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Synthesis of new hexahydro-1H-isoindole-1,3(2H)-dione derivatives from 2-ethyl/phenyl-3a,4,7,7a-tetrahydro-1H-isoindole-1,3-(2H)-dione

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Abstract: A new and appropriate synthesis for hexahydro-1H-isoindole-1,3(2H)-dione derivatives has been developed starting from 3-sulfolene. The epoxidation of 2-ethyl/phenyl-3a,4,7,7a-tetrahydro-1H-isoindole-1,3-(2H)-dione and then the opening of the epoxide with nucleophiles gave hexahydro-1H-isoindole-1,3(2H)-dione derivatives. Amino and triazole derivatives of hexahydro-1H-isoindole-1,3(2H)-dione were synthesized from the formed product by the opening reaction of the epoxide with sodium azide. Hydroxyl analogues were obtained from cis-hydroxylation of 2-ethyl/phenyl-3a,4,7,7a-tetrahydro-1H-isoindole-1,3-(2H)-dione. The hydroxyl groups were converted to acetate.

Key words: Norcantharimide, cis-hydroxylation, epoxidation, ring opening epoxide, reduction of azide

1. Introduction

Norcantharimides, which are derivatives of cantharidine (**1**), are composed of a tricyclic imide skeleton. Cantharidine (**1**) and norcantharimide (**3**) derivatives are important potential anticancer agents.[1–3] The effect of norcantharimide (**3**) derivatives was observed in a large number of cancer types.[4] For this reason, in recent years much effort has been devoted to the synthesis of N-derivatives of norcantharimide (**3**).[4] Norcantharimide (**3**) derivatives can be obtained by attaching different functional groups to the imide nitrogen or cyclohexane ring.[5,6] Some of the synthesized derivatives have been investigated for their effects on different carcinomas. For example, McCluskey et al. investigated the anticancer activity of norcantharimide derivatives of different groups attached to the imide nitrogen.[5,6] Lin et al. have also studied the N-substituted cantharimides (aliphatic, aryl, and pyridyl groups) in vitro against HepG2 and HL-60 cells.[7,8] Chan and Tang reported the synthesis and cytotoxicity of some cantharimide derivatives.[9] More recently, we have reported the first ever successful synthesis of a new type of norcantharimide derivative[10,11] containing a substituted group on the cyclohexane ring. We also explored the fluorescence properties of isoindole derivatives of norcantharimide.[12]

Hexahydro-1H-isoindole-1,3(2H)-dione's structure was similar to that of norcantharimide. Therefore, in this study, our objective was to synthesize different norcantharimide derivatives via functionalization of the cyclohexane ring. Two methods, based on epoxidation and cis − hydroxylation, were used for the preparation of synthetic derivatives of norcantharimide (**3**).

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** To whom inquiries concerning the X-ray structure should be directed.

2. Results and discussion

The key compound in this study was 3a,4,7,7a-tetrahydro-isobenzofuran-1,3-dione, which was prepared via cycloaddition of 3-sulfolene and maleic anhydride. The reaction of the primary amine with 3a,4,7,7a-tetrahydro-isobenzofuran-1,3-dione in the presence of a toluene and triethylamine mixture gave the corresponding hexahydro-1H-isoindole-1,3(2H)-dione **4** in 80% yield (Figure 2).[13]

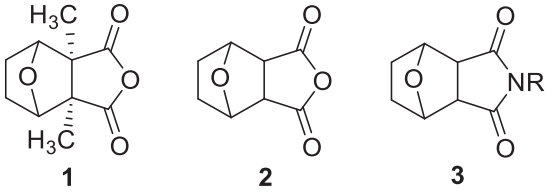


Figure 1. Structure of cantharidine (**1**), norcantharidine (**2**), and norcantharimide (**3**).

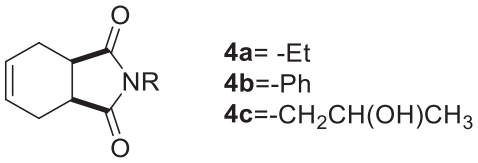
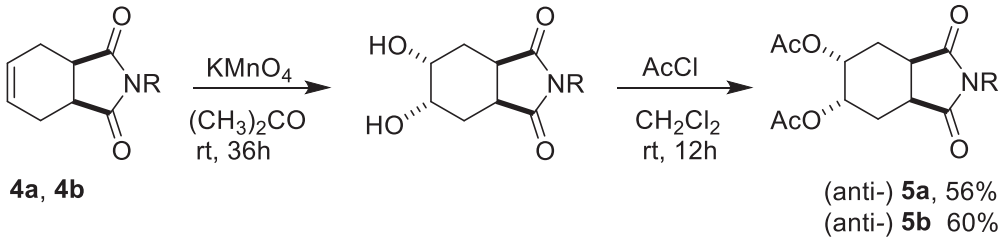


Figure 2. 2-Alkyl/aryl-3a,4,7,7a-tetrahydro-1H-isoindole-1,3-(2H)-dione (**4**).

In this research, we initially investigated the hydroxylation reaction of hexahydro-1H-isoindole-1,3(2H)-dione **4**.[4] $KMnO_4$ was used for the synthesis of cis-diol. Therefore, compounds **4a** and **4b** were treated with $KMnO_4$ at room temperature, followed by acetylation to give **5a** in 56% and **5b** 60% yield (Scheme 1). The ^{1}H NMR spectrum analysis of the crude product revealed the formation of a single isomer. As seen, the two faces of the double bond in **4(a, b)** are not symmetrical and so the double bond could be attacked from both sides. NMR experiments showed that the anti-isomer is formed in this reaction.

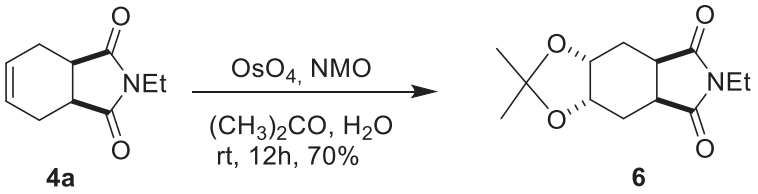


Scheme 1. Synthesis of cis-diacetate **5a** and **5b**.

The structure of the product formed in this reaction was determined by NMR spectrum analysis and it was anti-isomer. Here, the formation of anti-isomers **5a** and **5b** may be explained by considering the steric effects of the imide group. Therefore, $KMnO_4$ approached compound **4a** and **4b** exclusively from the sterically less crowded face of the molecule.

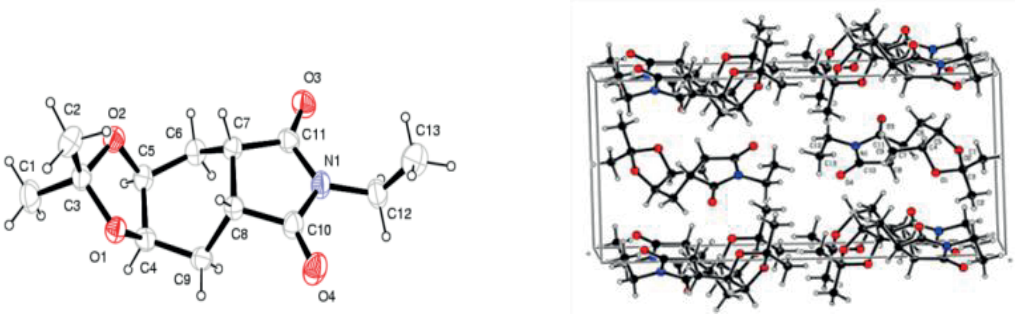
The hydroxylation of hexahydro-1H-isoindole-1,3(2H)-dione **4a** with OsO_4 gave a very interesting sole product. ^{1}H and ^{13}C NMR spectroscopic data confirmed the hydroxylation of the double bond. In fact, we supposed that the OsO_4 would add to the double bond to give a cis$-$diol. However, ^{1}H NMR spectrum analysis showed no hydroxyl groups. In addition, two methyl peaks were observed in this spectrum. In addition, in the DEPT spectrum of the molecule **6**, 26.0 and 23.6 ppm signals of the methyl carbon and 107.5 ppm signal of the

quaternary carbon were determined. In this case we assumed that ketal was formed in this reaction. This can be explained by the fact that the resulting diols were converted to ketal, depending on the reaction conditions (Scheme 2).



Scheme 2. Synthesis of ketal **6**.

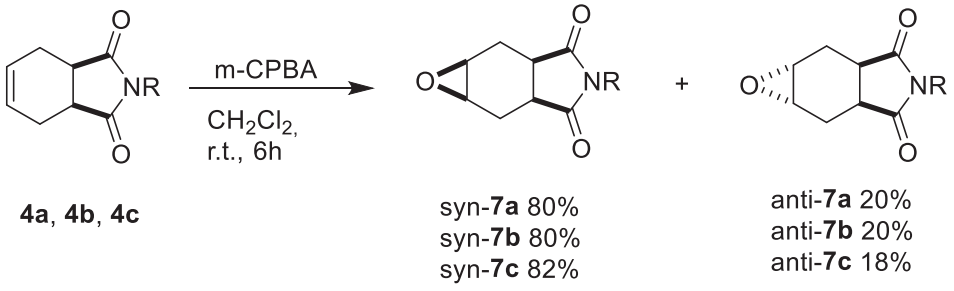
Further structure analysis of **6** was achieved by single crystal analysis (Figures 3a and 3b). Furthermore, the single crystal analysis of ketal **6** showed that an anti-product formed with respect to the imide ring. Thus, the X-ray of structure **6** can inform us about the approach of OsO_4. As in the $KMnO_4$ reaction, it approached compound **4a** exclusively from the sterically less crowded face of the molecule.



a) b)

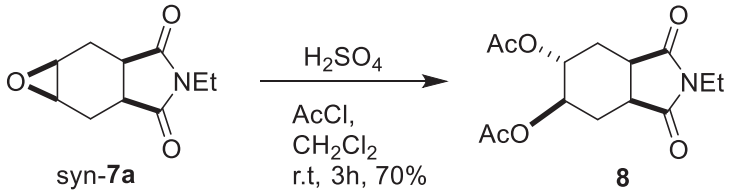
Figure 3. a) ORTEP diagram of **6**. Thermal ellipsoids are shown at 50% probability level. b) Stacking geometry of the compound **6** along the a-axis in the unit cell.

On the other hand, in our previous studies, the epoxidation of hexahydro-1H-isoindole-1,3(2H)-diones **4a** and **4b** was carried out with m-CPBA. A mixture of syn− and anti-isomers in a ratio of 4:1 was obtained from this reaction.[10,11] In addition, we also studied the epoxidation reaction of **4c** with m-CPBA and achieved similar results (Scheme 3). These results showed that the rates of product formation are not affected by the groups attached to the nitrogen atom in the imide ring. In these reactions, the greater formation of syn-isomer than of anti-isomer is explained by the dipole–dipole interaction between the RCO_3H and the imide moieties of the compounds by comparison with similar studies.[10,14]



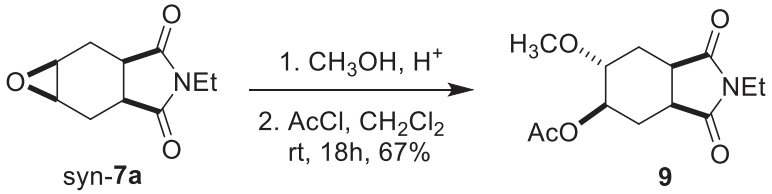
Scheme 3. Synthesis of epoxide isomers.

For further functionalization of the hexahydro-1H-isoindole-1,3(2H)-dione **4**, the epoxide syn-**7a** was converted to trans-diacetate derivatives **8**, by using acetic anhydride in concentrated H_2SO_4 (Scheme 4). The exact structure was determined by ^1H and ^{13}C NMR experiments.[10,11]



Scheme 4. Synthesis of trans-diacetate **8**.

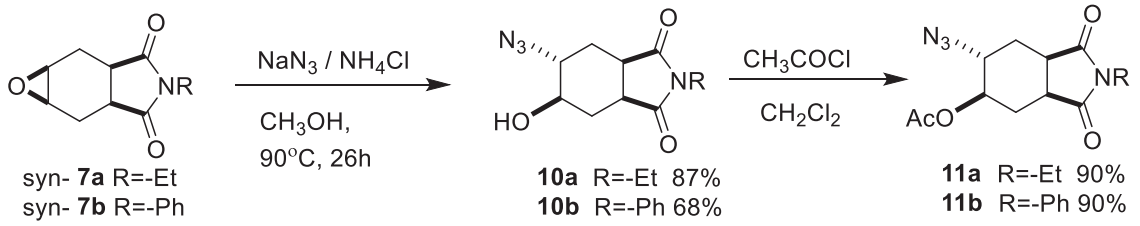
The epoxide ring opening of syn$-$**7a** was achieved with MeOH in the presence of H_2SO_4 (Scheme 5), followed by the acetylation of the hydroxyl group with acetyl chloride. The structure of **9** was elucidated according to the ^1H NMR spectrum.



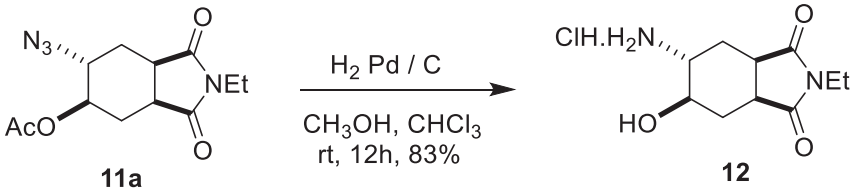
Scheme 5. Synthesis of trans-methoxy acetate **9**.

Epoxide syn$-$**7a** was opened with NaN_3[15] in CH_3OH to give azido-alcohol **10a** (R = –Et), as a single stereoisomer in a yield of 80%. The sharp signal belonged to the azide group at 2109 cm^{-1} and the broad hydroxyl group signal was observed to be 3454 cm^{-1} in the IR spectrum.

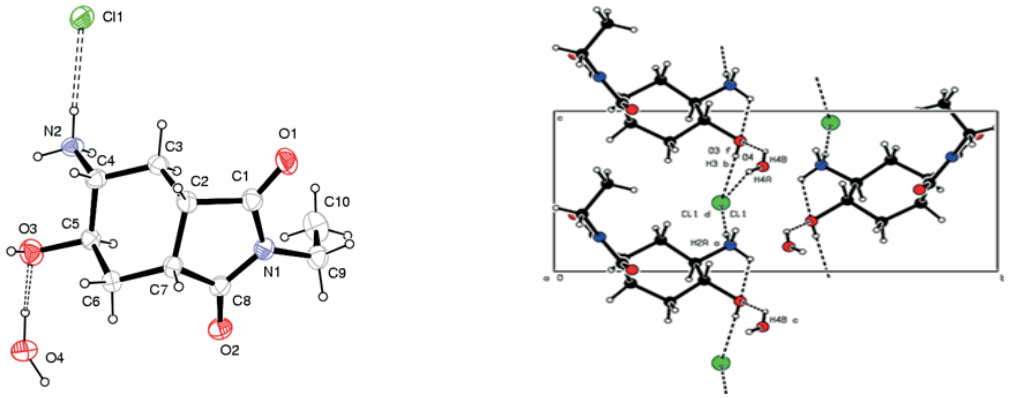
The resulting azido-alcohol derivative was converted to corresponding acetate **11** (Scheme 6). Then compound **11a** was converted to its amine derivative **12** with Pd/C catalyzed hydrogenation in the presence of $CHCl_3$ (Scheme 7). ^1H NMR and IR spectrum data confirmed the reduction of the azide group. However, ^1H NMR and ^{13}C NMR spectral analyses showed no acetyl group. The ^1H NMR and ^{13}C NMR spectra showed that other reactions occurred in the course of reduction of the azide group. Thus the exact structure of **12** was determined by X-ray crystal analysis (Figure 4).



Scheme 6. Synthesis of azido-alcohol **10** and azido-acetate **11**.



Scheme 7. Synthesis of amine **12**.

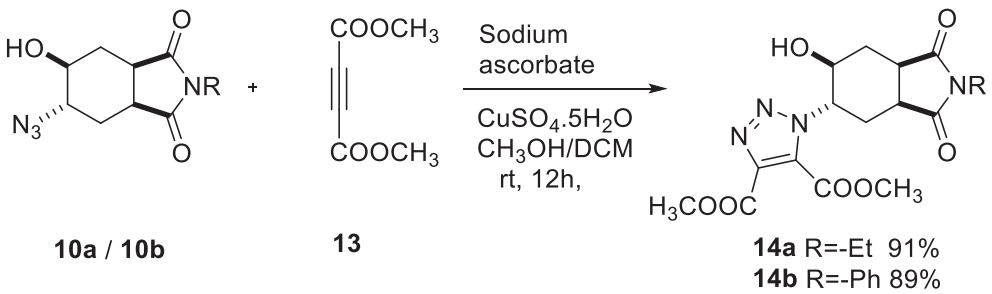


a) b)

Figure 4. a) ORTEP diagram of **12**. Thermal ellipsoids are shown at 50% probability level; b) H-bonding pattern (dashed lines) along the a-axis in the unit cell. O_4–H$\cdots$Cld = 3.159(5)Å, $<$ (O_4–H$\cdots$Cld) = 160°; O_3–H$\cdots$Clf = 3.243(5)Å, $<$ (O_2–H$\cdots$O1f) = 174°; N_2–H$\cdots$Cl = 3.113(5)Å, $<$ (N_2–H$\cdots$Cl) = 173°; O_4–H$\cdots O_3$ = 2.728(7)Å, $<$ (O_4–H$\cdots O_3$) = 141°. (Symmetry code: $\delta = -1 + x, y, z; f = x, y, 1 + z$).

The acetyl group is removed during hydrogenation of **11a** according to the crystal structure of **12**, and H-bonding was observed between the –OH group and the H_2O molecule. Moreover, the amine group (–NH_2) resulting from reduction of the azide group transforms into its amine salt following hydrogenation (Figures 4a and 4b).

Triazoles can act as the functional group and as attractive linker units, and are important in constructing bioactive and functional molecules.[16–19] Triazole and its derivatives have been synthesized by various groups and used in different areas.[20] 1,2,3-Triazoles are commonly prepared by the Huisgen 1, 3-dipolar cycloaddition of azides with alkynes. Therefore, as a part of our study we synthesized triazoles from azides **10a** and **10b**. Compounds **10a** and **10b** were reacted with acetylene dicarboxylate cycloaddition in a solution of sodium ascorbate and $CuSO_4.5H_2O$. Consequently, the norcantharimide derivatives **14a** and **14b** containing a triazole skeleton were synthesized (Scheme 8). The structure of product **14a** was elucidated by using NMR and X-ray analysis (Figures 5a and 5b).

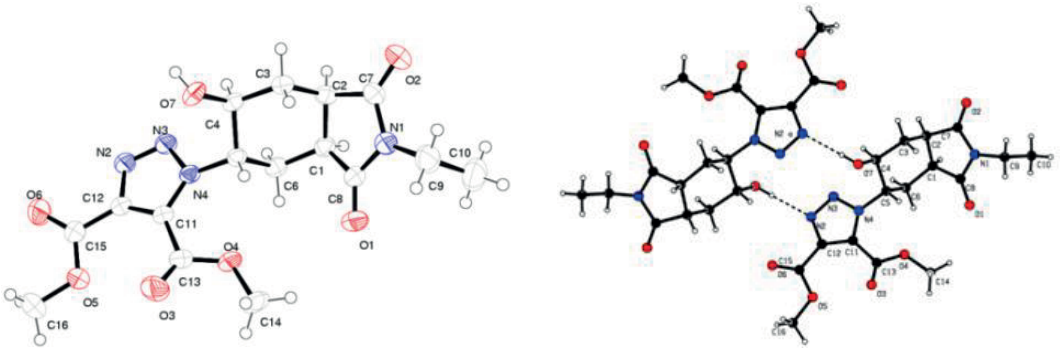


Scheme 8. The synthesis of triazole derivatives **14.**

3. Conclusions

We have accomplished the synthesis of modified hexahydro-1H-isoindole-1,3(2H)-dione derivatives. These derivatives comprise hydroxyl, acetate, amino, azido, and triazole groups. We think that while the dipole–dipole interaction plays a role in m-CPBA oxidation, in the case of oxidation with OsO_4 or $KMnO_4$ the steric

effects are directing the derivative outcome of the products. Thus, the configuration of the other carbon atoms, C-5 and C-6, was controlled by epoxidation and cis-hydroxylation reactions. Application of this methodology may provide opportunities for the synthesis of other hexahydro-1H-isoindole-1,3(2H)-dione derivatives.



a) b)

Figure 5. a) ORTEP diagram of compound **14a**. Thermal ellipsoids are shown at 50% probability level; b) Dimeric structure of **14a** with O–H$\cdots$N bonding O_7–H$\cdots N_{2a}$ = 2.828(2)Å, < (O7–H$\cdots N_{2a}$) = 169°. (Symmetry code: $\alpha = 3 - x, 1 - y, -z$).

4. Experimental

4.1. General

Column chromatography (CC): silica-gel 60 (70–230 mesh) and AlO_x (neutral Al_2O_3, type-III). Solvents were purified and dried by standard procedures before use. Mp: Büchi-539 cap. Melting point apparatus; uncorrected. ^{1}H and ^{13}C NMR spectra: Varian spectrometer; δgin ppm, J in Hz. Elemental analyses: Leco CHNS-932 instrument.

4.2. Synthesis of 5,6-diacetoxy-2-ethyl-1,3-dioxo-octahydro-isoindole (5a)

To a magnetically stirred acetone solution (25 mL) of 2-ethyl-3a,4,7,7a-tetrahydro-isoindole-1,3-dione (**4a**) (0.27 g, 1.5 mmol) was added a solution of $KMnO_4$ (0.48 g, 3.00 mmol) and $MgSO_4$ (0.36 g, 3.00 mmol) in water (25 mL) at –5 °C over 30 min. After the addition was complete, the reaction mixture was stirred for an additional 36 h at the given temperature and then filtered. The precipitate was washed several times with hot water. The combined filtrates were concentrated to 20 mL by rotoevaporation. The aqueous solution was extracted with ethyl acetate (3 × 30 mL) and the extracts were dried (Na_2SO_4). Evaporation of the solvent gave 2-ethyl-5,6-dihydroxy-hexahydro-isoindole-1,3-dione. The crude product was dissolved in DCM (25 mL) and acetyl chloride (1.2 g, 15 mmol) was added. The reaction mixture was stirred at room temperature for 12 h. The mixture was cooled to 0 °C and then water (100 mL) and DCM (50 mL) were added. The organic phase was separated, washed with saturated $NaHCO_3$ and water (2 × 50 mL), and dried (Na_2SO_4). Removal of the solvent under reduced pressure gave 6a,5,6-diacetoxy-2-ethyl-1,3-dioxo-octahydro-isoindole. Recrystallization of the residue from EtOAc/hexane gave 0.25 g, 56%, pale yellow liquid. ^{1}H NMR (400 MHz, $CDCl_3$): 5.05 (m, 2H), 3.54 (q, 2H, J = 7.1 Hz), 3.03 (m, 2H), 2.25 (m, 2H), 2.07 (s, 6H), 1.95 (m, 2H), 1.16 (t, 3H, J = 7.1 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 178.0, 170.2, 67.9, 37.8, 33.9, 25.7, 21.1, 13.2. Anal. calc. for. $C_{14}H_{19}NO_6$, (297.30), C 56.56; H 6.44; N 4.71. Found: C 56.65; H 6.53; N 4.83.

4.3. Synthesis of 5,6-diacetoxy-2-phenyl-1,3-dioxo-octahydro-isoindole (5b)

To a magnetically stirred acetone solution (25 mL) of 2-phenyl-3a,4,7,7a-tetrahydro-isoindole-1,3-dione (**4b**) (0.27 g, 1.2 mmol) was added a solution of $KMnO_4$ (0.38 g, 2.4 mmol) and $MgSO_4$ (0.29 g, 2.4 mmol) in water (25 mL) at –5 °C for 30 min. After the addition was complete, the reaction mixture was stirred for additional 36 h at the given temperature and then filtered. The precipitate was washed several times with hot water. The combined filtrates were concentrated to 20 mL by rotoevaporation. The aqueous solution was extracted with ethyl acetate (3 × 30 mL), and the extracts were dried (Na_2SO_4). Evaporation of the solvent gave 2-phenyl-5,6-dihydroxy-hexahydro-isoindole-1,3-dione. The crude product was dissolved in DCM (25 mL). Then acetyl chloride (1.2 g, 15 mmol) was added to the solution. The reaction mixture was stirred at room temperature for 12 h. The mixture was cooled to 0 °C and then water (100 mL) and DCM (50 mL) were added. The organic phase was separated, washed with saturated $NaHCO_3$ and water (2 × 50 mL), and dried (Na_2SO_4). Removal of the solvent under reduced pressure gave 6a,5,6-diacetoxy-2-phenyl-1,3-dioxo-octahydro-isoindole (**5b**). Recrystallization of the residue from EtOAc/hexane gave 0.25 g, 60%, colorless crystal, mp: 291–292 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 7.50–7.26 (m, 5H), 5.09 (m, 2H), 3.24 (m, 2H), 2.33 (m, 2H), 2.12 (m, 2H), 2.09 (s, 3H), 2.08 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 177.2, 170.2, 131.8, 129.5, 128.9, 126.4, 67.8, 38.2, 25.8, 21.2. Anal. calc. for. $C_{18}H_{19}NO_6$, (297.30), C 62.60; H 5.55; N 4.06. Found: C 61.59; H 5.77; N 4.01.

4.4. Synthesis of 6-ethyl-2,2-dimethyl-hexahydro-[1,3]dioxolo[4,5-f]isoindole -5,7-dione (6)

To a stirred solution of syn-2-ethyl-3a,4,7,7a-tetrahydro-isoindole-1,3-dione (**4a**) (280 mg, 1.56 mmol) in $(CH_3)_2CO/H_2O$ (2 mL, 1:1) were added NMO (189 mg, 1.87 mmol) and OsO_4 (4.0 mg, 0.016 mmol) at 0 °C. The resulting mixture was stirred vigorously under nitrogen at room temperature for 12 h. During the stirring the reaction mixture became homogeneous. Sodium hydrogensulfide (0.2 g) and florisil (0.5 g) slurried in water (2 mL) were added, the slurry was stirred for 10 min and the mixture was filtered through a pad of Celite (0.5 g) in a 50-mL sintered-glass funnel. The Celite cake was washed with acetone (3 × 10 mL). The filtrate was neutralized to pH 7 with H_2SO_4. The organic layer was removed in vacuo. The resulting aqueous solution was adjusted to pH 5 with sulfuric acid. Then the crude product was separated from *N*-methylmorpholine hydrosulfate by extraction with ethyl acetate (4 × 20 mL). The combined ethyl acetate extracts were washed with 5 mL of 25% NaCl solution and three times with water and dried (Na_2SO_4). Evaporation of the solvent and crystallization of the residue from EtOAc/n-hexane gave 6-ethyl-2,2-dimethyl-hexahydro-[1,3]dioxolo[4,5-f]isoindole-5,7-dione (**6**) (0.277 g, 70%). Colorless crystal, mp: 111–112 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 4.33 (s, 2H), 3.40 (q, 2H, J = 7.3 Hz), 2.91 (m, 2H), 2.21 (dd, 2H, J = 14.5, 3.5 Hz), 1.32 (s, 3H), 1.2 (s, 3H), 1.02 (t, 3H, J = 7.3 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 179.8, 71.3, 34.1, 33.5, 26.0, 26.0, 23.6, 13.1. Anal. calc. for. ($C_{13}H_{19}NO_4$), (253.13), C 61.64; H 7.56; N 5.53. Found: C 61.51; H 7.46; N 5.63. IR (KBr, cm^{-1}): 3453, 3054, 2982, 2938, 1772, 1705, 1444, 1405, 1378, 1352, 1296, 1260, 1228, 1164, 1137, 1076, 1040.

4.5. Synthesis of 5-acetoxy-2-ethyl-6-methoxy-1,3-dioxo-octahydro-isoindole (9)

To a solution of syn-4-ethyl-tetrahydro-1aH-oxireno[f]isoindole-3,5(2H,4H)-dione (**7a**) (2.2 mmol, 0.42 g) in DCM (15 mL) were added methanol (5 mL) and a catalytic amount H_2SO_4. The mixture was stirred at room temperature and the reaction's progress was monitored until it was completed. After 18 h, 2 g of $NaHCO_3$ was added to the reaction mixture, followed by stirring at 40 min. Then the mixture was filtered for removal of the

solid phase. The solvent was removed under reduced pressure. The residue was solved with ethyl acetate (50 mL). The organic phase was washed with $NaHCO_3$ solution (50 mL) and water (3 × 50 mL), and then dried over $MgSO_4$, and ethyl acetate was removed under reduced pressure. The residue was purified by thin layer chromatography (TLC) eluting with AcOEt/hexane (3:7) (R_f = 0.57) to give **9** (390 mg, 67%) as a pale yellow liquid. ^{1}H NMR (400 MHz, $CDCl_3$ 4.95 (m, 1H), 3.49 (q, 2H, J = 7.1 Hz), 3.37 (m, 1H), 3.35 (s, 3H), 2.86 (m, 1H), 2.81 (m, 1H), 2.06 (m, 3H), 1.92 (s, 3H), 1.80 (m, 1H), 1.11 (t, 3H, J = 7.3 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 179.7, 178.7, 169.9, 75.5, 69.5, 57.1, 36.5, 36.1, 33.7, 25.2, 23.3, 21.3, 13.2. Anal. calc. for. $C_{13}H_{19}NO_5$ (269.13): C 57.98; H 7.11; N 5.20. Found: C 57.70; H 7.23; N 4.83.

4.6. Synthesis of 5-acetoxy-6-azido-2-ethyl-1,3-dioxo-octahydro-isoindole (11a)

To a stirred solution of syn-4-ethyl-tetrahydro-1aH-oxireno-[f]isoindole-3,5(2H,4H)-dion (**7a**) (1.3 g, 6.65 mmol) in 20 mL of methyl alcohol was added a solution of NH_4Cl (0.72 g, 13.2 mmol) and NaN_3 (1.73 g, 26.6 mmol) in water (10 mL) dropwise at 0 °C over 15 min. The mixture was stirred at 90 °C for 26 h. After the filtration of the reaction mixture, the solvent was removed. The reaction mixture was cooled to room temperature and methanol was evaporated. Then H_2O (10 mL) and ether (60 mL) were added. The organic layer was separated and washed with H_2O (3 × 50 mL). The organic layer was dried over Na_2SO_4 and ether was evaporated. Removal of the solvent under reduced pressure gave azido-alcohol derivative isoindoline (5-azido-6-hydoxy-2-ethyl-1,3-dioxo-octahydro-isoindole) (**10a**) (1.4 g, 87%, yellow liquid). ^{1}H NMR (400 MHz, $CDCl_3$): 3.68 (m, 1H), 3.54 (q, 2H, J = 7.3 Hz), 3.41 (m, 1H), 2.97 (td, 1H, A part of AB_1 system, J= 7.6, 2.2 Hz), 2.91 (q, 1H, B part of AB_1 system, J= 7.6 Hz), 2.45 (dt, 1H, A part of AB_2 system, J= 14.6, 4.8 Hz), 2.37 (bs, 1H, OH), 2.29 (ddd, 1H, A part of AB_3 system, J= 14.6, 7.3, 3.7 Hz), 1.80 (ddd, 1H, B part of AB_2 system, J= 14.6, 8.5, 7.3 Hz), 1.64 (dt, 1H, B part of AB_3 system, J= 14.6, 8.5 Hz), 1.15 (t, 3H, J= 7.3 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 178.6, 178.1, 69.4, 61.9, 38.2, 38.1, 33.9, 30.3, 25.4, 13.0. Anal. calc. for. $C_{10}H_{14}N_4O_3$ (238.11): C 50.41; H 5.92; N 23.52. Found: C 50.53; H 6.03; N 21.61. IR (KBr, cm^{-1}): 3454, 2928, 2109, 1774, 1697, 1445, 1404, 1378, 1350, 1360, 1221. Next the azido-alcohol **10a** was dissolved in DCM (25 mL) and acetyl chloride (1.2 g, 15 mmol) was added. The reaction mixture was stirred at room temperature for 12 h. The mixture was cooled to 0 °C. Then water (100 mL) and DCM (50 mL) were added successively. The organic phase was separated, washed with saturated $NaHCO_3$ and water (2 × 50 mL), and dried (Na_2SO_4). Removal of the solvent under reduced pressure gave acetoxy-azide derivative isoindoline 5-acetoxy-6-azido-2-ethyl-1,3-dioxo-octahydro-isoindole (**11a)** (1.26 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$): 4.80 (m, 1H), 3.57 (m, 1H), 3.53 (m, 2H), 2.94 (dm, J = 14.0, Hz, 2H), 2.22 (m, 2H), 2.06 (m, 1H), 1.99 (s, 3H), 1.86 (m, 1H) 1.12 (t, J = 7.2 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 178.1, 177.9, 169.8, 70.8, 70.2, 58.6, 37.4, 37.2, 26.3, 25.8, 21.1, 13.1.

4.7. Synthesis of 5-acetoxy-6-azido-2-phenyl-1,3-dioxo-octahydro-isoindole (11b)

To a stirred solution of syn-4-phenyl-tetrahydro-1aH-oxireno-[f]isoindole-3,5(2H,4H)-dion (**7b**) (1.35 g, 5.56 mmol) in 20 mL of methyl alcohol was added a solution of NH_4Cl (0.595 g, 11.12 mmol) and NaN_3 (1.45 g, 22.24 mmol) in water (10 mL) dropwise at 0 °C over 15 min. The mixture was stirred at 90 °C for 26 h. After the filtration of the reaction mixture, the solvent was removed. The reaction mixture was cooled to room temperature and methanol was evaporated. Then H_2O (10 mL) and ether (60 mL) were added. The organic layer was separated and washed with H_2O (3 × 50 mL). The organic layer was dried over Na_2SO_4 and

ether was evaporated. Removal of the solvent under reduced pressure gave azido-alcohol derivative isoindoline (5-azido-6-hydoxy-2-phenyl-1,3-dioxo-octahydro-isoindole) (**10b**) (1.08 g, 68%, yellow liquid). ^{1}H NMR (400 MHz, $CDCl_3$): 7.50–7.26 (m, 5H), 3.73 (m, 1H), 3.52 (m, 1H), 3.18 (m, 1H), 3.07 (m, 1H), 2.56 (dt, J = 9.4, 4.3 Hz, 1H), 2.51 (bs, 1H, OH), 2.38–2.26 (m, 1H), 1.88 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): 178.8, 178.2, 129.4, 129.3, 128.9, 126.4, 69.3, 61.7, 38.4, 38.3, 30.1, 25.4. IR (KBr, cm^{-1}): 3457, 2930, 2115, 1772, 1693, 1444, 1400, 1377, 1352, 1225. Anal. calc. for. $C_{14}H_{14}N_4O_3$ (286.11): C, 58.74; H, 4.93; N, 19.57; Found: C 58.63; H 4.52; N 19.61. Then the azido-alcohol **10b** was dissolved in DCM (25 mL) and acetyl chloride (0.86 g, 11 mmol) was added. The reaction mixture was stirred at room temperature for 12 h. The mixture was cooled to 0 °C. Then water (100 mL) and DCM (50 mL) were added successively. The organic phase was separated, washed with saturated $NaHCO_3$ and water (2 × 50 mL), and dried (Na_2SO_4). Removal of the solvent under reduced pressure gave acetoxy-azide derivative isoindoline 5-acetoxy-6-azido-2-phenyl-1,3-dioxo-octahydro-isoindole (**11b)** (1.1 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$): 7.39 (m, 5H), 5.10 (m, 1H), 3.55 (m, 1H), 3.18 (td, J = 8.1, 2.0 Hz, 1H), 3.06 (dd, J = 9.9, 8.1, 2H), 2.50 (ddd, J = 13.6, 5.5, 2.0 Hz, 1H), 2.32 (m, 1H), 2.12 (m, 1H) 2.08 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 179.5, 179.0, 168.7, 134.4, 129.2, 128.7, 126.7, 69.5, 62.0, 38.7, 38.3, 30.1, 25.4, 20.1.

4.8. Synthesis of 5-amino-2-ethyl-6-hydroxy-hexahydro-isoindole-1,3-dione HCl salt (12)

Into a 50-mL flask were placed Pd/C (20 mg) and 5-acetoxy-6-azido-2-ethyl-1,3-dioxo-octahydro-isoindole (**11a**) (0.2 g, 0.78 mmol) in MeOH (6 mL) and $CHCl_3$ (1 mL). A balloon filled with H_2 gas (3 L) was fitted to the flask. The mixture was deoxygenated by flushing with H_2 and then hydrogenated at room temperature for 26 h. The catalyst was removed by filtration. Recrystallization of the residue from EtOAc/n-hexane gave 5-amino-2-ethyl-6-hydroxy-hexahydro-isoindole-1,3-dione HCl salt (**12**) (0.14 g, 83%). Colorless crystal, mp: 85–87 °C. ^{1}H NMR (400 MHz, D_2O): 3.62 (td, 1H, J = 10.3, 4.0 Hz), 3.34 (q, 2H, J = 7.3 Hz), 3.14 (td, 1H, J = 7.3, 1.8 Hz), 3.04 (dt, 1H, J = 10.3, 7.7 Hz), 2.84 (m, 1H), 2.48 (dm, 1H), 2.21 (m, 1H), 1.71 (ddd, 1H, J = 19.6, 12.5, 7.4 Hz), 1.31 (dt, 1H, J = 13.8, 10.3 Hz), 0.93 (t, 3H, J = 7.3 Hz). ^{13}C NMR (100 MHz, D_2O): 181.6, 180.4, 67.7, 52.2, 38.6, 38.4, 34.1, 32.5, 24.0, 11.8. IR (KBr, cm^{-1}): 3501, 3161, 2955, 2926, 2854, 1697, 1462, 1405, 1349, 1261, 1224, 1090, 1017.

4.9. Synthesis of triazole derivative 14a

To a stirred solution of **10a** (0.42 g, 1.76 mmol) in 20 mL of methyl alcohol were added consecutively a solution of sodium ascorbate [ascorbic acid (0.3 g, 1.7 mmol), 4 mL of H_2O + $NaHCO_3$ (0.1 g, 1.2 mmol), 4 mL H_2O)], $CuSO_4.5H_2O$ [0.04 g, 0.16 mmol + 1 ml H_2O], and dimethyl-acetylene dicarboxylate (0.46 g, 3.52 mol) in DCM (2 mL) at room temperature. The mixture was stirred at room temperature for 12 h and monitored by TLC. Then the reaction mixture was solved with DCM (50 mL). The organic layer was separated and washed with H_2O (3 × 50 mL). The organic layer was dried over Na_2SO_4. Evaporation of the solvent followed by crystallization of the residue from DCM/hexane (1:1) gave **14a**. Colorless crystal, 0.6 g, 91%, mp: 155–156 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 4.62 (m, 1H), 4.23 (m, 1H) 3.99 (s, 3H), 3.94 (s, 3H), 3.56 (q, 2H, J = 7.3 Hz), 3.20 (m, 1H), 3.19 (bs, 1H, OH), 3.12 (td, 1H, J = 9.2, 7.0 Hz), 2.77 (ddd, 1H, J = 14.3, 5.1, 3.3 Hz), 2.50 (m, 2H), 1.67 (dt, 1H, J = 14.3, 9.5 Hz), 1.18 (t, 3H, J = 7.3 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 178.5, 177.5, 160.5, 159.2, 139.9, 131.4, 70.0, 62.6, 54.0, 53.0, 38.9, 38.6, 34.1, 32.7, 26.4, 13.0. Anal. calc. for. $C_{16}H_{20}N_4O_7$ (380.35), C 50.52; H 5.30; N 14.73; found: C 49.59; H 5.187; N 14.28.

4.10. Synthesis of triazole derivative 14b

To a stirred solution of **10b** (0.5 g, 1.75 mmol) in 20 mL of t-BuOH-H_2O (1:1) were added consecutively a solution of sodium ascorbate [ascorbic acid (0.3 g, 1.7 mmol), 4 mL of H_2O + $NaHCO_3$ (0.1 g, 1.2 mmol), 4 mL of H_2O)], $CuSO_4.5H_2O$ [0.04 g, 0.17 mmol + 1 mL of H_2O], and dimethyl-acetylene dicarboxylate (0.5 g, 3.52 mol) at room temperature. The mixture was stirred at room temperature for 12 h and monitored by TLC. Then the reaction mixture was solved with DCM (50 mL). The organic layer was separated and washed with H_2O (3 × 50 mL). The organic layer was dried over Na_2SO_4. Evaporation of the solvent followed by crystallization of the residue from DCM/hexane (1:1) gave **14b**. Colorless crystal, 0.67 g, 89%, mp: 129–131 °C. ^{1}H NMR (400 MHz, $CDCl_3$): 7.50–7.26 (m, 5H), 4.74 (m, 1H), 4.26 (bs, 1H) 3.98 (s, 3H), 3.95 (s, 3H), 3.41 (m, 1H), 3.27 (q, 1H, J = 8.4 Hz), 3.20 (bs, 1H, OH), 2.85 (dd, 1H, J = 14.3, 5.3 Hz), 2.57 (m, 2H), 1.93 (dt, 1H, J = 14.3, 8.4 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): 177.4, 176.6, 160.3, 159.0, 139.8, 131.8, 131.1, 129.2, 128.7, 126.3, 69.8, 62.2, 53.8, 52.8, 38.8, 38.3, 32.1, 25.9. HRMS: (ESI/[M^+/Na]) m/z found: 429.1421, requires: 428.13.

4.11. Crystallography

For the crystal structure determination, the single crystals of the compounds **6**, **12**, and **14a** were used for data collection on a four-circle Rigaku R-AXIS RAPID-S diffractometer (equipped with a two-dimensional area IP detector). The graphite-monochromatized Mo Kα radiation (λ = 0.71073 Å) and oscillation scans technique with $\Delta w = 5°$ for each image were used for data collection. The lattice parameters were determined by the least-squares methods on the basis of all reflections with $F^2 > 2\sigma g(F^2)$. Integration of the intensities, correction for Lorentz and polarization effects, and cell refinement were performed using Crystal Clear (Rigaku/MSC Inc., 2005) software.[21] The structures were solved by direct methods using the program SHELXS-97[22] and refined by a full-matrix least-squares procedure using the same program.[22] Hydroxyl and water H molecules were positioned from the difference Fourier map, all other H atoms were positioned geometrically and refined using a riding model. The final difference Fourier maps showed no peaks of chemical significance. Crystal data for **6**: $C_{13}H_{19}NO_4$, crystal system, space group: monoclinic, P21/n; (no: 14); unit cell dimensions: α = 7.6753(3), β = 9.1962(3), γ = 19.6112(8) Å, β = 98.00(2); volume: 1370.73(9) Å^3; Z = 4; calculated density: 1.227 g/cm^3; absorption coefficient: 0.091 mm^{-1}; F(000): 544; θ-range for data collection 2.1–26.5°; refinement method: full-matrix least-square on F^2; data/parameters: 2813/165; goodness-of-fit on F^2: 1.043; final R indices [I > 2 σg(I)]: R_1 = 0.065, wR_2 = 0.152; R indices (all data): R_1 = 0.142, wR_2 = 0.190; largest diff. peak and hole: 0.317 and –0.185 e Å^3; Crystal data for **12**: $C_{10}H_{17}N_2O_3{\cdot}Cl{\cdot}H_2O$, crystal system, space group: monoclinic, Pc; (no: 7); unit cell dimensions: α = 6.7478(2), β = 15.0516(3), γ = 6.8591(2) Å, β = 108.13(2); volume: 662.04(3) Å^3; Z = 2; calculated density: 1.338 g/cm^3; absorption coefficient: 0.294 mm^{-1}; F(000): 284; θ-range for data collection 2.7–26.4°; refinement method: full-matrix least-square on F_2; data/parameters: 2030/166; goodness-of-fit on F_2: 1.04; final R indices [I > 2σg(I)]: R_1 = 0.065, wR_2 = 0.182; R indices (all data): R_1 = 0.079, wR_2 = 0.210; largest diff. peak and hole: 0.421 and –0.264 e Å^3; Crystal data for **14a**: $C_{16}H_{20}N_4O_7$, crystal system, space group: monoclinic, P-1; (no: 2); unit cell dimensions: α = 8.4612(2), β = 9.7794(3), γ = 11.6188(3) Å, α = 100.42(2) β = 93.72(2), γ = 109.34(2); volume: 884.10(4) Å^3; Z = 2; calculated density: 1.429 g/cm^3; absorption coefficient: 0.114 mm^{-1}; F(000): 400; θ-range for data collection 1.8–26.4°; refinement method: full-matrix least-square on F^2; data/parameters: 3165/248; goodness-of-fit on

F^2: 1.044; final R indices [$I > 2\sigma g(I)$]: $R_1 = 0.036$, $wR_2 = 0.096$; R indices (all data): $R_1 = 0.042$, $wR_2 = 0.101$; largest diff. peak and hole: 0.258 and –0.166 e $Å^3$; CCDC-973056 (**6**), CCDC-973328 (**12**), and CCDC-972561 (**14a**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: + 44 1223 336033; e-mail: deposit@ccdc.cam.ac.uk).

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