

Stereo- and regio-selectivity in acetonitrile oxide cycloaddition to norethisterone acetate

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Dedicated to Prof. Giovanni Desimoni on the occasion of his 90th birthday

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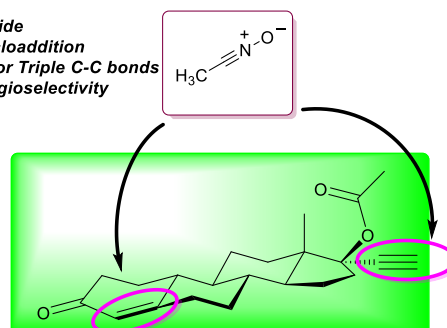
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Abstract

Acetonitrile oxide underwent a 1,3-dipolar cycloaddition with 17 α -acetate norethisterone affording the expected isoxazoline and isoxazoles derivatives in good yields. Mono- and bis-cycloadducts were obtained and the structures of the new compounds were elucidated based on the corresponding analytical and spectroscopic data, which were presented and discussed in detail. The stereo- and regio-chemical outcome of the cycloadditions was also accounted on the basis of 1,3-dipolar cycloaddition theory. Frontier Orbital theory (FO) and steric effects are assessed in orienting the selectivity in the cycloaddition to steroids.

Acetonitrile Oxide
1,3-Dipolar Cycloaddition
to Double and/or Triple C-C bonds
Stereo- and Regioselectivity



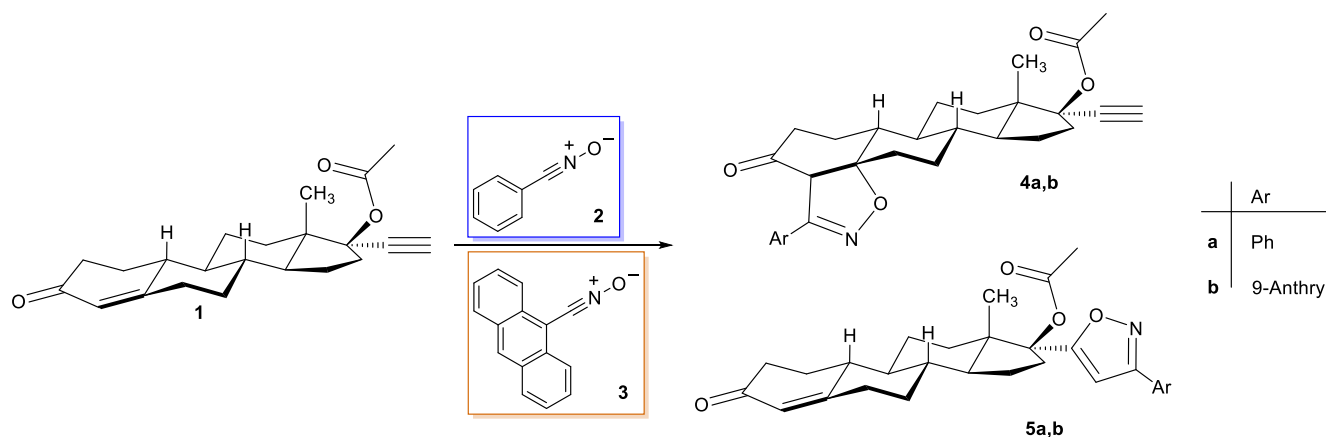
Keywords: Norethisterone acetate, acetonitrile oxide, cycloadditions, dipolarophile, regioselectivity, stereoselectivity.

Introduction

Steroidal compounds still hold immense potential for the development of potential pharmaceutical products. As in nature widely distributed compounds with diverse pharmacological effects, the discovery of natural steroidal molecules, not only expands the boundaries of knowledge regarding bioactive natural products, but also provides an abundant library of compounds and bioactive fragments for early-stage drug development, covering humans, marine organisms, plants and more.¹ The exploration of drug repurposing strategies and studies on the inherent properties of existing steroidal drug molecules is also significant. The similarity and variability in the structures of different steroids mean that minor changes in substituents on certain skeleton or ring sites can introduce entirely new pharmacological activities and even new mechanisms of action or the discovery of new biological targets.²

Among the various classes of steroids, we were attracted by those containing suitable and highly reactive functional groups for further derivatization, aiming to prepare new potentially active derivatives. Specifically, our interest concentrates on steroids containing double and triple C-C bonds, able to act as dipolarophiles in 1,3-dipolar cycloadditions. In this field, not much is reported in the literature, confining these compounds to chemical curiosities as dipolarophilic partners in cycloaddition derivatizations.³ Our research represents not only a mechanistic investigation on reactivity/selectivity of steroids in cycloadditions but also a potential search for novel structures with tuned-up biological activities. Our studies were oriented towards the derivatization of steroids with nitrile oxides for the isoxazoline or isoxazole rings introduction on the steroid skeleton in a selective manner.⁴ The use of nitrile oxides represents a general method for the synthesis of these five-membered heterocycles and can be undoubtedly considered a pillar of the synthesis of heterocyclic compounds. Several efforts are constantly made to widen and renew the heterocyclic syntheses through 1,3-dipoles, discovering new approaches to unveil molecules for modern and unexpected applications.^{4,5}

In a recent “proof of concept”, we have studied 19-norethisterone acetate **1**, as dipolarophile in the 1,3-dipolar cycloaddition reactions with aromatic nitrile oxides (Scheme 1).

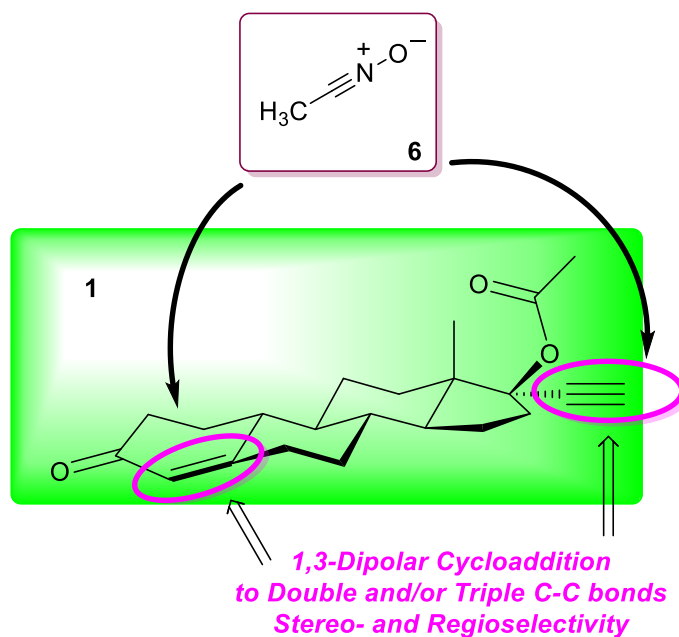


Scheme 1. Regio- and stereo-chemical outcome in 19-norethisterone as 17 α -acetate derivative **1** 1,3-dipolar cycloaddition with benzo- and anthracene-nitrile oxides **2** and **3**.

The double and triple C-C bonds in **1** can capture aromatic nitrile oxides, with subsequent chemical functionalization of the two distinctive dipolarophilic sites of the pristine structure, affording the respective isoxazoline and isoxazole derivatives. In both cases, singular chemical functionalizations were observed

(Scheme 1) when benzonitrile oxide **2**, generated *in situ* from the corresponding hydroxymoyl chloride and triethylamine or anthracenenitrile oxide **3**, previously prepared being a stable 1,3-dipole, were employed in cycloaddition reactions, *i.e.* the simultaneous addition of the nitrile oxides to the double and triple bond (formation of biscycloadducts) was never obtained. This was attributed reasonably to two main factors: the steric demand of the aromatic nitrile oxides used as well as their low reactivity as 1,3-dipoles vs. the selected steroid and the subsequent modified reactivity of the functionalized products that were less prone to undergo a further cycloaddition.⁶

On the basis of these findings, we wonder about the potential results obtainable when other and more reactive nitrile oxides with different steric demand are used, *e.g.* aliphatic nitrile oxides, in these type of derivatizations. Acetonitrile oxide **6** could be the right choice to strengthen the cycloaddition protocol (Scheme 2).



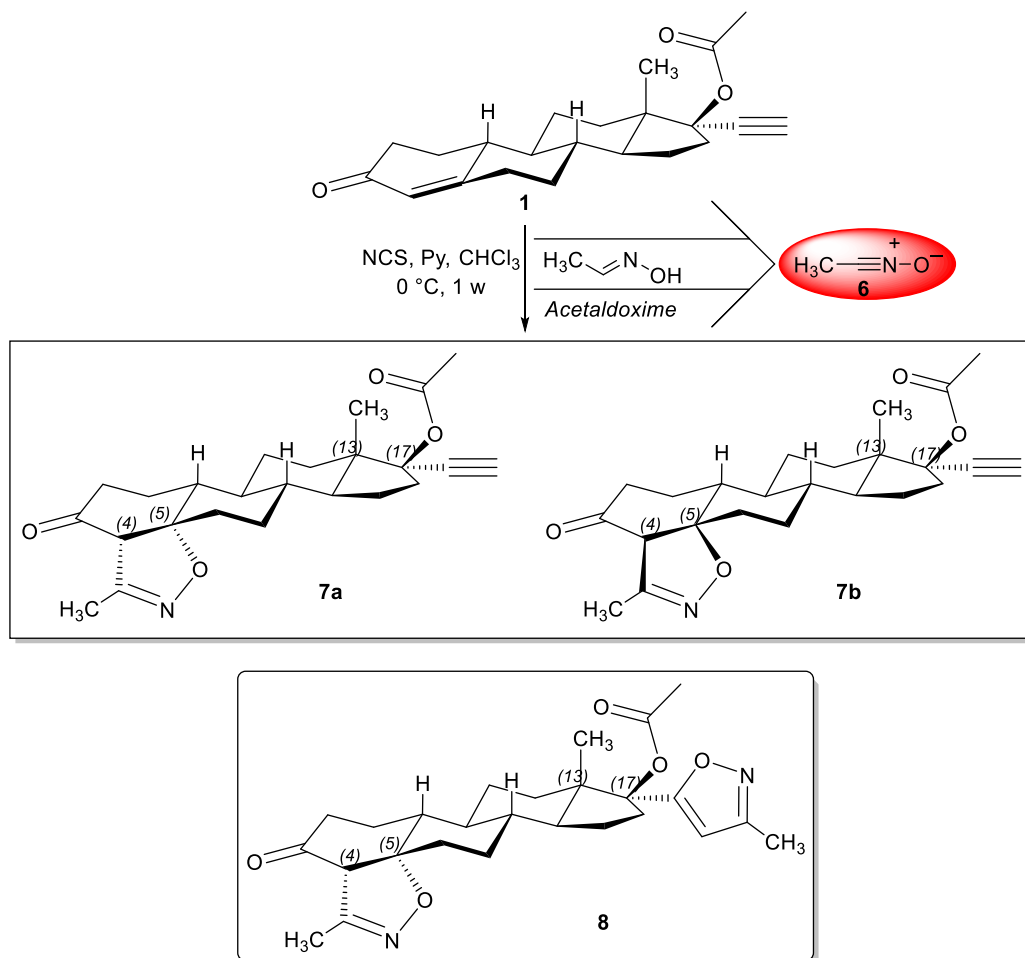
Scheme 2. Synthetic strategy from the norethisterone acetate **1** to the corresponding 1,3-dipolar cycloaddition with acetonitrile oxide **6**.

Here, we wish to demonstrate the versatility of the chemistry of traditional 1,3-dipolar probes that can be easily modeled for the preparation of valuable isoxazolines and isoxazoles with steroids acting as dipolarophiles. The synthetic strategy takes advantage of the well-established chemistry of nitrile oxides, introduced by means of a simple and reliable method, relying on a very adjustable and adaptable chemistry. The resulting novel norethisterone derivatives will enter in the family of heterocyclic steroids whose biological activity can be easily investigated.⁷

Results and Discussion

Norethisterone acetate **1** was allowed to react with acetonitrile oxide **6** upon treatment of the steroid with acetaldoxime, with NCS as chlorinating agent and pyridine at 0 °C in chloroform, in accordance with the classical methods reported in the literature (Scheme 3) and leaving the reaction at room temperature for 48

h.^{8,9} The reaction progress is monitored by TLC, checking in particular the consumption of the starting steroid. After one day under stirring, the derivatization of norethisterone proceeded slowly and a strong competition in the fast dimerization of the highly reactive acetonitrile oxide was observed. For this reason, further amounts (two runs) of a solution containing acetaldoxime/NCS/pyridine (see experimental section) were added at the third and at the fourth reaction day to improve the cycloadducts yields and the reaction time was prolonged to 1 week (total excess nitrile oxide = 21 equiv.). After typical work-up procedure needed to reduce or get rid of the excess of pyridine and succinimide and dessication of the resulting organic phase, the reaction mixture was submitted to column chromatography for the isolation of the products. Compounds **7a/7b** and **8** were obtained in fair yields (40% and 33%, respectively) as white solids and were fully characterized.



Scheme 3. Synthesis of acetonitrile oxide derivatives **7a/7b** and **8** from norethisterone acetate **1**.

An inseparable mixture of the diastereoisomeric compounds **7a** and **7b** was obtained in accordance with the characterization derived from the corresponding analytical and spectroscopic data, which need to be commented here below.

From the first exam of the FT-IR spectrum, the presence of a sharp band at 3259 cm⁻¹ is easily attributable to the C-H stretching of the C≡C-H bond of the norethisterone skeleton; the stretching band of the C≡C triple bond is also observed at 2355 cm⁻¹. The IR spectrum suggests that the products are derivatized on the C=C double bond, only and the confirm comes from the ¹H NMR spectrum (CDCl₃), compared with that of norethisterone acetate **1**.

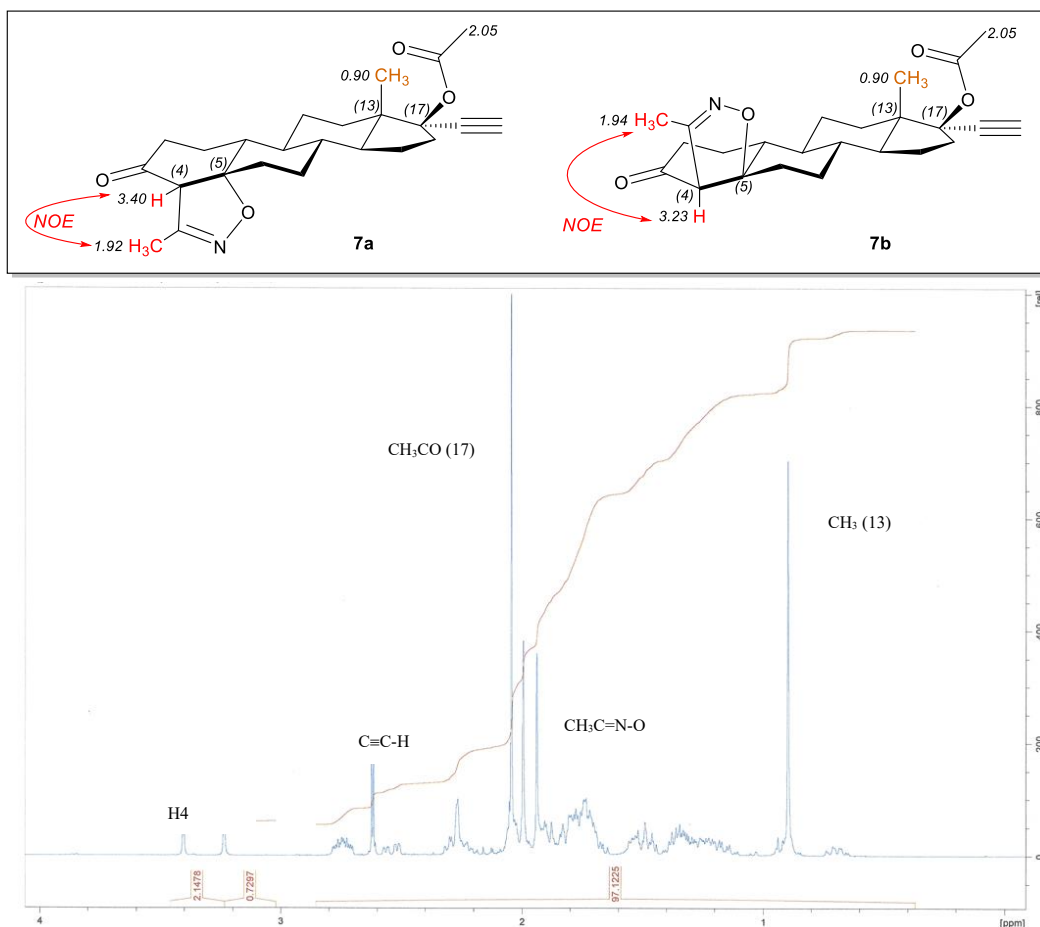


Figure 1. ^1H NMR spectrum of diastereoisomers **7a** and **7b**.

Specifically, a singlet at 0.90 ppm corresponding to 6 protons belongs to the methyl groups in position (13) of the steroid skeletons; similarly, it happens for the acetyl groups found at 2.05 ppm. The two isoxazolinic methyls, introduced through the acetonitrile oxide addition, showed different chemical shifts and are found at 1.92 and 1.94 ppm and are referred to the two diastereoisomeric compounds having different stereochemistry at the positions (4) and (5) of the steroid. Two distinct $\text{C}\equiv\text{C}-\text{H}$ proton signals (singlets) are found at 2.61 and 2.62 ppm and, finally, two singlets at 3.23 and 3.40 ppm were attributed to the H4 isoxazolinic protons.

Figure 1 reports the structures of the diastereoisomers **7a/7b** along with the corresponding ^1H NMR spectrum. Further support to the existence of a diastereoisomeric mixture of cycloadducts came from the ^{13}C NMR and 135-DEPT spectra (see Supplementary Material, SM) as well as from the NOESY and HSQC experiments. The number of carbon atoms is nearly double with respect to the required for a single compound and, besides those overlapped, the spectral pattern showed couples of signals with close chemical shifts in nice keeping with a diastereoisomeric mixture of parent compounds.

The NOESY and HSQC spectra also served to confirm the above statements but did not help in any manner to make a distinction of the signals attributable to one stereoisomer or the other. These experiments confirmed that the 1,3-dipolar cycloaddition of acetonitrile oxide **6** occurred at the olefinic dipolarophilic site of enorethisterone **1**.

To complete the characterization, the NOESY spectrum gives correlation spots between the isoxazolinic CH in position (4) at 3.40 ppm and the isoxazolinic CH₃ at 1.92 ppm. The isoxazolinic proton in position (4) at

3.23 ppm shows also a correlation with the isoxazolinic CH₃ at 1.94 ppm. Furthermore, the two closely spaced singlets corresponding to $\equiv$ CH at 2.61 and 2.62 ppm correlate perfectly with the relative carbon atoms, with closely spaced chemical shifts, 75.2 and 76.7 ppm.

The second compound obtained from the reaction with norethisterone **1** is the biscycloadduct **8** (Scheme 3), which was obtained in 33% yield, in the form of a crystalline solid whose structure, based on its relevant analytical and spectroscopic data, showed significantly the absence of the triple bond as confirmed simply by the IR spectrum in which the bands relating to this functional group are absent. In the ¹H NMR spectrum (CDCl₃) it is clear that the sample is representative of a single stereoisomer and four methyl groups are clearly present (Figure 2): the first at 1.07 ppm corresponds to the methyl in (13) of the steroid structure; subsequently a methyl appears at 2.08 ppm corresponding to the acetyl group in (17) followed by two methyl groups at 1.96 and 2.32 ppm, relating to the isoxazolinic and isoxazole substituents, respectively, as resulting from the acetonitrile oxide cycloaddition reaction. These first data undoubtedly indicate that the cycloaddition of the 1,3-dipole occurred on both the C=C and C $\equiv$ CH bonds. Indeed, the presence of the isoxazoline ring is confirmed by the singlet signal of the proton in (4) of the steroid structure found at 3.23 ppm, while the presence of the isoxazole ring is confirmed by a more deshielded singlet proton (H₄_{Isox}) at 5.87 ppm. The ¹³C NMR (CDCl₃) confirmed the presence of 26 carbon atoms, in line with the molecular formula and the HRMS data, supported by the DEPT 135 experiment, which precisely defines the number of methylene groups with respect to CH₃ and CH moieties (see SM).

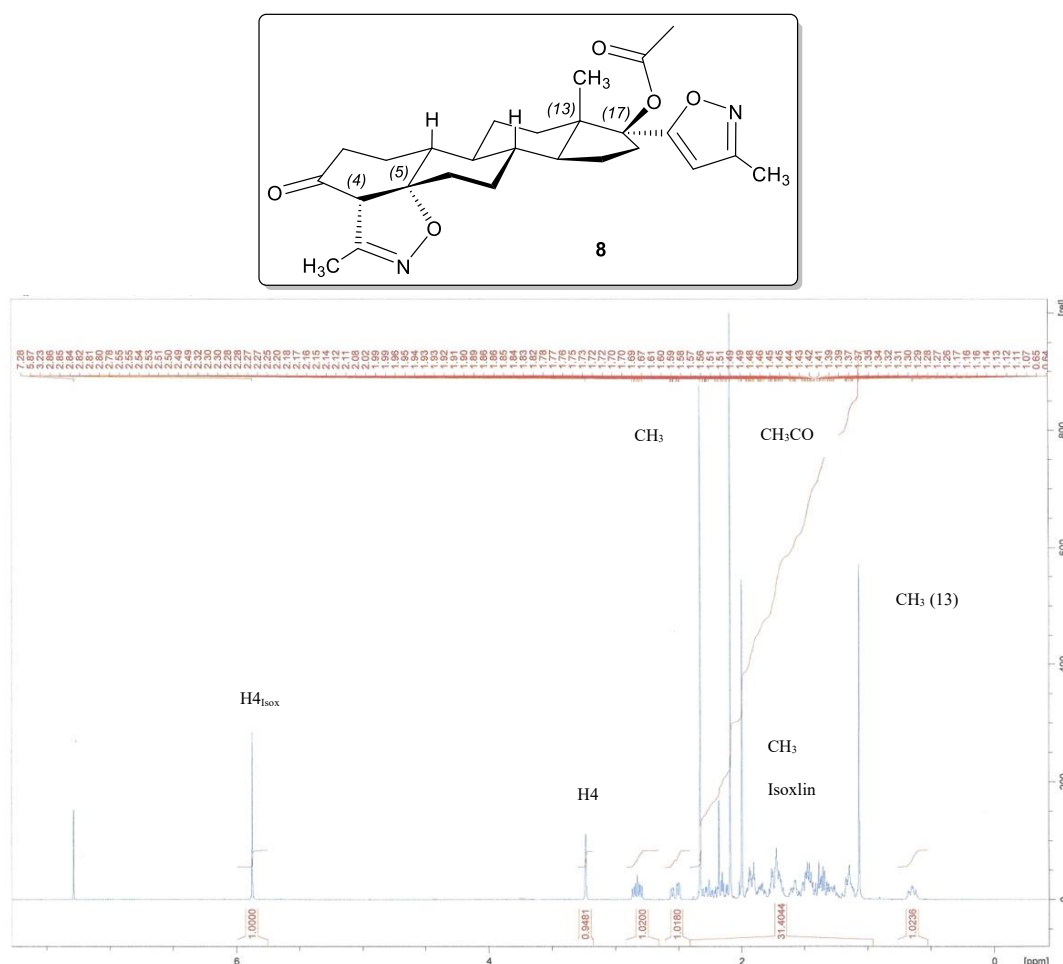


Figure 2. ¹H NMR spectrum of the biscycloadduct **8**.

The NOESY and HSQC experiments corroborated the above reported structural descriptions. In particular, the HSQC spectrum allowed to attribute the protons at 1.07 ppm of the ^1H NMR spectrum to the C in position (13) found at 14.6 ppm. The acetyl group, whose methyl is found at 2.08 ppm in the proton spectrum, connects with the carbon at 21.2 ppm; the two heterocyclic methyls at 1.96 and 2.32 ppm are bonded to the carbons at 11.5 and 12.9 ppm, respectively. In the NOESY spectrum, the isoxazoline proton H4 at 3.23 ppm showed a correlation with the methyl atom at 1.96 ppm, which is therefore confirmed to belong to that heterocyclic ring. Conversely, the isoxazole methyl atom at 2.32 ppm did not show any NOE correlation with other steroid protons, except for the proton at 5.87 ppm, clearly indicating its location far from the steroid structure and, therefore, also confirming the regiochemistry of the cycloaddition at the triple bond.

During the crystallization process for the purification of compound **8**, an agglomerate of single crystals of the biscycloadduct was obtained, thus allowing for the collection of X-ray diffraction data; the final and definitive structure of compound **8** was provided (Figure 3), nicely answering all the questions relative to the regio- and stereo-chemistry of the cycloaddition process on norethisterone. Table S1 in SM reports the crystal data of compound **8**.

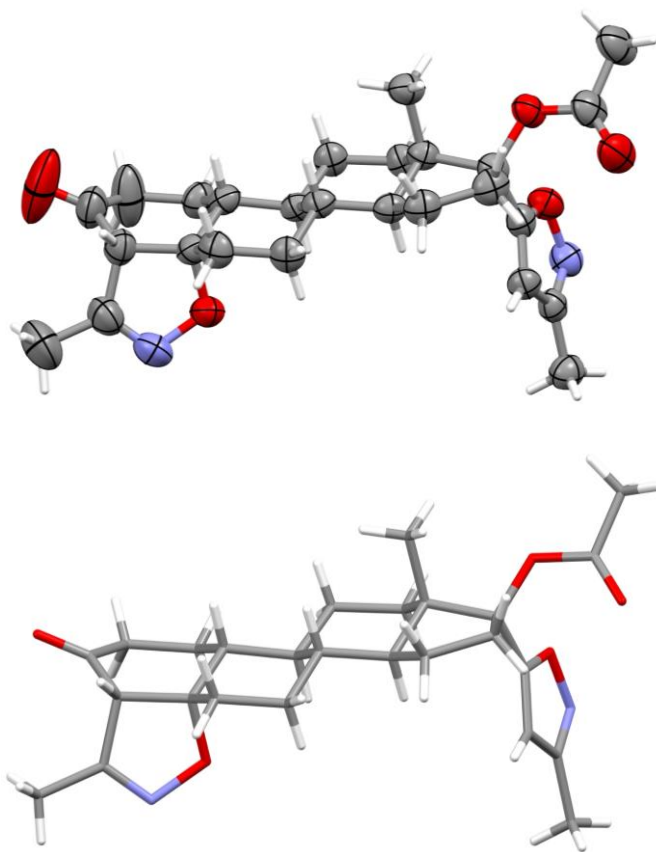


Figure 3. ORTEP view (top) and stick representation (bottom) of biscycloadduct **8**.

The importance of steroids in many significant physiological processes continues to attract research activities in this field.¹ Pharmacological compounds based on natural steroid derivatives are constantly developed, demonstrating numerous applications in the treatment and diagnosis of various diseases. It is expected that different steroid structures can be optimized to exert a wide range of pharmacological activities. Furthermore, the addition of new pharmacophores or the introduction of new substituents may lead to entirely new functions and modes of action in steroid drugs.⁶

In this view, the use of the 1,3-dipolar cycloaddition chemistry represents an intriguing method for diverse functionalization on steroid skeletons bearing suitable dipolarophilic sites. The thorough studies on this type of pericyclic reactions to unsaturated compounds conducted by Rolf Huisgen (LMU, Munich) in the last century are the obligatory references and canon for any regiochemical investigation in 1,3-dipolar cycloaddition reactions.¹⁰ In the present work along with previous results⁶ we have demonstrated that norethisterone (**1**)¹¹ can act as dipolarophile in 1,3-dipolar cycloaddition with nitrile oxides. Norethisterone contains a double and a triple C-C bond potentially apt to be functionalized through the cycloaddition protocol for the insertion of variable heterocyclic rings, specifically isoxazolines and isoxazoles.¹²

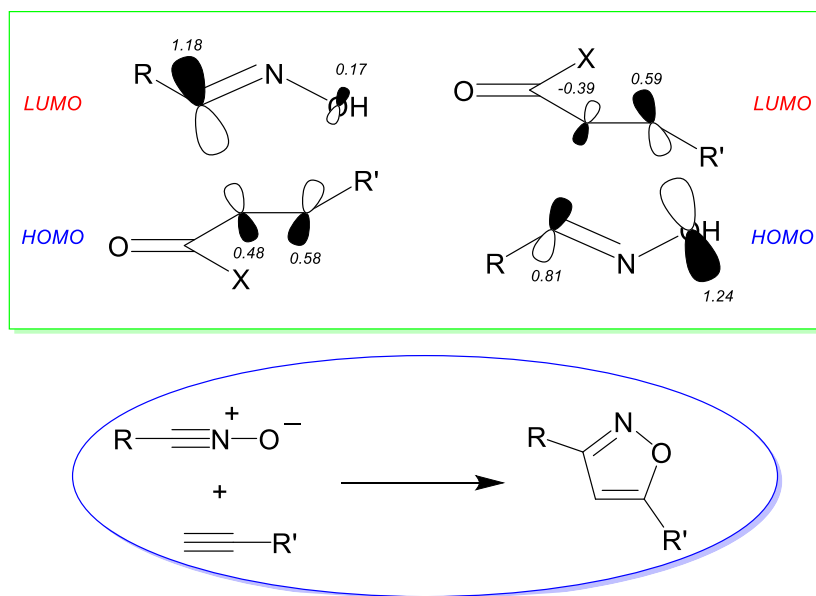


Figure 4. Nitrile oxide and unsaturated compound FOs shapes in HOMO/LUMO and reverse interactions (top). Numbers close to the orbitals are the coefficients given for fulminic acid ($R = H$) and for acrolein ($R' = X = H$).¹³ Regiochemical outcome in nitrile oxide and alkynes cycloadditions (bottom).

The regioselectivity in the cycloaddition reactions of benzonitrile oxide **2** and a series of α,β -unsaturated compounds typically afford mixtures of 4- and 5-acyl-isoxazolines where the 4-acyl isomers are the major products in keeping with the preferred binding of the nitrile oxide oxygen atom that possesses the highest *HOMO* coefficient to the β carbon atom of the unsaturated dipolarophiles.¹⁴ The regioselectivity in nitrile oxide cycloadditions to unsaturated compounds finds in the shape and energies of frontier orbitals (FOs) a valuable help for a better understanding. Even at a semiempirical level of calculation (AM1),¹⁵ the shape of the FOs (Figure 4, top) does not change sizeably upon changes in the X substituent in keeping with the modest influence of the acyl substituents on the conjugation of the carbonyl and the C=C bond in cross-conjugated systems. Hence, a high regioselection should occur in these cycloadditions, since the two FOs interactions favor the same 4-acyl regioisomers. However, in some cases the regioselection diminishes due to the steric drift operating at the X substitution level of steric demand.¹⁴

In the case at hand, norethisterone adds acetonitrile oxide on the C=C double bond at the position (4-5) of the ring A with the predicted regioselectivity in full accordance with the FO theory. Two isoxazoline derivatives as inseparable mixture of compounds were isolated having the nitrile oxide moiety directed on the two faces α and β of the steroid skeleton (Scheme 3).

Concerning the selectivity in the nitrile oxides cycloaddition to alkynes, prevalence of the 5-substituted isoxazole derivatives is clearly reported in literature¹⁶ in nice keeping with the FO theory accounts and experimental observations (Figure 4, bottom). The seminal papers by K. N. Houk (UCLA, USA) significantly reflect the relevance of the principle of maximal overlap that orientates the prediction of the preferred isomers derived by the union of the two sites having the largest coefficients in FOs.¹⁷ These observations are also at work in norethisterone cycloaddition with acetonitrile oxide with the exceptional case of the double cycloaddition leading to the biscycloadduct never observed in the case of the reactions conducted with aromatic 1,3-dipoles. The results reported in this work obey to these theoretical statements.

Curiously enough, the biscycloadduct **8** shows the same stereochemistry at the double bond of compound **7a**; among the fractions collected from the chromatographic separation of the reaction mixture, no other compounds could be detected and in particular not a second biscycloadduct displaying the stereochemistry of adduct **7b**.

We have already accounted the stereochemical features in the cycloaddition reactions of norethisterone **1** with aromatic nitrile oxides;⁶ the use of benzonitrile oxide **2** or anthracenenitrile oxide **3** proved the not-negligible steric effects^{18,19} in the cycloaddition outcome; the stereo- and regio-chemistry of the products was in keeping with the FO theory and the possibility to host large or very large aromatic moieties on a steroid skeleton. We discussed these features through computational derived model of the cycloadducts. Furthermore, these two 1,3-dipoles displayed a smooth reactivity towards norethisterone affording only monocycloadducts at the double and triple C-C bond; simultaneous cycloaddition was never observed, even in the presence of excess dipoles; reasonably this behavior was somewhat attributed to changes in dipolarophilic activity of the monofunctionalized steroids.

Acetonitrile oxide **6** is characterized by two main topics: high reactivity with respect to aromatic 1,3-dipoles and minimal steric demand of the methyl group. The high reactivity has, nevertheless, a considerable drawback due to the highly competitive dimerization reaction of the nitrile oxide to furnish the corresponding furoxan. In view of this fact, large amounts of the 1,3-dipole were used in this cycloaddition process, much more than required as reported in the experimental section. The reaction, conducted under these conditions, afforded the isoxazoline cycloadducts (derived from the addition of the acetonitrile oxide to the C=C double bond) with variable stereochemistry, where the 1,3-dipole moiety is found on both the steroid faces α and β . More intriguing was the result that led to the biscycloadduct to both the dipolarophilic sites of norethisterone, whose structure was not only elucidated through spectroscopic analyses but with an X-ray structure, confirming all the assumptions described on the basis of the FOs theory and stereochemical considerations.

Conclusions

In conclusion, this work confirms the possible and reliable application of steroids containing double or/and triple C–C bonds as dipolarophiles in nitrile oxides 1,3-dipolar cycloaddition reactions. The chemistry of isoxazoles,²⁰ dating since many decades ago, seems to be promising and valuable for the introduction of heterocyclic modifications to steroidal structures, with potential effects on the biological activities of known or novel compounds sharing a steroid skeleton. The chemistry of nitrile oxides can be clean and safe as the applied experimental protocol demonstrates; the construction of the isoxazoline and isoxazole heterocyclic ring is simple, reliable and robust.

Regarding the nitrile oxides, the methodology can be applied by taking advantage of the variety of structures having the aldehydes as starting compounds. In this light, we have indeed demonstrated the

possibility to expand the application of 1,3-dipolar cycloaddition reactions in order to obtain a group of easy-derivatizable steroids.²¹ In parallel, the set-up protocol is easily applied to design and prepare new steroids with variable and tunable biological activities. From this study a remarkable selectivity outcome emerged; the steroid skeleton orientates both regio- and stereo-chemistry leading to easily predictable structures on the basis of enforced selection rules well-known from the 1,3-dipolar cycloaddition chemistry.

Experimental Section

General. Melting points (mp) are uncorrected. NMR experiments were registered on Bruker AVANCE 400 spectrometers in deuterated solvent solutions as indicated. The chemical shifts (δ) are expressed in ppm, using tetramethylsilane as internal reference. IR spectra (ATR) were recorded on an Agilent Technologies Cary 630 ZnSe Engine spectrophotometer available at the Department and absorptions (ν are in cm^{-1}). HRMS were done on a X500B QTOF system (Sciex, Framingham, MA 01701 USA) available at the CGS of the University of Pavia. Column chromatography and tlc: silica gel H60 and GF₂₅₄, respectively; eluants: cyclohexane/ethyl acetate 9:1 to pure ethyl acetate.

Reference and starting materials. Generous amounts of 17 α -Acetate norethisterone (**1**) were offered by Steroid S.p.A. Acetonitrile oxide (**6**) was generated *in situ* from the corresponding oxime (commercially available) in the presence of NCS and pyridine, in accordance to the literature reported procedures.⁸ Solvents and other reagents were purchased from suitable chemical suppliers and used without any further purification.

Reaction of the 17 α -acetate norethisterone (1**) with acetonitrile oxide (**6**).** To a chloroform solution (100 mL) of 2.56 g (43.3 mmol) of acetaldoxime, 3.88 mL (47.9 mmol) of freshly 87 g (distilled pyridine) were added and the solution cooled to 0 °C with an ice bath and stirred. Solid NCS 5.43 g (43.9 mmol) was added portionwise within 30 minutes; the solution is left under stirring for further 30 minutes and then 1.36 g (3.90 mmol) of norethisterone acetate (**1**) were added and the resulting solution left under stirring for 48 h leaving the temperature to rise at ambient values. The reaction evolution was monitored by TLC verifying the consumption of the starting steroid. To ensure this and getting better reaction yields, two subsequent additions of acetaldoxime (1.00 g)/NCS (2.29 g)/pyridine (1.50 mL) in 50 mL chloroform were done, the first after the first reaction day and the second at the third reaction day. The overall reaction time corresponded to 1 week. After this period of time, the organic solution was washed with brine (3x200 mL) and dried over anhydrous Na₂SO₄. Upon concentration under reduced pressure of the organic phase, a residue was obtained that was submitted to chromatographic separation for the isolation of the products.

Hereby, we report the full characterization of the isolated products. In the case of compounds **7a,b**, the spectral data correspond to the inseparable mixture of diastereoisomers.

(3a*S*,6a*R*,6b*S*,8a*S*,9*R*,11a*S*,11b*R*,13a*S*)-9-ethynyl-3,8a-dimethyl-4-oxo-3a,4,5,6,6a,6b,7,8,8a,9,10,11,11a,11b,12,13-hexadecahydrocyclopenta[7,8]phenanthro[1,10a-*d*]isoxazol-9-yl acetate (**7a,b**): Yield: 40%. Mp: 199-204 °C from diisopropyl ether/ethanol. FT-IR: $\nu_{\text{C-H}}$ 3259; $\nu_{\text{C}\equiv\text{C}}$ 2355; $\nu_{\text{C=O}}$ 1738, $\nu_{\text{C=N}}$ 1701 cm^{-1} . ¹H NMR (300 MHz, δ , CDCl₃, 25°C): 0.90 (s, 6H, CH₃), 0.68-2.75 (m, 40H, CH and CH₂), 1.92 (s, 3H, CH₃), 1.94 (s, 3H, CH₃), 2.05 (s, 6H, COCH₃), 2.61 (s, 1H, $\equiv\text{CH}$), 2.62 (s, 1H, $\equiv\text{CH}$), 3.23 (s, 1H, CH-C=N), 3.40 (s, 1H, CH-C=N). ¹³C NMR (75 MHz, δ , CDCl₃, 25°C): 12.5, 12.9, 13.3, 13.4, 17.9, 20.6, 21.4, 23.28, 23.3, 25.9, 26.6, 30.7, 32.4, 32.9, 35.4, 36.6, 37.3, 40.2, 41.8, 42.4, 45.0, 46.6, 47.3, 47.5, 47.7, 66.7, 69.0, 75.2, 76.7, 83.1, 83.2, 84.2, 84.3, 88.2, 90.7, 150.8, 152.7, 169.5, 205.7, 205.9. HRMS: Calculated for C₂₄H₃₁NO₄: 398.2326. Found: 398.2316.

(3aS,6aR,6bS,8aS,9S,11aS,11bR,13aS)-3,8a-dimethyl-9-(3-methylisoxazol-5-yl)-4-oxo-3a,4,5,6,6a,6b,7,8,8a,9,10,11,11a,11b,12,13-hexadecahydrocyclopenta[7,8]phenanthro[1,10a-d]isoxazol-9-yl acetate (**8**): Yield: 33%. Mp: 201-205 °C from diisopropyl ether/ethanol. FT-IR: $\nu_{\text{C=O}}$ 1735; $\nu_{\text{C=N}}$ 1705 cm^{-1} . ^1H NMR (300 MHz, δ , CDCl_3 , 25°C): 0.64 (m, 1H, CH), 1.07 (s, 3H, CH_3), 1.10-2.85 (m, 18H, CH and CH_2), 1.96 (s, 3H, CH_3), 2.08 (s, 3H, COCH_3), 2.32 (s, 3H, CH_3), 3.23 (s, 1H, CH-C=N), 5.87 (s, 1H, $\text{H}_{4\text{isox}}$). ^{13}C NMR (75 MHz, δ , CDCl_3 , 25°C): 11.5, 12.9, 14.6, 20.5, 21.2, 23.7, 25.5, 26.0, 33.1, 35.6, 36.6, 38.3, 40.7, 42.1, 44.8, 46.6, 48.7, 68.9, 87.9, 88.2, 103.4, 152.7, 159.3, 170.0, 172.8, 205.9. HRMS: Calculated for $\text{C}_{26}\text{H}_{34}\text{N}_2\text{O}_5$: 455.2540. Found: 455.2533.

X-Ray crystallographic analysis. Crystal data of compound **8** are given in Table 1. Diffraction data for an irregular pale yellow single crystal were collected at ambient temperature using a Rigaku XtaLab SuperNova (Rigaku Europe SE, Neu-Isenburg, Germany) four-circle diffractometer equipped by a microfocus X-ray source ($\lambda = 0.7107 \text{ \AA}$) and a Dectris Pilatus 200K hybrid pixel array detector (DECTRIS AG, Baden-Daettwil, Switzerland). Data collection of diffraction frames, data reduction and final accurate determination of the unit cell parameters were performed by means of the CrysAlisPro software suite, ver. 1.171.41.²² Empirical absorption effects (based on a multi-scan approach using spherical harmonics) were estimated by the SCALE3 ABSPACK scaling algorithm implemented in the CrysAlisPro crystallographic suite and absorption correction was applied to the data. Crystal structure was solved by direct methods (SIR 97)²³ and refined by full-matrix least-square procedures on F^2 using all reflections (SHELXL 2019/2).²⁴ Full anisotropic structure refinement was performed for all non-H atoms, whereas hydrogen atoms were placed at calculated positions with the appropriate AFIX instructions and refined using a riding model.

CCDC deposition number 2534219 contains the supplementary crystallographic data for this paper. These data are provided free of charge by the joint Cambridge Crystallographic Data Centre and Fachinformationszentrum Karlsruhe Access Structures service.

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Supplementary Material

^1H , ^{13}C , 135-DEPT, NOESY, HSQC, HMBC and HRMS spectra of all the new synthesized compounds. CIF file for X-ray structure. Supplementary data to this article can be found online. NMR spectroscopy experiments (^1H , ^{13}C , HSQC, HMBC, 135-DEPT, NOESY) conducted on norethisterone base and its acetate **1** as well as the spectroscopic investigations commissioned by Steroid S.p.A. on other derivatives allowed for the precise identification of proton and carbon atoms in the steroid skeletons.

References

1. Klyne, W. The chemistry of the steroids. J. Wiley and Sons Inc., New York, 1957, pp. 13–230.
Zhu, J.; Luo, G.; Xiang, H. *J. Med. Chem.* **2026**, *69*, 765–791.
2. <https://doi.org/10.1021/acs.jmedchem.5c0300>
3. Huisgen, R. 1,3-Dipolar Cycloadditions – Introduction, Survey, Mechanism. Padwa, A. Ed., Wiley, J. & Sons Inc., New York, 1984, vol. 1, pp. 1–176.
4. Quadrelli, P. Modern applications of cycloaddition chemistry. Elsevier Eds., Amsterdam (2019) 1–152.
5. Grünanger, P.; Vita Finzi, P. Isoxazoles Part 1. P. Grünanger, P. Vita Finzi, Ed. J. Wiley Sons, Inc., New York, 1991.
6. Faita, G.; Mella, M.; Quadrelli, P. *Steroids* **2025**, *217*, 109598.
<https://doi.org/10.1016/j.steroids.2025.109598>
7. Singh, R.; Panda, G. *Tetrahedron* **2013**, *69*, 2853–2884.
<http://dx.doi.org/10.1016/j.tet.2013.02.018>
8. Grundmann, C.; Grünanger, P. The nitrile oxide. Springer-Verlag, Heidelberg, Germany, 1971.
9. Grundmann, C.; Dean, J. M. V. *J. Org. Chem.* **1965**, *30*, 2809–2812.
<https://doi.org/10.1021/jo01019a074>
10. Christl, M.; Huisgen, R. *Chem. Ber.* **1973**, *106*, 3345–3367.
<https://doi.org/10.1002/cber.19731061019>
11. Djerassi, C.; Miramontes, L.; Rosenkranz, G.; Sondheimer, F. *J. Am. Chem. Soc.* **1954**, *76*, 4092–4094.
<https://doi.org/10.1021/ja01645a010>
12. Syed, Y. Y. *Drugs* **2022**, *82*, 1549–1556.
<https://doi.org/10.1007/s40265-022-01790-4>
13. Fleming, I. Frontier Orbitals and Organic Chemical Reactions. J. Wiley and Sons, Ed., Chichester, 1976, pp. 141–161.
14. Caramella, P.; Reami, D.; Falzoni, M.; Quadrelli, P. *Tetrahedron* **1999**, *55*, 7027–7044.
15. Phelan, N. F.; Orchin, M. *J. Chem. Ed.* **1968**, *45*, 633–637.
<https://doi.org/10.1021/ed045p633>
16. Caramella, P.; Grünanger, P. 1,3-Dipolar Cycloaddition Chemistry. Padwa, A. Ed., Wiley, J. & Sons, New York, 1984, vol. 1, pp. 291–392 and references therein.
17. Houk, K. N.; Sims, J.; Watts, C. R.; Luskus, L. J. *J. Am. Chem. Soc.* **1973**, *95*, 7301–7315.
<https://doi.org/10.1021/ja00803a018>
18. Quadrelli, P.; Mella, M.; Carosso, S.; Bovio, B. *J. Org. Chem.* **2013**, *78*, 516–526.
<https://doi.org/10.1021/jo302346a>
19. Moggio, Y.; Legnani, L.; Bovio, B.; Memeo, M. G.; Quadrelli, P. *Tetrahedron* **2012**, *68*, 1384–1392.
<https://doi.org/10.1016/j.tet.2011.12.047>
20. Jäger, V.; Colinas, P. A. Nitrile Oxides in Synthetic Applications of 1,3-Dipolar Cycloaddition Chemistry Towards Heterocycles and Natural Products. Padwa, A.; Pearson, W. H. Ed., J. Wiley Sons, Inc., Hoboken (NJ), 2003, pp. 361–472.
<https://doi.org/10.1002/0471221902.ch6>
21. Gandolfi, R.; Grünanger, P. Steroids in Isoxazoles Part 2. Grünanger, P.; Vita Finzi, P. Ed. Wiley, J. Sons, Inc., New York, 1999, pp. 723–724.
22. Rigaku Oxford Diffraction, 2021. CrysAlisPro Software system, version 1.171.41.104a, Rigaku Corporation, Wroclaw, Poland.

23. Altomare, A.; Burla, M. C.; Camalli, M.; Cascarano, G. L.; Giacovazzo, C.; Guagliardi, A.; Moliterni, A. G. G.; Polidori, G.; Spagna, R. *J. Appl. Crystallogr.* **1999**, 32, 115-119.
<https://doi.org/10.1107/S0021889898007717>
24. Sheldrick, G. M. *Acta Cryst.* **2015**, C71, 3-8.
<https://doi.org/10.1107/S2053229614024218>

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