In situ nitrosonium ion generation — α-Oximinylation of enol ethers from steroidal spiroketals: Introduction of C23 (*R*)-OH in cephalostatin intermediates[1]

Seongmin Lee and Philip L. Fuchs

Abstract: Nitrosonium ion generated in situ from the reaction of *t*-$BuNO_2$ and $BF_3{\cdot}Et_2$ is an effective oximinylating agent for enol ethers derived from steroidal spiroketals. The scope and limitations of this method has been studied. The difficult reduction of C23 ketone to C23 (*R*)-alcohol has now been selectively achieved via L-Selectride® reduction. Application to cephalostatin intermediates is discussed.

Key words: oximinylation, nitrosonium ion, steroid spiroketal, cephalostatins.

Résumé : L'ion nitrosonium généré in situ par réaction du *t*-$BuNO_2$ avec le $BF_3{\cdot}OEt_2$ est un agent d'oximinylation efficace pour les éthers énoliques dérivés de spirocétals stéroïdaux. On a étudié la portée et les limitations de cette méthode. Faisant appel à une réduction à l'aide de L-Selectride®, on a maintenant réalisé la difficile réduction stéréosélective de la cétone C23 en alcool-(*R*) C23. On discute de l'application à des intermédiaires de la céphalostatine.

Mots clés : oximinylation, ion nitrosonium, spirocétal stéroïdal, céphalostatines.

[Traduit par la Rédaction]

Introduction

The 45 members of the cephalostatin and ritterazine family, along with analogs, afford the basis for elucidating some of the structure–activity relationships (SAR) of these potent cytotoxins (1). All members of the cephalostatin family possess two steroidal spiroketals connected by a pyrazine. The most active compounds bear a highly oxygenated north unit and a substantially less polar south unit. In terms of bioactivity, cephalostatin **1** (Fig. 1) shows average 1 nmol/L GI_{50}s in 2 day tests in the NCI 60-line screen and 10^{-14} mol/L GI_{50}s in 6day tests in the Purdue 6 line minipanel (2). Cephalostatin **1**, cephalostatin **7**, ritterazine **M**, and ritterazine **K** have been synthesized by our group (3), while we and others (4) have also been active in the synthesis and testing of analogs.

The mechanism of action of the cephalostatins and ritterazines is currently unknown, although recent data indicates that ritterazine **B** is an apoptotic agent like OSW-1 (5) and cephalostatin **7** (6). The role of spiroketals and sugars as hydrogen bond donors and (or) acceptors has been suggested (7). Although it is evident from the NIH 60-cell line COMPARE studies that cephalostatin 1 and OSW-1 are related, significant biological differences[3] and recent bioactivity data of C22 deoxy OSW-**1** analogs (8) suggest that they may have a modified mechanism of action. Correlation of cytotoxicity with energy for E-ring oxacarbenium ion access in cephalostatins and ritterazines has been proposed (9). SAR studies indicate that the hydroxyl groups of both north and south units of the cephalostatin family play an essential role in tumor eradication. Hydroxyl groups provide "polarity match" (10), a requirement for the exhibition of bioactivity, and affect the heat of formation of the E-ring oxacarbenium ion,[4] thus adjusting bioactivity. When the C17 alcohol, for instance, in the north unit of cephalostatin **1** was replaced by a hydrogen, the antitumor activity dropped dramatically.[5] The presence of the C23 hydroxyl group also contributes to the anticancer activity. The recently synthesized C23′-deoxy cephalostatin **1**(11) is about 10 times less potent than cephalostatin **1**.

Our second generation synthesis of cephalostatin **1** (**4**) uses a "redox" strategy(12), requiring a scalable protocol ca-

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[3]P. Huang. Unpublished results.
[4]T.G. LaCour and P.L. Fuchs. Unpublished results.
[5]Compare, for example, ritterazine **A** (17 -OH, 24 nmol/L) and ritterazine **T** (17 -H, 590 nmol/L).

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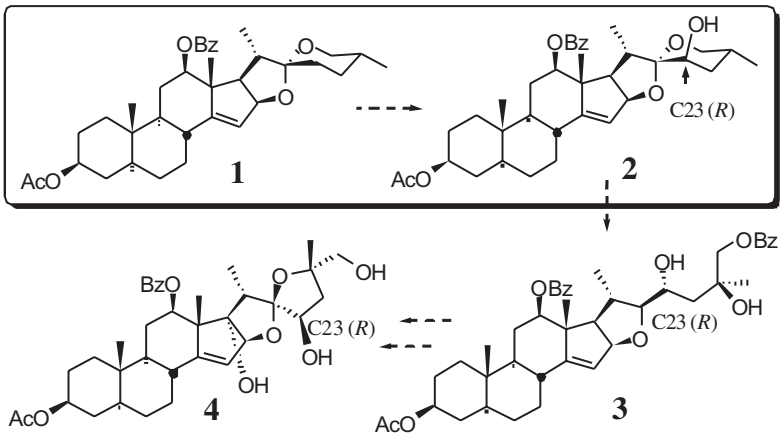
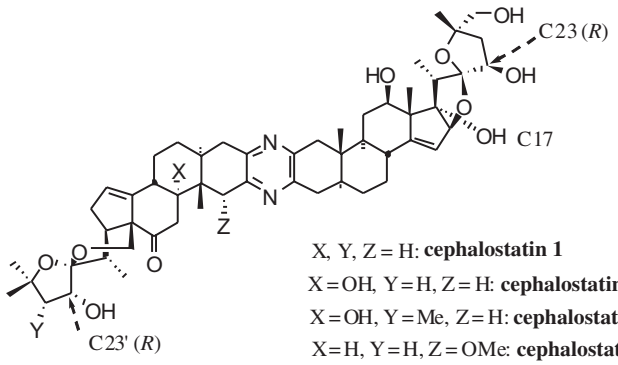
Scheme 1.



North unit of cephalosatin 1

Fig. 1.



pable of efficient introduction of the C23 (*R*)-alcohol (Scheme 1). Eighteen of nineteen known cephalostatins possess the north C23 (*R*)-alcohol and seventeen of those bear the south C23 (*R*)-alcohol (Fig. 1).

Many efforts have been made to introduce the C23 (*R*)-alcohol (13), such as the reduction of the C23 carbonyl group (14), sulfinylation of a C22,C23 enol ether with TFAA-activated DMSO followed by allylic rearrangement (15), and addition of allyl stannane to an aldehyde (16). However, none of these methods provided the requisite C23 (*R*)-alcohol in workable excess (de < 30%). At present, the only acceptable method is the dimethyldioxirane (DMDO) oxidation of an enone – vinyl ether to quantitatively achieve stereospecific cyclization to the prized (*R*)-alcohol. However, this method employs 750 mL of DMDO for the production of 15.7 g of C23 alcohol (17) and also requires the use of stoichiometric amounts of toxic selenium and stannyl reagents to prepare **2**. Herein, we describe a scalable pathway leading to C23 (*R*)-OH.

Results and discussion

The known methods for α-oxidation of ketones, enols, and spiroketals (Scheme 2) were initially surveyed to access the desired C23 ketone **7**. We first attempted the synthesis of a masked C23 ketone, such as nitroimine 5 or oxime 6. Although Suàrez and co-workers (18) recently demonstrated that the C14,C15-saturated spiroketal forms the nitroimine in acceptable yield, our attempts to use this procedure on substrate **1** only afforded the C23 nitroimine **5** in very low yield. We also investigated S_N2 chemistry with equatorial C23 bromide **8** and iodide **9**, prepared from **1** with phenyltrimethylammonium bromide (PTAB) or iodine monochloride, respectively. Unfortunately, numerous reagents that convert alkyl halides to alkoxy groups, including superoxide (19), KNO_2 (20), O_2–Bu_3SnH (21), $AgBF_4$–glyme–H_2O (22), AgOAc (23), and CsF–BzOH (24), were either unreactive or severely destructive.

The known methods for α-oximinylation of ketones were not applicable to our substrate (25). Thus, we were delighted that the formation of the desired oxime spiroketal **12** took place smoothly from the reaction of rockogenin diacetate **11** with *t*-$BuNO_2$ and $BF_3{\cdot}Et_2$. Optimization of this new procedure is summarized in Table 1. The choice of Lewis acid is critical for this event, as $BF_3{\cdot}Et_2$ gave the best yields while $FeCl_3$, $TiCl_4$, $SnCl_4$, $ZnBr_2$, $Sc(OTf)_3$, and TMSOTf failed to give reasonable conversions, probably because of their decreased oxophilic character relative to $BF_3{\cdot}OEt_2$.

Minimization of undesired lactone **13**, which comes from oxime spiroketal **12**, requires using excess *t*-BuONO (5 equiv.) and catalytic $BF_3{\cdot}OEt_2$ (0.5 equiv.) in acetic acid at ambient temperature for the minimum time. The use of less *t*-BuONO (Table 1, entries 1–5 and 7) and $BF_3{\cdot}Et_2$ (Table 1, entry 11), solvents other than AcOH (Table 1, entries 1, 3, and 4), lower temperature (Table 1, entry 2), or prolonged reaction time (Table 1, entry 10) results in the formation of significant amounts of lactone **13**.

The proposed mechanism of oximinylation involves nitrosonium ion (26) mediated nitrosylation of the enol ether **11a** formed via Lewis acid opening of the steroid spiroketal (Scheme 3). First, nitrosonium ion ($NO^+BF_4^-$) is generated in situ from a reaction of *t*-$BuNO_2$ and $BF_3{\cdot}OEt_2$. Then, the attack of enol ether **11a** on the nitrogen atom of nitrosonium ion takes place to produce nitroso spiroketal **12a**. Finally, the nitroso spiroketal **12a** is rapidly isomerized to give oxime spiroketal **12**. The presence of a $NOBF_4$ (–141.6 ppm, CD_3CN) peak in the ^{19}F NMR supports this mechanism. The reaction of spiroketal **11** with 3 equiv. of commercial $NOBF_4$ (Table 1, entry 19) in acetic acid affords the same

Scheme 2.

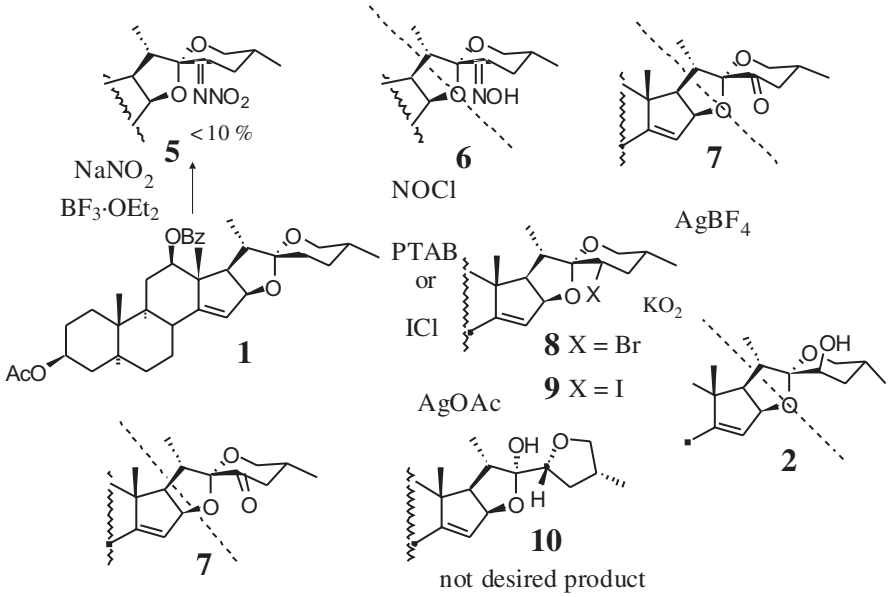
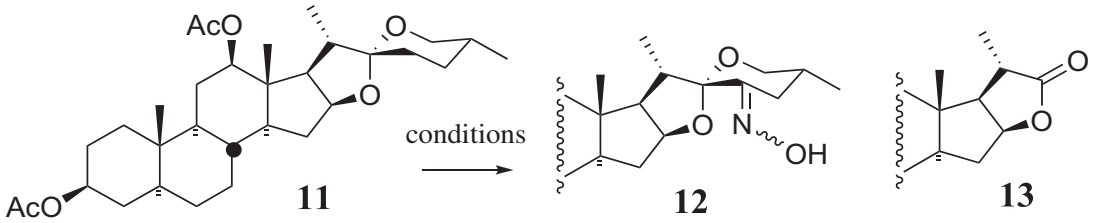


Table 1. Optimization of α-oximinylation.



Entry	Lewis acid (equiv.)	*t*-$BuNO_2$ (equiv.)	Solvent	*T* (°C)	Time (h)	Yields of **12** and **13** (%)
1	$BF_3{\cdot}OEt_2$ (1)	3	CH_2Cl_2	25	3	45, 31
2	$BF_3{\cdot}OEt_2$ (1)	3	CH_2Cl_2	0	3	NR
3	$BF_3{\cdot}OEt_2$ (1)	3	Toluene	25	3	40, 23
4	$BF_3{\cdot}OEt_2$ (1)	3	MeCN	25	3	35, 48
5	$BF_3{\cdot}OEt_2$ (1)	3	AcOH	25	0.5	62, 17
6	—	3	AcOH	25	1	NR
7	$BF_3{\cdot}OEt_2$ (2)	2	AcOH	25	1	37, 45
8	$BF_3{\cdot}OEt_2$ (0.5)	3	AcOH	25	1	32, 52
9	$BF_3{\cdot}OEt_2$ (0.5)	5	AcOH	25	0.2	92, 0
10	$BF_3{\cdot}OEt_2$ (0.5)	5	AcOH	25	2	50, 36
11	$BF_3{\cdot}OEt_2$ (0.2)	5	AcOH	25	2	41, 44
12	$NOBF_4$ (3)		AcOH	25	0.2	82, 0

Note: NR = No reation.

oxime spiroketal **12** in 82% yield after 10 min, thus exhibiting similar reactivity and product distribution pattern to that observed in the reaction of spiroketal **11** with $BF_3{\cdot}Et_2$ and *t*-BuONO (Table 1, entry 16). The Lewis acid promoted second-order Beckmann fragmentation (27) of α-oxygenated oxime spiroketal **12** appears to account for the formation of lactone **13**. The scope and limitation of the oximinylation of steroid spiroketals was then explored.[6] The results in Table 2 reveal some interesting features. First, C23 oximinylation works for only 5/6 spiroketals, not for 5/5 and 6/5 spiro systems, such as **21** and **22**. Hecogenin acetate (starting material for **15**) containing a C12 ketone, which is diffi-

[6] Supplementary data for this article are available on the journal Web site (http://canjchem.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Ottawa, ON K1A 0R6, Canada. DUD 5081. For more information on obtaining material refer to http://cisti-icist.nrc-cnrc.gc.ca/irm/unpub_e.shtml.

Scheme 3.

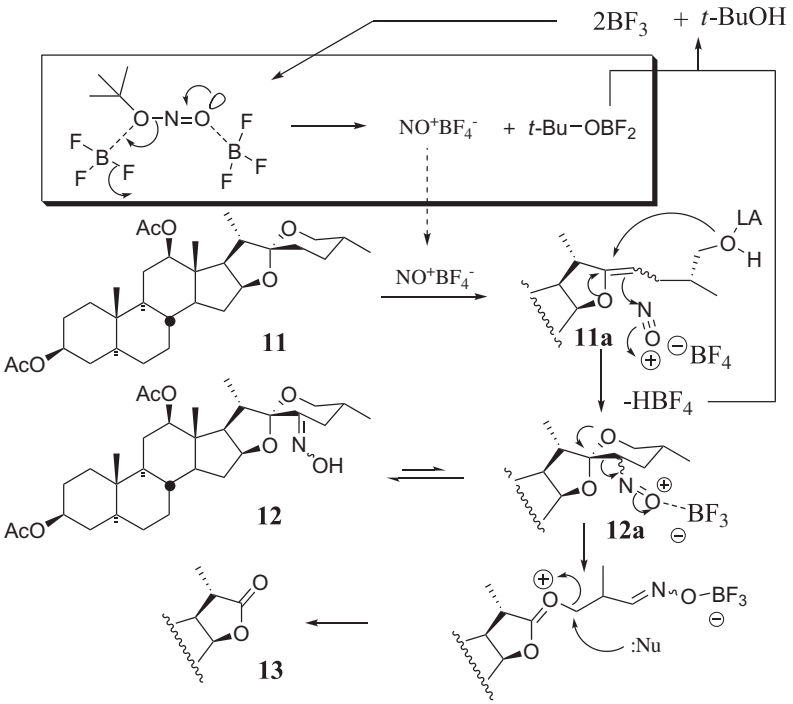
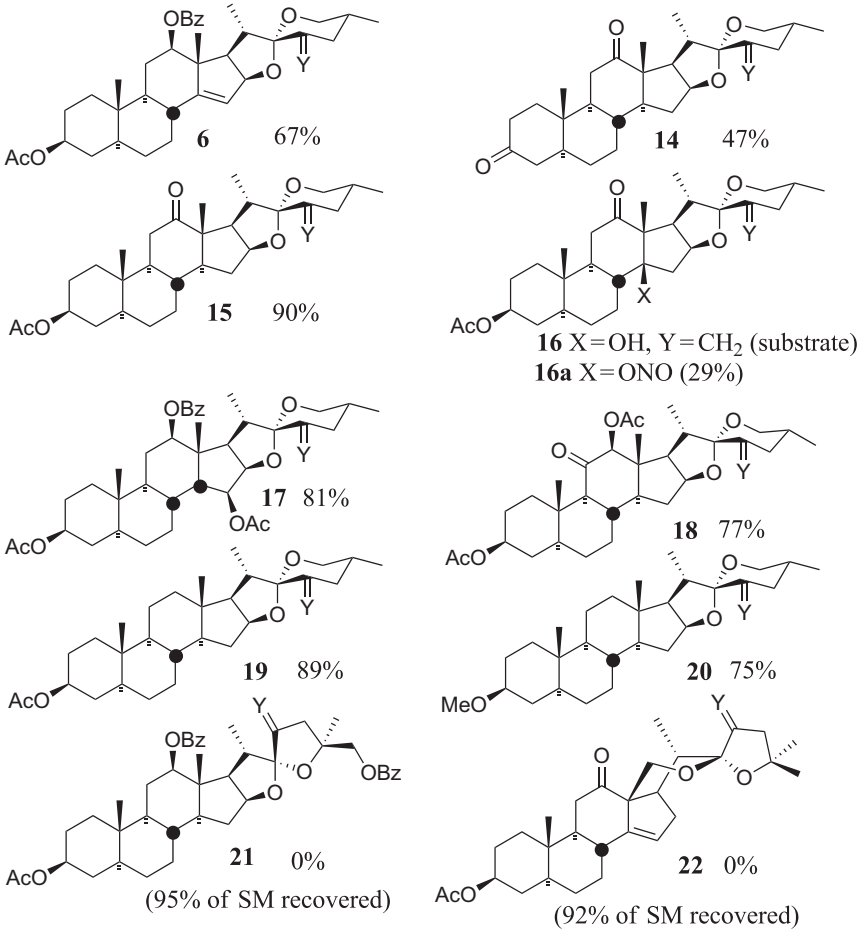
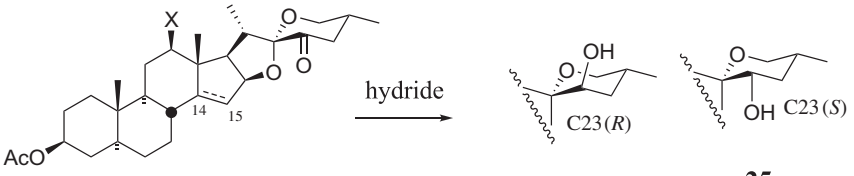


Table 2. α-Oximinylation of steroid spiroketals.



Note: isolated yields. Y = H_2 (substrates) or Y = NOH (products).

Table 3. Reduction of the C23 ketone.



23 X = OAc, C14–15 = 14α–H **24** **25**

7 X = OBz, C14–15 = Δ^{14} **2** **26**

Entry	Substrate	Hydride	Conditions	Products	Yield (%)	C23 (*R*):C23 (*S*)
1	23	$LiAl(OtBu)_3H$	0 °C, THF	**24**, **25**	93	1:20
2	23	$NaBH_4$	25 °C, MeOH	**24**, **25**	97	1:19
3	23	(*R*)-CBS/BH_3	25 °C, THF	**24**, **25**	85	1:11
4	23	$CeCl_3$/$NABH_4$	–78 °C, 2 h	**24**, **25**	94	1:4.2
5	23	LS-Selectride®	–78 °C, THF	**24**, **25**	81	1:1.4
6	23	L-Selectride®	–78 °C, THF	**24**, **25**	89	2.5:1
7	23	K-Selectride®	–78 °C, THF	**24**, **25**	83	4.6:1
8	23	(*S*)-CBS/BH_3	25 °C, 0.5 h, THF	**24**, **25**	91	6.2:1
9	7	L-Selectride®	–78 °C, 3 h, THF	**2**, **26**	87	10.3:1
10	7	(*S*)-CBS/BH_3	25 °C, 0.5 h, THF	**2**, **26**	92	7.3:1
11	7	L-Selectride®	–78 °C, 3 h, PhMe	**2**, **26**	85	5.5:1

cult to enolize, smoothly gave oxime spiroketal **15**. The presence of more readily enolized C3 ketone resulted in low yield of **14** because of the formation of by-products. It is notable that olefin moieties (**6** and **22**) are intact under the reaction conditions. Alcohol functionality was converted to nitrite **16a**. Most importantly, employing the new protocol, we were able to obtain a key intermediate (5/6 spiroketal **6**) in reasonable yield.

C23 ketones **23** and **7** were readily prepared by *p*-toluenesulfonic acid catalyzed deoximinylation. We next turned our attention to establishing the requisite C23 (*R*) stereochemistry (Table 3). In the case of C14,C15-saturated C23 ketone **23**, (*S*)-CBS best effected the desired reduction to afford C23 (*R*) and C23 (*S*) in a reproducible ratio of 6.2:1[7] (Table 3, entry 8, C23 (*R*) (78%) and C23 (*S*) (13%), isolated yield, 50 mmol scale; previous results (28) show formation of C23 (*R*) and C23 (*S*) in a ratio of 1.7:1 (72%)). We were pleased that the L-Selectride® reduction of C14,C15-unsaturated ketone **7** (Table 3, entry 9) delivered C23 axial alcohol **2** in a highly sterereoselective fashion.

In conclusion, an efficient and economical in situ method to generate nitrosonium fluoroborate is presented. This chemistry provides a large-scale pathway for the establishment of the C23 (*R*) stereochemistry of steroidal sapogenins. Further research is ongoing regarding the application of this method for the synthesis of the north unit of cephalostatin **1**.

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[7] Preincubation (~30 min) of (*S*)-CBS and BH_3 Me_2 in THF before the addition of **23** is critical to obtain good stereoselectivity.

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