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Partial synthesis of 14-deoxy-14-aminotriptolide

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Abstract

Triptolide is a diterpene triepoxide isolated from *Tripterygium wilfordii*, a chinese medicinal plant and possessing a wealth of biological activities. In order to produce more soluble derivatives of triptolide, its 14-amino analogue was prepared. Initial attempts at its preparation using classical displacements led to rearranged compounds and success was finally met using carefully controlled Borch conditions. 14-Deoxy-14-aminotriptolide was thus prepared for the first time and it was found 10 times less cytotoxic than the parent compound.

Keywords

Triptolide, Reductive amination, Aminotriptolide, Cytotoxicity

Triptolide **(1)** is a fascinating molecule embedding a highly reactive array of functionalities, i.e. three contiguous oxiran rings and one α, β unsaturated lactone. It was isolated in the seventies by Kupchan *et al.* from the root of *Tripterygium wilfordii* Hook, a plant used as a Chinese traditional medicine.[1] The compound displays a broad spectrum of biological activities and shows promises in the treatment of various disorders such as rheumatoid arthritis, chronic hepatitis and chronic nephritis, ankylosing spondylitis, polycystic kidney disease and cancer.[2] Triptolide is not a very soluble molecule, it is highly toxic and therefore, derivatives have been prepared to select candidates for clinical investigations. An excellent review has been published in 2012 on the subject and as far as SAR is concerned, it is concluded that the triepoxide and the lactone ring are necessary for the activity.[3] Worthy of note is the fact that because of the extreme sensitivity of triptolide, most derivatives have to be prepared by *de novo* total synthesis, with installation of the triepoxide in the last stages. In the natural product, the most accessible function left for chemical modification, is the alcohol at position C-14 and it has been the object of numerous derivatisations. Among rare compounds which went into clinical trials, one can mention Omtriptolide[4,5] formed by succinylation and the prodrug Minnelide[6,7,8]

Numerous efforts have been deployed to transform the alcohol function of triptolide into an amino group in order to increase water solubility. The most obvious route implying amine or azide displacement of a sulfonate led to rearranged products, while reductive amination of triptonide (the ketone derived from triptolide) gave triptolide back, due to sluggish imine formation. Alternatively, Ritter reaction was

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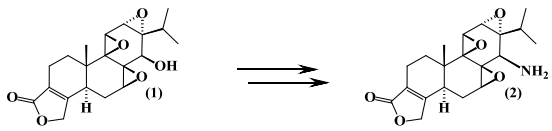
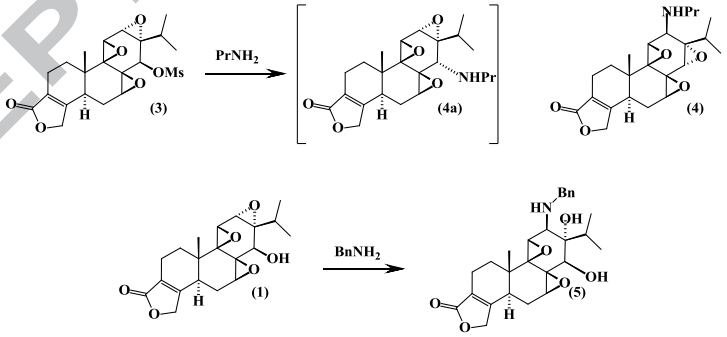
found unsuitable due to the acid sensitive character of the triepoxide. This Letter describes particular conditions, which allowed the preparation of the title compound (**2**) through reductive amination, along with preliminary chemical derivatisations and biological properties of this new molecule.

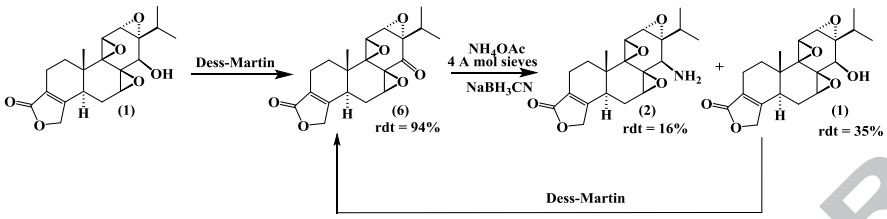


In a first series of reactions, triptolide mesylate **(3)** was treated with a variety of primary amines and in all cases, secondary amines were obtained. As an example, reaction with N-propylamine gave compound **(4)**, mass and NMR spectra of which showed disappearance of the mesyl group and presence of a N-propyl chain. The ^{13}C NMR spectrum presented signals for the three epoxides (C at δ 65.1, 64.3, 56.3 and CH at 61.0, 58.5, 58.4) and a CH at δ 53.6 corresponding to the attachment of the amino group. The structure was established by identification of the signals for the amino side chain and by observation of a HMBC correlation between the NCH_2 and the CH at δ 53.6. This CH corresponded to a proton resonance at δ 3.20 appearing as a double doublet with J= 3.6 and 0.9 Hz, the larger coupling of which was shared with a doublet at δ 3.67. Clearly, this spectrum did not fit expected structure **(4a)** and subsequently isomeric structure **(4)** appeared as the most reasonable alternative. Configuration was deduced from coupling constants analysis and further demonstrated by NOE correlations between H-14 and the isopropyl group and between H-11 and the NCH_2. It is presumed that, due to steric hindrance around the mesylate, the opening of the epoxide by the amine is faster than the expected SN_2 on C-14. This is in accordance with results published by Liu *et al.,* for the reaction between benzylamine and triptolide,[9] where the addition brings about C-12-C-13 epoxide opening, leading to diol **(5)** in the absence of a nucleofuge.



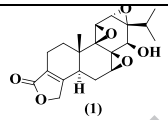
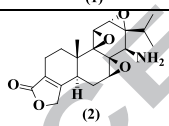
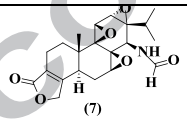
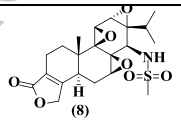
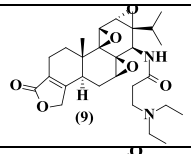
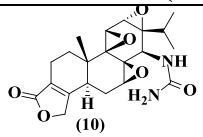
An alternative pathway for the formation of the title compound, was the reductive amination of triptonide **(6),** easily prepared by Dess-Martin oxidation of **(1)**.[10] Under normal conditions ($NaBH_4$, NH_3, MeOH, 60°C), it essentially led to reduction back to triptolide, due to difficulties in forming the intermediate imine. However, when the reaction was performed with ammonium acetate as the nitrogen source and in the presence of molecular sieves, **(2)** was obtained in a moderate 16% yield but accompanied with a fair amount of triptolide (35%), which was recycled.[11] Compound **2** was identified by its spectral properties and in particular by 2D NMR experiments, which showed the expected C-14 - H-14 correlations and indicated that no rearrangement had occurred.[12] The lactone ring proved to be untouched

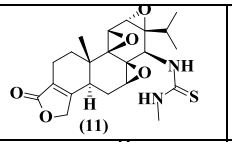
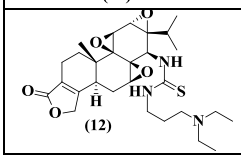
and H-11 and H-12, detected by their coupling, were found attached to oxygen bearing carbon. H-7 and H-14 had too similar chemical shifts to yield useful NOE correlations. The configuration of C-14 was based on the fact that, the hydride could only approach from the back side (exclusive reduction of triptonide into triptolide).



With 14-deoxy-14-aminotriptolide **(2)** in hand, a series of novel triptolide analogues were prepared, by using the nucleophilicity of this nitrogen atom. *N*-formyl derivative (**7**) was prepared using ethyl formate in 40% yield and classical conditions were used to prepare *N*-mesyl **(8)**, amide derivative **(9),** urea (**10**) and thioureas **(11** and **12)**.

To examine whether the substitution affected their biological activities, we evaluated the anticancer effects of these target compounds against five human tumour cell lines. The results suggested that the position 14 had to be free from large substituent. Compounds **8-12** with bulky group lost all the activity. Only *N*-formyl-14-aminotriptolide (**7**) retained significant cytotoxicity, and was found as equipotent as unsubstituted aminotriptolide **(2)**. However, both were found to be less potent than the original triptolide **(1)**, which might be useful for certain applications.

Compound	yield	A375 IC50 µM	A549 IC50 µM	HCT116 IC50 µM	MCF7 IC50 µM	Namalwa IC50 µM
 (1)		0.0050	0.0052	0.0047	0.0017	0.0100
 (2)	16%	1.32	NT	0.772	1.34	2.05
 (7)	40%	0.420	0.354	0.356	NT	1.34
 (8)	24%	>10	>10	>10	NT	>10
 (9)	37%	>10	>10	>10	NT	>10
 (10)	62%	>10	>10	>10	NT	>10

 (11)	68%	>10	>10	>10	NT	>10
 (12)	6%	>10	>10	>10	NT	>10

In summary, this work presents the preparation of C-14 aza triptolide and derivatives using a reductive-amination procedure, and also the preparation of some substituted derivatives. This work also demonstrates that high substitution on this position C14 yields to the loss of biological activity. Interestingly, in two recent publications Xu *et al*. described the preparation of novel derivatives of triptolide, including a series of compounds in which an heterocycle was placed onto C-14 via an additional methylene and which retained the full cytotoxicity of the natural product.[13,14]

Acknowledgements:

This work was supported by Institut de Recherche Pierre Fabre, whose support is gratefully acknowledged. The authors thank Drs Christian Bailly, Philippe Schmitt, Frédéric Marion and Nguyen Thien for encouragements and fruitful discussions.

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[11] Procedure for reductive amination of triptonide: ammonium acetate (4.3 g, 55.8 mmol) and sodium cyanoborohydride (0.658 g, 10.46 mmol) were dissolved in 6 mL methanol in a sealed tube, at room temperature and 3.53 g 4Å molecular sieves were added. Triptonide (500 mg, 1.395 mmol) dissolved in 120 mL THF was added under stirring and the reaction mixture was degassed by bubbling with argon for 15 minutes and sealed. After 3 days of sonication, the suspension was filtered over Celite®, washed with DCM and concentrated under reduced pressure. The residue was purified by HPLC to give 89 mg (0.223 mmol, 16 % yield) of (**2**) as a white amorphous solid.

[12] White powder, $[\alpha]^{20}_{D}$ -118.4 (*c* 0.17, MeOH); IR (film) ν_{max} 3406, 3339, 2941, 2901, 1758, 1676, 1029, 887, 701cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.82 (2H, *m*, H-19), 3.87 (1H, *d*, *J* = 3.2 Hz, H-11), 3.65 (1H, *s*, H-14), 3.62 (1H, *d*, *J* = 5.5 Hz, H-7), 3.44 (1H, *d*, *J* = 3.1 Hz, H-12), 2.79 (1H, *m*, H-5), 2.45 (1H, *qt*, *J* = 7 Hz, H-15), 2.29-2.22 (2H, *m*, H-2, H-6), 2.09 (1H, *m*, H-2), 1.97 (1H, *dd*, J = 15.0, 13.2 Hz, H-6), 1.50 (1H,m , H-1), 1.35 (1H, m, H-1), 1.09 (3H, *s*, H-20), 1.06 (3H, *d*, *J* = 6.6 Hz, H-16), 0.76 (3H, *d*, *J* = 7.1 Hz, H-17); ^{13}C NMR (MeOH, 125 MHz) δ 176.3 (C-18), 164.4 (C-4), 125.6 (C-3), 72.1 (C-19), 67.4 (C-13), C-9 (66.8), 63.3 (C-8), 57.3 (C-11), 56.0 (C-7), 53.0 (C-12), 50.9 (C-14), 41.7 (C-5), 37.3 (C-10), 31.2 (C-1), 28.2 (C-15), 24.6 (C-6), 19.6 (C-16), 18.2 (C-2), 15.9 (C-17), 14.1 (C-20); HRESIMS *m/z* 360.1793 (calcd for $C_{20}H_{26}NO_5$: 360.1805).

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Graphical Abstract

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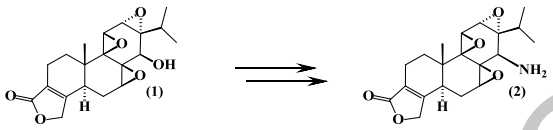

Highlights

Nucleophilic attack on triptolide mesylate yields epoxide opening and rearrangement

Borch reduction of triptonide yields aminotriptolide

Replacement of OH in triptolide by a primary amine strongly decreases cytotoxicity