New strategy of synthesis of *Peramivir* analogues as potential Neuraminidase inhibitors

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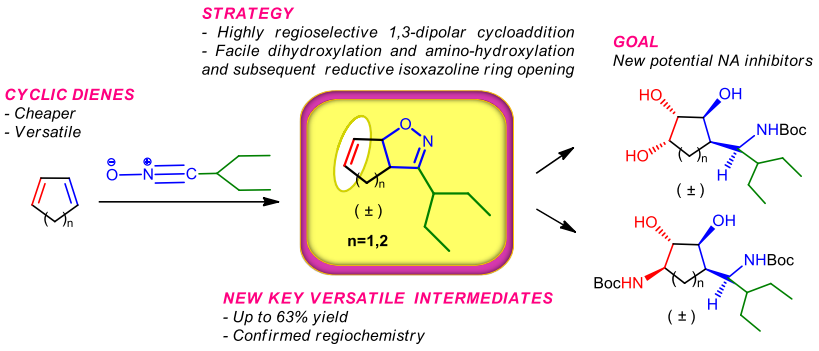


Abstract: Highly functionalised potential Neuraminidase (NA) inhibitors, analogues of peramivir, were synthesised *via* a new and versatile method starting from a stereoselective 1,3-dipolar cycloaddition reaction between the nitrile oxide derived from 2-ethylbutanal and the commercially available and inexpensive cyclopentadiene and 1,3-cyclohexadiene, which afforded the isoxazolino-cyclopentene or cyclohexene intermediates respectively. The subsequent reaction of the C=C bond in different conditions allowed the functionalisation of the five (or six) membered carbon nucleus. Further functionalised derivatives displaying an amino and a hydroxyl group were achieved *via* the final opening of the isoxazoline ring.

Key words *peramivir; neuraminidase inhibitors; 1,3-dipolar cycloaddition; isoxazolines; cyclopentadiene; 1,3-cyclohexadiene.*

Influenza is an acute viral infection of the first respiratory tract caused by RNA virus of the Orthomyxoviridae family that affects an enormous number of people each year,[1] and causes substantial costs for the community and a high mortality rate.[2] Annual flu vaccination remains the primary preventive measure to reduce influenza epidemics. However, it provides limited control, because influenza viruses mutate very rapidly and there is a continuous emergence of new strains to which even previously vaccinated subjects remain sensitive .[3] Thus, despite the implementation of a vaccination program organized and monitored by the World Health Organization, the flu remains a serious public health problem.

The most recently used target in influenza prophylaxis is neuraminidase (NA), a viral surface enzyme belonging to the family of glycoside hydrolases.[4] Neuraminidase catalyses the cleavage of the glycosidic bond between a terminal sialic acid and hemagglutinin, thus allowing the virion to escape from infected host cells and spread the infection in the respiratory tract.

The Neuraminidase inhibitors, such as zanamivir[5], oseltamivir[6] and peramivir[7], which halt the viral replication thus to slowing down the spread of the infection, have been developed as anti-influenza drugs in clinical treatment to date (Fig. 1).

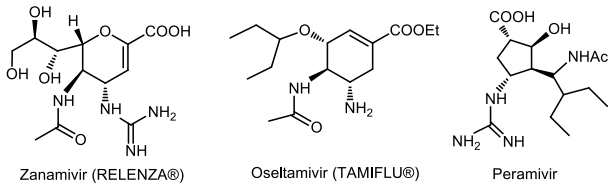
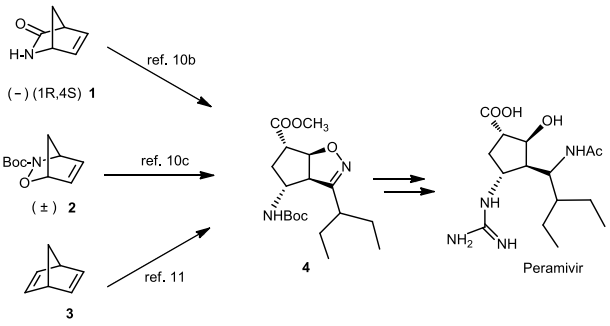


Figure 1 Structure of three neuraminidase inhibitors.

The clinical use of zanamivir is hampered by its limited oral bioavailability thus to be administered only by oral inhalation, whereas the second-generation of neuraminidase inhibitor oseltamivir has a good oral activity as an ester pro-drug that is converted to the active carboxylate metabolite by endogenous esterase. Both products have some side effects: headache and diarrhoea in the case of zanamivir, as well as nausea and vomit in the case of oseltamivir. The new neuraminidase inhibitor peramivir[7] has recently proved to be very potent against a variety of strains of influenza, and more effective than zanamivir or oseltamivir,[8] but its poor bioavailability has aroused

considerable interest in the design and the synthesis of analogous structures.[9] Finding a new, versatile and efficient synthetic strategy could promote further structure-activity relationship (SAR) studies through the development of new molecules as potential NA inhibitors showing a better bioavailability than the NA inhibitors known so far.

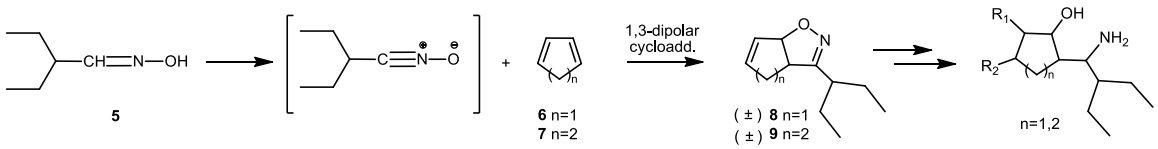
A number of studies of peramivir synthesis have been published.[10] Recently we proposed an alternative synthesis of peramivir.[11] The starting material in the first studies[10a,b,d] was the (-)-(1R,4S)-2-azabicyclo[2.2.1]hept-5-en-3-one **1** (Vince's lactam),[12] which is commercially available as an expensive pure enantiomer. Using a different approach, Miller[10c] started with the racemic 2-oxa-3-aza-bicyclo[2.2.1]hept-5-ene-3-carboxylic acid tert-butyl ester **2**. Finally, in our approach,[11] we started from the inexpensive bicyclo[2.2.1]hepta-2,5-diene (2,5-norbornadiene-NBD) **3**. All the syntheses share the bicyclic isoxazoline moiety **4** as a key intermediate achieved *via* a 1,3-dipolar cycloaddition between the 2-ethylbutanenitrile *N*-oxide and the respective alkenes (Scheme 1).



Scheme 1 Previous synthesis of Peramivir.

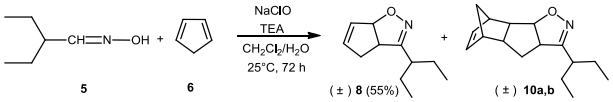
Considering both the interest in the synthesis of analogues of the above mentioned neuraminidase inhibitors and our expertise in the stereoselective synthesis of highly functionalised heterocycles using the 1,3-dipolar cycloaddition reactions,[13] we focused on the study of a new general synthetic pattern in order to obtain some peramivir analogues, showing variously functionalised five or six membered aliphatic ring cores, achievable from common intermediates. These intermediates could be generated by an initial 1,3-dipolar cycloaddition between the nitrile oxide, obtained from the oxime **5**, and commercially available and inexpensive cyclic dienes as cyclopentadiene **6** or 1,3-cyclohexadiene **7** (Scheme 2).

According to Scheme 2, cycloadducts **8** and **9** could be used as versatile, common intermediates taking advantage of the C=C bond. Our current aim is the synthesis of neuraminidase inhibitors showing a cyclopentane or cyclohexane ring as central nucleus, highly functionalised in different relative positions compared to peramivir. Our approach may provide a good versatility in synthesising structural analogues of this compound, not only in relation to the R substitution (R≠1-ethy-propyl) but also to the introduction of different R_1/R_2 groups.



Scheme 2 Synthetic approach to peramivir analogues

Differently from the reported synthesis of peramivir,[11] racemic compounds **8** and **9** were synthesized by starting from a 1,3-dipolar cycloaddition between the oxime **5** and cyclic dienes **6** and **7**. Oxime **5**, the precursor of the nitrile oxide, was prepared from the corresponding commercially available 2-ethylbutanal and hydroxylamine at reflux in MeOH,[11,14] and then converted into the corresponding 1,3-dipole *via* treatment with NaOCl and TEA in a biphasic system. In the presence of an excess of freshly distilled cyclopentadiene **6** the reaction can lead to a mixture of the expected cycloadduct **8** and two isomers of the tricyclic compound **10** with variable yields depending on the different ratios of reagents (Scheme 3, Table 1).



Scheme 3 Synthesis of compounds **8** or **10a,b**.

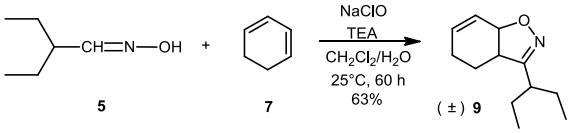
The analytical data of the inseparable by-products **10a,b**, obtained with a ratio of 67:33, have allowed only to clarify that they derive from a subsequent Diels-Alder cycloaddition reaction between cyclopentadiene **6** and the already formed product **8**. Compounds **10a,b** are the only products obtained, or the predominant ones, when an excess of cyclopentadiene is used compared to the oxime, or when an amount of TEA higher than the catalytic amount is employed (Table 1).

Table 1 1,3-Cycloaddition reaction conditions to afford compounds **8** or **10a,b**.

5 (eq.)	6 (eq.)	TEA (eq.)	Ratio 8:10
1	1	0.3	100:0
1	2	0.3	17:83
1	1	1	25:75
1	2	1	10:90
1	3	2	0:100

Product **8** was purified by column chromatography and obtained with a moderate yield ranging from 50 to 55%. As already known in the literature, also in this case the formation of the only regioisomer **8** was observed, whose regiochemistry was confirmed by comparison with similar compounds.[15]

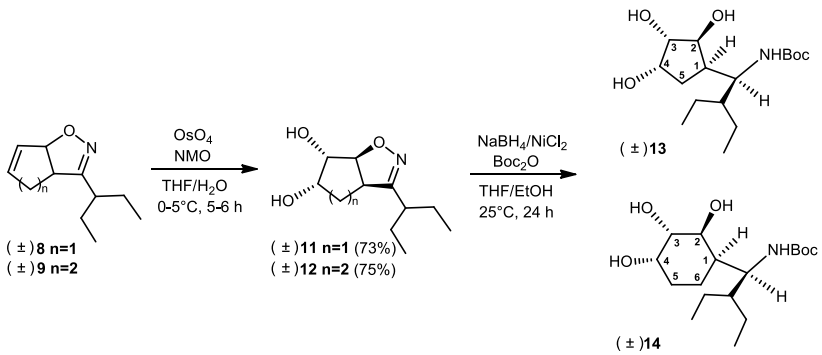
The reaction of the 1,3-cyclohexadiene **7** with oxime **5** led to the formation of only the regioisomer **9** analogously to what reported in the literature for the reactions between 1,3-cyclohexadiene and nitrile oxides (Scheme 4).[16]



Scheme 4 Synthesis of compound **9**.

Compound **9** was purified by column chromatography and obtained in an overall yield of 63% by using a **5**:**7**:TEA=1:2:1 ratio. The regiochemistry of **9** was confirmed by comparison with similar compounds.[16a]

As the next step, different functional groups were introduced on the cyclopentane or cyclohexane ring taking advantage of the presence of the carbon-carbon double bond. First, two hydroxyl groups were introduced by performing a dihydroxylation reaction. The osmium catalysed dihydroxylation on compounds **8** and **9**, in the presence of 4-methylmorpholine *N*-oxide as a co-oxidant, resulted in the formation of only the trans-diastereomers **11** and **12** in 73 and 75% yield respectively (Scheme 5).



Scheme 5 Synthesis of compounds **13** and **14**.

The trans relationship between the isoxazoline ring and the hydroxylic groups was determined by comparison with similar molecules for compound **11**,[17] and by accurate NMR spectra analysis for compound **12** (Figure 2).

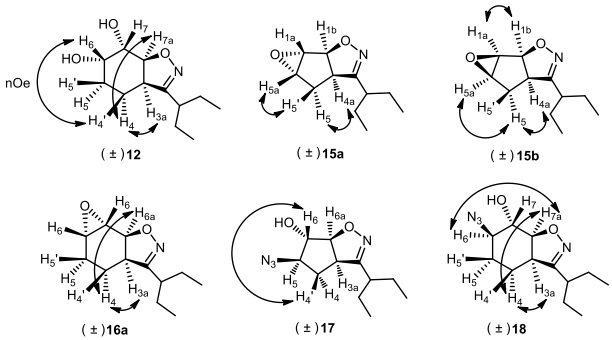
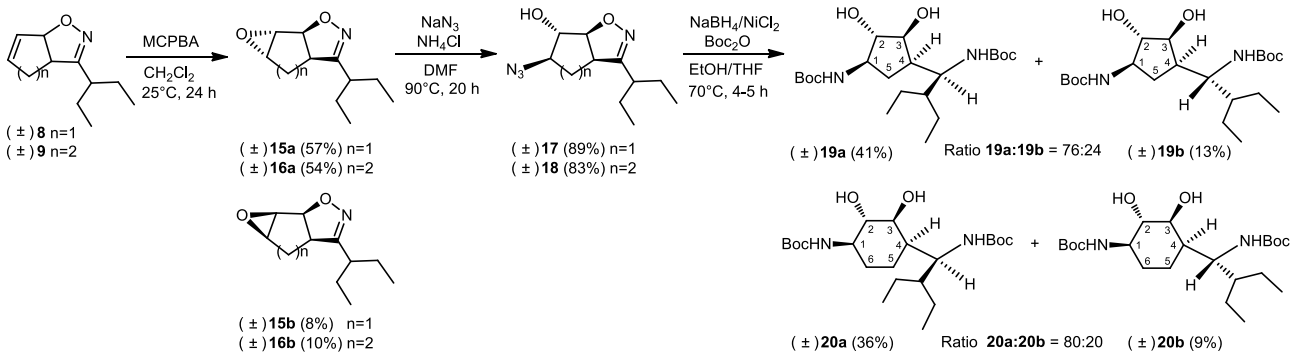


Figure 2 Main NOE effects of compounds **12**, **15a,b, 16a, 17, 18**.

In this latter, the COSY showed the correlation of H-7a (at δ 4.49-4.53) with the protons H-3a (at δ 3.19) and H-7 (at δ 3.78-3.82) as well as the correlation between H-7 and H-6 (at δ 3.99-4.03) and between H-3a and the two protons H-4 and H-4' (at δ 2.01-2.12 and 1.46-1.72). This allowed to assign the correct chemical shifts to protons H-6 and H-7. The NOESY experiment displayed a nuclear Overhauser effect between H-7a and H-4 (at δ 2.01-2.12) confirming their cis relationship, and between H-6 and H-4' (at δ 1.59) validating the trans relationship between H-7a and the protons H-6 and H-7. Therefore, it is clear that the preference for the attack by osmium tetroxide is from the opposite side to the one of the isoxazoline ring, which is less sterically hindered.

On the diols thus obtained, the best reaction conditions for the isoxazoline ring opening have been developed, based on the reduction with sodium borohydride in the presence of nickel chloride.[10d,18] Among these, the conditions that allowed to obtain the final products **13** and **14** were those using Boc anhydride to acylate the amino group that is generated after reduction[18b] (Scheme 5). The isoxazoline opening led to the formation of a new stereocentre adjacent to the nitrogen atom. In both cases the reactions afforded only one of the two possible diastereomers, namely 13 and 14 respectively. The relative stereochemistry of the new stereocentre could not be assigned, but, most probably, it corresponds to the one observed in similar structures, also reduced in analogous manner, that is, with the hydride attack from the sterically less hindered side, as expected.[15e,18b,19]

To introduce functional groups besides hydroxyls, the corresponding epoxides of intermediates **8** and **9** were prepared to be subsequently opened with a nucleophile. The epoxidation reaction was conducted using 3-chloroperbenzoic acid (MCPBA) at room temperature and provided the corresponding epoxides in an overall yield of 65 and 64% respectively (Scheme 6).



Scheme 6 Synthesis of compounds **19a,b** and **20a,b**.

The reaction led to the formation of mixtures of the epoxides *trans*/*cis* **15a**/**15b** and **16a**/**16b** in a ratio of 87:13 and 84:16 respectively. The epoxides were purified by flash chromatography; compounds **15a**, **15b** and **16a** were fully characterized by means of NMR analysis (^{1}H, ^{13}C, COSY, HSQC, NOESY) and by comparison with similar compounds reported in literature,[20] so their relative configuration was completely assigned (Figure 2).

The nucleophile so far used to open the epoxide was sodium azide which was added to a DMF solution of epoxide **15a** or **16a** in the presence of ammonium chloride (Scheme 6). The reaction afforded the corresponding products **17** or **18** in 89 and 83% yields respectively, with complete regio- and stereoselectivity.[20b] In both cases the only product formed was the one resulting from the attack by the azide on the carbon 5 from the opposite side of the epoxide ring, which means in *cis* relatively to isoxazoline ring. In fact, the COSY of compound **17** showed the correlation of H-6a (at δ 4.78) with the proton H-3a (at δ 3.66) and with H-6 (at δ 4.20) as well as the correlation between H-6 and H-5 (at δ 3.85). The protons H-3a and H-5 also showed a correlation with the two protons H-4 and H-4' (at δ 2.35-2.41 and 1.72-1.82). This allowed to assign the correct chemical shifts to protons H-6, H-5, H-4 and H-4'. The trans relationship between the protons H-5 and H-6 was confirmed by means of the NOESY experiment of compound **17**. The positive Overhauser effect between H-6 and H-4' confirmed their *cis* relationship and, therefore, the *trans* relationship between H-6 and H-6a, H-6 and H-5 (Figure 2). Similarly, the exact steric relationship of the compound **18** was determined (Figure 2).

The catalytic hydrogenation of the azide **17** (Pd/C, r.t., 1 atm) failed. Instead, by heating (80°C) of **17** with $NaBH_4$ in a toluene/water biphasic system and in the presence of tributylhexadecylphosphonium bromide as phase transfer catalyst,[21] the corresponding amine was obtained in a very poor yield though. Since $NaBH_4$ was overall efficient both in reducing the azide group and in opening the isoxazoline ring, it was considered to carry out both steps together by treating the azides **17** and **18** at the same conditions through which diols were achieved (Scheme 5), but differently heating at 70°C. In fact the reactions with sodium borohydride, in the presence of nickel chloride and Boc anhydride, led to the reduction of both the azide and the isoxazoline ring with formation of a mixture of two diastereoisomeric compounds **19a/19b** and **20a/20b** in a total yield of 54 and 45% and a ratio of 76:24 and 80:20 respectively (Scheme 6). The couples of stereoisomers were separated by means of column chromatography. Despite an accurate NMR analysis conducted on **20a** and **20b**, it was not possible to determine the stereochemistry of the new stereocentre. However, also in this case the new configuration of the more abundant stereoisomers **19a** and **20a** should be the same as that obtained in other examples that utilized the same experimental conditions of isoxazoline opening.[22]

In conclusion, we have developed a process for the synthesis of new molecules as potential Neuraminidase inhibitors, analogues of peramivir. The strategy for obtaining them is based on a 1,3-dipolar cycloaddition reaction of the appropriate nitrile oxide with cyclic dienes as cyclopentadiene and 1,3-cycloexadiene, inexpensive and commercially available reagents. This method represents an alternative approach to the previous ones reported in the literature.[10b-c,11] It is potentially very versatile, allowing the access to different products by varying the diene, the 1,3-dipole and, consequently, the subsequent reactions used.

Experimental

TLC separations were performed on precoated Merck silica gel 60-F_{254}. Melting points were determined by the capillary method with a *Büchi* B-540 apparatus and are uncorrected. IR spectra were measured with a Jasco FT/IR 5300 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded using a *Varian-Mercury 300 MHz* and a *Bruker-Avance 300 MHz* spectrometers. Chemical shifts (δ) are given in ppm in relation to residual solvent peaks ($CDCl_3$) as the internal reference. The solvent was $CDCl_3$ unless otherwise specified. ^{13}C NMR spectra are ^1H-decoupled and the determination of the multiplicities was achieved from the APT pulse sequence. Two-dimensional experiments (COSY-NOESY-HSQC) were performed with 2048 points in the F2 direction and 256 points in F1. The spectra were acquired with 8-16 scans. Two-dimensional nuclear Overhauser effect spectroscopy experiments were performed with mixing time values range of 400ms-2s depending on the spectra acquired. Elemental analyses were performed on a Perkin-Elmer CHN Analyzer Series II 2400. The MS spectra were recorded with a *Thermo-Finnigan LCQ Advantage* AP electrospray and a *Thermo-Scientific LCQ fleet* ion trap mass spectrometers using a syringe pump device to directly inject sample solutions. Cyclopentadiene **6**, 1,3-cyclohexadiene **7** and 2-ethylbutanal were obtained from commercial sources. Oxime **5**[11,14] was prepared following the reported method.

3-(1-Ethyl-propyl)-4,6a-dihydro-3aH-cyclopenta[d]isoxazole (**8**)

A 4.5% aqueous solution of NaClO (80.0 mL, 50 mmol) was added dropwise to a solution of freshly distilled cyclopentadiene **6** (1.5 g, 22.7 mmol, 1 equiv.), oxime **5** (2.62 g, 22.7 mmol, 1 equiv.) and TEA (1.05 mL, 7.56 mmol, 0.3 equiv.) in CH_2Cl_2 (30 mL). The mixture was stirred for 72 h at room temperature, the solvents were separated, and the aqueous phase was extracted using CH_2Cl_2 (3x15 mL). The combined extracts were dried over anhydrous Na_2SO_4 and concentrated at reduced pressure. Compounds **8** was purified by means of column chromatography on silica gel (from hexane = 100 to hexane/ethyl acetate = 50:50).

Yellow oil; yield: 2.23 g, (55%). R_f = 0.5 (hexane/ethyl acetate = 98:2, I_2).

IR (film) ν_{max}: 701 (ν_{C-H}, CH=CH), 1611 ($\nu_{C=N}$, C=N).

^1H NMR: δ 0.87 (t, *J*=7.7 Hz, 6H, $2CH_3$); 1.51-1.66 (m, 4H, $2CH_2$); 2.20-2.30 (m, 1H, CH); 2.48-2.64 (m, 2H, 2H-4); 3.72-3.79 (dt, *J*=8.5, 2.1 Hz, 1H, H-3a); 5.55 (d, *J*=9.1 Hz, 1H, H-6a); 5.77-5.83 (m, 1H, H-5), 5.91-5.96 (m, 1H, H-6).

^{13}C NMR: δ 11.1, 12.2 ($2CH_3$); 24.3, 25.7 ($2CH_2$); 35.8 (C-4); 41.0 (CH); 51.4 (C-3a); 89.4 (C-6a); 130.4, 133.2 (C-5, C-6); 162.3 (C-3).

MS-ESI$^+$ (m/z): 180 $[M+H]^+$, 202 $[M+Na]^+$.

Anal. Calcd for $C_{11}H_{17}NO$: C, 73.70; H, 9.56; N, 7.81. Found: C, 73.56; H 9.35; N, 7.73.

3-(1-Ethyl-propyl)-4,4a,5,8,8a,8b-hexahydro-3aH-5,8-methanoindeno[2,1-d]isoxazole (**10a,b**)

A 4.5% aqueous solution of NaClO (35.0 mL, 22 mmol) was added dropwise to a solution of freshly distilled cyclopentadiene **6** (1.72 g, 26.1 mmol, 3 equiv.), oxime **5** (1.0 g, 8.7 mmol, 1 equiv.) and TEA (2.4 mL, 17.4 mmol, 2 equiv.) in CH_2Cl_2 (30 mL). The mixture was stirred for 24 h at room temperature, the solvents were separated, and the aqueous phase was extracted using CH_2Cl_2 (3x15 mL). The combined extracts were dried over anhydrous Na_2SO_4 and concentrated at reduced pressure. The inseparable mixture of compounds **10a,b** was purified by means of column chromatography on silica gel (hexane/ethyl acetate = 95:5).

Colourless oil; yield: 1.5 g, (70%). R_f = 0.6 (hexane/ethyl acetate = 95:5, I_2).

^1H NMR: δ 0.85 (t, *J*=7.5 Hz, 6H); 1.44-1.62 (m, 7H); 2.18-2.70 (m, 5H); 3.00, 3.07 (2d, *J*=8.0 Hz, *J*=8.5 Hz, 1H); 3.13-3.19 (m, 1H); 4.30, 4.41 (2d, *J*=8.0 Hz, *J*=8.5 Hz, 1H); 5.50-5.78 (m, 2H).

^{13}C NMR: δ 11.27, 11.29, 12.0, 12.03, 24.4, 24.6, 25.8, 26.0, 31.5, 32.5, 34.9, 35.4, 39.7, 40.9, 41.2, 41.3, 42.1, 43.7, 45.5, 47.3, 50.5, 51.3, 51.9, 54.8, 81.5, 83.9, 130.5, 131.1, 131.4, 132.0, 161.7.

MS-ESI$^+$ (m/z): 246 $[M+H]^+$, 268 $[M+Na]^+$.

Anal. Calcd for $C_{16}H_{23}NO$: C, 78.32; H, 9.45; N, 5.71. Found: C, 78.11; H 9.35; N, 5.69.

3-(1-Ethyl-propyl)-3a,4,5,7a-tetrahydrobenzo[d]isoxazole (9)

A 4.5% aqueous solution of NaClO (120.0 mL, 76 mmol) was added dropwise to a solution of cyclohexadiene **7** (5.6 g, 70.0 mmol, 2 equiv.), oxime **5** (4.0 g, 35.0 mmol, 1 equiv.) and TEA (4.84 mL, 35.0 mmol, 1 equiv.) in CH_2Cl_2 (60 mL). The mixture was stirred for 60 h at room temperature. The solvents were separated, and the aqueous phase was extracted using CH_2Cl_2 (4x20 mL). The combined extracts were dried over anhydrous Na_2SO_4 and concentrated at reduced pressure. Compound **9** was purified by means of column chromatography on silica gel (hexane/ethyl acetate = 90:10).

Yellow oil; yield: 4.24 g, (63%). R_f = 0.3 (hexane/ethyl acetate = 90:10, I_2).

IR (film) ν_{max}: 694 (ν_{C-H}, CH=CH), 1601 ($\nu_{C=N}$, C=N).

^{1}H NMR: δ0.88 (t, *J*=7.7 Hz, 6H, $2CH_3$); 1.42-1.78 (m, 5H, $2CH_2$+H-4); 1.80-1.90 (m, 1H, H-4); 1.90-2.00 (m, 1H, H-5); 2.00-2.18 (m, 1H, H-5); 2.22-2.38 (m, 1H, CH); 2.98-3.04 (m, 1H, H-3a); 4.61-4.65 (m, 1H, H-7a); 5.89-5.95 (m, 1H, H-6), 6.06-6.12 (m, 1H, H-7).

^{13}C NMR: δ11.4, 12.4 ($2CH_3$); 21.9, 23.0 (C-4, C-5); 24.5, 26.1 ($2CH_2$); 41.6 (CH); 45.6 (C-3a); 75.7 (C-7a); 123.3, 132.5 (C-6, C-7); 165.4 (C-3).

MS-ESI$^+$ (m/z): 194 $[M+H]^+$, 216 $[M+Na]^+$.

Anal. Calcd for $C_{12}H_{19}NO$: C, 74.57; H, 9.91; N, 7.25. Found: C, 74.42; H 9.84; N, 7.17.

3-(1-Ethyl-propyl)-4,5,6,6a-tetrahydro-3aH-cyclopenta[d]isoxazole-5,6-diol (11)

4-Methylmorpholine N-oxide (0.13 g, 1.11 mmol) was added to a solution of compound **8** (0.1 g, 0.56 mmol) in THF/H_2O=9/1 (3mL) cooled to 0°C. Osmium tetroxide (2.5% in tBuOH, 0.7 mL, 0.06 mmol) was added dropwise while maintaining the temperature between 0 and 5°C. The mixture was stirred for 5 h at 0°C after that it was treated with Na_2SO_3 (0.126 g, 1.0 mmol) and stirred for 0.5 h at room temperature. The solvent was evaporated *in vacuo*, the residue was treated with CH_2Cl_2, the insoluble was filtered and the filtrate was evaporated off. Compound **11** was purified by means of column chromatography on silica gel ($CHCl_3/CH_3OH$ = 98:2).

Colourless amorphous glass; yield: 0.087 g, (73%). R_f = 0.45 ($CHCl_3/CH_3OH$ = 90:10, I_2).

^{1}H NMR: δ0.87 (t, *J*=7.7 Hz, 6H, $2CH_3$); 1.51-1.68 (m, 4H, $2CH_2$); 1.89-2.18 (m, 2H, 2H-4); 2.22-2.31 (m, 1H, CH); 2.70 (bs, 2H, 2 OH); 3.62-3.76 (dt, *J*=9.6 Hz, 4.1 Hz, 1H, H-3a); 4.10-4.19 (m, 2H, H-5 + H-6); 4.80 (d, *J*=9.8 Hz, 1H, H-6a).

^{13}C NMR: δ11.0, 12.0 ($2CH_3$); 24.2, 25.6 ($2CH_2$); 33.8 (C-4); 40.8 (CH); 50.5 (C-3a); 72.8, 78.1 (C-5, C-6); 88.4 (C-6a); 163.5 (C-3).

MS-ESI$^+$ (m/z): 236 $[M+Na]^+$.

Anal. Calcd for $C_{11}H_{19}NO_3$: C, 61.95; H, 8.98; N, 6.57. Found: C, 61.88; H 8.95; N, 6.53.

3-(1-Ethyl-propyl)-3a,4,5,6,7,7a-hexahydrobenzo[d]isoxazole-6,7-diol (12)

4-Methylmorpholine N-oxide (0.607 g, 5.18 mmol) was added to a solution of compound **9** (0.5 g, 2.59 mmol) in THF/H_2O = 9/1 (15mL) cooled to 0°C. Osmium tetroxide (2.5% in tBuOH, 3.02 mL, 0.29 mmol) was added dropwise while maintaining the temperature between 0 and 5°C. The mixture was stirred for 6 h at 0°C after that it was treated with Na_2SO_3 (0.583 g, 4.63 mmol) and stirred for 0.5 h at room temperature. The solvent was evaporated *in vacuo*, the residue was treated with CH_2Cl_2, the insoluble was filtered and the filtrate was evaporated off. Compound **12** was purified by means of column chromatography on silica gel (from $CHCl_3$ to $CHCl_3/CH_3OH$ = 98:2).

Colourless amorphous glass; yield: 0,443 g, (75%). R_f = 0.40 ($CHCl_3/CH_3OH$ = 90:10, I_2).

^{1}H NMR: δ0.90, 0.93 (2t, *J*=7.4 Hz, 6H, $2CH_3$); 1.46-1.72 (m, 6H, $2CH_2$ + H-4' + H-5); 1.74-1.88 (m, 1H, H-5); 2.01-2.12 (m, 1H, H-4); 2.27-2.36 (m, 1H, CH); 2.99 (bs, 2H, 2 OH); 3.19 (q, *J*=7.7 Hz, 1H, H-3a); 3.78-3.82 (m, 1H, H-7); 3.99-4.03 (m, 1H, H-6); 4.49-4.53 (m, 1H, H-7a).

^{13}C NMR: δ11.0, 12.2 ($2CH_3$); 20.0 (C-4); 23.9, 25.4 ($2CH_2$); 26.1 (C-5); 41.0 (CH); 45.7 (C-3a); 68.5, 70.2 (C-6, C-7); 83.3 (C-7a);166.5 (C-3).

MS-ESI$^+$ (m/z): 228 $[M+H]^+$, 250 $[M+Na]^+$.

Anal. Calcd for $C_{12}H_{21}NO_3$: C, 63.41; H, 9.31; N, 6.16. Found: C, 63.38; H 9.22; N, 6.07.

***tert*-Butyl [2-ethyl-1-(2,3,4-trihydroxycyclopentyl)butyl]carbamate (13)**

$NiCl_2$ (0.30 g, 2.34 mmol) and Boc_2O (0.51 g, 2.34 mmol) were added to a stirred solution of **11** (0.25 g, 1.17 mmol) in EtOH/THF (10 mL, 3/1). Subsequently, $NaBH_4$ (0.09 g, 2.34 mmol) was added in small portions. The mixture was stirred at room temperature for 24 h, then treated with H_2O (5 mL) and filtered through a layer of silica gel using a Büchner funnel. The organic solvent was evaporated under reduced pressure and the aqueous residue was extracted using AcOEt (3x10 mL). The organic phase was separated, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. Compound **13** was purified by repeated column chromatography on silica gel (eluent: toluene/ethyl acetate = 90/10 to 50/50).

Colourless amorphous glass; yield: 0.116g, (31%). R_f = 0.30 (toluene/ethyl acetate = 75:25, I_2).

^{1}H NMR: δ0.90-0.97 (m, 6H, $2CH_3$); 1.01-1.54 (m, 2H, CH_2); 1.19-1.55 (m, 2H CH_2), 1.20-1.26 (m, 1H CH), 1.45 (s, 9H, *t*Bu); 1.51-1.56 (m, 1H, H-5); 1.89-1.93 (m, 1H, H-5); 2.39-2.44 (m, 1H, H-1); 2.90 (bs, 3H, 3 OH); 3.72-3.76 (m, 1H, CH-N); 3.97-3.41 (m, 1H, H-2), 4.07-4.11 (m, 1H, H-3), 4.53 (bs, 1H, N-H), 4.55-4.59 (m, 1H, H-4).

^{13}C NMR: δ 12.0, 12.7 ($2CH_3$); 21.2, 23.3 ($2CH_2$); 28.3 (CH_3*t*Bu); 34.2 (C-5 ring); 42.8 (C-2 chain); 45.2 (C-1 ring); 50.7 (C-1 chain); 71.9 (C-4 ring); 76.7 (C-3 ring); 77.6 (C-2 ring); 80.3 (C Boc); 157.9 (C=O Boc).

MS-ESI$^+$ (m/z): 340 $[M+Na]^+$.

Anal. Calcd for $C_{16}H_{31}NO_5$: C, 60.54; H, 9.84; N, 4.41. Found: C, 60.49; H 9.71; N, 4.38.

tert-Butyl [2-ethyl-1-(2,3,4-trihydroxycyclohexyl)butyl]carbamate (14)

$NiCl_2$ (0.38 g, 2.92 mmol) and Boc_2O (0.64 g, 2.92 mmol) were added to a stirred solution of **12** (0.33 g, 1.46 mmol) in EtOH/THF (10 mL, 3:1). Subsequently, $NaBH_4$ (0.11 g, 2.92 mmol) was added in small portions. The mixture was stirred at room temperature for 24 h, then treated with H_2O (7 mL) and CH_2Cl_2 (10 mL) and filtered through a layer of silica gel using a Büchner funnel. The organic phase was evaporated under reduced pressure and the aqueous residue was extracted using CH_2Cl_2 (3x10 mL). The organic phase was separated, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. Compounds **14** was purified by column chromatography on silica gel ($CHCl_3/CH_3OH$ = 98/2).

Colourless solid; yield: 0.253 g, (52%); mp 99-100°C. R_f = 0.42 ($CHCl_3/CH_3OH$ = 98/2, I_2).

^{1}H NMR: δ 0.93 (t, *J*=6.9 Hz, 6H, $2CH_3$); 1.20-1.40 (m, 2H, CH_2); 1.22-1.43 (m, 2H, CH_2); 1.33-137 (m, 1H, CH); 1.38-1.56 (m, 2H, H-6, H-6'); 1.45 (s, 9H, *t*Bu); 1.58-1.75 (m, 2H, H-5, H-5'); 1.83 (bs, 1H, OH); 1.91-1.97 (m, 1H, H-1); 2.15 (bs, 1H, OH); 2.52 (bs, 1H, OH); 3.62-3.67 (m, 1H, CH-N); 3.78-4.05 (m, 3H, H-2, H-3, H-4); 4.55 (bd, *J*=10.2 Hz, 1H, N-H).

^{13}C NMR: δ 12.0, 12.3 ($2CH_3$); 20.9, 21.5, 22.9, 28.1 ($2CH_2$ + C-5, C-6 ring); 28.6 ($3CH_3$ *t*Bu); 37.9, 44.4 (C-1 ring, C-2 chain); 53.0 (C-1 chain); 68.3, 72.7, 73.1 (C-2, C-3, C-4 ring); 80.0 (C Boc); 157.3 (C=O Boc).

MS-ESI$^+$ (m/z): 354 $[M+Na]^+$.

Anal. Calcd for $C_{17}H_{33}NO_5$: C, 61.60; H, 10.04; N, 4.23. Found: C, 61.51; H 9.97; N, 4.17.

4-(1-Ethyl-propyl)-1b,4a,5,5a-tetrahydro-1aH-oxireno[2',3':3,4]cyclopenta[1,2-d]isoxazole (15a,b)

3-Chloroperbenzoic acid (1.88 g, 10.9 mmol) in anhydrous CH_2Cl_2 (10 mL) was added dropwise to a solution of **8** (1.7 g, 9.5 mmol) in anhydrous CH_2Cl_2 (10 mL). The mixture was stirred for 24 h at room temperature and then basified with a 5% aqueous solution of $NaHCO_3$. The organic phase was separated and dried over anhydrous Na_2SO_4, and then evaporated under reduced pressure. Compounds **15a** and **15b** were purified and separated by flash chromatography on silica gel (hexane/ethyl acetate = 70/30).

Compound **15a**: light yellow oil; yield: 1.06 g, (57%). R_f = 0.41 (toluene/ethyl acetate = 90:10, I_2).

^{1}H NMR: δ 0.89, 0.90 (2t, *J*=7.6 Hz, 6H, $2CH_3$); 1.50-1.58 (m, 4H, $2CH_2$); 1.85 (ddd, *J*=14.5, 5.7, 2.0 Hz, 1H, H-5'); 2.24-2.29 (m, 1H, CH); 2.34 (dd, *J*=14.5, 9.2 Hz, 1H, H-5); 3.25-3.31 (m, 1H, H-4a); 3.65 (t, *J*=2.2 Hz, 1H, H-5a); 3.71 (d, *J*=2.2 Hz, 1H, H-1a); 5.05 (d, *J*=8.6 Hz, 1H, H-1b).

^{13}C NMR: δ 11.1, 11.9 ($2CH_3$); 24.3, 25.6 ($2CH_2$); 32.7 (C-5); 40.9 (CH); 51.0 (C-4a); 58.7 (C-5a); 59.3 (C-1a); 85.9 (C-1b); 163.6 (C-4).

MS-ESI$^+$ (m/z): 196 $[M+1]^+$.

Anal. Calcd for $C_{11}H_{17}NO_2$: C, 67.66; H, 8.78; N, 7.17. Found: C, 67.59; H 8.65; N, 7.1.

Compound **15b**: light yellow oil; yield: 0.15 g, (8%). R_f = 0.16 (toluene/ethyl acetate =90:10, I_2).

^{1}H NMR: δ 0.88, 0.94 (2t, *J*=7.5 Hz, 6H, $2CH_3$); 1.41-1.79 (m, 4H, $2CH_2$); 2.09 (ddd, *J*=16.7, 9.9, 1.7 Hz, 1H, H-5); 2.17-2.22 (m, 1H, CH); 2.37 (d, *J*=16.7 Hz, 1H, H-5'); 3.60 (t, *J*=1.7 Hz, 1H, H-5a); 3.69 (s, 1H, H-1a); 3.75 (t, *J*=9.9 Hz, 1H, H-4a); 5.00 (dd, *J*=10.2, 1.6 Hz, 1H, H-1b).

^{13}C NMR: δ 10.8, 12.0 ($2CH_3$); 24.0, 25.1 ($2CH_2$); 29.4 (C-5); 40.6 (CH); 51.8 (C-4a); 58.8 (C-5a); 59.8 (C-1a); 82.6 (C-1b); 162.1 (C-4).

MS-ESI$^+$ (m/z): 196 $[M+1]^+$, 218 $[M+23]^+$.

Anal. Calcd for $C_{11}H_{17}NO_2$: C, 67.66; H, 8.78; N, 7.17. Found: C, 67.56; H 8.63; N, 7.08.

3-(1-Ethyl-propyl)-3a,4,5,5a,6a,6b-hexahydrooxireno[2',3':3,4]benzo[1,2-d]isoxazole (16a,b)

3-Chloroperbenzoic acid (1.05 g, 6.08 mmol) in anhydrous CH_2Cl_2 (6 mL) was added dropwise to a solution of **9** (1.02 g, 5.3 mmol) in anhydrous CH_2Cl_2 (6 mL). The mixture was stirred for 24 h at room temperature and then basified with a 5% aqueous solution of $NaHCO_3$. The organic phase was separated and dried over anhydrous Na_2SO_4, and then evaporated under reduced pressure. Compound **16a** was purified by column chromatography on silica gel (hexane/ethyl acetate = 80/20). It was not possible to isolate the compound **16b** pure enough.

Compound **16a**: colourless oil; yield: 0.6 g, (54%). R_f = 0.43 (toluene/ethyl acetate = 90:10, I_2).

^{1}H NMR: δ 0.87, 0.98 (2t, *J*=7.4 Hz, 6H, $2CH_3$); 1.42-1.66 (m, 2H, CH_2); 1.58-1.87 (m, 2H, CH_2); 1.52-1.58 (m, 1H, H-4'); 1.62-1.67 (m, 1H, H-5'); 1.85-1.93 (m, 1H, H-4); 2.02-2.09 (m, 1H, H-5); 2.18-2.24 (m, 1H, CH); 3.09 (d, *J*=2.6 Hz, 1H, H-7); 3.24 (s, 1H, H-6); 3.30 (dt, *J*=10.5, 4.2 Hz, 1H, H-3a); 4.87 (d, *J*=10.5 Hz, 1H, H-7a).

^{13}C NMR: δ 10.5, 12.7 ($2CH_3$); 14.8 (C-4); 21.2 (C-5); 23.3, 25.1 ($2CH_2$); 39.5 (CH); 45.7 (C-3a); 51.4 (C-7); 52.0 (C-6); 74.2 (C-7a); 162.3 (C-3).

MS-ESI$^+$ (m/z): 210 $[M+1]^+$, 232 $[M+23]^+$.

Anal. Calcd for $C_{12}H_{19}NO_2$: C, 68.87; H, 9.15; N, 6.69. Found: C, 68.77; H 9.04; N, 6.58.

Compound **16b**: It was not possible to isolate compound **16b** pure enough; yield (10%) was calculated from ^{1}H NMR analysis of the mixed fractions. The ^{1}H and ^{13}C NMR spectra were recorded on a mixed sample of **16a** and **16b**.

^{1}H NMR: δ 0.95 (t, *J*=7.4 Hz, 6H, $2CH_3$); 1.58-1.71 (m, 2H, CH_2); 1.64-1.79 (m, 2H, CH_2); 1.87-2.07 (m, 4H, 2H-4, 2H-5); 2.34-2.42 (m, 1H, CH); 2.97 (d, *J*=2.4 Hz, 1H, H-7); 3.26 (bs, 1H, H-6); 3.36 (m, 1H, H-3a); 4.4 (bd, *J*=10.2 Hz, 1H, H-7a).

^{13}C NMR: δ 11.1, 12.22 ($2CH_3$); 18.0 (C-4); 22.9 (C-5); 26.3, 24.4 ($2CH_2$); 41.3 (CH); 45.8 (C-3a); 49.1 (C-7); 52.5 (C-6); 76.9 (C-7a); 161.9 (C-3).

5-Azido-3-(1-Ethyl-propyl)-4,5,6,6a-tetrahydro-3aH-cyclopenta[d]isoxazol-6-ol (17)

NaN_3 (1.0 g, 15.4 mmol) and NH_4Cl (1.07 g, 20.0 mmol) were added to a solution of **15a** (0.3 g, 1.54 mmol) in DMF (15 mL). The mixture was stirred for 20 h at 90°C and then treated with water and ethyl acetate. The organic phase was separated, dried over anhydrous Na_2SO_4, and then evaporated under reduced pressure. Compounds **17** was purified by column chromatography on silica gel (toluene/ethyl acetate = 90/10).

Colorless oil; yield: 0.325 g, (89%). R_f = 0.15 (toluene/ethyl acetate = 90:10, I_2).

IR (film) ν_{max}: 1611 ($\nu_{C=N}$, C=N); 2100 (ν_{N3}, N_3); 3369 (ν_{OH}, OH).

^{1}H NMR: δ 0.93, 0.95 (2t, *J*=7.4 Hz, 6H, $2CH_3$); 1.51-1.70 (m, 4H, $2CH_2$); 1.72-1.82 (m, 1H, H-4'); 2.29-2.34 (m, 1H, CH); 2.35-2.41 (m, 1H, H-4); 2.68 (broad s, 1H, OH); 3.66 (ddd, *J*=9.8, 9.8, 6.3 Hz, 1H, H-3a); 3.85 (dd, *J*=13.8, 6.4 Hz, 1H, H-5); 4.20 (dd, *J*=6.4, 3.5 Hz, 1H, H-6); 4.78 (dd, *J*=10.4, 3.5 Hz, 1H, H-6a).

^{13}C NMR: δ 11.0, 11.9 ($2CH_3$); 24.0, 25.3 ($2CH_2$); 32.4 (C-4); 40.7 (CH); 49.9 (C-3a); 65.4 (C-5); 82.2 (C-6); 89.0 (C-6a); 162.5 (C-3).

MS-ESI$^+$ (m/z): 239 $[M+1]^+$, 261 $[M+23]^+$.

Anal. Calcd for $C_{11}H_{18}N_4O_2$: C, 55.44; H, 7.61; N, 23.51. Found: C, 55.38; H 7.55; N, 23.46.

6-Azido-3-(1-Ethyl-propyl)-3a,4,5,6,7,7a-hexahydrobenzo[d]isoxazol-7-ol (**18**)

NaN_3 (0.98 g, 15.1 mmol) and NH_4Cl (1.05 g, 19.6 mmol) were added to a solution of **16a** (0.316 g, 1.51 mmol) in DMF (15 mL). The mixture was stirred for 20 h at 90°C and then treated with water and ethyl acetate. The organic phase was separated and dried over anhydrous Na_2SO_4, and then evaporated under reduced pressure. Compounds **18** was purified by column chromatography on silica gel (toluene/ethyl acetate = 90/10).

Colourless oil; yield: 0.315 g, (83%). R_f = 0.18 (toluene/ethyl acetate=90:10, I_2).

^{1}H NMR: δ 0.89, 0.95 (2t, *J*=7.5 Hz, 6H, $2CH_3$); 1.18-1.32 (m, 1H, H-5'); 1.55-1.68 (m, 4H, $2CH_2$); 1.70-1.86 (m, 1H, H-4); 1.85-2.00 (m, 1H, H-5); 2.01-2.12 (m, 1H, H-4'); 2.25-2.35 (m, 1H, CH); 3.25 (broad s, 1H, OH); 3.30 (dd, 1H, H-6); 3.35-3.40 (m, 1H, H-3a); 3.46 (t, *J*=7.5 Hz, 1H, H-7); 4.43 (t, *J*=7.5 Hz, 1H, H-7a).

^{13}C NMR: δ 11.1, 12.6 ($2CH_3$); 20.9 (C-4); 23.8, 25.3 ($2CH_2$); 27.2 (C-5); 40.6 (CH); 48.1 (C-3a); 61.8 (C-6); 75.4 (C-7); 84.4 (C-7a); 164.4 (C-3).

MS-ESI$^+$ (m/z): 253 $[M+1]^+$, 275 $[M+23]^+$.

Anal. Calcd for $C_{12}H_{20}N_4O_2$: C, 57.12; H, 7.99; N, 22.21. Found: C, 57.07; H 7.88; N, 22.16.

Carbamic acid, [4-[1-[[(1,1-dimethylethoxy)carbonyl]amino]-2-ethylbuthyl]-2,3-dihydroxycyclopentyl]-, 1,1-dimethylethyl ester (**19a,b**)

$NiCl_2$ (0.26 g, 2 mmol) and Boc_2O (0.48 g, 2.2 mmol) were added to a stirred solution of **17** (0.24 g, 1 mmol) in EtOH/THF (10 mL, 3:1). Subsequently, $NaBH_4$ (0.15 g, 4 mmol) was added in small portions. The mixture was stirred for 5 h at 70°C, cooled down to room temperature then treated with H_2O (5 mL) and filtered through a layer of silica gel using a Büchner funnel. The organic phase was evaporated under reduced pressure. The residue was treated with water (15 mL) and extracted using ethyl acetate (3x10 mL). The organic phase was separated, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The mixture of stereoisomers **19a** and **19b** was purified and separated by column chromatography on silica gel (toluene/ethyl acetate = 75/25).

Compound **19a**: colourless solid; yield: 0.17 g, (41%); mp 148-150°C. R_f = 0.22 (toluene/ethyl acetate = 62:38, I_2).

^{1}H NMR: δ 0.93, 0.94 (2t, *J*=7.5 Hz, 6H, $2CH_3$); 1.09-1.47 (m, 2H, CH_2); 1.19-1.45 (m, 2H, CH_2); 1.33-1.37 (m, 1H, CH); 1.44 (s, 18H, 2*t*Bu); 1.61-1.65 (m, 1H, H-5); 2.13-2.17 (m, 1H, H-5); 2.25-2.30 (m, 1H, H-4); 2.95 (bs, 1H, OH); 3.53 (bs, 1H, OH); 3.62-3.67 (m, 1H, H-1); 3.84-3.88 (m, 1H, H-2); 3.89-3.93 (m, 1H, H-3); 3.95-4.02 (m, 1H, CH-N); 4.50 (bd, 1H, NH); 5.13 (bs, 1H, NH).

^{13}C NMR: δ 11.8, 12.3 ($2CH_3$); 21.4, 23.0 ($2CH_2$); 28.4 (CH_3 *t*Bu); 32.2 (C-5); 43.7, 44.0, 51.0, 58.0, 77.6, 84,8 (6 C-H); 79.4, 79.5 (C Boc); 156.4, 156.6 (C=O Boc).

MS-ESI$^+$ (m/z): 417 $[M+1]^+$, 439 $[M+23]^+$.

Anal. Calcd for $C_{21}H_{40}N_2O_6$: C, 60.55; H, 9.68; N, 6.73. Found: C, 60.49; H 9.58; N, 6.7.

Compound **19b**: colourless solid; yield: 0.055 g, (13%); m.p. 156-158°C. R_f = 0.33 (toluene/ethyl acetate = 62:38, I_2).

^{1}H NMR: δ 0.91, 0.95 (2t, *J*=7.5 Hz, 6H, $2CH_3$); 1.17-1.53 (m, 2H, CH_2); 1.17-1.23 (m, 1H, CH); 1.19-1.54 (m, 2H, CH_2); 1.31-1.36 (m, 1H, H-5); 1.43 (s, 18H, 2*t*Bu); 1.45-1.52 (m, 2H, 2 OH); 2.10-2.16 (m, 1H, H-5); 2.18-2.24 (m, 1H, H-4); 3.75-3.81 (m, 1H, CH-N); 3.79-3.84 (m, 1H, H-1); 3.85-3.90 (m, 1H, H-3); 3.93-3.98 (m, 1H, H-2); 4.55 (bd, 1H, NH); 5.06 (bd, 1H, NH).

^{13}C NMR: δ 11.9, 12.6 ($2CH_3$); 21.2, 23.3 ($2CH_2$); 28.3, 28.4 (CH_3*t*Bu); 33.1 (C-5); 42.9, 46.4, 50.6, 59.1, 77.4, 83,4 (6 C-H); 79.5, 80.2 (C Boc); 156.1, 157.8 (C=O Boc).

MS-ESI$^+$ (m/z): 439 $[M+23]^+$.

Anal. Calcd for $C_{21}H_{40}N_2O_6$: C, 60.55; H, 9.68; N, 6.73. Found: C, 60.48; H 9.56; N, 6.67.

Carbamic acid, [4-[1-[[(1,1-dimethylethoxy)carbonyl]amino]-2-ethylbuthyl]-2,3- dihydroxycyclohexyl]-, 1,1-dimethylethyl ester (**20a,b**)

$NiCl_2$ (0.144 g, 1.112 mmol) and Boc_2O (0.267 g, 1.22 mmol) were added to a stirred solution of **18** (0.14 g, 0.556 mmol) in EtOH/THF (5 mL, 3:1). Subsequently, $NaBH_4$ (0.084 g, 2.22 mmol) was added in small portions. The mixture was stirred for 4 h at 75°C, cooled down to room temperature then treated with H_2O (2.5 mL) and filtered through a layer of silica gel using a Büchner funnel. The organic phase was evaporated under reduced pressure. The residue was treated with water (10 mL) and extracted using ethyl acetate (3x7 mL). The organic phase was separated, dried over anhydrous Na_2SO_4 and evaporated under reduced pressure. The mixture of stereoisomers **20a** and **20b** was purified and separated by column chromatography on silica gel (toluene/ethyl acetate = 75/25).

Compound **20a**: colourless amorphous glass; yield: 0.086 g, (36%). R_f = 0.26 (toluene/ethyl acetate = 62:38, I_2).

^{1}H NMR: δ 0.91 (t, *J*=7.3 Hz, 6H, $2CH_3$); 1.10-1.46 (m, 2H, CH_2); 1.23-1.43 (m, 2H, CH_2); 1.24-1.28 (m, 1H, H-5); 1.33-1.40 (m, 1H, CH); 1.42 (s, 9H, *t*Bu); 1.45 (s, 9H, *t*Bu); 1.56-1.62 (m, 1H, H-5); 1.63-1.68 (m, 1H, H-6); 1.82-1.89 (m, 1H, H-6); 1.96-2.20 (m, 1H, H-4); 3.30 (bs, 1H, OH); 3.62-3.68 (m, 1H, CH-N); 3.69-3.77 (m, 1H, H-1); 3.79-3.85 (m, 1H, H-3); 3.80-3.86 (m, 1H, H-2); 4.15 (bs, 1H, OH); 4.50 (bd, 1H, NH); 6.13 (bd, 1H, NH).

^{13}C NMR: δ 11.7, 11.9 ($2CH_3$); 18.0 (C-5); 21.4, 22.6 ($2CH_2$); 26.0 (C-6); 28.4, 28.4 (CH_3*t*Bu); 38.7 (C-4); 44.7 (CH-Et$_2$); 50.6 (C-1); 53.0 (C-N); 71.0 (C-2); 73.7 (C-3); 79.0, 79.9 (C Boc); 155.8, 157.3 (C=O Boc).

MS-ESI$^+$ (m/z): 431 $[M+1]^+$, 453 $[M+23]^+$.

Anal. Calcd for $C_{22}H_{42}N_2O_6$: C, 61.37; H, 9.83; N, 6.51. Found: C, 61.29; H 9.76; N, 6.47.

Compound **20b**: colourless solid; yield: 0.022 g, (9%); mp 124-126°C. R_f = 0.33 (toluene/ethyl acetate = 62:38, I_2).

^{1}H NMR: δ 0.91, 0.96 (t, *J*=7.2 Hz, 6H, $2CH_3$); 1.11-1.20 (m, 1H, 1H of CH_2); 1.31-1.35 (m, 1H, H-5); 1.42-1.47 (m, 1H, CH); 1.44 (s, 18H, 2*t*Bu); 1.47-1.50 (m, 2H, CH_2); 1.48-1.54 (m, 1H, H-5); 1.52-1.58 (m, 1H, 1H of CH_2); 1.63-1.72 (m, 1H, H-6); 1.74-1.83 (m, 1H, H-4); 1.80-1.87 (m, 1H, H-6); 2.56 (bs, 1H, OH); 3.67 (t, *J*=9.5 Hz, 1H, CH-N); 3.85 (bs, 2H, H-1, H-3); 4.02 (bs, 1H, H-2); 4.44 (bd, 1H, NH); 4.52 (bd, 1H, OH); 6.30 (bd, 1H, NH).

^{13}C NMR: δ11.9, 12.6 ($2CH_3$); 18.3 (C-5); 21.0, 23.3 ($2CH_2$); 25.4 (C-6); 28.3, 28.5 (CH_3*t*Bu); 38.1 (C-4); 40.6 (CH-Et_2); 50.0 (C-1); 51.9 (C-N); 70.3 (C-2); 70.4 (C-3); 78.9, 80.2 (C Boc); 155.7, 158.3 (C=O Boc).

MS-ESI$^+$ (m/z): 431 $[M+1]^+$, 453 $[M+23]^+$.

Anal. Calcd for $C_{22}H_{42}N_2O_6$: C, 61.37; H, 9.83; N, 6.51. Found: C, 61.32; H 9.77; N, 6.45.

Funding Information

Università degli Studi di Milano is acknowledged for financial support.

Acknowledgment

The authors thank Dr. Gerald Edward Lazo Reyna for his helpful technical assistance.

Supporting Information

YES (this text will be updated with links prior to publication)

Primary Data

NO (this text will be deleted prior to publication)

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