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CHEMISTRY

Peptide Derivatives of Some Physiologically Active Substances

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Abstract—The synthesis of Boc-Gly-Pro-Dox, Boc-Gly-Pro-DOPA, and Boc-Gly-Pro-Srt and their deuterated analogues was carried out. The condensation is accompanied by side reactions, which could be minimized by optimizing the reaction conditions. The most versatile approach to the synthesis of Boc-Gly-Pro-Dox, Boc-Gly-Pro-DOPA, and Boc-Gly-Pro-Srt and their deuterated analogues is condensation of Boc-Gly-Pro or Boc-Gly-[^{2}H]Pro with the amino groups of dopamine, serotonin, and doxorubicin. For the introduction of hydrogen isotopes into ΔPro, hydrogenation of its aqueous solution followed by condensation of the reduced proline with Boc-GlyOSu is recommended. Mass spectrometry was used to determine the content of isotopomers in the deuterated products.

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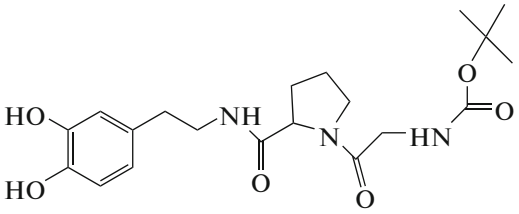
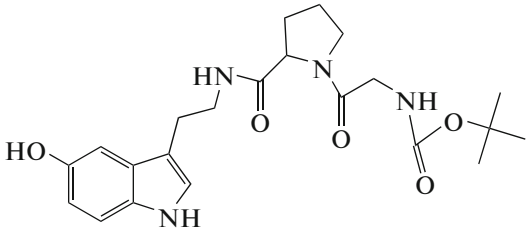
The targeted delivery of a biologically active substance (drug) to a particular organ, i.e., directly to the body tissue to be treated, is a challenge. Here we synthesized peptide derivatives of doxorubicin (Boc-Gly-Pro-Dox), dopamine (Boc-Gly-Pro-DOPA), and serotonin (Boc-Gly-Pro-Srt), which play an important role in functioning of various body systems.



Boc-Gly-Pro-DOPA



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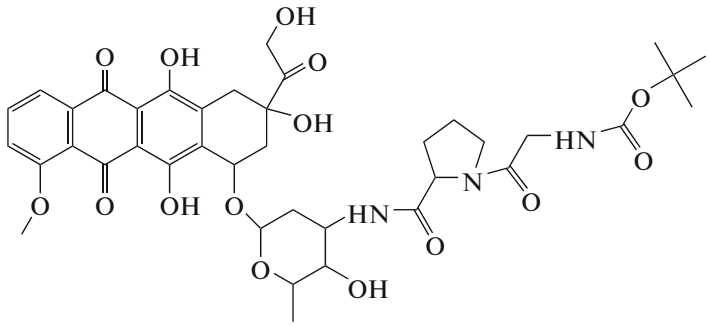

Boc-Gly-Pro-Dox

The relevance of this synthesis can be exemplified by approaches to development of synthetic routes to doxorubicin (Dox) derivatives. It is known that the use of Dox in the anticancer therapy is mainly restricted by its cardiotoxicity [1–3]. Cases of severe cardiac failure, which could even cause death, appearing in patients were noted upon the used of Dox [1]. Therefore, the search for Dox derivatives that would be toxic

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Table 1. Conditions of synthesis of Boc-Gly-Pro-Dox, Boc-Gly-Pro-DOPA, and Boc-Gly-Pro-Srt

Reaction conditions	Product analysis and identification
Dox (10 μmol), or DOPA (52 μmol), or serotonin (70 μmol) were taken. First, a solution of peptide in DMF was treated with DCHC and Et_3N in the presence of 1-hydroxybenzotriazole. After 10 min, the amine-containing component was added. The amine : 1- hydroxybenzotriazole : Boc-Gly-Pro : DCHC : Et_3N ratio was 5.0 : 1.0 : 1.1 : 1.5 : 2.0. The reaction was conducted for 4 to 17 h, and DMF was removed by freeze-drying. The reaction mixtures were first purified by solid-phase extraction on Diapak C16, the product was eluted from the stationary phase by aqueous methanol with 0.05% acetic acid, with methanol percentage being gradually increased from 50 to 100%. The products were isolated by HPLC. The yields were 60–70%.	Pronto SIL-120-5-C18 AQ DB-2003 column (2.0 × 75 mm size; 5 μm particle diameter) in the system: A, 0.1% CH_3COOH; B, acetonitrile, with 0 to 100% gradient of B in 12.5 min; the eluent flow rate was 0.2 mL/min; retention times: Boc-Gly-Pro-Dox, 8.32 min; Boc-Gly-Pro-DOPA, 6.26 min; or in the system: A, 0.2 M $LiClO_4$ + 0.005 M $HClO_4$ buffer, pH 2.24; B, methanol; the retention time of Boc-Gly-Pro-Srt was 8.37 min. Reprosil pur C18aq column (150 × 20 mm size; 10 μm particle diameter) in the system: A, 75% methanol with 0.05% acetic acid; B, methanol (detection at 280 nm); the eluent flow rate was 20 mL/min; the retention time of Boc-Gly-Pro-Dox was 3.58 min.

only against tumors with a minimum damage of healthy tissues of the human body is still in progress.

In this respect, of interest is the publication [4] in which Dox-Pro-Gly-Z was proposed as a prodrug. The essence of this work is in the fact that an enzyme that hydrolyzes Dox-Pro-Gly-Z at the Dox-Pro bond is present in tumor cells. As a result, Dox is released and kills the cell. This procedure increases the therapeutic efficacy of cytostatic agents in the treatment of cancer by minimizing the systemic adverse effects related to high toxicity of Dox.

The purpose of this work was to synthesize Boc-Gly-Pro-Dox, Boc-Gly-Pro-DOPA, and Boc-Gly-Pro-Srt and their deuterated analogues.

The catalysts, reagents, and solvents were commercial chemicals. Boc-Gly-Pro was synthesized at the Institute of Molecular Genetics, Russian Academy of Sciences, by condensation of Boc-Gly with Pro-OBzl followed by removal of the benzyl group by hydrogenation. The starting compounds and products were characterized by high performance liquid chromatography (HPLC) and mass spectrometry. The mass spectrometric data were collected on an LCQ Advantage MAX instrument (Thermo Electron Corp., United States) with electrospray ionization, direct sample injection (10 μg/mL concentration in 0.1% acetic acid), and further ion collision-induced fragmentation of the molecular ion at 35 eV.

The synthesis was carried out according to the following scheme (Table 1) [5, 6]. Optimization of reaction conditions demonstrated that with excess dicyclohexylcarbodiimide (DCHC), treatment of a mixture containing Boc-Gly-Pro-Dox with acetic acid affords Boc-Gly-Pro-14-Ac-Dox. The use of a twofold excess of DCHC under these conditions may give Boc-Gly-Pro-14-Ac-Dox in up to 40% yield.

In the case of serotonin, Boc-Gly-Pro present in excess reacts also with the indole moiety of serotonin. When the serotonin to Boc-Gly-Pro ratio is 1 : 1.3, the amount of $(Boc\text{-}Gly\text{-}Pro)_2$-Srt may reach 30% relative to Boc-Gly-Pro-Srt. The formation of Boc-Gly-Pro-14-Ac-Dox and $(Boc\text{-}Gly\text{-}Pro)_2$-Srt was confirmed by mass spectrometry. The chromatographic retention time of Boc-Gly-Pro-14-Ac-Dox on an analytical column was 9.05 min, that of $(Boc\text{-}Gly\text{-}Pro)_2$-Srt was 10.47 min; in other words, the retention times of these products differ from the retention times of Boc-Gly-Pro-Dox and Boc-Gly-Pro-Srt by almost 30 s and 2 min, respectively.

When the reactions were carried out under optimal conditions (Table 1), the yields of Boc-Gly-Pro-Dox, Boc-Gly-Pro-DOPA, and Boc-Gly-Pro-Srt reached 60–70%. This allowed us to prepare sufficient amounts of the products to carry out their biological tests as potential sources of doxorubicin, dopamine, and serotonin.

For the preparation of deuterated analogues of the above compounds, it was necessary to synthesize the precursors with proline being replaced by dehydroproline (ΔPro). Hydrogenation of the precursors resulted in deuterated Boc-Gly-Pro-Dox, Boc-Gly-Pro-DOPA, and Boc-Gly-Pro-Srt.

The ΔPro-containing derivatives can be obtained in the following way. Solutions of ΔPro in water, deuterium water, and dimethylformamide (DMF) were hydrogenated with deuterium gas. Hydrogenation of ΔPro was also carried out without a solvent at 140°C. As a result, proline obtained by the four listed procedures contained 1.73, 1.85, 1.73, and 1.76 deuterium atoms, respectively (Table 2).

As can be seen (Table 2), the best results were obtained using water or deuterium water. Hydrogenation in DMF cannot be used to obtain preparative amounts of labeled proline, because its solubility in this solvent is too low. The formation of large amounts of isotopomers containing more than two deuterium atoms upon solvent-free hydrogenation of ΔPro with heating is probably attributable to deuterium inclusion

Table 2. Distribution of proline isotopomers for different conditions of deuteration (deuterium gas pressure of 400 hPa)

^2H atoms	Content of isotopomers in hydrogenated ΔPro, %				
	H_2O*	^2H_2O*	DMF**	140°C***	EtAc****
0	13.2	7.9	5.4	29.8	3.5
1	17.2	14.9	24.5	18.9	20.9
2	69.6	64.2	63.6	23.4	60.2
3	4.0	10.6	4.8	12.4	13.5
4	1.1	2.5	1.7	8.1	2.2
5	–	–	–	4.4	–
6	–	–	–	2.2	–
7	–	–	–	0.8	–

* ΔPro (80 mg), H_2O or ^2H_2O (0.4 mL), 5% $PdO/BaSO_4$ (20 mg), 2 h; ** ΔPro (2 mg), DMF (0.4 mL), 5% $PdO/BaSO_4$ (4 mg), 2 h; *** 5% $PdO/BaSO_4$ (117 mg) was impregnated with ΔPro (11.7 mg) in water (40 μL) and freeze-dried, the reaction was carried out for 15 min at 140°C; **** Boc-Gly-ΔPro (5 mg), ethyl acetate (0.4 mL), 5% $PdO/BaSO_4$ (11 mg), 2 h.

Table 3. Calculated distributions of Dox, DOPA, and serotonin between blood and brain tissues

Compound	AUC_{brain}/AUC_{blood}
Boc-Gly-Pro-DOPA	0.085
Boc-Gly-Pro-Dox	0.019
Boc-Gly-Pro-Srt	0.079
Z-Gly-Pro-DOPA	0.059
Z-Gly-Pro-Dox	0.013
Z-Gly-Pro-Srt	0.054

due to isotope exchange under these conditions. This is also indicated by the formation of a large amount of Pro isotopomers containing less than two deuterium atoms per amino acid molecule. It is evident that some double bonds are reduced by protons activated via isotope exchange. For the above reasons, preparative amounts of hydrogen isotope-labeled proline should better be obtained using an aqueous solution, because, as indicated above, the isotope dilution is minimized and the labeling procedure is markedly more facile and does not depend on many factors that should be borne in mind to conduct the solid-phase hydrogenation.

For the synthesis of Boc-Gly-[^{2}H]Pro, equimolar amounts of Boc-Gly, *N*-hydroxysuccinimide, and DCHC (a chloroform solution) were stirred overnight to give Boc-GlyOSu. The solvent was evaporated. The residue was extracted with ethyl acetate and centrifuged. The condensation of Boc-GlyOSu (without additional purification) with [^{2}H]Pro was carried out by a reported procedure [4]. For this purpose, [^{2}H]Pro hydrochloride (40 mg, 0.26 mmol) in water (0.2 mL) containing Et_3N (0.1 mL) was treated with an ethanol solution of Boc-GlyOSu (1 mL) (1 : 2 molar ratio). The reaction was carried out with stirring for 12–17 h at room temperature. The reaction mixture was worked-up by the procedure described in [6]. The Boc-Gly-[^{2}H]Pro dipeptide was condensed with Dox, DOPA, or serotonin, as described in Table 1. The yields reached values of about 30–40%. Apparently, this is due to the necessity of preliminary preparation of glycine N-hydroxysuccinimide ester and conduction of condensation in the presence of water.

A similar procedure was used to prepare Boc-Gly-ΔPro. Hydrogenation of Boc-Gly-ΔPro was conducted in ethyl acetate in the presence of 5% $PdO/BaSO_4$ in a deuterium atmosphere (400 hPa). Hydrogenation in aprotic solvent made it possible to introduce, on average, 1.91 deuterium atoms into the peptide. The contents of isotopomers are summarized in Table 2. Furthermore, isotope labeling at a later stage minimizes the overall loss of the label during the synthesis of isotope-modified compounds. High-performance liquid chromatography of Boc-Gly-[^{2}H]Pro and Boc-Gly-ΔPro was conducted on a ProntoSIL-120-5-C18 AQ DB-2003 column (2.0×75 mm; particle diameter of 5 μm) in the system of A, 0.1% CH_3COOH, and B, acetonitrile, with 0 to 100% gradient of B in 12.5 min; the eluent flow rate was 0.2 mL/min. The retention time was 5.76 min.

Thus, we obtained and identified Boc-Gly-Pro-Dox, Boc-Gly-Pro-DOPA, and Boc-Gly-Pro-Srt and their deuterated analogues.

With a large number of compounds at hand, their comprehensive biological testing presents a certain problem. This takes a lot of time and is experimentally sophisticated, for example, when it is necessary to find out how efficiently these compounds overcome the blood–brain barrier (BBB). The time is required for the preparation of rats, injection of the agent, isolation of the biological material, analysis of the obtained specimens, etc. This work can be simplified by using recently developed approaches [7, 8]. These approaches provide a tentative idea of the brain content of the test compound (AUC_{brain}) if the blood level of the compound is known (AUC_{blood}) (Table 3).

As can be seen from the presented data, this value is affected by not only the molecular groups responsible for the biological activity of the compound, but also groups attached to free amine of the amino acid. The Boc-protection is somewhat more advantageous than Z-protection for penetration of the same agent through the BBB. Among the groups determining the biological activity, the best results were found for DOPA, which was followed by Srt; the Dox-containing compounds were 4 to 5 times inferior.

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