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Study of addition of difluorocarbene on double bond of triterpenes

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ABSTRACT

In this work, we describe synthesis and characterization of novel triterpenic derivatives formed by the addition of difluorocarbene on activated double bonds of natural and semisynthetic triterpenes. Despite the general trend that introducing hydrophobicity into the triterpenic molecule often causes decrease in cytotoxic activity, there are examples in literature where addition of dichlorocarbene to a terpenic double bond had the opposite effect. That sparked our interest to synthesize similar analogues from our active triterpenes. The reaction only worked with limited amount of substrates and the cytotoxicity of resulting products was very low. We obtained several interesting structures and the major problem was to accurately determine their structures, especially the configuration at newly introduced chiral carbon atoms. For that, we used 2D-NMR methods combined with molecular modeling and one structure was also confirmed by X-ray crystallography.

1. Introduction

Triterpenoids are a large group of natural compounds particularly prevalent in plants and often have a variety of biological activities [1,2]. Commonly, however, the pharmacological properties of those active compounds are not suitable for their use in medicine. Many research groups have tried to improve the properties of the most promising terpenoids that would make them useful candidates for HIV and cancer treatment. These studies have either explored new plant species or modified the structures of known active compounds. Among hundreds of new compounds, we have synthesized many derivatives of lupane that are highly cytotoxic on a variety of cancer cell lines *in vitro*, including those that are resistant to current cytostatics [3–9]. This allowed us to make structure–activity relationship studies in order to find some trends of how the chemical structure may affect the anti tumor activity. We found that in general, increasing hydrophobicity leads to less active compounds. However, within this trend, we found many examples where just a slight modification of the structure with minimal impact on hydrophobicity had a much more dominant impact on cytotoxicity than the general trend [7]. There are many examples in the literature in which a small modification of a barely active triterpene tremendously increases the biological activity, and there are also instances where highly active compounds are rendered less active by a small modification. Examples with anti-HIV activity have been previously reported [10–12]. These findings suggest that a simple prediction of the structure–activity relationship is difficult, especially with compounds that contain more complicated structures, such as triterpenes.

Previously, we studied a possibility of introduction of fluorine into the terpenic structures using DAST agent and as expected, the result was a group of hydrophobic triterpenes where fluorine replaced carboxyl, carbonyl or hydroxy groups [13]. In agreement with the general trend, there was no cytotoxic activity within this group of hydrophobic fluoroderivatives. However, another research group tested the activity of adducts of dichloro- and dibromocarbene to double bonds of some terpenic structures against human melanoma cells Colo 38 and Bro.92. The best results were achieved with a dichlorocyclopropyl derivative with the activity on Bro.92 line slightly better than the activity of the starting material, nevertheless the authors claimed that the high lipophilicity of synthesized derivatives complicated *in vitro* tests, therefore further tests after the derivatization of these non-polar compounds were suggested [14]. This and also interesting chemistry of difluorocyclopropanes [15,16] sparked an idea to synthesize analogous adduct of difluorocarbene to the activated double bonds of several triterpenes that we prepared earlier. Despite the expected hydrophobicity and problems associated

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with it, we anticipated that the cyclopropane motif may be a strong pharmacophore and if the products show activity, we would always be able to modify another part of the molecule to decrease the overall hydrophobicity later. There are various methods to create a difluorocarbene species, an intermediate for difluorocyclopropane ring preparation [17–19] and we chose thermal decomposition of sodium chlorodifluoroacetate [20]. From our previous research of carbene additions [21] and after several unsuccessful experiments at the beginning of this work, we realized that double bonds (except the 20(29) exocyclic) in triterpenes are not reactive enough to give a product of difluorocarbene addition (*e.g.* Δ^2 bond). Therefore we selected compounds with less sterically hindered exocyclic double bonds activated by conjugation with a carbonyl group that proved to be reactive with carbenes in our earlier work [21]. This however limited the amount of possible substrates.

2. Results and discussion

2.1. Chemistry

We obtained the starting triterpenoids **1**–**4** from Betulinines Chemical Group. By the reaction of betulin diacetate (**1**) with difluorocarbene generated *in situ* by heating sodium chlorodifluoroacetate in diglyme, we obtained difluorocyclopropyl derivative **5**. Applying the same reaction conditions to methyleneketone **2**, we obtained a mixture of two compounds that were separated as pure isomers **6a** and **b**. Using methyleneketone **4** as a starting material, compound 7 was the major product (Scheme 1).

Triterpenoid skeletons in general are very rigid structures sterically hindered by methyl substituents and they contain multiple centers of chirality. Those features direct most of the addition reactions predominantly from beta side of the skeleton and here we were able to observe exactly this effect. In case of compounds **1** and **4**, we were only able to isolate single product of the beta addition and in case of compound **2**, the product of alpha addition was a minor part of the reaction mixture. Unfortunately, the reactions were not very clean and contained multiple side-product that we were unable to isolate. Therefore we were unable to directly measure the diastereomeric eccess from the reaction mixture. For the reaction of compound **2**, we can approximately estimate it from the isolated yields of compounds **6a** and **b**. In this case, the de value was 37%.

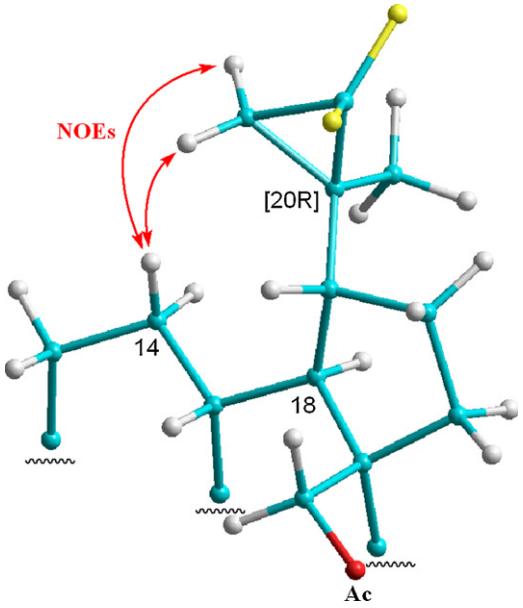
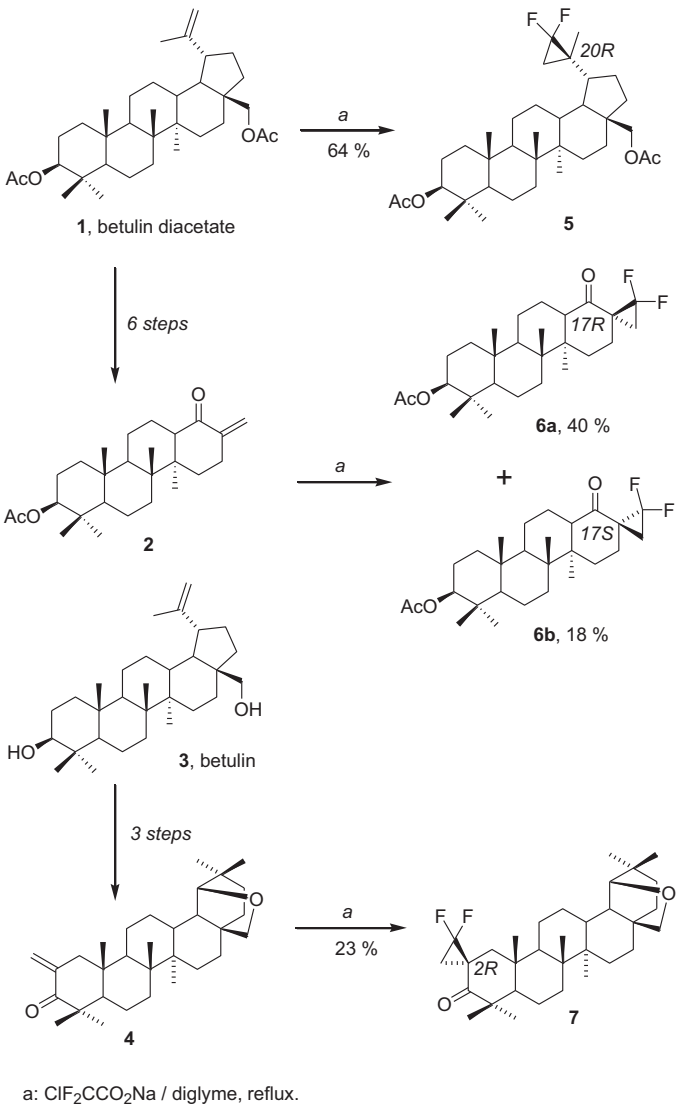


Fig. 1. Partial stereostructure of compound **5**. The NOEs observed in energy minimized conformation of [20R]-diastereomer are shown with red arrows. (For interpretation of the references to color in the artwork, the reader is referred to the web version of the article.)



2.2. Configuration at newly introduced chiral carbon atom

Complete structural assignment of proton and carbon signals was done using homonuclear and heteronuclear 2D-NMR spectra (^{1}H and ^{13}C NMR data are summarized in Tables 1 and 2) with support from literature [22]. Then the observed NOE contacts in 2D-H,H-ROESY spectra together with molecular modeling of both theoretically possible diatereoisomers were used to determine the configuration at newly introduced chiral carbon atoms.

2.2.1. Compound 5

Partial overlap of CH_3 and CH_2 signal (1.075 and 1.06 ppm) in C(19)-difluoromethyl-cyclopropyl substituent in $CDCl_3$ did not allowed the observation of selective NOEs of these protons. The better separation of these signals (1.02 and 0.92 ppm) was then achieved in C_6D_6 solution where we observed NOE contacts between H-12β proton (at 1.42 ppm) and CH_2 protons of cyclopropyl ring (at 0.92 ppm) but not with CH_3 protons (at 1.02 ppm). Since the new chiral carbon atom appears in a side