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CONJUGATES OF HYALURONIC ACID AND CHONDROITIN SULFATES WITH 4-AMINOANTIPYRINE AND THEIR ANALGESIC PROPERTIES

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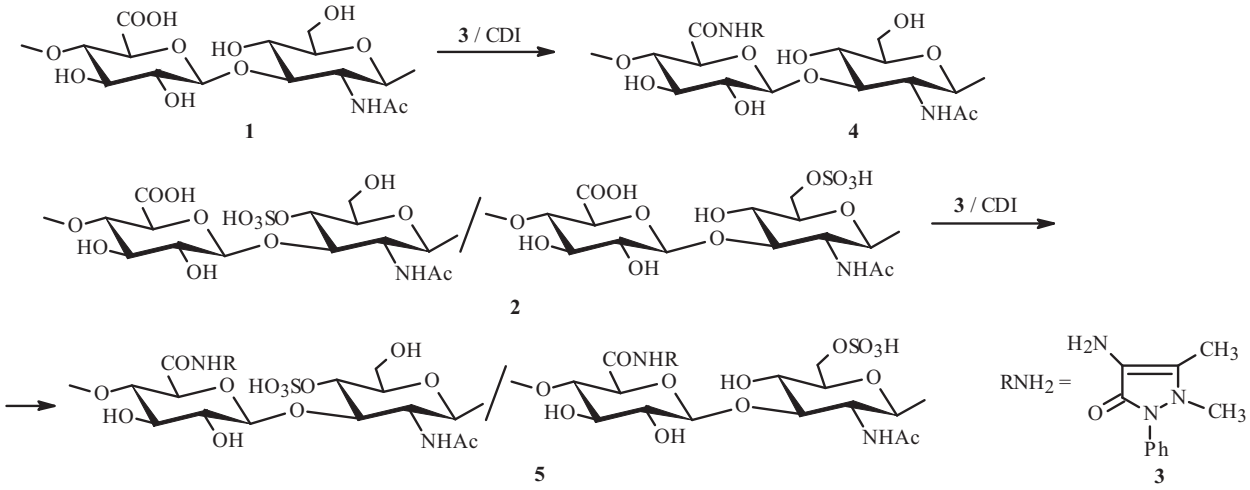
Heteropolysaccharides of the glucosaminoglycan class, e.g. hyaluronic acid (HA) and chondroitin sulfates (CS), are known for their reparative and regenerative properties, on the basis of which several drugs for treating diseases of the musculoskeletal system were developed [1–3]. HA and CS stimulate regeneration of cartilage and bone tissue and exhibit anti-inflammatory and analgesic activity. High-molecular-weight HA is the principal component in the sinovial fluid replacements Sinokrom, Sinvisk, etc. These are highly viscous hydrogels for intra-joint administration that act as a lubricant for painful joints [2]. The active substance CS in chondro-protector drugs (Chondroxide etc.) normalizes the exchange of cartilage tissue substances including phosphorus and calcium, decreases the pain level, and increases the flexibility of afflicted joints [3, 4].

In order to enhance the anti-inflammatory and analgesic activity of HA (**1**) and CS [**2**, a 3:2 mixture of CS-4 and CS-6], we incorporated into their structures 1-phenyl-2,3-dimethyl-4-aminopyrazol-5-one (4-aminoantipyrine) (**3**). The corresponding conjugates **4** and **5** (Scheme 1) were synthesized by the reaction of glucosaminoglycans **1** and **2** with amine **3** through the action of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (CDI) [5].



Scheme 1

Resonances of CH_3CON methyl protons in PMR spectra of conjugate **4** were found in the range δ 1.86–2.01 ppm; the corresponding resonances of CH_3CON protons of conjugate **5**, δ 1.93–2.04. Resonances appeared at stronger field δ 1.86 (for **4**) and 1.93 (for **5**) because of the shielding effect of the magnetically anisotropic phenyl ring of the aminoantipyrine moiety [5]. The content of **3** in each of the conjugates was determined from the intensity ratio per proton of resonances for Ph– and CH_3CON– protons, which was 83 and 87% for **4** and **5**, respectively.

Analgesic activity was determined as before [6] using the model of "acetic-acid writhing" induced by i.p. administration of CH_3COOH (1%, 1 mL) to mice (20–25 g). Conjugates **4** and **5** at a dose of 50 mg/kg, which corresponded to 18.9 and 16.2 mg of **3**, were administered i.p. 1 h before injecting CH_3COOH. The reference drug was analgin. The results are given below (*n* is the number of mice in the experiment; the dose for all experiments except the control was 50 mg/kg):

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Compound	*Number of writhings**	*Compound*	*Number of writhings**
Control	17.4 ± 1.3	**4**	8.2 ± 0.9
$n = 10$		$n = 10, p < 0.01$	
Analgin	7.0 ± 0.7	**5**	5.6 ± 0.7
$n = 10, p < 0.01$		$n = 10, p < 0.01$	

*Data were processed statistically using the Student coefficient. Differences from the control were considered statistically significant for $p < 0.01$.

Preventive administration of **4** reduced the frequency of painful manifestations by 1.9 times compared with the control of untreated animals; **5**, by 2.7 times. This slightly exceeded the effect of analgin, application of which at the same dose reduced the frequency of painful manifestations by 2.3 times.

Hyaluronic acid (1) was isolated by the literature method [5].

Chondroitin sulfates (2) were obtained from cartilage (1 kg, bovine) by analogy with HA [5]. The CS fraction (mixture of CS-4 and CS-6) was eluted by NaCl solution (0.8 M) and precipitated by addition to the eluate of three times the amount of EtOH. The precipitate was centrifuged, washed successively with EtOH (50 mL) and Et_2O (50 mL), and dried at ≤60°C at reduced pressure to afford a white powder (6.1 g) containing 4.8–5.9 mass% S. Protein was not observed (according to the Lowry method). The CS molecular weight after basic extraction averaged 16.91 kDa [7]. The CS-4/CS-6 ratio was determined as 3:2 (from the ratio of integrated intensities of resonances for the MeCON methyl protons at δ 2.01 (CH_3CON in CS-4) and 2.03 (CH_3CON in CS-6). PMR spectrum (400 MHz, D_2O, δ, ppm): 2.01 (CH_3CON in CS-4), 2.03 (CH_3CON in CS-6). ^{13}C NMR spectrum (100 MHz, D_2O, δ, ppm): 23.7 ($\underline{C}H_3CON$), 52.0 (GalNAc4S C-2), 52.7 (GalNAc6S C-2), 62.2 (GalNAc4S C-6), 68.6 (GalNAc6S C-6), 73.4–82.9 (GlcA C-2, C-3–C-5), 102.0 (GalNAc6S C-1), 102.6 (GalNAc4S C-1), 104.9 (GlcA C-1 in CS-6), 105.4 (GlcA C-1 in CS-4), 175.5, 175.6, 175.9 and 176.1 (C=O) [8, 9].

Conjugate 4 (127 mg) was prepared by the literature method [5] from **1** (120 mg, 0.3 mmol) with **1**:**3**:CDI = 1:3:3 in H_2O (40 mL). UV spectrum (H_2O, pH 7): 262 nm. PMR spectrum (400 MHz, D_2O, δ, ppm): 1.86 and 2.01 ($C\underline{H}_3CON$), 2.33 ($C{=}CCH_3$), 3.40 (NCH_3), 7.5 and 7.7 (Ph). ^{13}C NMR spectrum (100 MHz, D_2O, δ, ppm): 24.0 ($\underline{C}H_3CON$), 36.2, 130.7, 132.5, 135.2, 151.0, 163.3, 173.4 (characteristic resonances of 4-aminoantipyrine).

Conjugate 5 (145 mg) was prepared from **2** (150 mg, ~0.3 mmol) by analogy with **4**. UV spectrum (H_2O, pH 7 nm): 261. PMR spectrum (400 MHz, D_2O, δ, ppm): 1.93 and 2.04 ($C\underline{H}_3CON$), 2.26–2.33 ($C{=}CCH_3$), 3.36 (NCH_3), 7.5 and 7.7 (Ph). ^{13}C NMR spectrum (100 MHz, D_2O, δ, ppm): 24.0 ($\underline{C}H_3CON$), 36.0, 130.8, 132.5, 135.1, 151.0, 163.0, 173.4

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