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Stereocontrolled synthesis and biological activity of two diastereoisomers of the potent HIV-1 protease inhibitor saquinavir

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Abstract—A general enantioselective synthesis of new *syn*-hydroxyethylamine isosteres has been developed. The approach, based on the controlled opening of functionalized optically active 2,3-epoxy amines, can be conveniently used for the preparation of new peptidomimetics with various residues. Finally the total synthesis of two diastereoisomer analogues of HIV-Protease inhibitor Saquinavir has been achieved and their biological activity evaluated.

1. Introduction

The human immunodeficiency virus (HIV), isolated for the first time in 1983 at the Institute Pasteur in France and a year later in USA, is the causative agent of the acquired immunodeficiency syndrome (AIDS). The pharmaceutical industry and a great number of researchers all over the world are in continuous search for new and more potent drugs to suppress or slow down the lethal infection.[1] Essential to replication, maturation, and infectivity of HIV are three viral enzymes: reverse transcriptase (RT), integrase (IN) and protease (PR). Inhibition of each of them can be used against HIV infection through an antiretroviral therapy.[2] Actually, synergy of the two RT and PR inhibitors represents the most efficacious therapy for the treatment of this disease, called Highly Active Antiretroviral Therapy (HAART).

In spite of the good results so far obtained, the widespread diffusion of the disease and the development of numerous mutant resistant viruses to the used therapy justify the research towards new and selective inhibitors of HIV-PR, which could overcome the virus resistant mutagenesis.[3,4]

As well as for other aspartyl proteases, the guideline adopted to design drugs to block the HIV-PR has focused on synthetic peptidic analogues which mimic the transition state (TS) of the catalytic reaction.[5,6]

Possible TS mimetics for inclusion in new potential inhibitors may be all non-hydrolyzable central cores $\psi(P_1 - P_1')$ depicted in Figure 1, where it is always recognizable vicinal amino alcoholic sequence.

The first HIV-PR inhibitor to be marketed in 1995 was Saquinavir,[7] (Ro 31-8959), which showed excellent activity against HIV-PR with IC_{50} value of 0.4 nM, and synergy with other HIV agents, such as RT inhibitors. However the insurgence of resistant viruses to Saquinavir as well as to other drug,[8] led to the need of developing new HIV-PR inhibitors, both peptido and non-peptidomimetics.[9]

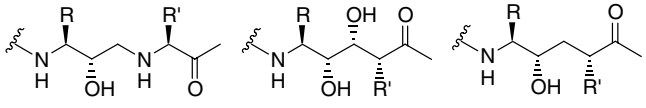

Figure 1. TS mimetics for HIV-PR.

Keywords: Peptidomimetic; HIV-1 proteasi inhibitor; Saquinavir; Syn hydroxyethylamine isoster; Epoxy amine.
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X-ray structures of HIV-PR co-crystallized with Saquinavir demonstrated that the hydroxy group of the drug is located between the catalytic aspartic acids and that the *R* configuration at the hydroxyl-bearing carbon atom is preferred.[10] This hydroxyl group is in an *anti*relationship (see Fig. 2) with the vicinal amino group. The [3*S*-(4a*S*, 8a*S*)]-*N*-(*tert*-butyl)-decahydro-3-isoquinolincarboxy amide (DIQ) occupies almost the entire subsite S_1', while the *tert*-butyl amide group tightly fits into the S_2' subsite. Finally, the *S*,*S*,*S* stereochemistry of the DIQ ring is optimal for good inhibitory activity.[11]

The potency of Saquinavir prompted further design and synthesis of its analogues as candidates to HIV-PR inhibition. Numerous derivatives have been synthesized making structural changes, like incorporating different side chains or modifying the stereochemistry at the CHOH of the core,[12] and several efforts have been made to synthesize HEA in a stereocontrolled manner with the correct relative and absolute stereochemistry.

Considering our experience in the stereo- and regiocontrolled preparation of such backbones via epoxides and aziridines chemistry, [13] we focused our attention on peptidomimetics synthesis with a hydroxyethylamine isoster *core* (*syn*-HEA) (see Fig. 3). In this paper, we report a highly stereocontrolled synthesis of two new Saquinavir analogues, with modification in the relative and absolute configuration at the amino alcohol *core*. Preliminary biological activity as HIV-PR potential inhibitors has been also evaluated (see Fig. 4).

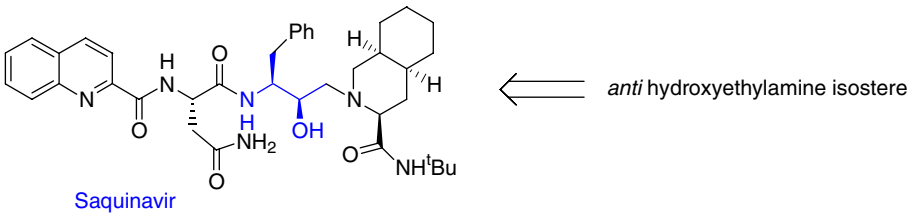


Figure 2. Saquinavir: A peptididomimetic with an *anti*HEA core.

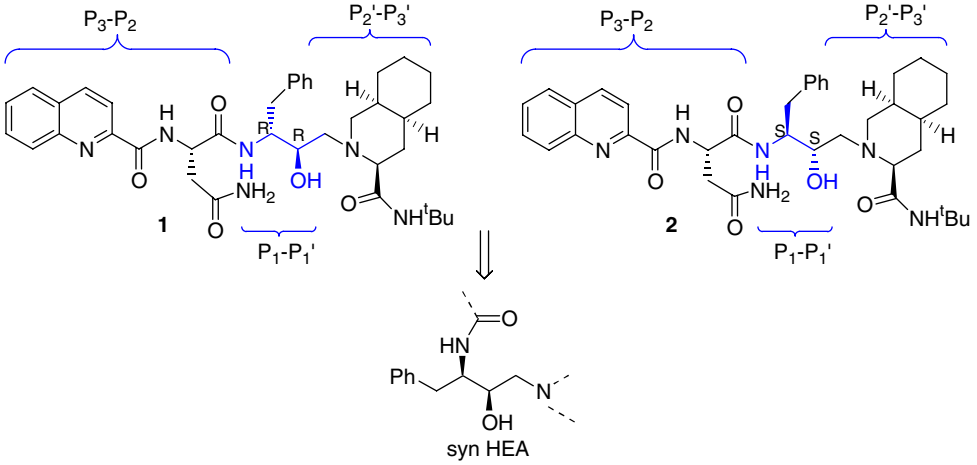


Figure 3. Epimers of Saquinavir and *syn*-HEA.

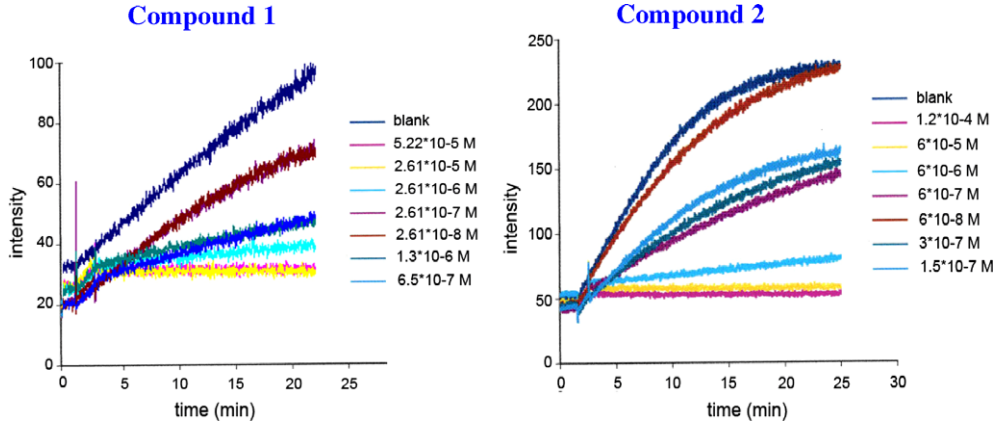


Figure 4. Variation of fluorescence at different concentration of substrate.

2. Chemistry

Our general synthetic approach, which will be described initially for known compound **1**[14] (see retrosynthetic Scheme 1), relays on the stereo- and regioselective opening of the properly prepared epoxy amine **6**, having the (*S*,*S*,*S*)-decahydroisoquinoline-3-carboxylamide residue (DIQ) already introduced. The key epoxy amine **6** in turn can be easily obtained from the known (*2R*,*3R*)-epoxy alcohols **4**.

2.1. Synthesis of epoxy amine 6

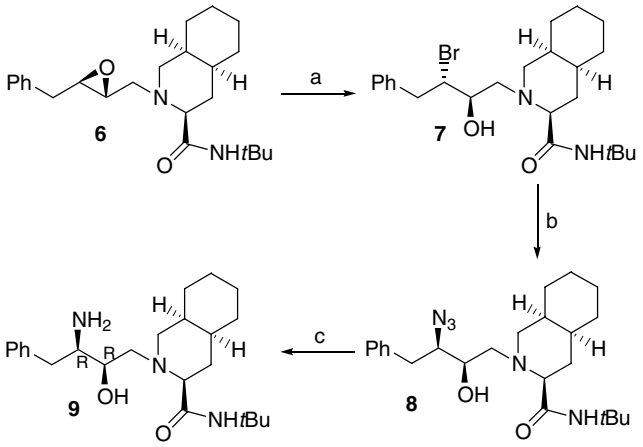
The enantiomerically pure (2*R*,3*R*)-4-phenyl-2, 3-epoxybutanol **4** was promptly prepared by known procedure, starting from alcohol **3**.[15] Then the DIQ residue was readily introduced via mesylate derivative **5**, affording the epoxyamine **6** with an overall yield of 77.4% from **3** (Scheme 2).

2.2. Synthesis of the syn HEA isoster 9

The *syn*-relationship of the amino alcohol fragment was introduced following an already reported stereocontrolled opening of epoxide by halide and subsequent substitution of the halogen by azide.[16]

The regio- and stereoselective opening of the epoxy amine **6** was performed utilizing the LiBr/Amberlyst 15 reagent;[17,18] the bromo derivative **7** was thus obtained with good chemical yield and at satisfactory regioisomeric ratio (92:8), as determined by spin–spin decoupling experiments on the acetylate derivative.

The following displacement of bromine by azide ion was not a trivial one, because of unwanted elimination reactions which lowered the overall yield. After several attempts in various reaction conditions (solvent, temperature, azide reagent), by employing NaN_3 in DMSO/THF = 1/1 as solvent, in the presence of 18-crown-6 at room temperature,[19] the desired azido alcohol **8** was obtained as single diastereoisomer in satisfactory chemical yield (72%).



Scheme 3. Reagents and conditions: (a) LiBr/Amberlyst 15, CH_3CN, −20 °C, 81%; (b) NaN_3, 18-crown-6, DMSO/THF 1/1, rt, 72%; (c) H_2, Pd/C, MeOH, rt, 86%.

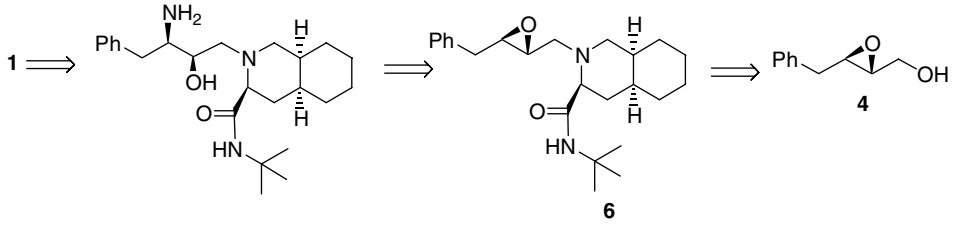
The subsequent catalytic hydrogenation afforded the *syn*(*R*,*R*)-hydroxyethylene isostere **9**, as epimer of the *anti*(*S*,*R*) Saquinavir core (Scheme 3).

2.3. Synthesis of saquinavir analogue 1

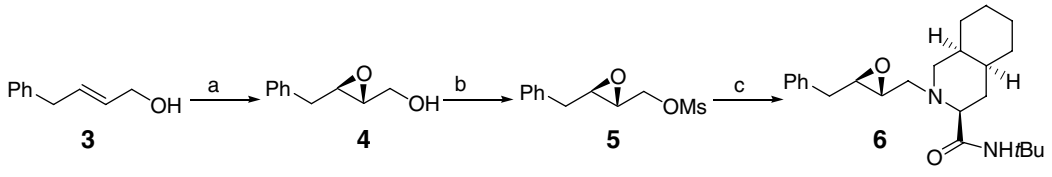
Finally, the coupling between the known dipeptide Quin-Asn-OH of the P_3–P_2 portion of Saquinavir and the HEA isostere **9** afforded the target Saquinavir analogue **1** characterized by the novel *syn*-HEA isostere (Scheme 4).

2.4. Synthesis of saquinavir analogue 2

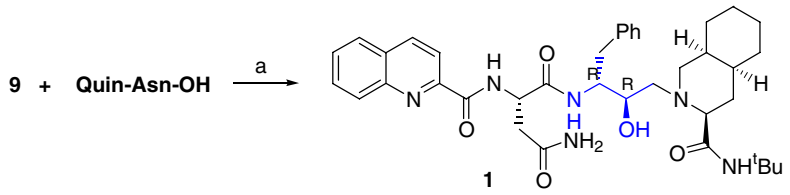
The same synthetic sequence (starting from *ent*-**4**, Scheme 5) was then applied to the preparation of the new Saquinavir analogue **2**, with the *S* configuration



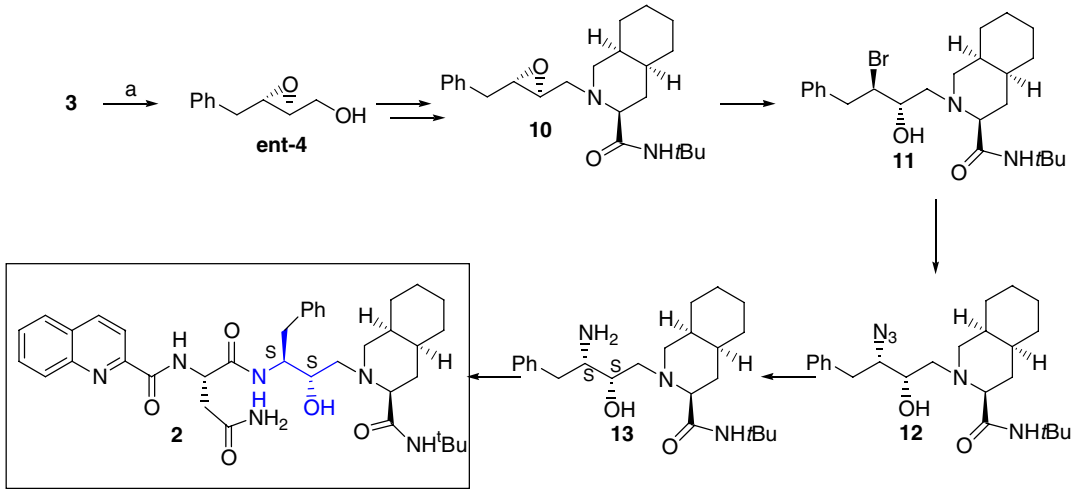
Scheme 1. Retrosynthetic pathway.



Scheme 2. Reagents and conditions: (a) TBHP, (−)DET, Ti(*i*-OPr)$_4$, CH_2Cl_2, −20 °C, 88%; (b) MsCl, Et_3N, DMAP, CH_2Cl_2, rt, 90%; (c) DIQ, DMSO, 45 °C, rt, 86%.



Scheme 4. Reagents and condition: (a) EDC, HOBt, Et_3N, THF, rt, 72%.



Scheme 5. Reagents and conditions: (a) TBHP, (+)DET, Ti(*i*-OPr)$_4$, CH_2Cl_2, −20 °C, 88%.

at the CH(OH) in the *syn* HEA core. The reaction yields of the single steps were found to be identical to the previous preparation of compound **1** (see Schemes 2–4), and the spectroscopical data are reported in Section 4.

The synthesis of the target compounds **1** and **2** was thus performed in total stereo- and regiocontrolled fashion, with satisfactory overall yield and total control of stereoselectivity with respect to previous preparation (≈ 20%, nine steps from the commercially available phenylacetaldehyde).[20]

2.4.1. Biological activity. For both **1** and **2** the extent of inhibition of the activity of the HIV-1 aspartic protease was determined as previously reported for other series of mono- and dihydroxy pseudopeptide inhibitors[21] through 'intramolecular fluorescence resonance energy transfer'.[22] IC_{50} values were evaluated on the inhibition of a recombinant wild-type HIV-PR (purchased from Bioczech, Prague, Czech Republic) by measuring the initial velocities of hydrolysis of the fluorogenic hexapeptidic substrate Abz-Thr-Ile-Nle-Phe(*p*-NO_2)-Gln-Arg-NH_2 in absence and with variable concentrations of the two epimers of Saquinavir.

Table 1. IC_{50} values

Compound	IC_{50} (nM)
Saquinavir	0.4
1	483
2	290

Both **1** and **2** show a clear activity against HIV-PR, with IC_{50} values in the nanomolar range (483 and 290 nM, respectively), but they are less active than Saquinavir which shows a lower IC_{50} (Table 1).

3. Conclusion

In conclusion we developed a general strategy for the synthesis of peptidomimetics with *syn*-hydroxyethylene isosteres, where the key step was the regio- and stereocontrolled opening of an optically active 2,3-epoxy amine. A distinct feature of this approach is the possibility of introducing different side chains as P_2–P_n and P_2'–P_n' residues. The final Saquinavir analogues, obtained in overall good yield and total stereocontrol, were also evaluated as HIV-PR inhibitors, showing a modest activity compared to the original Saquinavir.

4. Experimental

^1H and ^{13}C NMR spectra were recorded at 200 and 50.3 MHz, respectively. Reactions were monitored by TLC using Merck silica gel 60 F-254 plates with UV indicator; spots were also visualized with phosphomolybdic acid (10% solution in EtOH). Flash column chromatography on silica gel was normally used for purification of the reaction mixtures. ESI–MS analyses were performed using a commercial API 365 triple-quadrupole mass spectrometer from Perkin Elmer Sciex Instruments, equipped with an ESI source and a syringe

pump. The experiments were conducted in the positive ion mode. Optical rotations were recorded at the Sodium D line with a polarimeter at room temperature.

Compounds **3**,[23] **4**, and *ent*-**4**[24] are known. *Quin-Asn-OH* ((Quinolin-2-ylcarbonyl)-L-asparagine) has been prepared in according to Ref. 20c.

4.1. (2′*R*,3′*R*)-Methanesulfonic acid 2-[2′,3′-epoxy-4′-phenyl-butyl]-ester 5

To a solution of **4** (1 mmol) in 1.6 mL of CH_2Cl_2, 2 mmol of Et_3N (0.27 mL) and a catalytic amount of DMAP were added. After being stirred at 0 °C, 0.08 mL (1 mmol) of methane sulfonyl chloride was added dropwise. After 2 h (TLC monitoring), the reaction was quenched with ice cold water, the organic layers were extracted with CH_2Cl_2, washed with ice cold HCl 1N, saturated aqueous $NaHCO_3$, and then brine. Organic extracts were dried over Na_2SO_4 and then evaporated to dryness in vacuo to give **5**, which was used without any purification (90%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.36–7.14 (m, 5H, *Ph*); 4.44 (dd, 1H, J = 2.9, 12.4 Hz, CH_AOMs); 4.08 (dd, 1H, J = 6.2, 12.4 Hz, CH_BOMs); 3.18–3.04 (m, 2H, C*H*OC*H*); 3.01 (s, 3H, CH_3); 2.89 (d, 2H, J = 5.1 Hz, CH_2Ph). ^{13}C NMR (50 MHz, $CDCl_3$): δ 136.7, 128.8, 128.4, 126.8, 69.5, 56.4, 54.5, 37.5, 37.3. HR-MS (ES Q-TOF) Calcd for $C_{11}H_{15}O_4S$ $[M + H]^+$: 243.0691. Found: 243.0697.

4.2. (−)-(3*S*, 4*aS*, 8*aS*, 2′*R*, 3′*R*)-2-[2′,3′-Epoxy-4′-phenyl-butyl]-*N*-*tert*-butyldecahydro-isoquinoline-3-carboxamide 6

To a solution of **5** (1 mmol) in 1 mL di DMSO, 2 mmol of DIQ was added. The reaction mixture was stirred at 50 °C overnight. Then the reaction was diluted with EtOAc (8 mL), washed several times with water and brine, dried over Na_2SO_4 and concentrated in vacuo. The crude mixture was purified by flash chromatography (petroleum ether/AcOEt 6:4) affording **6** as a light brown oil in 86% yield; $[\alpha]_D^{20}$ −103.9 (c 1.7, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.38–7.13 (m, 5H, *Ph*); 6.50 (bs, 1H, N*H*); 3.11–2.6 (m, 5H, CH_2Ph + C*H* N^{DIQ} + CH_AOCH_B); 2.58–2.48 (m, 2H, CH_2N^{DIQ}); 2.18–1.99 (m, 2H, CH_2N); 1.78–1.02 (m, 12H, DIQ); 1.33 (s, 9H, *tert*-But). ^{13}C NMR (50 MHz, $CDCl_3$): δ 173.6, 137.1, 128.7, 128.5, 126.5, 69.4, 59.6, 56.8, 56.2, 55.7, 50.3, 38.2, 35.8, 33.0, 30.8, 30.6, 28.5, 26.3, 25.6, 20.3. HR-MS (ES Q-TOF) Calcd for $C_{24}H_{37}N_2O_2$ $[M + H]^+$: 385.2855. Found: 385.2861.

4.3. (−)-(3*S*, 4*aS*, 8*aS*, 2′*R*, 3′*S*)-2-[3′-Bromo-2′-hydroxy-4′-phenyl-butyl]-*N*-*tert*-butyldecahydro-isoquinoline-3-carboxamide 7

To a stirred solution of **6** (1 mmol) in CH_3CN (10 mL) at −20 °C, LiBr (348 mg, 4 mmol) and Amberlyst15 (217 mg, 1 mmol) were added. The mixture was stirred for 6 h (TLC monitoring) and filtered. The filtrate solution, diluted with EtOAc, was washed with saturated aqueous $NaHCO_3$ and brine; the organic layer, dried over Na_2SO_4 was concentrated in vacuo. The crude mixture was purified by column chromatography (petroleum ether/AcOEt 8:2) affording **7** as a soft white powder, yield 81%. $[\alpha]_D^{20}$ −64.0 (c 2.3, $CHCl_3$), ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.37–7.15 (m, 5H, *Ph*); 6.10 (bs, 1H, N*H*); 4.21 (ddd, 1H, J = 2.9, 6.6, 9.5 Hz C*H*Br); 3.85 (bq, 1H, J = 6.6 Hz, C*H*OH); 3.48 (dd, 1H, J = 2.9, 14.6 Hz, CH_APh); 3.00 (dd, 1H, J = 9.5, 14.6 Hz, CH_BPh); 2.86 (dd, 1H, J = 4.4, 13.9 Hz, CH_AN); 2.73 (dd, 1H, J = 2.9, 10.2 Hz, C*H*N^{DIQ}); 2.50 (dd, 1H, J = 6.6, 13.9 Hz, CH_BN); 2.4 (dd, 1H, J = 2.9, 11.7 Hz, C$H_A N^{DIQ}$); 2.08–1.22 (m, 14H, DIQ + O*H*); 1.32 (s, 9H, *tert*-But).^{13}C NMR (50 MHz, $CDCl_3$): δ 173.2, 138.2, 129.2, 128.05, 126.4, 72.2, 69.9, 60.6, 60.3, 59.03, 50.9, 39.9, 35.9, 33.1, 30.7, 30.4, 28.5, 25.9, 25.6, 20.5. HR-MS (ES Q-TOF) Calcd for $C_{24}H_{38}BrN_2O_2$ $[M + H]^+$: 465.2116. Found: 465.2121.

4.4. (−)-(3*S*, 4*aS*, 8*aS*, 2′*R*, 3′*R*)-2-[3′-Azido-2′-hydroxy-4′-phenyl-butyl]-*N*-*tert*-butyldecahydro-isoquinoline-3-carboxamide 8

To a solution of **7** (1 mmol) in the mixed solvent DMSO/THF = 1/1 (4 mL), NaN_3 260 mg (4 mmol) and 18-crown-6 (264 mg, 1 mmol) were added, and the mixture was stirred at room temperature for 48 h. Then the reaction was diluted with EtOAc (8 mL), washed several times with water and brine. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The crude mixture was purified by flash chromatography (petroleum ether/EtOAc, 7:3) to afford **8** yield 72%. $[\alpha]_D^{20}$ 121.3 (c 1.1, $CHCl_3$), ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.40–7.16 (m, 5H, *Ph*); 6.15 (s, 1H, N*H*); 3.71 (bt, 1H, J = 7.3 Hz, C*H*OH); 3.47 (bt, 1H, J = 7.3 Hz, C*H*N_3); 3.11 (bd, 2H, J = 7.3, CH_2Ph); 2.62 (dd, 1H, J = 8.0; 12.4 Hz, CH_AN); 2.58–2.39 (m, 2H, C*H*N^{DIQ} + C$H_A N^{DIQ}$); 2.19 (dd, 1H, J = 6.6, 12.4 Hz, CH_BN); 2.11–1.99 (m, 1H, C$H_B N^{DIQ}$); 1.81–1.08 (m, 13H, DIQ + OH); 1.32 (s, 9H, *tert*-But). ^{13}C NMR (50 MHz, $CDCl_3$): δ 173, 4; 137, 3; 129, 1; 128, 6; 126, 7; 70, 6; 68, 7; 62, 9; 58, 8; 58, 6; 50, 7; 36, 1; 35, 5; 33, 1; 30, 8; 30, 5; 28, 5; 26, 0; 25, 7; 20, 4. HR-MS (ES Q-TOF) Calcd for $C_{24}H_{38}N_5O_2$ $[M + H]^+$: 428.3025. Found: 428.3031.

4.5. (−)-(3*S*, 4*aS*, 8*aS*, 2′*R*, 3′*R*)-2-[3′-Ammino-2′-hydroxy-4′-phenyl-butyl]-*N*-*tert*-butyldecahydro-isoquinoline-3-carboxamide 9

A solution of **8** (1 mmol) in MeOH (2 mL) was hydrogenated at atmospheric pressure over 10% Pd/C (51 mg) for 3 h at room temperature; then the solution was filtered on a Celite pad and concentrated in vacuo. Flash chromatography (chloroform/methanol, 9:1) furnished **9** yield 86%. $[\alpha]_D^{20}$ −50.9 (c 1.0, $CHCl_3$), ^{1}H NMR (200 MHz, CD_3OD): δ 7.9 (s, 1H, NH); 7.42–7.20 (m, 5H, *Ph*); 3.82 (dt, 1H, J = 8.0, 1.5, C*H*OH); 3.65 (dt, 1H, J = 8.8, 1.5 Hz, C*H*NH_2); 3.11 (dd, 1H, J = 13.2, 8.8 Hz, CH_APh); 2.96 (dd, 1H, J = 13.2, 5.9 Hz, CH_BPh); 2.74 (dd, 1H, J = 10.2, 2.9 Hz, CH_AN); 2.37–2.22 (m, 3H, CH_BN + C*H*N^{DIQ} + C$H_A N^{DIQ}$); 2.19-2.07 (m, 2H, C$H_B N^{DIQ}$ + N*H*); 2.0–1.09 (m, 13H, DIQ + N*H*); 1.34 (s, 9H, *tert*-But). ^{13}C NMR (50 MHz, $CDCl_3$): δ 174, 6; 136, 7; 129, 4; 129, 0; 127,

1; 69, 3; 66, 2; 58, 2; 56, 8; 55, 8; 52, 1; 33, 5; 35, 6; 35, 3; 29, 6; 29, 2; 28, 7; 26, 2; 25, 6; 20, 1. HR-MS (ES Q-TOF) Calcd for $C_{24}H_{40}N_3O_2$ $[M + H]^+$: 402.3120. Found: 402.3126.

4.6. (−)-(3*S*, 4*aS*, 8*aS*, 2′*R*, 3′*R*)-2-[2′-Hydroxy-4′-phenyl-3′[*N*-(2-quinolylcarbonyl)-L-asparaginyl]amino]-butyl]-*N*-*tert*-butyldecahydro-isoquinoline-3-carboxamide 1

To a solution of 1 mmol Quin-Asn-OH, EDC (1.1 mmol, 210 mg) and HOBt (1.1 mmol, 148 mg) in THF (15 mL), Et_3N was (2 mmol, 0.27 mL) was added at 0 °C. After 30 min **9** (1 mmol) was added and the mixture was allowed to stir at room temperature overnight. Then the mixture was filtered and evaporated under reduced pressure. The residue was diluted with EtOAc and was washed with 10 mL of 10% aqueous citric acid, 10 mL of saturated $NaHCO_3$and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The crude mixture was purified by flash chromatography ($CHCl_3$/MeOH, 95:5) to afford **1** as a brown oil. $[\alpha]_D^{20}$ −10.1 (c 0.7, $CHCl_3$), ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.42–8.08 (m, 3H, Quin); 7.89 (d, 1H, J = 8.0 Hz, Quin); 7.85–7.71 (m, 1H, Quin); 7.66 (d, 1H, J = 8.0 Hz, Quin); 7.45–7.02 (m, 5H, *Ph*); 6.15 (bs, 2H, NH_2^{Asn}; 5.87 (bs, 1H, $NH^{Quin\text{-}Asn}$); 5.56 (bs, 1H, NH^{DIQ}); 5.17 (m, 2H, CH^{Asn} + N*H*); 4.13–3.86 (m, 1H, C*H*OH); 3.77–3.70 (m, 1H, C*H*CH_2Ph); 3.11–2.55 (m, 5H, CH_2Ph + C*H*N^{DIQ} + CH_2^{Asn}); 2.49–2.11 (m, 4H, CH_2N + CH_2N^{DIQ}); 1.92–0.80 (m, 13H, DIQ + O*H*); 1.14 (s, 9H, *tert*-But). ^{13}C NMR (50 MHz, $CDCl_3$): δ174.8; 173.1; 170.5; 164.8; 152.2; 149.0; 137.4; 137.3; 130.5; 130.1; 129.4; 128.5; 128.1; 127.6; 126.3; 125.7; 118.7; 69.4; 67.2; 58.9; 60.6; 53.1; 50.7; 50.0; 36.9; 35.5; 32.9; 31.8; 30.3; 29.6; 28.4; 25.8; 25.6; 20.8. HR-MS (ES Q-TOF) Calcd for $C_{38}H_{51}N_6O_5$ $[M + H]^+$: 671.3921. Found: 671.3896. Calcd for $C_{38}H_{50}N_6O_5Na$ $[M + Na]^+$: 693.3740. Found: 693.3736.

4.7. (−)-(3*S*, 4*aS*, 8*aS*, 2′*S*, 3′*S*)-2-[2′, 3′-Epoxy-4′-phenyl-butyl]-*N*-*tert*-butyldecahydro-isoquinoline-3-carboxamide 10

$[\alpha]_D^{20}$ −91.5 (c 0.86, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.41–7.22 (m, 5H, *Ph*); 6.60 (bs, 1H, N*H*); 3.03–2.74 (m, 5H, CH_2Ph + C*H*N^{DIQ} + CH_AOCH_B); 2.66–2.50 (m, 2H, CH_2N^{DIQ}); 2.34–2.16 (m, 2H, CH_2N); 1.89–1.18 (m, 12H, DIQ); 1.39 (s, 9H, *tert*-But). ^{13}C NMR (50 MHz, $CDCl_3$): δ 173.2, 136.7, 128. 8, 128.5, 126.6, 69.2, 59.61, 58.6, 58.4, 58.1, 55.6, 50.3, 38.20, 35.92, 33.01, 30.71, 28.52, 26.25, 25.49, 20.21. HR-MS (ES Q-TOF) Calcd for $C_{24}H_{37}N_2O_2$ $[M + H]^+$: 385.2855. Found: 385.2859.

4.8. (−)-(3*S*, 4*aS*, 8*aS*, 2′*S*, 3′*R*)-2-[3′-Bromo-2′-hydroxy-4′-phenyl-butyl]-*N*-*tert*-butyldecahydro-isoquinoline-3-carboxamide 11

$[\alpha]_D^{20}$ −99.07 (c 0.94, $CHCl_3$), ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.45–7.15 (m, 5H, *Ph*); 6.11 (bs, 1H, N*H*); 4.18 (ddd, 1H, J = 8.8, 5.1, 4.4 Hz C*H*Br); 3.91–3.72 (m, 2H, C*H*OH + O*H*); 3.35 (dd, 1H, J = 4.4, 14.6 Hz, CH_APh); 3.04 (dd, 1H, J = 8.8, 14.6 Hz, CH_BPh); 2.89–2.52 (m, 3H, CH_2N^{DIQ} + C*H*N^{DIQ}); 2.47 (dd, 1H, J = 2.9, 12.4 Hz, CH_AN); 2.27 (dd, 1H, J = 2.9, 11.7 Hz, CH_BN); 1.99–1.12 (m, 12H, DIQ); 1.37 (s, 9H, *t*-Bu).^{13}C NMR (50 MHz, $CDCl_3$): δ 173.4, 138.0, 129.1, 128.1, 126.5, 69.8, 69.1, 59.9, 58.9, 58.3, 50.9, 40.4, 35.7, 33.2, 30.9, 30.7, 28.6, 26.2, 25.3, 20.1. HR-MS (ES Q-TOF) Calcd for $C_{24}H_{38}BrN_2O_2$ $[M + H]^+$: 465.2116. Found: 465.2119.

4.9. (−)-(3*S*, 4*aS*, 8*aS*, 2′*S*, 3′*S*)-2-[3′-Azido-2′-hydroxy-4′-phenyl-butyl]-*N*-*tert*-butyldecahydro-isoquinoline-3-carboxamide 12

$[\alpha]_D^{20}$ −93.6 (c 1.1, $CHCl_3$). ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.47–7.16 (m, 5H, *Ph*); 6.01 (s, 1H, N*H*); 3.81 (ddd, 1H, J = 2.9, 5.9, 10.2 Hz, C*H*OH); 3.41 (bs, 1H, O*H*); 3.26–3.13 (m, 1H, C*H*N_3); 3.12–2.98 (m, 2H, CH_2Ph); 2.93–2.62 (m, 3H, CH_2N^{DIQ} + C*H*N^{DIQ}); 2.26–2.11 (dd, 1H, J = 2.9, 11.7 Hz, CH_AN); 2.09–1.91 (m, 1H, CH_BN); 1.94–1.18 (m, 12H, DIQ); 1.32 (s, 9H, *tert*-Bu). ^{13}C NMR (50 MHz, $CDCl_3$): δ 173, 0; 137, 6; 129, 1; 128, 7; 126, 8; 70, 2; 68, 3; 65.1; 58, 5; 58, 1; 50, 9; 36, 7; 35, 8; 33, 3; 30, 8; 30, 5; 28, 6; 26, 3; 25, 6; 20, 3. HR-MS (ES Q-TOF) Calcd for $C_{24}H_{38}N_5O_2$ $[M + H]^+$: 428.3025. Found: 428.3029.

4.10. (−)-(3*S*, 4*aS*, 8*aS*, 2′*R*, 3′*R*)-2-[3′-Ammino-2′-hydroxy-4′-phenyl-butyl]-*N*-*tert*-butyldecahydro-isoquinoline-3-carboxamide 13

$[\alpha]_D^{20}$ −61.3 (c 0.63, $CHCl_3$), ^{1}H NMR (200 MHz, $CDCl_3$): δ: 7.34–7.19 (m, 5H, *Ph*); 6.06–5.50 (bs, 2H, O*H* + NH^{DIQ}); 3.97–3.82 (m, 1H, C*H*OH); 3.69–3.51 (m, 1H, C*H*NH_2); 3.49–3.21 (m, 3H, CH_2Ph + N*H*); 3.21–2.85 (m, 3H, NH + CH_AN^{DIQ} + C*H*N^{DIQ}); 2.84–2.66 (m, 1H, CH_AN); 2.66–2.49 (m, 1H, CH_BN^{DIQ}); 2.21–1.12 (m, 13H, DIQ + CH_BN); 1.34 (s, 9H, *t*-Bu). ^{13}C NMR (50 MHz, $CDCl_3$): δ 172.4; 137.5; 129, 3; 128, 6; 126, 6; 69, 0; 67, 0; 60.7; 59, 6; 56, 6; 51, 1; 39.1; 35, 6; 32.5; 30.6; 30.0; 28, 5; 26, 2; 25, 2; 20, 1. HR-MS (ES Q-TOF) Calcd for $C_{24}H_{40}N_3O_2$ $[M + H]^+$: 402.3120. Found: 402.3128.

4.11. (−)-(3*S*, 4*aS*, 8*aS*, 2′*S*, 3′*S*)-2-[2′-Hydroxy-4′-phenyl-3′[*N*-(2-quinolylcarbonyl)-L-asparaginyl]ammino]butyl]-*N*-*tert*-butyldecahydro-isoquinoline-3-carboxamide 2

$[\alpha]_D^{20}$ −55.9 (c 1.0, MeOH). ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.37–8.05 (m, 3H); 7.93–7.53 (m, 3H); 7.34–6.89 (m, 5H); 6.72 (bs, 2H); 6.44 (bs, 1H); 5.85 (bs, 1H, NH^{DIQ}); 5.05–4.86 (m, 1H, CH^{Asn}); 4.07–3.88 (m, 1H, C*H*OH); 3.87–3.70 (bd, 1H, J = 9.5 Hz, C*H*NCH_2Ph); 2.99–2.43 (m, 7H); 2.20–1.94 (m, 2H); 1.87–1.04 (m, 12H); 1.36 (s, 9H). ^{13}C NMR (50 MHz, $CDCl_3$): δ 174, 0; 172, 9; 171, 0; 164, 7; 149, 8; 148, 8; 137, 9; 137, 3; 130, 1; 130, 0; 129, 3; 129, 1; 128, 2; 128, 0; 127, 5; 126, 1; 118, 6; 69, 5; 68, 1; 59, 8; 58, 1; 54, 2; 50, 9; 50, 1; 37, 8; 37, 0; 35, 7; 33, 1; 30, 9; 30, 7; 28, 6; 26, 1; 25, 4; 20, 2. HR-MS (ES Q-TOF) Calcd for $C_{38}H_{51}N_6O_5$ $[M + H^+]$: 671.3921. Found: 671.3925.

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