SOCH_3$	21	187	25.1	$C_{22}H_{22}N_4O_{10}S_2$	46.64	3.91	9.89
						46.66	3.93	9.97
4d	$C_6H_3OCH_3OH$	21.5	217	5.3	$C_{34}H_{30}N_4O_{12}$	59.47	4.40	8.16
						59.50	4.41	8.14
4e	$C_7H_5O_2$	23	184	19.0	$C_{34}H_{26}N_4O_{12}$	59.83	3.84	8.21
						59.82	3.87	8.20
5a	C_6H_5	21	207	21.6	$C_{44}H_{36}N_6O_6$	70.96	4.87	11.28
						70.94	4.88	11.26
5b	C_6H_4Cl	20	>300	18.2	$C_{44}H_{34}N_6O_6Cl_2$	64.95	4.21	10.33
						64.99	4.23	10.28
5c	$C_6H_3Cl_2$	22	>300	28.8	$C_{44}H_{32}N_6O_6Cl_4$	59.88	3.65	9.52
						59.85	3.67	9.54
5d	$C_8H_5N_2$	23	191	27.4	$C_{48}H_{36}N_{10}O_6$	67.92	4.27	16.50
						67.91	4.29	16.53
5e	$C_8H_6N_3$	22.5	203	11.7	$C_{48}H_{38}N_{12}O_6$	65.60	4.36	19.12
						65.63	4.34	19.11

$C_{18}H_{16}N_2O_6$: C, 60.67; H, 4.53; N, 7.86. Found: C, 60.65; H, 4.55; N, 7.87.

6,6′-(Ethane-1,2-diyl)bis[exo,exo-4,8-epoxy-3a,4,4a,7a,8,8a-hexahydro-3-(4-fluorophenyl)-pyrrolo[3,4-f]-1,2-benzisoxazole-5,7(1H,3aH)-dione] (4a). This compound was obtained as beige crystals, yield 37.4%, m.p. >300°C; ^{1}H NMR($CDCl_3$) δ: 7.83–7.25(m, 8H, Ar-H), 5.23 (d, J = 7.92 Hz, 2H, C_5—H, C_5'—H), 4.98 (s, 2H, C_4—H, C_4'—H), 4.76 (s, 2H, C_1—H, C_1'—H), 4.51 (d, J = 7.90 Hz, 2H, C_6—H, C_6'—H), 3.59 (d, J = 7.91 Hz, 2H, C_3—H, C_3'—H), 3.74 (s, 4H, $(CH_2)_2$), 3.38 (d, J = 7.90 Hz, 2H, C_2—H, C_2'—H). Anal. Calcd. for $C_{32}H_{24}N_4O_8F_2$: C, 60.95; H, 3.84; N, 8.89. Found: C, 60.94; H, 3.87; N, 8.91.

6,6′-(Ethane-1,2-diyl)bis[exo,exo-4,8-epoxy-3a,4,4a,7a,8,8a-hexahydro-3-(4-chlorophenyl)-pyrrolo[3,4-f]-1,2-benzisoxazole-5,7(1H,3aH)-dione] (4b). This compound was obtained as beige crystals, yield 51.8%, m.p. >300°C; ^{1}H NMR($CDCl_3$) δ: 7.74–7.06(m, 8H, Ar-H), 5.18 (d, J = 7.91 Hz, 2H, C_5—H, C_5'—H), 4.95 (s, 2H, C_4—H, C_4'—H), 4.73 (s, 2H, C_1—H, C_1'—H), 4.48 (d, J = 7.92 Hz, 2H, C_6—H, C_6'—H), 3.59 (d, J = 7.91 Hz, 2H, C_3—H, C_3'—H), 3.75 (s, 4H, $(CH_2)_2$), 3.36 (d, J = 7.90 Hz, 2H, C_2—H, C_2'—H). Anal. Calcd. for $C_{32}H_{24}N_4O_8Cl_2$: C, 57.93; H, 3.65; N, 8.44. Found: C, 57.89; H, 3.66; N, 8.42.

6,6′-(Ethane-1,2-diyl)bis[exo,exo-4,8-epoxy-3a,4,4a,7a,8,8a-hexahydro-3-(4-(methylsulfinyl)phenyl)-pyrrolo[3,4-f]-1,2-benzisoxazole-5,7(1H,3aH)-dione] (4c). This compound was obtained as beige crystals, yield 25.1%, m.p. 187°C; ^{1}H NMR($CDCl_3$) δ: 7.83–7.20(m, 8H, Ar-H), 5.24 (d, J = 7.90 Hz, 2H, C_5—H, C_5'—H), 4.98 (s, 2H, C_4—H, C_4'—H), 4.75 (s, 2H, C_1—H, C_1'—H), 4.52 (d, J = 7.90 Hz, 2H, C_6—H, C_6'—H), 3.59 (d, J = 7.91 Hz, 2H, C_3—H, C_3'—H), 3.77 (s, 4H, $(CH_2)_2$), 3.38 (d, J = 7.92 Hz, 2H, C_2—H, C_2'—H), 3.27(s, 6H, $2SOCH_3$). Anal. Calcd. for $C_{22}H_{22}N_4O_{10}S_2$: C, 46.64; H, 3.91; N, 9.89. Found: C, 46.66; H, 3.93; N, 9.87.

6,6′-(Ethane-1,2-diyl)bis[exo,exo-4,8-epoxy-3a,4,4a,7a,8,8a-hexahydro-3-(3-hydroxy-4-methoxyphenyl)-pyrrolo[3,4-f]-1,2-benzisoxazole-5,7(1H,3aH)-dione] (4d). This compound was obtained as beige crystals, yield 5.3%, m.p. 217°C; ^{1}H NMR($CDCl_3$) δ: 8.37–7.49 (m, 6H, Ar-H), 5.25 (d, J = 7.90 Hz, 2H, C_5—H, C_5'—H), 5.02 (s, 2H, C_4—H, C_4'—H), 4.80 (s, 2H, C_1—H, C_1'—H), 4.53 (d, J = 7.92 Hz, 2H, C_6—H, C_6'—H), 3.66 (d, J = 7.91 Hz, 2H, C_3—H, C_3'—H), 3.83 (s, 6H, $2OCH_3$), 3.74 (s, 4H, $(CH_2)_2$), 3.44 (d, J = 7.91 Hz, 2H, C_2—H, C_2'—H). Anal. Calcd. for $C_{34}H_{30}N_4O_{12}$: C, 59.47; H, 4.40; N, 8.16. Found: C, 59.50; H, 4.41; N, 8.14.

6,6′-(Ethane-1,2-diyl)bis[exo,exo-4,8-epoxy-3a,4,4a,7a,8,8a-hexahydro-3-(benzo[d][1,3]dioxol-5-yl)-pyrrolo[3,4-f]-1,2-benzisoxazole-5,7(1H,3aH)-dione] (4e). This compound was obtained as beige crystals, yield 19.0%, m.p. 184°C; ^{1}H NMR($CDCl_3$) δ: 7.91–7.33 (m, 6H, Ar-H), 6.07 (s, 4H, $2OCH_2O$), 5.27 (d, J = 7.90 Hz, 2H, C_5—H, C_5'—H), 5.12 (s, 2H, C_4—H, C_4'—H), 4.81 (s, 2H, C_1—H, C_1'—H), 4.51 (d, J = 7.90 Hz, 2H, C_6—H, C_6'—H), 3.56 (d, J = 7.91 Hz, 2H, C_3—H, C_3'—H), 3.73 (s, 4H, $(CH_2)_2$), 3.41 (d, J = 7.90 Hz, 2H, C_2—H, C_2'—H). Anal. Calcd. for $C_{34}H_{26}N_4O_{12}$: C, 59.83; H, 3.84; N, 8.21. Found: C, 59.82; H, 3.87; N, 8.20.

6,6′-(Ethane-1,2-diyl)bis[rel-(3aR,4S,4aR,7aS,8S,8aR)-4,8-epoxy-1,3-diphenyl-4,4a,6,7a,8,8a-hexahydropyrrolo[3,4-f]indazole-5,7(1H,3aH)-dione] (5a). This compound was obtained as

beige crystals, yield 21.6%, m.p. 207°C; ^{1}H NMR($CDCl_3$) δ: 7.77–6.91 (m, 20H, Ar-H), 5.33 (s, 2H, C_4—H, C_4'—H), 5.27 (s, 2H, C_1—H, C_1'—H), 4.66–4.64 (d, J = 9.60 Hz, 2H, C_5—H, C_5'—H), 4.16–4.14 (d, J = 9.60 Hz, 2H, C_6—H, C_6'—H), 3.74 (s, 4H, $(CH_2)_2$), 3.39–3.37 (d, J = 7.20 Hz, 2H, C_3—H, C_3'—H), 3.34–3.32 (d, J = 7.20 Hz, 2H, C_2—H, C_2'—H). Anal. Calcd. for $C_{44}H_{36}N_6O_6$: C, 70.96; H, 4.87; N, 11.28. Found: C, 70.94; H, 4.88; N, 11.26.

6,6′-(Ethane-1,2-diyl)bis[rel-(3aR,4S,4aR,7aS,8S,8aR)-4,8-epoxy-1-phenyl-3-(2-chlorophenyl)-4,4a,6,7a,8,8a-hexahydropyrrolo[3,4-f]indazole-5,7(1H,3aH)-dione] (5b). This compound was obtained as beige crystals, yield 18.2%, m.p. >300°C; ^{1}H NMR($CDCl_3$) δ: 7.81–6.96 (m, 18H, Ar-H), 5.31 (s, 2H, C_4—H, C_4'—H), 5.01 (s, 2H, C_1—H, C_1'—H), 4.65 (s, 4H, C_5—H, C_5'—H, C_6—H, C_6'—H), 3.74 (s, 4H, $(CH_2)_2$), 3.30 (s, 4H, C_2—H, C_2'—H, C_3—H, C_3'—H). Anal. Calcd. for $C_{44}H_{34}N_6O_6Cl_2$: C, 64.95; H, 4.21; N, 10.33. Found: C, 64.99; H, 4.23; N, 10.28.

6,6′-(Ethane-1,2-diyl)bis[rel-(3aR,4S,4aR,7aS,8S,8aR)-4,8-epoxy-1-phenyl-3-(2,3-dichlorophenyl)-4,4a,6,7a,8,8a-hexahydropyrrolo[3,4-f]indazole-5,7(1H,3aH)-dione] (5c). This compound was obtained as beige crystals, yield 28.8%, m.p. >300°C; ^{1}H NMR($CDCl_3$) δ: 7.65–6.94 (m, 16H, Ar-H), 5.31 (s, 2H, C_4—H, C_4'—H), 5.00 (s, 2H, C_1—H, C_1'—H), 4.67–4.65 (m, 4H, C_5—H, C_5'—H, C_6—H, C_6'—H), 3.75 (s, 4H, $(CH_2)_2$), 3.31–3.29 (m, 4H, C_2—H, C_2'—H, C_3—H, C_3'—H). Anal. Calcd. for $C_{44}H_{32}N_6O_6Cl_4$: C, 59.88; H, 3.65; N, 9.52. Found: C, 59.85; H, 3.67; N, 9.54.

6,6′-(Ethane-1,2-diyl)bis[rel-(3aR,4S,4aR,7aS,8S,8aR)-4,8-epoxy-1-phenyl-3-(quinoxalin-2-yl)-4,4a,6,7a,8,8a-hexahydropyrrolo[3,4-f]indazole-5,7(1H,3aH)-dione] (5d). This compound was obtained as beige crystals, yield 27.4%, m.p. 191°C; ^{1}H NMR($CDCl_3$) δ: 9.54 (s, 2H, 2H—C=N), 8.14–7.00 (m, 18H, Ar-H), 5.58 (s, 2H, C_4—H, C_4'—H), 5.38 (s, 2H, C_1—H, C_1'—H), 4.70–4.68 (d, J = 9.20 Hz, 2H, C_5—H, C_5'—H), 4.26–4.24 (d, J = 9.20 Hz, 2H, C_6—H, C_6'—H), 3.76 (s, 4H, $(CH_2)_2$), 3.49–3.47 (d, J = 7.20 Hz, 2H, C_3—H, C_3'—H), 3.38–3.36 (d, J = 7.20 Hz, 2H, C_2—H, C_2'—H). Anal. Calcd. for $C_{48}H_{36}N_{10}O_6$: C, 67.92; H, 4.27; N, 16.50. Found: C, 67.91; H, 4.29; N, 16.53.

6,6′-(Ethane-1,2-diyl)bis[rel-(3aR,4S,4aR,7aS,8S,8aR)-4,8-epoxy-1-phenyl-3-(2-phenyl-2H-1,2,3-triazol-4-yl)-4,4a,6,7a,8,8a-hexahydropyrrolo[3,4-f]indazole-5,7(1H,3aH)-dione] (5e). This compound was obtained as beige crystals, yield 11.7%, m.p. 203°C; ^{1}H NMR($CDCl_3$) δ: 8.53 (s, 2H, 2H—C=N), 8.20–6.93 (m, 20H, Ar-H), 5.51 (s, 2H, C_4—H, C_4'—H), 5.34 (s, 2H, C_1—H, C_1'—H), 4.65–4.62 (d, J = 9.60 Hz, 2H, C_5—H, C_5'—H), 4.21–4.18 (d, J = 9.60 Hz, 2H, C_6—H, C_6'—H), 3.75 (s, 4H, $(CH_2)_2$), 3.41—3.39 (d, J = 7.20 Hz, 2H, C_3—H, C_3'—H), 3.30–3.28 (d, J = 7.20 Hz, 2H, C_2—H, C_2'—H). Anal. Calcd. for $C_{48}H_{38}N_{12}O_6$: C, 65.60; H, 4.36; N, 19.12 Found: C, 65.63; H, 4.34; N, 19.11.

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