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Facile preparation of a symmetric hexavalent oleanolic acid/per-*O*-methylated α-cyclodextrin conjugate with two conformations in solution and unambiguous NMR analysis

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ABSTRACT

A newly synthesized hexavalent oleanolic acid/per-*O*-methylated α-cyclodextrin conjugate shows significant differences in NMR spectra with previously synthesized analogs. Characterization of the product by ^{1}H NMR, ^{13}C NMR, COSY, HSQC, HMBC, TOCSY, NOE, and ROESY experiments were performed. Detailed investigations revealed that the compound has two conformations in solution and the ratio of them was 1:1. Further variable-temperature NMR study revealed that the two conformations were stable at temperature range of 273−323 K in $CDCl_3$ solution.

1. Introduction

Pentacyclic triterpene acids, such as oleanolic acid (OA), betulinic acid (BA) and echinocystic acid (EA), and their glycosides are widely distributed in the plant kingdom.[1] Many compounds of this group are reported to have various interesting biological, pharmacological, or medicinal activities including antiviral,[2] antitumor,[3] anti-inflammation,[4] and so on.[5] However, in many cases, the potency of these triterpenoids is relatively weak. Therefore, the semi-synthetic derivatives of pentacyclic triterpene could be of great value in discovering highly potent bioactive compounds. Several synthetic derivatives of OA, 2-cyano-3,12-dioxoolean-1,9-dien-28-oic acid (CDDO), and its C-28 methyl ester (CDDO-Me) and C-28 imidazole (CDDO-Im), have shown potent antitumor activity against a wide range of cancer cell lines in vitro and inhibited the growth of several cancer in vivo.[6] In our previous study, a series of multivalent pentacyclic triterpene-cyclodextrin conjugates have been synthesized and a dramatic multivalent effect for their anti-influenza virus entry activity has been found.[7]

Recently, per-*O*-methylated cyclodextrins and their derivatives have attracted considerable attention due to their improved solubility both in water and in organic solvents.[8] Inclusion complexes of methylated cyclodextrins are usually more stable than the corresponding complexes of unmodified cyclodextrins. As a further step to our previous work towards the development of novel antiviral inhibitors,[7,9] we would like to synthesize the similar conjugates of compound **a**-**b** (Fig. 1) in which the acetyl or hydroxyl groups of α-cyclodextrin were replaced by methyl groups. To our surprise, the obtained hexavalent oleanolic acid/per-*O*-methylated α-cyclodextrin conjugate **8** showed significant differences in NMR spectra with previously synthesized analogs. Herein, we would like to report the synthesis and unambiguous structure characterization of this compound with two conformations in solution by one- and two-dimensional NMR experiments.

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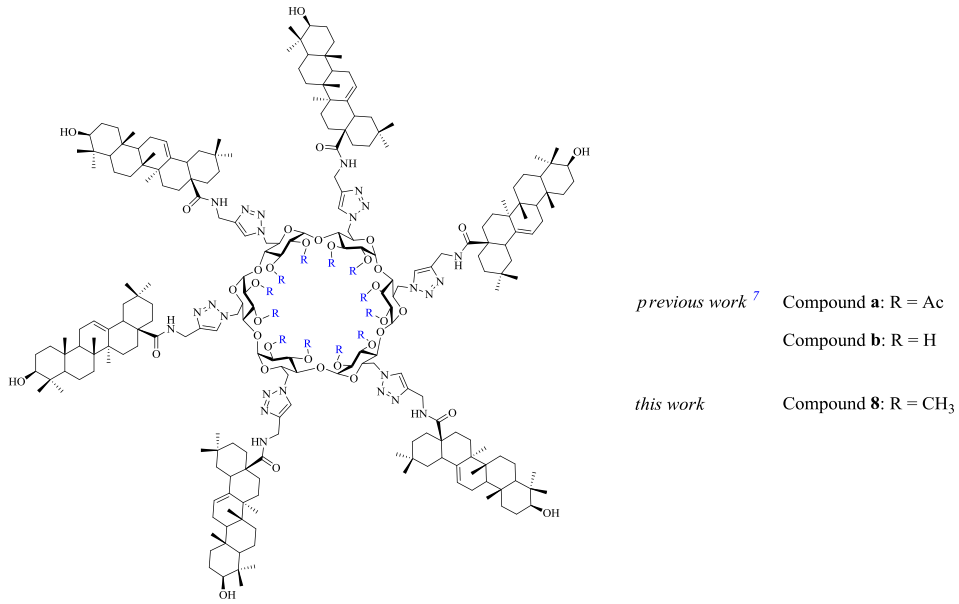


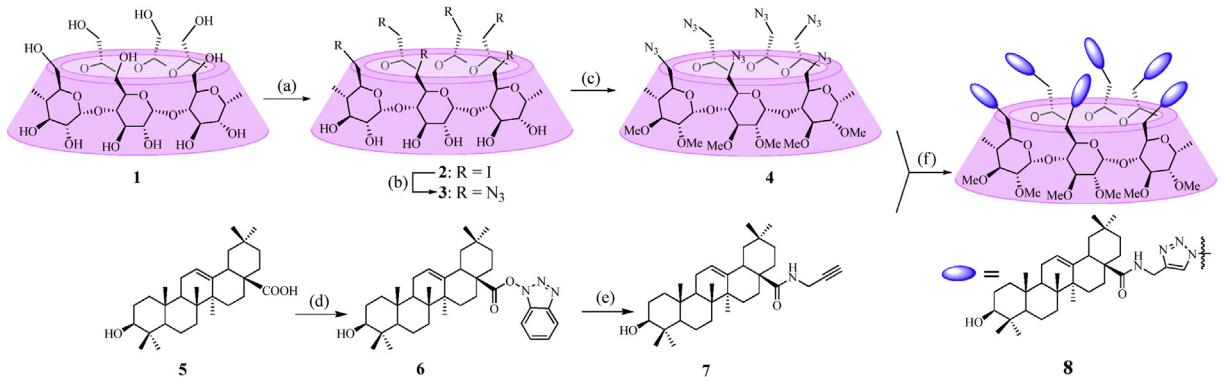
Fig. 1. Chemical structures of three hexavalent oleanolic acid/α-cyclodextrin conjugates.

2. Results and discussion

The synthesis route of the unusual oleanolic acid/per-*O*-methylated α-cyclodextrin conjugate **8** was summarized in Scheme 1. Briefly, α-cyclodextrin was converted into hexakis (2,3-di-*O*-methyl-6-deoxy-6-azide)-α-CD **4** in 51% yield through a three-step procedure described by Baer et al.[10] *N*-propargyl 3β-hydroxyolean-12-en-28-amide **7** was prepared according to our previously reported method.[9b] When compound **4** was reacted with 6.6 equiv of compound **7** (1.1equiv per N_3) in the present of $CuSO_4$ and *L*-ascorbic acid under microwave condition at 100 °C for 1 h, conjugate **8** was separated out by conventional silica gel column chromatography in 62% yield.

The MALDI-TOF mass spectrum for compound **8** showed an $[M+Cu]/2^+$ ion at *m*/*z* 2156.9030 Da (Calcd for $C_{246}H_{384}CuN_{24}O_{36}$, 4313.8251); and an $[M + Na + Cu]/2^+$ ion at *m*/*z* 2168.4004 Da (Calcd for $C_{246}H_{384}CuN_{24}NaO_{36}$, 4336.8149) (SI Fig. 1), indicating that it is a hexavalent conjugate. However, compound **8** demonstrated complex ^1H and ^{13}C NMR spectra and significant differences were found between compound **8** and its analogs **a** and **b**. ^1H NMR spectrum of the compounds **a** and **b** in $CDCl_3$ solution showed only a broad signal at 6.80 and 7.15 ppm with the integration of six (6 × N*H*), respectively.[7] However, the ^1H NMR spectrum of compound **8** consists of two sets of peaks of nearly equal amplitude for N*H*, trizolyl-C*H*, OA-H_{18}, methoxy, etc. For example, the signal of compound **8** corresponding to the (N*H*) proton splits into two broad signals appearing at δ 8.23 and 6.75 ppm, and each signal has a integration value of six (6 × N*H*). Similar results were also observed in the ^{13}C NMR spectrum of compound **8** (see Supporting information).

The structure of **8** was further assigned through detailed analysis of one- and two-dimensional NMR experiments. The lower field region spanning 2.5–8.5 ppm of **8** was relatively well-resolved and fully assigned with the aid of $^1H-^1H$ COSY, $^1H-^{13}C$ HSQC and $^1H-^{13}C$ HMBC spectra. The chemical shifts of ^1H and ^{13}C NMR of compound **8** at 298 K are listed in Table 1. In the aromatic region, the signals at δ = 7.36 and 7.80 ppm could be assigned to two triazole-CHs according to its $^1H-^1H$ and $^1H-^{13}C$ correlation spectra (Fig. 2A). Additional signals were observed for two amide protons at 6.75 and 8.23 ppm. From the $^1H-^{13}C$ HSQC spectrum, two sets of signals at 5.46 and 5.37 ppm, overlapped with the signals of CD-H_1 and CD-H_6, respectively, could be assigned to two H_{12} of OA. In addition, compound **8** shows two sets of CD-H_1 signals appearing at 5.36 and 5.01 ppm and two CD-C_1 signals appearing at 100.86 and



Scheme 1. Synthesis of the hexavalent oleanolic acid/per-*O*-methylated α-cyclodextrin conjugate **8**. Reagents and condition: (a) Ph_3P, I_2, DMF, 80 °C, overnight; (b) NaN_3, H_2O, 60 °C, 12 h; (c) CH_3I, NaH, DMF; (d) TBTU, DIEA, THF, rt, overnight; (e) 2-propynylamine, Na_2CO_3, DMF, rt, 1 h; (f) $CuSO_4$, Na-*L*-ascorbate, THF-H_2O (1:1, v/v), 100 °C, microwave.

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