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Diversity-oriented syntheses of 7-substituted lentiginosines

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ABSTRACT

Diversity-oriented synthesis of derivatives of the potent glycosidase inhibitor lentiginosine can be achieved in an efficient and versatile way by two modular approaches on key intermediates. After assembling the indolizidine ring system through 1,3-dipolar cycloaddition of a dihydroxylated pyrroline *N*-oxide with 4-butenol followed by elaboration of the isoxazolidine moiety, the 7-amino and 7-azido derivatives synthesized can be conjugated with functionalised chains by coupling, respectively, with an amino acid, or an alkyne in copper-catalyzed Huisgen cycloadditions.

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1. Introduction

(+)-Lentiginosine [(1*S*,2*S*,8a*S*)-octahydro-1,2-indolizinediol, (+)-**1**, Fig. 1], an indolizidine alkaloid isolated for the first time by Elbein et al. from the leaves of *Astragalus lentiginosus* in 1990,[1] is a small molecule (MW 157) exhibiting very interesting properties. In particular, (+)-**1**, with only two hydroxyl groups, is the least oxygenated iminosugar that mimics glucosidase natural substrates and indeed it is a selective inhibitor of amyloglucosidases, i.e., enzymes that hydrolyze 1,4- and 1,6-α-glucosidic linkages. The inhibitory power is good (K_i=2.0 and 3.0 μM toward *Aspergillus niger* amyloglucosidase and *Rhizopus* mold amyloglucosidase, respectively)[2] and the selectivity is remarkably high in comparison with other important glucosidase inhibitors such as 1-deoxynojirimycin and castanospermine.[3] The non-natural enantiomer, (−)-lentiginosine [(−)-**1**, Fig. 1], is a weaker inhibitor of amyloglucosidases compared to (+)-lentiginosine (K_i=70 and 98 μM toward *A. niger* amyloglucosidase and *Rhizopus* mold amyloglucosidase, respectively),[2] and was recently shown to be an appealing caspase-dependent apoptosis inducer on tumor cells of different origin.[4] The high interest in (+)- and (−)-lentiginosine[5] is also testified by the large number of their syntheses published since the discovery of **1**.[6–9]

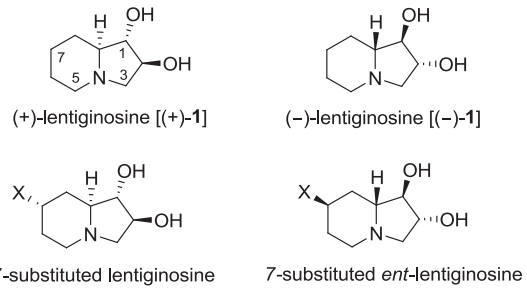

Fig. 1. Natural (+)-lentiginosine, non-natural (−)-lentiginosine, and general structure of their 7-substituted derivatives.

Based on the biological properties of (+)-**1** and (−)-**1**, our goal is to design diversity-oriented syntheses for the preparation of 7-substituted lentiginosine in both enantiomeric forms to get a deeper insight into ligand receptor interactions regarding their glycosidase inhibition and pro-apoptotic activity.

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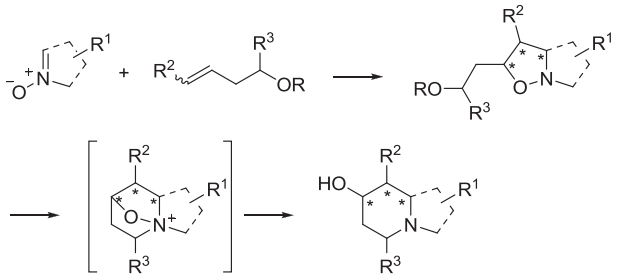
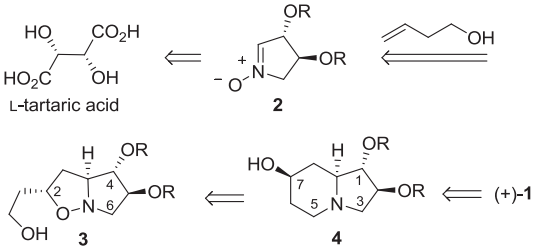
The 1,3-dipolar cycloaddition (1,3-DC) of nitrones with dipolarophiles containing the 3-buten-1-ol moiety followed by conversion of cycloadducts into 4-piperidinols was proved to be a reliable approach to azaheterocyclic compounds. The method, first proposed by Tufariello and Tegeler,[10] has been applied to the preparation of functionalized piperidines,[11] indolizidines,[12] and quinolizidines[13] (Scheme 1).



Scheme 1.

Taking advantage of the very well-known, highly reliable, and selective nitrone 1,3-DC reaction, the planning of the synthesis of these compounds is quite simple, and a nice control of the relative and absolute configuration of up to three new stereocenters in the final products can be achieved employing a suitable nitrone, an (*E*)- or (*Z*)-disubstituted dipolarophile and the inter- or intramolecular approach between the two reagents.[14]

The use of pyrroline *N*-oxides derived from chiral pool molecules such as malic acid and tartaric acid allowed the stereoselective synthesis of optically active hydroxyindolizidines[14,15] including (+)-lentiginosine [(+)-**1**] (Scheme 2).[2b,16]



Scheme 2.

The glycosidase inhibitor activity of (+)-**1** combined with the discovery of the pro-apoptotic action of its enantiomer (−)-**1** suggested the undertaking of a program to derivatize the lentiginosine structure by conjugation with aminoacidic, lipidic, aromatic and heteroaromatic, and fluorescent structures. In fact, the design, synthesis, and biological evaluation of analogs of glycosidase inhibitors are subjected to extensive studies due to the many potential medical applications of these compounds,[17] and general methods able to introduce groups having different structural and functional properties are highly desirable. Our intention is to create a class of possible bioactive compounds based on the lentiginosine structure that may be useful to shed light on the type of receptors involved and on the ligand–receptor interactions.

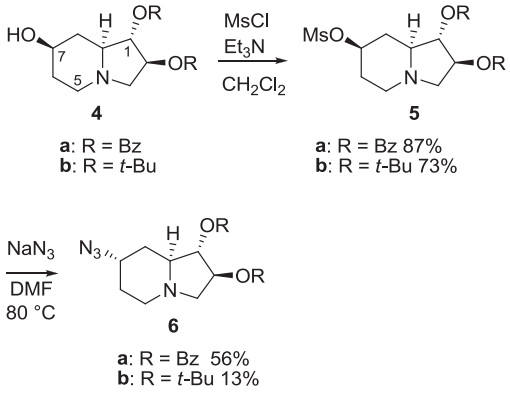
As a starting point of this ongoing programme, we envisioned that the exploitation of the 7-hydroxy group of intermediate **4** in the synthesis of lentiginosine (Scheme 2) would provide a rapid and flexible access to variously decorated final products.

Herein, we report the preparation of 7-azido and 7-amino derivatives of **1** and their use as common intermediates in the diversity-oriented syntheses of 7-substituted lentiginosines (Fig. 1).[18] Two modular and reliable processes, such as Cu(I)-catalyzed Huisgen cycloaddition and amino acylation, were used to conjugate lentiginosine and its enantiomer with polar moieties, lipophilic chains, natural amino acids, and markers such as biotin. The lentiginosine derivatives were also tested as inhibitors toward some commercially available glycosidases. Finally, docking studies into the active site of glucoamylase from *Aspergillus awamori* were carried out to investigate the interaction mode of the new synthesized lentiginosine derivatives.

2. Results and discussion

The indolizidinols **4** (**a**: R=Bz; **b**: R=*t*-Bu; **c**: R=TBDMS) used in this study were prepared according to the general synthetic methodology previously described[18,19] (see also Supplementary data). The introduction of the azido group in position 7 was achieved either through mesylation followed by nucleophilic substitution or directly by a Mitsunobu reaction.

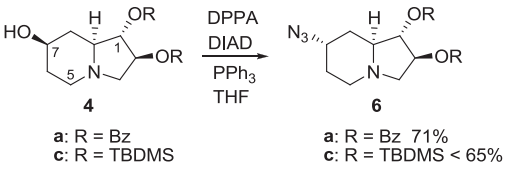
The 7-mesyl derivatives **5a** and **5b** were obtained from the corresponding alcohols **4a** and **4b** under standard conditions in good yields (73–87%) (Scheme 3). The nature of the R protecting group proved to be crucial during the nucleophilic substitution of the mesylate group. Treatment of **5a** with NaN_3 in DMF at 80 °C afforded the corresponding azide **6a** with complete inversion of configuration at C-7 in 56% yield (Scheme 3). Under the same conditions, the *tert*-butyl protected mesylate **5b** gave the expected azide **6b** in only 13% yield along with 1-(but-3-enyl)-3-*tert*-butoxy-1*H*-pyrrole[18] (21% yield), which is believed to form through a Grob fragmentation followed by aromatization of the *tert*-butyl derivative **5b**.



Scheme 3.

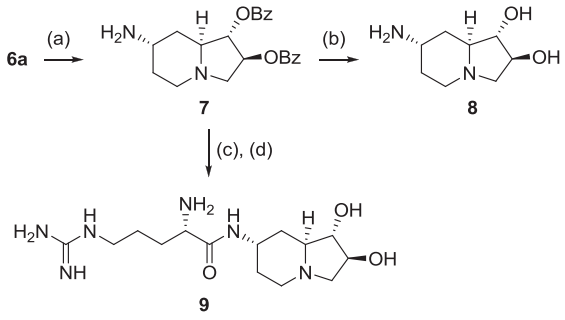
The relative configuration of C-7 in azides **6** was confirmed by the ^{1}H NMR resonance of 7-H (**6a**: 4.05 ppm; **6b**: 3.99 ppm), which appears as a pseudo quintet with a small coupling constant (J=3 Hz) consistent with an equatorial orientation. In compounds **4** and **5**, proton 7-H experiences a strong diaxial coupling generating a triplet of triplet (J_{ax-ax}=10.9–11.2; J_{ax-eq}=4.5–4.8) in the ^{1}H NMR spectrum. The observed stereochemical result is in accordance with analogous C-7 nucleophilic substitutions performed by Tyler et al.[20] on the indolizidine alkaloid castanospermine, whereas displacements with retention occur at C-8 and C-6 of castanospermine because of the participation of the ring nitrogen.

The one-step substitution of the 7-OH group under Mitsunobu conditions was tested on indolizidinols **4a** and **4c** (Scheme 4). Treatment of **4a** with diphenylphosphoryl azide (DPPA), diisopropylazodicarboxylate (DIAD), and Ph_3P in THF afforded **6a** with a better yield compared with the two-step procedure (71% vs 49% overall yield). On the contrary, purification of the crude azide **6c** obtained under the same conditions proved to be tricky, and the product was recovered with a very low purity grade after chromatography on silica gel (<65% yield). Accordingly, the benzoylated 7-azido lentiginosine was used as substrate in subsequent reactions.



Scheme 4.

The best procedure to convert azide **6a** into the corresponding amine **7** proved to be hydrogenation in the presence of Raney-Ni (Scheme 5). Other catalysts and methods, such as Pd/C, Zn/AcOH, and PPh_3/H_2O afforded the desired amine **7** in lower yield and poor reproducibility. Hydrolysis of the benzoyl groups with the basic resin Ambersep 900 OH in MeOH gave the 7-amino lentiginosine **8** in 66% yield (Scheme 5).

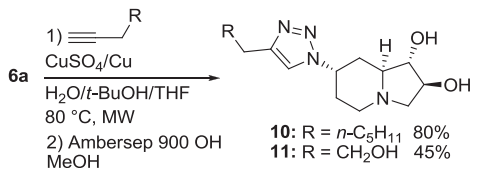


Scheme 5. (a): Raney-Ni, H_2, MeOH, 71%; or Pd/C, H_2, AcOH, MeOH, 26%; or Zn, AcOH, CH_2Cl_2, 37%; or Ph_3P, H_2O, THF, 65–75 °C, 61%; (b) Ambersep 900 OH, MeOH, 85%; (c) FmocHN–Arg(Pbf)–CO_2H, DIPEA, PyBrop, 75%; (d) i: TFA, ii: piperidine, iii: Ambersep 900 OH, MeOH, 49%.

To test the ability of the 7-amino group to undergo coupling with natural amino acids, the synthesis of the 7-arginilated lentiginosine **9** was carried out. The orthogonally protected arginine Fmoc–Arg(Pbf)–OH smoothly reacted with the benzoylated amine **7** under standard coupling conditions to give the expected amide in 75% yield (Scheme 5). The three protecting groups (Pbf, Fmoc, and Bz) could be sequentially removed with complete selectivity under the usual conditions and eventually the rather polar product **9** was obtained in 49% overall yield.

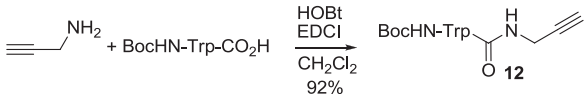
These results indicate the possibility of linking peptides and simple amino acids with the iminosugar lentiginosine and making conjugated amino acids suitably protected for peptide syntheses.

Azide **6a** was then evaluated as a tool for derivatization of lentiginosine, via the synthesis of triazole derivatives. In recent years, 1,4-disubstituted 1,2,3-triazoles have become very popular as stable linking units to connect molecular fragments through a modular approach. The reliability of the copper-(I)-catalyzed addition of azides with terminal alkynes (CuAAc)[21,22] and the structural analogies with the amide bond[21c,22a,22f,22m,23] makes the triazole moiety a privileged tether in drug discovery. This approach could be applied to azide **6a** to get different 7-substituted lentiginosines. An aliphatic chain, a polar chain, and an amino acid such as tryptophan were chosen as different model fragments to be appended on lentiginosine. The two commercial alkynes 1-octyne and 3-butyn-1-ol were reacted with **6a** in the presence of in situ generated Cu(I) to achieve, after hydrolysis of the benzoyl groups, the triazoles **10** and **11** in 80% and 45% overall yield, respectively (Scheme 6).

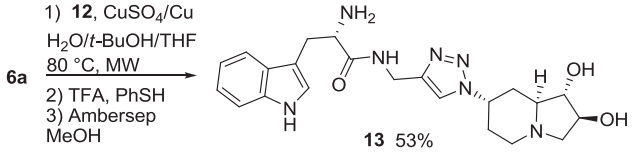


Scheme 6.

Analogously, the CuAAc reaction of **6a** with alkyne **12**,[24] prepared by condensation of 2-propynylamine with *N*-Boc protected Trp (Scheme 7), followed by hydrolysis of the carbamate and the benzoyl groups afforded triazole **13** in 53% overall yield (Scheme 8).

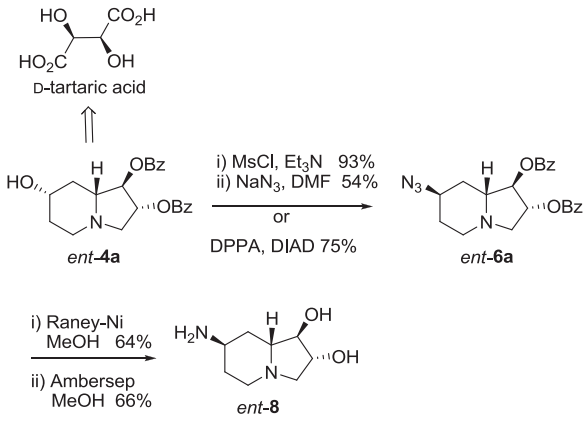


Scheme 7.



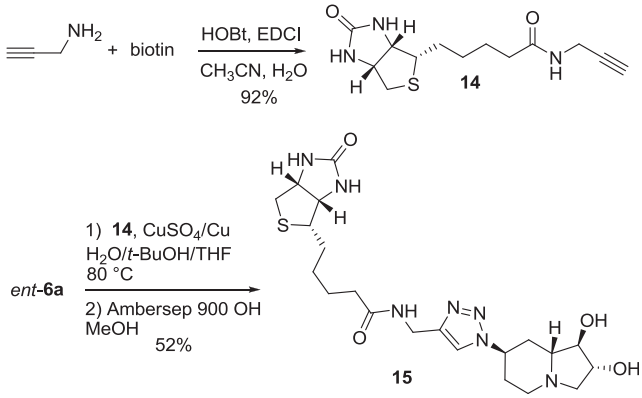
Scheme 8.

To address the pro-apoptotic activity of new derivatives also the enantiomeric protected indolizidinol *ent*-**4a** was prepared and converted into the 7-substituted D-lentiginosines[5] *ent*-**6a** and *ent*-**8** starting from D-tartaric acid (Scheme 9).



Scheme 9.

Moreover, to allow a study of the interaction of D-lentiginosines with cellular receptors, a biotinylated lentiginosine was synthesized. The amide of biotin and 2-propynylamine **14**[25] was prepared in high yield using the coupling reagents HOBt and EDCI in an acetonitrile/water mixture as a solvent and reacted with azide *ent*-**5a** under the usual conditions. After basic hydrolysis, the biotinylated D-lentiginosine **15** was obtained in 52% overall yield (Scheme 10).



Scheme 10.

The 7-substituted L-lentiginosines **8**, **9**, **10**, **11**, and **13** were evaluated as glycosidase inhibitors toward 12 commercially available enzymes.[26] All the inhibition values different from zero at 1 mM concentration are given in Table 1. At 1 mM concentration, the arginylated lentiginosine **9** did not inhibit any of the examined enzymes. Moderate inhibition was displayed by indolizidines **8**, **10**, and **11** toward amyloglucosidase (16–40%) and β-glucosidase (17–67%) and by **13** toward amyloglucosidase and β-glucosidase (Table 1).

Table 1
Inhibition (in %) toward glycosidases at 1 mM concentration of 7-substituted L-lentiginosines **8**, **10**, **11**, and **13**

	1	**8**	**10**	**11**	**13**
Amyloglucosidase EC 3.2.1.3 *Aspergillus niger*	100[a]	40	16	26	40
β-Glucosidase EC 3.2.1.21 Almonds	0	35	67	17	0
β-Xylosidase EC 3.2.1.37 *Aspergillus niger*	0	0	0	0	15

[a] K_i=2.0 μM[2].

Having established a rapid, reliable, and general functionalization on C-7 of lentiginosine through two modular approaches based on 7-amino and 7-azido-lentiginosine as building blocks and in order to interpret the biological data from a structural point of view, we decided to perform a modeling investigation to analyze the interactions between these new lentiginosine derivatives and the amyloglucosidase binding site. Docking calculations carried out on the *N*-protonated form of the (+)-lentiginosine revealed a good fit of the indolizidine ring into the binding cavity of the enzyme (Fig. 2a), thus confirming what was already shown with previous calculations show a slight change of position of the indolizidine ring in the binding cavity compared to (+)-**1**. In all these cases, the charged N–H···OWat501 hydrogen bond loses the optimal geometrical requirements both in term of distance *d* and angles *θ* (donor–H···acceptor) and *φ* (H···acceptor–Y) (Table 2). In most cases, the C1–OH does not H-bond to the D55 residue and unfavorable van der Waals contacts are formed between atoms of the 7-substituent and the binding site W120, W178, E179, E180, and Y311 residues. The increase in the internal torsional energy ($E_{internal}$), one of the terms of the docking scoring function, reflects the difficulty in the adaptation of the indolizidine ring into the binding cavity (Table 2, Fig. 2b–e). In summary, calculation results are consistent with a lower affinity of indolizidines **8**, **10**, **11**, and **13** for the enzyme amyloglucosidase and reaffirm the importance of the formation of a N–H···O hydrogen bond between the iminosugar and the water 501 molecule for inhibitory activity.

Table 2
Interaction features between amyloglucosidase and (+)-lentiginosine and its 7-substituted analogs **8**, **9**, **10**, **11**, and **13**

	N–H···OWat501				
Compd	*d* (Å)	*θ* (°) (D–H···A–)	*φ* (°) (H···A–Y)	$E_{internal}$ (kJ/mol)	Residue in unfavorable van der Waal contacts
(+)-**1**	2.172	163.3	94.7	1.15	
8	2.261	156.8	85.4	1.48	W178
9	2.55	148.9	75.1	4.6	
10	2.55	138.1	90.9	5.41	E180
11	2.6	135.9	88.3	5.25	E179, E180
13	3.5	121.4	76.7	8.5	E180, Y311, W120

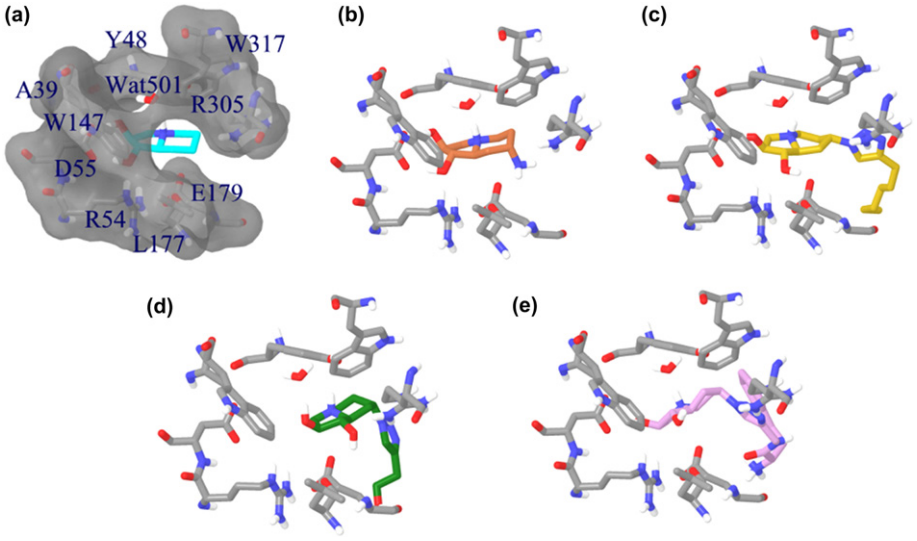


Fig. 2. Docking orientation of (a) (+)-lentiginosine and (b–e) 7-lentiginosine derivatives (**8**, **10**, **11**, and **13**) into the 1DOG glucoamylase binding site.

molecular dynamics simulations.[27] A favorable network of interactions are established involving a three center C1–O–H···(D55, R54) hydrogen bond and a C2–OH H-bond interaction with the D55 active site residue. Moreover (+)-**1** donates a N–H···O hydrogen bond to the water 501 molecule, which is known to have a catalytic role in the hydrolysis mechanism. The resulting charged H-bond is characterized by good geometrical parameters (Table 2). In the case of the 7-substituted derivatives **8**, **10**, **11**, and **13**, docking

3. Conclusion

A general method for the functionalization of lentiginosine on the7-position of the indolizidine ring has been reported using the 7-azido and 7-amino derivatives as common intermediates. The study has set the basis for the construction of a new class of lentiginosines conjugated with different groups for the study of their glycosidase inhibition (1*S*,2*S*,7*S*,8a*S* series) and pro-apoptotic

activity (1*R*,2*R*,7*R*,8a*R* series). None of the synthesized derivatives of (+)-**1** has reached the glycosidase inhibitor activity and selectivity of the parent lentiginosine. A preliminary computational study of the interaction of the new compounds with the amyloglucosidase enzyme cavity gives some hints to justify the reduced activity and sets the basis for selection of active candidates.

4. Experimental section

4.1. General experimental methods

All the reactions requiring anhydrous conditions were carried out under N_2, and the solvents were appropriately dried before use. R_f values refer to TLC on 0.25 mm silica gel plates. The NMR data are reported in δ (ppm) from TMS at 25 °C, and peak assignments were made on the basis of ^{1}H–^{1}H COSY, HMQC, HSQC, and HMBC experiments. Mass spectra: MS (ESI) were recorded on a LCQ Fleet Ion Trap Mass Spectrometer with Surveyor Plus LC System (Thermo Scientific); MS (EI) were recorded on a QP5050 Shimadzu spectrometer with a GC; relative percentages are shown in parentheses. Accurate mass spectra were recorded on a LTQ-Orbitrap high-resolution mass spectrometer (Thermo, San Jose, CA, USA), equipped with a conventional ESI source. Microwave-assisted reactions were carried out in a CEM Discover (TM) singlemode microwave reactor with IR temperature sensor. The two series of enantiomeric compounds were synthesized starting from, respectively, (2*R*,3*R*)-(+)-tartaric acid (ee ≥99.5%) and (2*S*,3*S*)-(−)-tartaric acid (ee 99%).

4.2. (1*S*,2*S*,7*S*,8a*S*)-7-Azido-2-(benzoyloxy)octahydro-1-indolizinyl benzoate (6a)

*Method A: (1S,2S,7R,8aS)-2-(Benzoyloxy)-7-[(methylsulfonyl)oxy] octahydro-1-indolizinyl benzoate (**5a**)*: MsCl (0.327 mL, 4.24 mmol) was added dropwise to a solution of **4a** (809 mg, 2.12 mmol, see Supplementary data) and TEA (1.462 mL, 10.5 mmol) in CH_2Cl_2 (3 mL) at 0 °C. The mixture was stirred under N_2 at rt for 2 h and the resulting suspension was diluted with CH_2Cl_2 (9 mL) and H_2O (9 mL). The two phases were separated and the aqueous phase extracted with CH_2Cl_2 (2×9 mL). The collected organic phases were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was filtered through a short pad of silica gel (eluent: EtOAc/petroleum ether 1:1) and evaporation of the solvent afforded **5a** (848 mg, 87%) as a white solid. Compound **5a**: R_f=0.35; mp 134.4–134.7 °C; $[\alpha]_D^{25}$ +97.5 (*c* 0.325, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ=8.11–8.02 (m, 4H; H_{Ph}), 7.61–7.54 (m, 2H; H_{Ph}), 7.48–7.41 (m, 4H; H_{Ph}), 5.67–5.40 (m, 2H; 1-H, 2-H), 4.72–4.62 (m, 1H; 7-H), 3.19 (br d, *J*=11.3 Hz, 1H; 3-H_a), 3.14–3.08 (m, 1H; 5-H_a), 3.03 (s, 3H; CH_3), 2.87 (dd, *J*=11.3, 6.5 Hz, 1H; 3-H_b), 2.47 (dm, *J*=11.6 Hz, 1H; 8-H_a), 2.41–2.33 (m, 1H; 8a-H), 2.27–2.12 (m, 2H; 5-H_b+6-H_a), 1.95 (dq, *J*=4.5, 12.1 Hz, 1H; 6-H_b), 1.88 (q, *J*=11.6 Hz, 1H; 8-H_b) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ=166.3 (s; C=O), 165.8 (s; C=O), 133.4 (d; CH_{Ph}), 133.2 (d; CH_{Ph}), 129.9 (d, 2C; CH_{Ph}), 129.8 (d, 2C; CH_{Ph}), 129.6 (s; C_{Ph}), 129.3 (s; C_{Ph}), 128.4 (d, 2C; CH_{Ph}), 128.3 (d, 2C; CH_{Ph}), 81.5 (d; C-1), 78.3 (d; C-7), 78.1 (d; C-2), 65.6 (d; C-8a), 58.6 (t; C-3), 49.3 (t; C-5), 38.9 (q, CH_3), 35.5 (t; C-8), 31.5 (t; C-6) ppm. IR ($CDCl_3$): ν=3050, 2948, 2942, 1720, 1332, 1279, 1178, 1112 cm^{-1}; $C_{23}H_{25}NO_7S$ (459.5): calcd C 60.12, H 5.48, N 3.05; found C 60.33, H 5.49, N 3.03. A mixture of the mesylate **5a** (591 mg, 1.29 mmol) and NaN_3 (209 mg, 3.2 mmol) in DMF (3.1 mL) was heated at 80 °C for 22 h. The reaction mixture was diluted with EtOAc (8 mL) and H_2O (9 mL), the two phases were separated, and the aqueous phase was extracted with EtOAc (9×8 mL). The collected organic phases were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (eluent: CH_2Cl_2) to afford azide **6a** (296 mg, 56%) as a colorless oil.

Method B: Diisopropyl azodicarboxylate (DIAD, 0.62 mL, 3.15 mmol) was added dropwise to a solution of triphenylphosphine (708 mg, 2.7 mmol) in dry THF (45 mL) under N_2 atmosphere. After 5 min under magnetic stirring at rt, the solution was added via cannula to a solution of **4a** (835 mg, 2.19 mmol, see Supplementary data) in dry THF (45 mL). Diphenyl phosphoryl azide (DPPA, 0.681 mL, 3.15 mmol) was added dropwise and the reaction mixture was stirred overnight at rt under N_2 atmosphere. After concentration under reduced pressure, the crude product was purified by chromatography on silica gel (eluent: first petroleum ether/EtOAc 10:1, then petroleum ether/EtOAc 4:1) to obtain **6a** as a colorless oil (694 mg, 71%).

*Compound **6a***: R_f=0.34 (eluent: petroleum ether/EtOAc 4:1); $[\alpha]_D^{25}$ +109.7 (*c* 0.500, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ=8.10–8.03 (m, 4H; H_{Ph}), 7.60–7.52 (m, 2H; H_{Ph}), 7.48–7.40 (m, 4H; H_{Ph}), 5.43–5.36 (m, 2H; 1-H+2-H), 4.07–4.02 (m, 1H; 7-H), 3.19 (d, *J*=11.2 Hz, 1H; 3-H_a), 2.95–2.86 (m, 2H; 3-H_b+5-H_a), 2.55 (ddd, *J*=11.1, 8.4, 2.4 Hz, 1H; 8a-H), 2.42 (dt, *J*=3.0, 11.6 Hz, 1H; 5-H_b), 2.13 (dm, *J*=13.6 Hz, 1H; 8-H_a), 1.98–1.77 (m, 3H; 6-H+8-H_b) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ=166.4 (s; C=O), 165.9 (s; C=O), 133.3 (d; CH_{Ph}), 133.1 (d; CH_{Ph}), 129.9 (d, 2C; CH_{Ph}), 129.8 (d, 2C; CH_{Ph}), 129.7 (s; C_{Ph}), 129.5 (s; C_{Ph}), 128.4 (d, 2C; CH_{Ph}), 128.3 (d, 2C; CH_{Ph}), 81.9 (d; C-1), 77.4 (d; C-2), 61.7 (d; C-8a), 59.5 (t; C-3), 55.5 (d; C-7), 47.3 (t; C-5), 33.2 (t; C-8), 28.6 (t; C-6) ppm; IR ($CDCl_3$): ν=2930, 2815, 2098, 1718, 1602, 1451, 1276, 1112 cm^{-1}; MS (EI): *m*/*z* (%)=405 (M^+−1, 3), 364 (2), 284 (2), 163 (23), 105 (100), 77 (31); HRMS: calcd for $C_{22}H_{23}N_4O_4$ $[M+H]^+$ 407.17138; found 407.17102; $C_{22}H_{22}N_4O_4$ (406.4): calcd C, 65.01; H, 5.46; N, 13.78; found C, 64.70; H, 5.08; N, 13.55.

4.3. (1*R*,2*R*,7*R*,8a*R*)-7-Azido-2-(benzoyloxy)octahydro-1-indolizinyl benzoate (*ent*-6a)

Azide *ent*-**6a** was prepared applying the same two procedures as for its enantiomer **6a**.

Method A: Starting from *ent*-**4a** (272 mg, 0.715 mmol), (1*R*,2*R*,7*S*,8a*R*)-2-(benzoyloxy)-7-[(methylsulfonyl)oxy]octahydro-1-indolizinyl benzoate (*ent*-**5a**) was obtained in 93% yield (306 mg). Mesylate *ent*-**5a** (283 mg, 0.6 mmol) was then converted into azide *ent*-**6a** (132 mg, 54% yield).

Method B: Starting from indolizidinol *ent*-**4a** (964 mg, 2.53 mmol), azide *ent*-**6a** was obtained in 75% yield (764 mg). *ent*-**6a**: $[\alpha]_D^{23}$ −111.4 (*c* 0.460, $CHCl_3$); $C_{22}H_{22}N_4O_4$ (406.4): calcd C 65.01, H 5.46, N 13.78; found C 64.73, H 5.26, N 13.55.

4.4. (1*S*,2*S*,7*S*,8a*S*)-7-Azido-1,2-bis{[*tert*-butyl(dimethyl)silyl]oxy}octahydroindolizine (6c)

Azide **6c** was prepared starting from alcohol **4c**[2b] (611 mg, 1.5 mmol) using Method B. Chromatography on silica gel (eluent: petroleum ether/EtOAc 4:1) of the crude product afforded **6c** (435 mg, 68%) as a colorless oil. Compound **6c**: R_f: 0.60; $[\alpha]_D^{24}$ +23.4 (*c* 0.780, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ=4.05–3.97 (m, 2H, 2-H+7-H), 3.71 (dd, *J*=8.4, 3.8 Hz, 1H, 1-H), 2.84 (dd, *J*=10.1, 1.3 Hz, 1H, 3-H_a), 2.75 (dm, *J*=11.0 Hz, 1H, 5-H_a), 2.64–2.56 (m, 1H, 3-H_b), 2.30–2.20 (m, 1H, 5-H_b), 2.15 (br t, *J*=9.2 Hz, 1H, 8a-H), 2.00 (dq, *J*=13.3, 2.5 Hz, 1H, 8-H_a), 1.94–1.80 (m, 1H, 6-H_a), 1.74 (dm, *J*=14.0 Hz, 1H, 6-H_b), 1.57–1.47 (m, 1H, 8-H_b), 0.89 [br s, 18H, C(CH_3)$_3$], 0.08 (s, 3H, SiCH_3), 0.06 (s, 3H, SiCH_3), 0.05 (s, 3H, SiCH_3), 0.04 (s, 3H, SiCH_3) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ=85.1 (d; C-1), 78.0 (d; C-2), 62.4 (d; C-8a), 61.8 (t; C-3), 55.5 (d; C-7), 47.7 (t; C-5), 32.5 (t; C-8), 28.7 (t; C-6), 25.9 [q; 3C, C(CH_3)$_3$], 25.8 [q; 3C, C(CH_3)$_3$], 17.9 (s; SiCMe$_3$), 17.8 (s; SiCMe$_3$), −4.1 (q; SiCH_3), −4.28 (q; SiCH_3), −4.31 (q; SiCH_3), −4.7 (q; SiCH_3) ppm; IR ($CDCl_3$): ν=2955, 2929, 2856, 2813, 2100, 1471, 1360, 1258, 1150 cm^{-1}; MS (EI): *m*/*z* (%)=426 (M^+, 6), 411 (3), 398 (3), 384 (11), 369 (11), 138 (76), 73 (100); $C_{20}H_{42}N_4O_2Si_2$ (426.74): calcd C, 56.29; H, 9.92; N, 13.13; found C, 56.10; H, 9.77; N, 13.52.

4.5. (1*S*,2*S*,7*S*,8a*S*)-7-Amino-2-(benzoyloxy)octahydro-1-indolizinyl benzoate (7)

A water suspension of activated Raney-Ni was added dropwise to an ice bath cooled solution of **6a** (395 mg, 0.97 mmol) in MeOH (11 mL). The mixture was stirred at 0 °C for 1 h and then at rt for 45 min under H_2 atmosphere (1 atm). The reaction mixture was filtered through a short pad of Celite and MeOH was evaporated under reduced pressure. The aqueous phase was extracted with CH_2Cl_2 (6×4 mL). The combined organic phases were dried on Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by chromatography on silica gel (eluent: CH_2Cl_2/MeOH 7:1) to give **7** in 71% yield (262 mg) as a colorless oil. Compound **7**: R_f: 0.24; $[\alpha]_D^{24}$+77.5 (*c* 0.390, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ=8.11–8.01 (m, 4H, H_{Ph}), 7.59–7.51 (m, 2H, H_{Ph}), 7.47–7.39 (m, 4H, H_{Ph}), 5.44–5.36 (m, 2H, 1-H+2-H), 3.47–3.39 (m, 1H, 7-H), 3.19 (d, *J*=11.3 Hz, 1H, 3-H_a), 2.95 (dd, *J*=11.3, 6.6 Hz, 1H, 3-H_b), 2.83 (ddd, *J*=11.2, 4.7, 2.2 Hz, 1H, 5-H_a), 2.70 (ddd, *J*=11.0, 8.4, 2.7 Hz, 1H, 8a-H), 2.57–2.49 (m, 1H, 5-H_b), 2.07–1.75 (m, 3H, 6-H_a+8-H), 2.70 (dm, *J*=13.9 Hz, 1H, 6-H_b) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ=166.4 (s, C=O), 165.9 (s, C=O), 133.2 (d, CH_{Ph}), 133.1 (d, CH_{Ph}), 129.9 (d, 2C, CH_{Ph}), 129.8 (d, 2C, CH_{Ph}+s, C_{Ph}), 129.6 (s, C_{Ph}), 128.4 (d, 2C, CH_{Ph}), 128.3 (d, 2C, CH_{Ph}), 82.2 (d, C-1), 77.6 (d, C-2), 61.0 (d, C-8a), 59.7 (t, C-3), 46.9 (t, C-5), 43.7 (d, C-7), 36.0 (t, C-8), 31.8(t, C-6) ppm; IR ($CDCl_3$): ν=3393, 3063, 2927, 2853, 1717, 1603, 1451, 1280, 1114 cm^{-1}; MS (EI): *m*/*z* (%)=362 (M^+−18, 1), 258 (6), 137 (75), 120 (77), 105 (100), 77 (60); HRMS: calcd for $C_{22}H_{25}N_2O_4$ $[M+H]^+$ 381.18088, found 381.18190.

4.6. (1*R*,2*R*,7*R*,8a*R*)-7-Amino-2-(benzoyloxy)octahydro-1-indolizinyl benzoate (ent-7)

Following the same procedure as for amine **7**, the enantiomer *ent*-**7** was obtained in 71% yield (32 mg) starting from *ent*-**6a** (48 mg, 0.118 mmol). *ent*-**7**: $[\alpha]_D^{23}$ −79.3 (*c* 0.265, $CHCl_3$); spectral properties are identical to those of **7**.

4.7. (1*S*,2*S*,7*S*,8a*S*)-7-Aminooctahydro-1,2-indolizinediol (7*S*-amino-lentiginosine, 8)

Ambersep 900 OH was added to a solution of **7** (40 mg, 0.10 mmol) in MeOH (2 mL) and the mixture was shaken at rt for 1 h on a flat shaker at 150 rpm. The reaction mixture was filtered through cotton wool and concentrated under reduced pressure. Chromatography on silica gel [eluent: MeOH (1% NH_4OH)] afforded **8** in 85% yield (15 mg). Compound **8** R_f: 0.19; $[\alpha]_D^{24}$ −2.4 (*c* 0.340, CH_3OH); ^{1}H NMR (CD_3OD, 400 MHz): δ=4.00 (ddd, *J*=7.2, 3.5, 1.5 Hz, 1H; 2-H), 3.62 (dd, *J*=8.5, 3.5 Hz, 1H; 1-H), 3.33–3.29 (obscured by solvent residual peak, 1H; 7-H), 2.85 (dd, *J*=10.8, 1.5 Hz, 1H; 3-H_a), 2.78 (ddd, *J*=11.5, 4.7, 2.7 Hz, 1H; 5-H_a), 2.66 (dd, *J*=10.8, 7.2 Hz, 1H; 3-H_b), 2.42–2.33 (m, 1H; 5-H_b), 2.21 (ddd, *J*=11.5, 8.5, 2.7 Hz, 1H; 8a-H), 1.97–1.82 (m, 2H; 6-H_a+8-H_a), 1.66–1.57 (m, 2H; 6-H_b+8-H_b) ppm; ^{13}C NMR (CD_3OD, 100 MHz): δ=85.1 (d; C-1), 77.8 (d; C-2), 64.1 (d; C-8a), 62.6 (t; C-3), 48.2 (t; C-5), 45.1 (d; C-7), 35.9 (t; C-8), 32.2 (t; C-6) ppm; MS (EI): *m*/*z* (%)=154 (M^+−H_2O, 27), 137 (49), 120 (33), 95 (33), 56 (100); HRMS: calcd for $C_8H_{17}N_2O_2$ $[M+H]^+$ 173.1294, found 173.1290.

4.8. (1*R*,2*R*,7*R*,8a*R*)-7-Aminooctahydro-1,2-indolizinediol (ent-8)

Following the same procedure as for **8**, the enantiomeric compound *ent*-**8** was obtained in 83% yield (19 mg) starting from *ent*-**7** (50 mg, 0.13 mmol). *ent*-**8**: $[\alpha]_D^{22}$ +3.7 (*c* 0.500, CH_3OH); spectral properties are identical to those of **8**.

4.9. (1*S*,2*S*,7*S*,8a*S*)-2-(Benzoyloxy)-7-({(2*S*)-2-{[(9*H*-fluoren-9-ylmethoxy)carbonyl]amino}-5-[(imino{[(2,2,4,5,6-pentamethyl-2,3-dihydro-1-benzofuran-7-yl)sulfonyl]amino}methyl)amino]pentanoyl}amino)octahydro-1-indolizinyl benzoate [FmocHN–Arg(Pbf)–HN-7]

Bromotripyrrolidinophosphonium hexafluorophosphate (PyBroP, 289 mg, 0.62 mmol) was added to a mixture of **7** (235.6 mg, 0.62 mmol), FmocHN–Arg(Pbf)–CO_2H (441.2 mg, 0.68 mmol), and *N*,*N*-diisopropylethylamine (0.106 mL, 0.62 mmol) in CH_2Cl_2 (0.62 mL, freshly distilled from P_2O_5) at 0 °C (ice/H_2O bath). The reaction mixture was stirred at rt under N_2 atmosphere overnight and then concentrated under reduced pressure. The residue was dissolved in EtOAc (5 mL), filtered, and concentrated under reduced pressure. Purification by chromatography on silica gel (eluent: EtOAc/TEA 98:2) afforded the title compound FmocHN–Arg(Pbf)–HN-**7** in 75% yield (471 mg) as a white solid. FmocHN–Arg(Pbf)–HN-**7**: R_f: 0.17; mp 127–130 °C; $[\alpha]_D^{22}$ +44.2 (*c* 0.445, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.08–8.03 (m, 2H, H_{Ar}), 7.97–7.91 (m, 2H, H_{Ar}), 7.76–7.67 (m, 2H, H_{Ar}), 7.57–7.50 (m, 3H, H_{Ar}), 7.45–7.17 (m, 10H, 9H_{Ar}+NH), 6.51–6.09 (m, 4H, NH), 5.32 (dd, *J*=8.1, 3.2 Hz, 1H, 1-H), 5.28 (dd, *J*=6.4, 2.9 Hz, 1H, 2-H), 4.29 (d, *J*=7.2 Hz, 2H, CH_2O), 4.31–418 (m, 2H, 1′-H+7-H), 4.11 (t, *J*=7.2 Hz, 1H, *CH*CH_2O), 3.28–3.16 (m, 2H, 4′-H), 3.05 (d, *J*=11.2 Hz, 1H, 3-Ha), 2.88 (s, 2H, *CH*$_2$$CMe_2$), 2.79–2.63 (m, 2H, 3-Hb+5-Ha), 2.58 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 2.53–2.40 (m, 1H, 8a-H), 2.36–2.24 (m, 1H, 5-Hb), 2.13–1.99 (m, 1H, 8-Ha), 2.05 (s, 3H, CH_3), 1.91–1.46 (m, 7H, 2′-H+3′-H+6-H+8-Hb), 1.40 (s, 6H, CH_3×2) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ=171.6 (s; C=O), 166.3 (s; C=O), 165.9 (s; C=O), 158.8 (s; C_{Ar}), 156.7 (s) and 156.4 (s) (C=N and C=O), 143.6 (s; 2C, C_{Ar}), 141.2 (s; 2C, C_{Ar}), 138.3 (s; C_{Ar}), 133.1 (d; 2C, CH_{Ar}), 132.6 (s; C_{Ar}), 132.2 (s; C_{Ar}), 129.8 (d; 2C, CH_{Ar}), 129.7 (d; 2C, CH_{Ar}), 129.6 (s; C_{Ar}), 129.4 (s; C_{Ar}), 128.3 (d; 4C, CH_{Ar}), 127.7 (d; 2C, CH_{Ar}), 127.0 (d; 2C, CH_{Ar}), 125.1 (d; 2C, CH_{Ar}), 124.7 (s; C_{Ar}), 120.0 (d; 2C, CH_{Ar}), 117.6 (s; C_{Ar}), 86.4 (s; CMe_2), 82.2 (d; C-1), 77.4 (d; C-2), 67.2 (t; CH_2O), 61.8 (d; C-8a), 59.2 (t; C-3), 54.6 (d; C-1′), 47.3 (t; C-5), 47.0 (d; *C*HCH_2O), 43.2 (d; C-7), 43.1 (t; *C*H_2CMe_2), 40.5 (t; C-4′), 33.0 (t; C-8), 29.6 (t; C-2′), 28.7 (t; C-6), 28.5 (q; 2C, CH_3×2), 25.6 (t; C-3′), 19.4 (q; CH_3), 18.0 (q; CH_3), 12.4 (q; CH_3) ppm; IR ($CDCl_3$): ν=3431, 3340, 3067, 2931, 2248, 1718, 1666, 1554, 1451, 1281 cm^{-1}; MS (ESI): *m*/*z*=1011 $[M+H]^+$, 1033 $[M+Na]^+$.

4.10. (2*S*)-*N*-[(1*S*,2*S*,7*S*,8a*S*)-1,2-Dihydroxyoctahydro-7-indolizinyl]-2-amino-5-{[amino(imino)methyl]amino}pentanamide {*N*^{1}-[(1*S*,2*S*,7*S*,8a*S*)-1,2-dihydroxyoctahydroindolizin-7-yl]-L-argininamide, 9}

A solution of FmocHN–Arg(Pbf)–HN-**7** (187.5 mg, 0.185 mmol) in a 95:5 mixture of TFA and H_2O (3 mL) was stirred at rt for 30 min. Et_2O (8 mL) was added to the reaction mixture, the precipitated white solid was filtered, and washed with Et_2O. MS-ESI analysis of the residue showed the complete removal of the Pbf protecting group {FmocHN–Arg–HN-**7**: MS (ESI): *m*/*z*=759 $[M+H]^+$, 781 $[M+Na]^+$}. The residue was dissolved in CH_2Cl_2 (2.2 mL), cooled in an ice bath, and treated with freshly distilled piperidine (0.43 mL, 0.004 mmol). The reaction mixture was stirred at 0 °C for 1 h and 30 min, concentrated under reduced pressure, and the residue was sequentially washed with petroleum ether (5 mL), Et_2O (5 mL), and EtOAc (5 mL). MS-ESI analysis of the residue showed the complete removal of the Fmoc protecting group {Arg–HN-**7**: MS (ESI): *m*/*z*=537 $[M+H]^+$}. The crude intermediate was dissolved in MeOH (15 mL) and treated with Ambersep 900 OH at rt for 2 h. Then, the mixture was filtered through a short pad of Celite, concentrated under reduced pressure, diluted with H_2O (0.5 mL), and acidified with 3 N HCl to pH=1 under magnetic stirring at 0 °C. The aqueous phase was sequentially washed with petroleum ether (0.5 mL×3), EtOAc (0.5 mL×3), and CH_2Cl_2(0.5 mL×3) and then concentrated

under reduced pressure. MS-ESI analysis of the residue showed the complete removal of the Bz protecting groups. The residue was dissolved in MeOH and the solution was basified with Ambersep 900 OH to pH=10, filtered through a short pad of Celite, and concentrated under reduced pressure. The product **9** was obtained in 49% overall yield over three steps (30 mg) as a white waxy solid and was characterized without further purification. ^{1}H NMR spectrum showed the presence of two isomers in ca. 1.7:1 ratio, which were tentatively assigned as the two epimers at Cα of Arg. Compound **9**: (isomer mixture) $[\alpha]_D^{24}$ −16.0 (*c* 0.760, CH_3OH/H_2O 1:1); ^{1}H NMR (400 MHz, D_2O) major isomer: δ=4.04–3.95 (m, 2H, 2-H+7-H), 3.57 (dd, *J*=8.4, 3.9 Hz, 1H, 1-H), 3.34–3.27 (m, 1H, 1′-H), 3.12–3.04 (m, 2H, 4′-H), 2.81–2.72 (m, 2H, 3-H_a+5-H_a), 2.56 (dd, *J*=11.2, 7.3 Hz, 1H, 3-H_b) 2.24–2.14 (m, 1H, 5-H_b), 2.05–1.91 (m, 2H, 8-H_a+8a-H), 1.80–1.36 (m, 7H, 2′-H+3′-H+6-H+8-H_b) ppm; ^{13}C NMR (D_2O, 100 MHz) major isomer: δ=162.6 (s, C=O), 156.7 (s, C=N), 82.5 (d; C-1), 75.4 (d; C-2), 63.2 (d; C-8a), 60.0 (t; C-3), 53.9 (d, C-1′), 47.4 (t; C-5), 43.1 (d; C-7), 40.7 (t, C-4′), 31.4 (t; C-8), 28.9 (t, C-2′), 27.7 (t; C-6), 24.3 (t, C-3′) ppm; MS (ESI): *m*/*z*=329 $[M+H]^+$; HRMS-ESI: calcd for $C_{14}H_{29}N_6O_3$ $[M+H]^+$ 329.22957; found 329.22899.

4.11. (1*S*,2*S*,7*S*,8a*S*)-2-(Benzoyloxy)-7-(4-hexyl-1*H*-1,2,3-triazol-1-yl)octahydro-1-indolizinyl benzoate (10-Bz_2)

Azide **6a** (61 mg, 0.150 mmol), 98% pure 1-octyne (0.020 mL, 0.135 mmol), H_2O (0.4 mL), *t*-BuOH (0.4 mL), THF (0.1 mL), copper powder (3.8 mg, 0.06 mmol), and copper sulfate (16.8 mg, 0.10 mmol) were mixed in a microwave reaction tube. The reaction mixture was heated at 80 °C (MW irradiation power of 100 W) under stirring for 30 min. Then, other three portions of the octyne (0.020 mL) were added and the mixture was heated at 80 °C for 30 min after each addition. The reaction mixture was filtered and concentrated under reduced pressure. The crude product was purified by chromatography on silica gel (EtOAc/petroleum ether 1:1) to afford the title compound **10**-Bz_2 in analytically pure form (76 mg, 98%) as a white solid. Compound **10**-Bz_2: R_f: 0.26; mp 119.5–120.7 °C; $[\alpha]_D^{22}$ +83.1 (*c* 0.76, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ=8.10–8.05 (m, 2H, H_{Ph}), 8.04–8.00 (m, 2H, H_{Ph}), 7.60–7.51 (m, 2H, H_{Ph}), 7.47–7.37 (m, 4H, H_{Ph}), 7.30 (s, 1H, $H_{triazole}$), 5.45 (dd, *J*=8.1, 3.1 Hz, 1H, 1-H), 5.41 (ddd, *J*=6.7, 3.1, 0.9 Hz, 1H, 2-H), 4.78–4.73 (m, 1H, 7-H), 3.24 (d, *J*=11.4 Hz, 1H, 3-Ha), 3.04 (ddd, *J*=11.3, 4.5, 2.5 Hz, 1H, 5-Ha), 2.96 (dd, *J*=11.4, 6.7 Hz, 1H, 3-Hb), 2.79–2.61 (m, 5H, 5-Hb, 8a-H, 8-Ha, 1′-H), 2.45–2.28 (m, 2H, 6-H), 2.24 (ddd, *J*=14.1, 11.3, 4.1 Hz, 1H, 8-Hb), 1.65 (pseudo quintet, *J*=7.7 Hz, 2H, 2′-H), 1.39–1.22 (m, 6H, 3′-H+4′-H+5′-H), 0.87 (t, *J*=7.0 Hz, 3H, 6′-H) ppm; ^{13}C NMR ($CDCl_3$, 50 MHz): δ=166.2 (s; C=O), 165.9 (s; C=O), 148.3 (s; $C_{triazole}$), 133.3 (d; CH_{Ph}), 133.1 (d; CH_{Ph}), 129.8 (d, 4C, CH_{Ph}), 129.6 (s; C_{Ph}), 129.3 (s; C_{Ph}), 128.3 (d; 2C, CH_{Ph}), 128.2 (d; 2C, CH_{Ph}), 119.9 (d; $CH_{triazole}$), 82.0 (d; C-1), 77.3 (d; C-2), 61.6 (d; C-8a), 59.3 (t; C-3), 53.5 (d; C-7), 47.7 (t; C-5), 33.5 (t; C-8), 31.5 (t; C-3′ or C-4′ or C-5′), 29.3 (t; C-2′), 29.1 (t; C-6), 28.9 (t; C-3′ or C-4′ or C-5′), 25.6 (t; C-1′), 22.6 (t; C-3′ or C-4′ or C-5′), 14.1 (q; C-6′) ppm; IR ($CDCl_3$): ν=3064, 2957, 2929, 2857, 1716, 1603, 1451, 1316, 1280, 1177, 1114, 1027 cm^{-1}; MS (EI): *m*/*z* 394 (M^+−BzOH, 2), 273 (5), 241 (2), 120 (100), 105 (38), 77 (15); $C_{30}H_{36}N_4O_4$ (516.63): calcd C 69.74, H 7.02, N 10.84; found C 69.46, H 6.92, N 10.64.

4.12. (1*S*,2*S*,7*S*,8a*S*)-7-(4-Hexyl-1*H*-1,2,3-triazol-1-yl) octahydro-1,2-indolizinediol (10)

Ambersep 900 OH was added to a solution of the diester **10**-Bz_2 (63 mg, 0.122 mmol) in MeOH (5 mL) and the mixture was stirred at rt for 4 h. Then, the reaction mixture was filtered and the solution was concentrated under reduced pressure. Chromatography on silica gel (eluent: MeOH) of the residue afforded **10** in 82% yield (31 mg) as a waxy solid. Compound **10**: R_f: 0.43; $[\alpha]_D^{24}$ +14.4 (*c* 0.965, CH_3OH); ^{1}H NMR (CD_3OD, 400 MHz): δ=7.82 (s, 1H, $H_{triazole}$), 4.83–4.78 (m, 1H, 7-H), 3.94 (ddd, *J*=7.0, 3.4, 1.4 Hz, 1H, 2-H), 3.64 (dd, *J*=8.2, 3.4 Hz, 1H, 1-H), 2.95–2.88 (m, 1H, 5-Ha), 3.88 (dd, *J*=10.6, 1.4 Hz, 1H, 3-Ha), 2.69 (t, *J*=7.7 Hz, 2H, 1′-H), 2.70–2.63 (m, 1H, 8-Ha), 2.56 (dd, *J*=10.6, 7.0 Hz, 1H, 3-Hb), 2.47–2.37 (m, 2H, 6-Ha+5-Hb), 2.26–2.16 (m, 1H, 6-Hb), 2.08 (ddd, *J*=11.5, 8.2, 2.3 Hz, 1H, 8a-H), 1.95 (ddd, *J*=13.9, 11.5, 4.1 Hz, 1H, 8-Hb), 1.67 (pseudo quintet, *J*=7.5 Hz, 2H, 2′-H), 1.41–1.27 (m, 6H, 3′-H+4′-H+5′-H), 0.90 (t, *J*=6.9 Hz, 3H, 6′-H) ppm; ^{13}C NMR (CD_3OD, 50 MHz): δ=149.0 (s; $C_{triazole}$), 122.5 (d; $CH_{triazole}$), 85.1 (d; C-1), 77.7 (d; C-2), 64.8 (d; C-8a), 62.5 (t; C-3), 55.3 (d; C-7), 49.4 (t; C-5), 34.1 (t; C-8), 32.7 (t; C-3′ or C-4′ or C-5′), 30.6 (t; C-2′), 30.0 (t; C-3′ or C-4′ or C-5′), 29.7 (t; C-6), 26.4 (t; C-1′), 23.6 (t; C-3′ or C-4′ or C-5′), 14.4 (q; C-6′) ppm; MS (EI): *m*/*z* (%)=290 (M^+−H_2O, 2), 206 (2), 191 (1), 154 (14), 152 (2), 137 (61), 120 (100); MS (ESI): *m*/*z*=309 $[M+H]^+$, 331 $[M+Na]^+$, 639 $[2M+Na]^+$. HRMS-ESI: calcd for $C_{16}H_{29}N_4O_2$ $[M+H]^+$ 309.22786; found 309.22850.

4.13. (1*S*,2*S*,7*S*,8a*S*)-2-(Benzoyloxy)-7-[4-(2-hydroxyethyl)-1*H*-1,2,3-triazol-1-yl]octahydro-1-indolizinyl benzoate (11-Bz_2)

Azide **6a** (70 mg, 0.172 mmol), 97% pure 3-butin-1-ol (0.012 mL, 0.155 mmol), H_2O (0.4 mL), *t*-BuOH (0.4 mL), THF (0.1 mL), copper powder (4.4 mg, 0.07 mmol), and copper sulfate (19.5 mg, 0.12 mmol) were mixed in a microwave reaction tube. The reaction mixture was heated at 80 °C (MW irradiation power of 100 W) under stirring for 30 min. Then, another portion of butanol (0.006 mL) was added and the mixture was heated at 80 °C for 15 min. The reaction mixture was diluted with CH_2Cl_2 (2 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by chromatography on silica gel (EtOAc/MeOH 14:1) to afford the title compound **11**-Bz_2 in 85% yield (70 mg) as a colorless oil. Compound **11**-Bz_2: R_f=0.26; $[\alpha]_D^{24}$ +119.0 (*c* 0.49, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ=8.10–8.06 (m, 2H; H_{Ph}), 8.04–8.01 (m, 2H; H_{Ph}), 7.59–7.54 (m, 2H; H_{Ph}), 7.46–7.41 (m, 5H; H_{Ph}+$H_{triazole}$), 5.46–5.39 (m, 2H; 1-H+2-H), 4.80–4.74 (m, 1H; 7-H), 3.94 (t, *J*=5.9 Hz, 2H; CH_2OH), 3.22 (d, *J*=11.2 Hz, 1H; 3-H_a), 3.01 (ddd, *J*=11.3, 4.7, 2.4 Hz, 1H; 5-H_a), 2.98–2.91 (m, 3H; 3-H_b+C$H_2$$CH_2$OH), 2.75–2.67 (m, 2H; 8-H_a+8a-H), 2.63 (dt, *J*=2.9, 11.8 Hz, 1H; 5-H_b), 2.43–2.27 (m, 2H; 6-H), 2.22 (ddd, *J*=14.5, 11.8, 4.4 Hz, 1H; 8-H_b) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): δ=166.3 (s; C=O), 166.0 (s; C=O), 145.4 (s, $C_{triazole}$), 133.3 (d; CH_{Ph}), 133.2 (d; CH_{Ph}), 129.8 (d, 4C; CH_{Ph}), 129.6 (s; C_{Ph}), 129.3 (s; C_{Ph}), 128.4 (d, 2C; CH_{Ph}), 128.3 (d, 2C; CH_{Ph}), 121.0 (d, $CH_{triazole}$), 82.1 (d; C-1), 77.4 (d; C-2), 61.6 (t; CH_2OH), 61.5 (d; C-8a), 59.3 (t; C-3), 53.8 (d; C-7), 47.6 (t; C-5), 33.5 (t; C-8), 29.2 (t; C-6), 28.6 (t, C$H_2$$CH_2$OH) ppm; IR ($CDCl_3$): ν=3627, 3456 br, 3064, 2957, 2836, 1718, 1603, 1451, 1280, 1113 cm^{-1}; HRMS-ESI: calcd for $C_{26}H_{29}N_5O_4$ $[M+H]^+$ 477.21325; found 477.21286; $C_{26}H_{28}N_4O_5$ (476.52): calcd C 65.53, H 5.92, N 11.76; found C 65.17, H 5.95, N 11.40.

4.14. (1*S*,2*S*,7*S*,8a*S*)-7-[4-(2-Hydroxyethyl)-1*H*-1,2,3-triazol-1-yl]octahydro-1,2-indolizinediol (11)

Ambersep 900 OH was added to a solution of the diester **11**-Bz_2 (70 mg, 0.15 mmol) in MeOH (5 mL) and the mixture was stirred at rt for 2 h, filtered, and the solution was concentrated under reduced pressure. Chromatography on silica gel [eluent: MeOH (2% NH_4OH)] of the residue afforded **11** in 52% yield (23.92 mg). Compound **11**: R_f: 0.40; $[\alpha]_D^{24}$ +13.5 (*c* 0.215, CH_3OH); ^{1}H NMR (CD_3OD, 400 MHz): δ=7.87 (s, 1H, $H_{triazole}$), 4.84–4.80 (m partially overlapped with water peak, 1H, 7-H), 3.94 (ddd, *J*=7.0, 3.4, 1.5 Hz, 1H, 2-H), 3.81 (t, *J*=6.7 Hz, 2H, CH_2OH), 3.65 (dd, *J*=8.2, 3.4 Hz,1H, 1-H), 2.96–2.87

(m, 2H, 5-Ha+3-Ha), 2.91 (t, J=6.7 Hz, 2H, CH_2CH$_2$OH), 2.65 (dq, J=13.9, 2.4 Hz, 1H, 8-Ha), 2.58 (dd, J=10.6, 7.0 Hz, 1H, 3-Hb), 2.49–2.40 (m, 2H, 6-Ha+5-Hb), 2.22 (ddt, J=15.0, 12.8, 4.7 Hz, 1H, 6-Hb), 2.12 (ddd, J=11.5, 8.2, 2.4 Hz, 1H, 8a-H), 1.97 (ddd, J=13.9, 11.5, 4.2 Hz, 1H, 8-Hb) ppm; ^{13}C NMR (CD_3OD, 50 MHz): δ=145.9 (s; $C_{triazole}$), 123.4 (d; $CH_{triazole}$), 85.0 (d; C-1), 77.7 (d; C-2), 64.8 (d; C-8a), 62.5 (t; C-3), 62.1 (t; CH_2OH), 55.3 (d; C-7), 49.4 (t; C-5), 34.0 (t; C-8), 30.0 (t; CH_2CH_2OH), 29.7 (t; C-6) ppm; MS (EI): m/z (%)=250 (M^+-H_2O, 8), 233 (2), 154 (19), 137 (60), 120 (100); HRMS-ESI: calcd for $C_{12}H_{21}N_4O_3$ $[M+H]^+$ 269.16082; found 269.16125.

4.15. (1*S*,2*S*,7*S*,8a*S*)-2-(Benzoyloxy)-7-[4-({[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-(1*H*-indol-3-yl)propanoyl]amino}methyl)-1*H*-1,2,3-triazol-1-yl]octahydro-1-indolizinyl benzoate (13-NBoc,OBz)

Azide **6a** (80.5 mg, 0.198 mmol), alkyne **12** (24 mg, 0.07 mmol, see Supplementary data), H_2O (0.5 mL), t-BuOH (0.5 mL), THF (0.3 mL), copper powder (5.2 mg, 0.08 mmol), and copper sulfate (22 mg, 0.14 mmol) were mixed in a microwave reaction tube. The reaction mixture was heated at 80 °C (MW irradiation power of 100 W) under stirring for 30 min. Then, other two portions of **12** (24 mg) were added and the mixture was heated at 80 °C for 30 min after each addition. The reaction mixture was filtered and concentrated under reduced pressure. The crude product was purified by chromatography on silica gel (eluent: CH_2Cl_2/MeOH 10:1) to afford the title compound **13**-NBoc,OBz in 61% yield (90 mg,) as a white solid. Compound **13**-NBoc,OBz: R_f: 0.26; mp 223–224 °C; $[\alpha]_D^{25}$ +74.7 (c 0.745, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.40 (br s, 1H, NH_{indole}), 8.10–8.06 (m, 2H, H_{Ph}), 8.03–7.99 (m, 2H, H_{Ph}), 7.61 (d, J=7.9 Hz, 1H, H_{indole}), 7.58–7.52 (m, 2H, H_{Ph}), 7.46–7.39 (m, 4H, H_{Ph}), 7.33 (d, J=8.1 Hz, 1H, H_{indole}), 7.19–7.14 (m, 1H, H_{indole}), 7.12–7.06 (m, 2H, H_{indole}+$H_{triazole}$), 6.88 (d, J=2.3 Hz, 1H, H_{indole}), 6.38 (br s, 1H, NH), 5.45–5.38 (m, 2H, 1-H+2-H), 5.17 (br s, 1H, NH), 4.62 (br s, 1H, 7-H), 4.42–4.30 (m, 3H, CH_2NH+CH$_2$C*H*NH), 3.26 (dd, J=14.5, 4.9 Hz, 1H, C*H*HCHNH), 3.20 (d, J=11.4 Hz, 1H, 3-Ha), 3.13 (dd, J=14.5, 7.4 Hz, 1H, C*H*HCHNH), 3.00–2.90 (m, 1H, 5-Ha), 2.93 (dd, J=11.4, 6.5 Hz, 1H, 3-Hb), 2.70–2.55 (m, 3H, 5-Hb+8-Ha+8a-H), 2.30–2.13 (m, 3H, 6-H+8-Hb), 1.38 (s, 9H, CH_3×3) ppm; ^{13}C NMR (50 MHz, $CDCl_3$): δ=171.7 (s; C=O), 166.3 (s; C=O), 166.2 (s; C=O), 155.3 (s; C=O), 144.2 (s; $C_{triazole}$), 136.2 (s; C_{indole}), 133.4 (d; CH_{Ph}), 133.2 (d; CH_{Ph}), 129.8 (d; 4C, CH_{Ph}), 129.7 (s; C_{Ph}), 129.3 (s; C_{Ph}), 128.5 (d; 2C, CH_{Ph}), 128.3 (d; 2C, CH_{Ph}), 127.5 (s; C_{indole}), 123.1 (d; CH_{indole}), 122.2 (d; CH_{indole}), 121.7 (d; $CH_{triazole}$), 119.7 (d; CH_{indole}), 118.9 (d; CH_{indole}), 111.2(d; CH_{indole}), 110.5 (s; C_{indole}), 82.3 (d; C-1), 80.2 (s; CMe_3) 77.4 (d; C-2), 61.5 (d; C-8a), 59.3 (t; C-3), 55.5 (d; CH_2CHNH), 53.8 (d; C-7), 47.5 (t; C-5), 35.0 (t; CH_2NH), 33.7 (t; C-8), 29.1 (t; C-6), 28.4 (t; CH_2CHNH), 28.2 (q; 3C, CH_3×3) ppm; IR ($CDCl_3$): ν=3464, 3411, 3054, 2980, 2933, 1716, 1672, 1491, 1316, 1281, 1114 cm^{-1}; MS (EI): m/z (%)=647 ($M^+-C_4H_8-CO_2$, 1), 441 (1), 359 (1), 316 (1), 134 (21), 98 (45), 57 (65), 43 (100); HRMS-ESI: calcd for $C_{41}H_{46}N_7O_7$ $[M+H]^+$ 748.34532; found 748.34467.

4.16. (2*S*)-*N*-({1-[(1*S*,2*S*,7*S*,8a*S*)-1,2-Dihydroxyoctahydro-7-indolizinyl]-1*H*-1,2,3-triazol-4-yl}methyl)-2-amino-3-(1*H*-indol-3-yl)propanamide (13)

A 1% solution of thiophenol in TFA (3 mL) was added to the protected intermediate **13**-NBoc,OBz (85 mg, 0.11 mmol) at 0 °C. The mixture was stirred at rt for 40 min and concentrated under reduced pressure. MS-ESI analysis of the residue showed the complete removal of the Boc protecting group {**13**-OBz: MS-ESI: m/z=562 $[M+Na]^+$}. Ambersep 900 OH was added to the solution of the residue in MeOH (10 mL) at 0 °C and the mixture was stirred overnight at rt and then filtered. The solution was concentrated under reduced pressure and the product was purified by chromatography on silica gel [eluent: CH_2Cl_2/MeOH (1% NH_4OH) 1:1] to give **13** in 73% yield (35.3 mg) as a white waxy solid. Compound **13**: R_f: 0.21, $[\alpha]_D^{24}$ +17.0 (c 0.995, CH_3OH); ^{1}H NMR (400 MHz, CD_3OD): δ=7.60 (d, J=7.9 Hz, 1H, H_{indole}), 7.35 (d, J=8.2 Hz, 1H, H_{indole}), 7.33 (s, 1H, $H_{triazole}$), 7.13–7.08 (m, 1H, H_{indole}), 7.06 (s, 1H, H_{indole}), 7.03–6.98 (m, 1H, H_{indole}), 4.72–4.67 (m, 1H, 7-H), 4.39 (A part of an AB system, J=15.3 Hz, 1H, C*H*HNH), 4.32 (B part of an AB system, J=15.3 Hz, 1H, C*H*HNH), 3.93 (ddd, J=7.0, 3.3, 1.5 Hz, 1H, 2-H), 3.66–3.61 (m, 2H, 1-H+C*H*NH$_2$), 3.15 (A part of an ABX system, J=14.1, 6.8 Hz, 1H, C*H*HCHNH$_2$), 3.04 (B part of an ABX system, J=14.1, 6.4 Hz, 1H, C*H*HCHNH$_2$), 2.92–2.84 (m, 2H, 3-Ha+5-Ha), 2.58–2.51 (m, 2H, 3-Hb+8-Ha), 2.44–2.27 (m, 2H, 5-Hb+6-Ha), 2.24–2.12 (m, 1H, 6-Hb), 2.06 (ddd, J=11.5, 8.2, 2.3 Hz, 1H, 8a-H), 1.93 (ddd, J=13.9, 11.5, 4.2 Hz, 1H, 8-Hb) ppm; ^{13}C NMR (50 MHz, CD_3OD): δ=177.2 (s; C=O), 145.8 (s; $C_{triazole}$), 138.0(s; C_{indole}), 128.9 (s; C_{indole}), 124.7 (d; C-2$_{indole}$), 123.4 (d; $CH_{triazole}$), 122.5 (d; CH_{indole}), 119.8 (d; CH_{indole}), 119.6 (d; CH_{indole}), 112.3 (d; CH_{indole}), 111.2 (s; C_{indole}), 85.0 (d; C-1), 77.7 (d; C-2), 64.7 (d; C-8a), 62.6 (t; C-3), 57.0 (d; $CHNH_2$), 55.4 (d; C-7), 49.3 (t; C-5), 35.7 (t; CH_2NH), 34.2 (t; C-8), 32.2 (t; CH_2CHNH_2), 29.7 (t; C-6) ppm; MS (ESI): m/z=440 $[M+H]^+$, 462 $[M+Na]^+$, 901 $[2M+Na]^+$; $C_{22}H_{29}N_7O_3$ (439.5): calcd C 60.12, H 6.65; N 22.31; found C 60.34, H 7.04, N 22.51.

4.17. (1*R*,2*R*,7*R*,8a*R*)-7-{4-[({5-[(3a*S*,4*S*,6a*R*)-2-Oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl]pentanoyl}amino)methyl]-1*H*-1,2,3-triazol-1-yl}-2-(benzoyloxy)octahydro-1-indolizinyl benzoate (15-Bz$_2$)

Azide *ent*-**6a** (141 mg, 0.347 mmol), alkyne **14** (35 mg, 0.123 mmol, see Supplementary data), H_2O (0.75 mL), t-BuOH (0.75 mL), THF (0.1 mL), copper powder (9 mg, 0.141), and copper sulfate (39 mg, 0.246 mmol) were mixed in a microwave reaction tube. The reaction mixture was heated at 80 °C (MW irradiation power of 100 W) under stirring for 30 min. Then, other three portions of **14** (35 mg) were added and the mixture was heated at 80 °C for 30 min after each addition. The reaction mixture was filtered and concentrated under reduced pressure. The crude product was purified by chromatography on silica gel (eluent: CH_2Cl_2/MeOH 10:1) to afford the title compound **15**-Bz$_2$ in 53% yield (126.3 mg) as a waxy solid. Compound **15**-Bz$_2$: R_f: 0.28; $[\alpha]_D^{25}$ −44.4 (c 0.565, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.09–8.04 (m, 2H, H_{Ph}), 8.04–7.99 (m, 2H, H_{Ph}), 7.71 (t, J=5.8 Hz, 1H, NH), 7.65 (s, 1H, $H_{triazole}$), 7.58–7.52 (m, 2H, H_{Ph}), 7.46–7.39 (m, 4H, H_{Ph}), 7.05 (br s, 1H, NH), 6.42 (br s, 1H, NH), 5.44–5.37 (m, 2H, 1-H+2-H), 4.79–4.73 (m, 1H, 7-H), 4.52 (dd, J=15.0, 6.0 Hz, 1H, C*H*HNH), 4.48 (dd, J=7.6, 4.9 Hz, 1H, 6a″-H), 4.37 (dd, J=15.0, 5.6 Hz, 1H, C*H*HNH), 4.26 (dd, J=7.6, 4.6 Hz, 1H, 3a″-H), 3.20 (d, J=11.2 Hz, 1H, 3-Ha), 3.05 (dt, J=4.6, 7.2 Hz, 1H, 4″-H), 3.02–2.94 (m, 1H, 5-Ha), 2.93 (dd, J=11.2, 6.5 Hz, 1H, 3-Hb), 2.82 (dd, J=12.8, 4.8 Hz, 1H, 6″-Ha), 2.76–2.62 (m, 2H, 8-Ha+8a-H), 2.70 (d, J=12.8 Hz, 1H, 6″-Hb), 2.52 (dt, J=3.1, 11.4 Hz, 1H, 5-Hb), 2.38–2.12 (m, 5H, 6-H, 8-Hb, 2′-H), 1.77–1.55 (m, 4H, 3′-H, 5′-H), 1.46–1.32 (m, 2H, 4′-H) ppm; ^{13}C NMR (50 MHz, $CDCl_3$): δ=173.3 (s; C=O), 166.2 (s; C=O), 165.9 (s; C=O), 164.5 (s; C=O), 144.8 (s; $C_{triazole}$), 133.3 (d; CH_{Ph}), 133.1 (d; CH_{Ph}), 129.8 (d; 4C, CH_{Ph}), 129.5 (s; C_{Ph}), 129.3 (s; C_{Ph}), 128.4 (d; 2C, CH_{Ph}), 128.3 (d; 2C, CH_{Ph}), 121.9 (d; $CH_{triazole}$), 82.0 (d; C-1), 77.3 (d; C-2), 61.6 (d; C-3a″), 61.5 (d; C-8a), 60.3 (d; C-6a″), 59.3 (t; C-3), 55.7 (d; C-4″), 53.9 (d; C-7), 47.5 (t; C-5), 40.6 (t; C-6″), 35.7 (t; C-2′), 34.3 (t; CH_2NH), 33.2 (t; C-8), 29.2 (t; C-6), 28.1 (t; C-4′), 27.9 (t; C-5′), 25.4 (t; C-3′) ppm; IR ($CDCl_3$): ν=3257 br, 2932, 1717, 1699, 1659, 1521, 1450, 1280, 1113 cm^{-1}; MS (ESI): m/z=710 $[M+Na]^+$; HRMS-ESI: calcd for $C_{35}H_{42}N_7O_6S$ $[M+H]^+$ 688.29118; found 688.29065.

4.18. 5-[(3a*S*,4*S*,6a*R*)-2-Oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl]-*N*-({1-[(1*R*,2*R*,7*R*,8a*R*)-1,2-dihydroxyoctahydro-7-indolizinyl]-1*H*-1,2,3-triazol-4-yl}methyl)pentanamide (15)

Ambersep 900 OH was added to a solution of **15**-Bz_2 (75.2 mg, 0.109 mmol) in MeOH (10 mL) and the mixture was stirred at rt for 3 h. The reaction mixture was filtered and the solution was concentrated under reduced pressure. Purification of the crude product by chromatography on silica gel (eluent: CH_2Cl_2/MeOH 5:1) afforded **15** in 98% yield (51.3 mg) as a waxy solid. Compound **15**: R_f: 0.29 (MeOH); $[\alpha]_D^{22}$ +29.9 (*c* 0.750, CH_3OH); ^1H NMR (CD_3OD, 400 MHz): δ=7.94 (s, 1H, $H_{triazole}$), 4.86–4.82 (obscured by water peak, 1H, 7-H), 4.49 (dd, *J*=7.9, 4.9 Hz, 1H, 6a″-H), 4.43 (s, 2H, CH_2NH), 4.30 (dd, *J*=7.9, 4.6 Hz, 1H, 3a″-H), 3.94 (ddd, *J*=7.1, 3.3, 1.4 Hz, 1H, 2-H), 3.64 (dd, *J*=8.2, 3.3 Hz, 1H, 1-H), 3.19 (ddd, *J*=8.7, 5.7, 4.6 Hz, 1H, 4″-H), 2.95–2.88 (m, 1H, 5-Ha), 2.93 (dd, *J*=12.7, 4.9 Hz, 1H, 6″-Ha), 2.88 (dd, *J*=10.6, 1.4 Hz, 1H, 3-Ha), 2.70 (d, *J*=12.7 Hz, 1H, 6″-Hb), 2.65 (dq, *J*=13.9, 2.3 Hz, 1H, 8-Ha), 2.56 (dd, *J*=10.6, 7.1 Hz, 1H, 3-Hb), 2.46–2.38 (m, 2H, 5-Hb+6-Ha), 2.27–2.19 (m, 1H, 6-Hb), 2.24 (t, *J*=7.3 Hz, 2H, 2′-H), 2.09 (ddd, *J*=11.5, 8.2, 2.3 Hz, 1H, 8a-H), 1.96 (ddd, *J*=13.9, 11.5, 4.2 Hz, 1H, 8-Hb), 1.78–1.53 (m, 4H, 3′-H+5′-H), 1.48–1.36 (m, 2H, 4′-H); ^{13}C NMR (CD_3OD, 50 MHz): δ=175.9 (s; C=O), 166.0 (s; C=O), 145.9 (s; $C_{triazole}$), 123.7 (d; $CH_{triazole}$), 85.0 (d; C-1), 77.6 (d; C-2), 64.8 (d; C-8a), 63.3 (d; C-3a″), 62.6 (t; C-3), 61.6 (d; C-6a″), 57.0 (d; C-4″), 55.5 (d; C-7), 49.4 (t; C-5), 41.1 (t; C-6″), 36.6 (t; C-2′), 35.6 (t; CH_2NH), 34.1 (t; C-8), 29.8 (t), 29.7 (t), and 29.5 (t) (C-6, C-4′, and C-5′), 26.8 (t; C-3′) ppm; MS (EI): *m*/*z* (%)=325 (1), 267 (2), 239 (2), 226 (1), 154 (2), 143 (3), 134 (6), 98 (13); HRMS-ESI: calcd for $C_{21}H_{34}N_7O_4S$ $[M+H]^+$ 480.23875; found 480.23767.

4.19. Molecular modeling

The ligand structures were built by using Maestro v8.5.[28] The protonation state for all the ligands was evaluated by means of Epik 1.6[29] (solvent water and pH=7.0±0.4) in order to better simulate the behavior at physiological conditions. All the molecules were subjected to conformational search and clusterization with Macromodel 9.6[30] in order to sample the most accessible conformations. All the docking calculations were performed by using Glide 5.0.[31] The crystal structure of glucoamylase-471 from *A. awamori* complexed with 1-deoxynojirimycin, available in the PDB (PDB ID: 1DOG),[32] was used in the docking calculations. This crystal structure corresponds to a proteolytic fragment of glucoamylase G2 from *A. awamori*, which has 95% sequence identity with *A. niger* amyloglucosidase and makes it possible the investigation of the interaction between ligands and the glucoamylase at atomic level. The structure 1DOG was prepared according to the recommended Protein Preparation module in Maestro 8.5 by using default input parameters (no scaling factors for the van der Waals radii of non-polar protein atoms, 0.8 scaling factor for non-polar ligand atoms). This procedure was used to remove water molecules (except for molecule Wat501, which is considered as part of the target structure), to assign missing hydrogen atoms, to optimize hydrogen-bonding interactions, and to reduce structural problems. The grids were prepared with the center of the site defined by the center of the complexed ligand. All the relevant conformations for the ligands were docked in the binding site by using the SP scoring function to score the ligand poses. After docking calculations, for each ligand, the poses with the lowest value of E_{model} (docking scoring functions) were chosen. Finally, a post-docking minimization was applied to the selected poses in order to remove ligand strains.

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Supplementary data

Preparations of **2a**, *ent*-**2a**, **4a**, *ent*-**4a**, **12**, and **14**; ^1H and ^{13}C NMR spectra. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2011.10.008. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

1. Pastuszak, I.; Molyneux, R. J.; James, L. F.; Elbein, A. D. *Biochemistry* **1990**, *29*, 1886–1891.
2. (a) Brandi, A.; Cicchi, S.; Cordero, F. M.; Frignoli, R.; Goti, A.; Picasso, S.; Vogel, P. *J. Org. Chem.* **1995**, *60*, 6806–6812; (b) Goti, A.; Cardona, F.; Brandi, A.; Picasso, S.; Vogel, P. *Tetrahedron: Asymmetry* **1996**, *7*, 1659–1674.
3. Borges de Melo, E.; da Silveira Gomes, A.; Carvalho, I. *Tetrahedron* **2006**, *62*, 10277–10302 and references therein.
4. Macchi, B.; Minutolo, A.; Grelli, S.; Cardona, F.; Cordero, F. M.; Mastino, A.; Brandi, A. *Glycobiology* **2010**, *20*, 500–506.
5. (+)-Lentiginosine and (−)-lentiginosine are also indicated as L-(+)-lentiginosine and D-(−)-lentiginosine, respectively, in consideration of the enantiomer of the tartaric acid used as chiral precursor in most of their syntheses (see Refs. 6–8).
6. Cardona, F.; Goti, A.; Brandi, A. *Eur. J. Org. Chem.* **2007**, 1551–1565 and references cited therein.
7. For some other recent syntheses of (+)-lentiginosine, see: (a) Chen, M.-J.; Tsai, Y.-M. *Tetrahedron Lett.* **2007**, *48*, 6271–6274; (b) Alam, M. A.; Vankar, Y. D. *Tetrahedron Lett.* **2008**, *49*, 5534–5536; (c) Lauzon, S.; Tremblay, F.; Gagnon, D.; Godbout, C.; Chabot, C.; Mercier-Shanks, C.; Perreault, S.; DeSève, H.; Spino, C. *J. Org. Chem.* **2008**, *73*, 6239–6250; (d) Shaikh, T. M.; Sudalai, A. *Tetrahedron: Asymmetry* **2009**, *20*, 2287–2292; (e) Cui, L.; Zhang, L.-M. *Sci. China Chem.* **2010**, *53*, 113–118; (f) Prasad, K. R.; Pawar, A. B. *Arkivoc* **2010**, *vi*, 39–46.
8. For some other recent syntheses of (−)-lentiginosine, see: (a) Ayad, T.; Genisson, Y.; Baltas, M. *Org. Biomol. Chem.* **2005**, *3*, 2626–2631; (b) Kim, I. S.; Zee, O. P.; Jung, Y. H. *Org. Lett.* **2006**, *8*, 4101–4104; (c) Angle, S. R.; Bensa, D.; Belanger, D. S. *J. Org. Chem.* **2007**, *72*, 5592–5597; (d) Azzouz, R.; Fruit, C.; Bischoff, L.; Marsais, F. *J. Org. Chem.* **2008**, *73*, 1154–1157; (e) Chandrasekhar, S.; Vijaykumar, B. V. D.; Pratap, T. V. *Tetrahedron: Asymmetry* **2008**, *19*, 746–750; (f) Liu, S.-W.; Hsu, H.-C.; Chang, C.-H.; Gavin Tsai, H.-H.; Hou, D.-R. *Eur. J. Org. Chem.* **2010**, 4771–4773.
9. For some lentiginosine analogs, see: (a) Muramatsu, T.; Yamashita, S.; Nakamura, Y.; Suzuki, M.; Mase, N.; Yoda, H.; Takabe, K. *Tetrahedron Lett.* **2007**, *48*, 8956–8959; (b) Kumar, K. S. A.; Chaudhari, V. D.; Dhavale, D. D. *Org. Biomol. Chem.* **2008**, *6*, 703–711; (c) Kumar, N. S.; Kuntz, D. A.; Wen, X.; Pinto, B. M.; Rose, D. R. *Proteins: Struct. Funct. Bioinf.* **2008**, *71*, 1484–1496; (d) Kim, I. S.; Li, Q. R.; Dong, G. R.; Kim, Y. C.; Hong, Y. J.; Lee, M.; Chi, K.-W.; Oh, J. S.; Jung, Y. H. *Eur. J. Org. Chem.* **2010**, 1569–1573; (e) Stecko, S.; Jurczak, M.; Staszewska-Krajewska, O.; Solecka, J.; Chmielewski, M. *Tetrahedron* **2009**, *65*, 7056–7063.
10. Tufariello, J. J.; Tegeler, J. J. *Tetrahedron Lett.* **1976**, *17*, 4037–4040.
11. Cordero, F. M.; Bonollo, S.; Machetti, F.; Brandi, A. *Eur. J. Org. Chem.* **2006**, 3235–3241.
12. (a) Otomasu, H.; Takatsu, N.; Honda, T.; Kametani, T. *Tetrahedron* **1982**, *38*, 2627–2633; (b) Brandi, A.; Cordero, F.; Querci, C. *J. Org. Chem.* **1989**, *54*, 1748–1750.
13. Cordero, F. M.; Faggi, C.; De Sarlo, F.; Brandi, A. *Eur. J. Org. Chem.* **2000**, 3595–3600.
14. (a) Socha, D.; Jurczak, M.; Chmielewski, M. *Carbohydr. Res.* **2001**, *336*, 315–318; (b) Cordero, F. M.; Pisaneschi, F.; Gensini, M.; Goti, A.; Brandi, A. *Eur. J. Org. Chem.* **2002**, 1941–1951; (c) Brandi, A.; Cardona, F.; Cicchi, S.; Cordero, F. M.; Goti, A. *Chem.—Eur. J.* **2009**, *15*, 7808–7821.
15. (a) Cicchi, S.; Goti, A.; Brandi, A. *J. Org. Chem.* **1995**, *60*, 4743–4748; (b) Cicchi, S.; Nunes, J., Jr.; Goti, A.; Brandi, A. *Eur. J. Org. Chem.* **1998**, 419–421; (c) McCaig, A. E.; Meldrum, K. P.; Wightman, R. H. *Tetrahedron* **1998**, *54*, 9429–9446; (d) Cicchi, S.; Ponzuoli, P.; Goti, A.; Brandi, A. *Tetrahedron Lett.* **2000**, *41*, 1583–1587.
16. Goti, A.; Cardona, F.; Brandi, A. *Synlett* **1996**, 761–763.
17. Lillelund, V. H.; Jensen, H. H.; Liang, X.; Bols, M. *Chem. Rev.* **2002**, *102*, 515–553.
18. For a preliminary communication, see: Cordero, F. M.; Bonanno, P.; Neudeck, S.; Vurchio, C.; Brandi, A. *Adv. Synth. Catal.* **2009**, *351*, 1155–1161. In this communication, the absolute configuration at C-2 of the minor adduct **3** (R=Bz) and at C-7 of the corresponding indolizidinol **4** (R=Bz) was incorrectly assigned (see Supplementary data).
19. Cardona, F.; Goti, A.; Picasso, S.; Vogel, P.; Brandi, A. *J. Carbohydr. Chem.* **2000**, *19*, 585–601.
20. (a) Furneaux, R. H.; Graeme, J. G.; Mason, J. M.; Tyler, P. C. *Tetrahedron* **1994**, *50*, 2131–2160; (b) Furneaux, R. H.; Gainsford, G. J.; Mason, J. M.; Tyler, P. C. *Tetrahedron* **1995**, *51*, 12611–12630; (c) Furneaux, R. H.; Gainsford, G. J.; Mason, J. M.; Tyler, P. C.; Hartley, O.; Winchester, B. G. *Tetrahedron* **1997**, *53*, 245–268.

21. (a) Tornøe, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057–3064; (b) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596–2599; (c) Kolb, H. C.; Sharpless, K. B. *Drug Discovery Today* **2003**, *8*, 1128–1137; (d) Tron, G. C.; Pirali, T.; Billington, R. A.; Canonico, P. L.; Sorba, G.; Genazzani, A. A. *Med. Res. Rev.* **2008**, *28*, 278–308; (e) Hein, C. D.; Liu, X.-M.; Wang, D. *Pharm. Res.* **2008**, *25*, 2216–2230.
22. For some other recent reviews, see: (a) Bock, V. D.; Hiemstra, H.; van Maarseveen, J. H. *Eur. J. Org. Chem.* **2006**, 51–68; (b) Lutz, J. F. *Angew. Chem., Int. Ed.* **2007**, *46*, 1018–1025; (c) Gil, M. V.; Arevalo, M. J.; Lopez, O. *Synthesis* **2007**, 1589–1620; (d) Moses, J. E.; Moorhouse, A. D. *Chem. Soc. Rev.* **2007**, *36*, 1249–1262; (e) Wu, P.; Fokin, V. V. *Aldrichimica Acta* **2007**, *40*, 7–17; (f) Angell, Y. L.; Burgess, K. *Chem. Soc. Rev.* **2007**, *36*, 1674–1689; (g) Meldal, M.; Tornøe, C. W. *Chem. Rev.* **2008**, *108*, 2952–3015; (h) van Dijk, M.; Rijkers, D. T. S.; Liskamp, R. M. J.; van Nostrum, C. F.; Hennink, W. E. *Bioconjugate Chem.* **2009**, *20*, 2001–2016; (i) Best, M. D. *Biochemistry* **2009**, *48*, 6571–6584; (j) Lu, K.; Duan, Q.-P.; Ma, L.; Zhao, D.-X. *Bioconjugate Chem.* **2010**, *21*, 187–202; (k) Kappe, C. O.; Van der Eycken, E. *Chem. Soc. Rev.* **2010**, *39*, 1280–1290; (l) Hein, J. E.; Fokin, V. V. *Chem. Soc. Rev.* **2010**, *39*, 1302–1315; (m) Holub, J. M.; Kirshenbaum, K. *Chem. Soc. Rev.* **2010**, *39*, 1325–1337; (n) Aragão-Leoneti, V.; Campo, V. L.; Gomes, A. S.; Field, R. A.; Carvalho, I. *Tetrahedron* **2010**, *66*, 9475–9492.
23. (a) Horne, W. S.; Yadav, M. K.; Stout, C. D.; Ghadiri, M. R. *J. Am. Chem. Soc.* **2004**, *126*, 15366–15367; (b) Angelo, N. G.; Arora, P. S. *J. Am. Chem. Soc.* **2005**, *127*, 17134–17135; (c) van Maarseveen, J. H.; Horne, W. S.; Ghadiri, M. R. *Org. Lett.* **2005**, *7*, 4503–4506; (d) Brik, A.; Alexandratos, J.; Lin, Y. C.; Elder, J. H.; Olson, A. J.; Wlodawer, A.; Goodsell, D. S.; Wong, C. H. *ChemBioChem* **2005**, *6*, 1167–1169; (e) Appendino, G.; Bacchiega, S.; Minassi, A.; Cascio, M. G.; De Petrocellis, L.; Di Marzo, V. *Angew. Chem., Int. Ed.* **2007**, *46*, 9312–9315.
24. For a synthesis of *ent*-**12**, see: Zemolka, S.; Nolte, B.; Linz, K.; Saunders, D. J.; Schroder, W.; Englberger, W.; Theil, F.; Schick, H.; Kaufmann, J.; Gebauer, J.; Sonnenschein, H. WO 2009118174, 2009. *Chem. Abstr.* **2009**, 1204244.
25. (a) Meier, J. L.; Mercer, A. C.; Rivera, H., Jr.; Burkart, M. D. *J. Am. Chem. Soc.* **2006**, *128*, 12174–12184; (b) Zhao, X. Z.; Semenova, E. A.; Liao, C.; Nicklaus, M.; Pommier, Y.; Burke, T. R., Jr. *Bioorg. Med. Chem.* **2006**, *14*, 7816–7825; (c) Lin, P.-C.; Ueng, S.-H.; Yu, S.-C.; Jan, M.-D.; Adak, A. K.; Yu, C.-C.; Lin, C.-C. *Org. Lett.* **2007**, *9*, 2131–2134; (d) Aoki, S.; Matsuo, N.; Hanaya, K.; Yamada, Y.; Kageyama, Y. *Bioorg. Med. Chem.* **2009**, *17*, 3405–3413.
26. α-L-Fucosidase from bovine kidney, α-galactosidase from coffee beans, β-galactosidase from *Escherichia coli* and from *Aspergillus oryzae*, α-glucosidase from yeast and from rice, amyloglucosidase from *Aspergillus niger*, β-glucosidase from 15-almonds, α-mannosidase from Jack beans, β-mannosidase from snail, β-xylosidase from *A. niger*, β-*N*-acetylglucosaminidase from Jack beans. The experiments were performed essentially as previously described: (a) Saul, R.; Chambers, J. P.; Molyneux, R. J.; Elbein, A. D. *Arch. Biochem. Biophys.* **1983**, *221*, 593–597 and Ref. 2a.
27. Cardona, F.; Goti, A.; Brandi, A.; Scarselli, M.; Niccolai, N.; Mangani, S. J. *Mol. Model.* **1997**, *3*, 249–260.
28. *Maestro, v. 8.5*; Schrödinger, L.L.C.: New York, 2008; http://www.schrodinger.com.
29. *Epik, v. 1.6*; Schrödinger, L.L.C.: New York, 2008; http://www.schrodinger.com.
30. *Macromodel, v. 9.6*; Schrödinger, L.L.C.: New York, 2008; http://www.schrodinger.com.
31. *Glide, v. 5.0*; Schrödinger, L.L.C.: New York, 2008; http://www.schrodinger.com.
32. Harris, E. M. S.; Aleshin, A. E.; Firsov, L. M.; Honzatko, R. B. *Biochemistry* **1993**, *32*, 1618–1626.