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Stereoselective synthesis of E-64 and related cysteine proteases inhibitors from 2,3-epoxyamides

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Abstract—The stereoselective synthesis of cathepsin inhibitors from indoline type epoxyamides is described. The use of this type of epoxyamides permitted the preparation of E-64 and CA-074 related compounds depending on the order in which the key steps, the oxidation of indoline amides to indole amides and oxidative acetal cleavage were undertaken. By taking advantage of the facile substitution of the indole of the corresponding indole epoxyamides, with various nucleophiles, we were able to prepare different epoxysuccinic acids derivatives as potential cathepsin inhibitors.

1. Introduction

The biological activity displayed by certain natural and synthetic compounds against cysteine-proteases, a large family of enzymes involved in the degradation of proteins, is due to the alkylation of the Cys-25 residue, an essential amino acid for their proteolytic action. Among the various electrophilic groups present in cysteine protease inhibitors, the oxirane ring represents one of the most common and efficient groups. Thus, compounds such as the natural E-64 (**1**)[1] and synthetic related products **2**–**6**,[2] as well as the designed compounds CA-074 (**8**), CA-030 (**9**) and CA-028 (**10**)[3] lead the list of these representative bioactive compounds (Figure 1). Particularly striking are the IC_{50} values for E-64c (**2**), CA-074 (**8**) and CA-030 (**9**) against cathepsin B of 3.36, 2.24 and 2.28 nM, respectively, or the value of E-64c (**2**) against cathepsin L of 0.09 nM. The recognition of E-64 (**1**) as an inhibitor of the cathepsins, proteases involved in degenerative diseases, including muscular distrophy, rheumatoid arthritis and metastasis, made it one of the most attractive targets in the pharmaceutical industry. The discovery prompted intense research activity directed towards the design of novel cathepsin inhibitors, with more potency and selectivity, and consequently potential therapeutical applications.[4a–n] In fact, E-64 (**1**) represents one of the compounds with the most complete and extensive structure–activity relationship studies, including rational design based on computational studies in pharmaceutical research. Associated with these studies, several syntheses of E-64, as well as analogues thereof, have been described in the literature.[5] In contrast to the nonselective inhibition displayed by E-64 against cathepsins, CA-074 (**8**) and related compounds display specific inhibition against cathepsin B.[6]

The stereoselective synthesis of epoxyamides via reaction of chiral aldehydes with stabilized sulfur ylides has occupied a central position in our research during the last years,[7] finding interesting synthetic applications in the field of carbohydrates.[8] In particular, the high stereofacial selectivity showed by 2,3-*O*-isopropylidene-D-glyceraldehyde **12** in its reactions with sulfur ylides presented an intriguing synthetic value with numerous applications in the synthesis of natural products.[9] In fact, we envisioned the corresponding epoxyamide **11** as a useful and common precursor for E-64 (**1**), CA-074 (**8**) and related compounds. The reason for using the indoline derived amide is justified by its facile oxidation to the corresponding indole,[10] which is amenable to the attack of nucleophiles as a mild method of amide hydrolysis.[11] This is in contrast to conventional amides, which require harsh conditions for hydrolysis, which could affect the integrity of the oxirane ring contained

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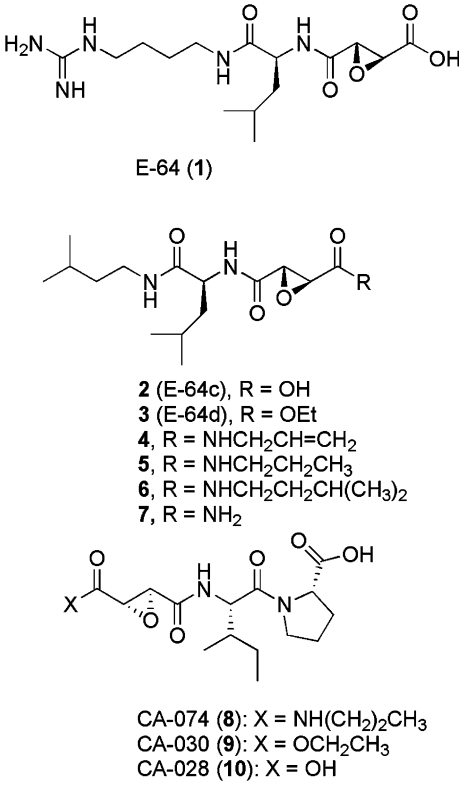
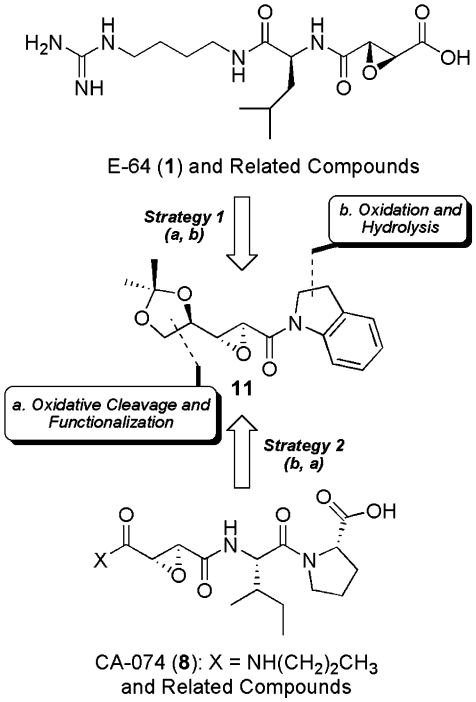


Figure 1. Structures of E-64 (**1**), CA-074 (**8**) and related cathepsin inhibitors.



Scheme 1. General approach to the synthesis of E-64 (**1**), CA-074 (**8**) and related compounds from a common intermediate, epoxyamide (**11**).

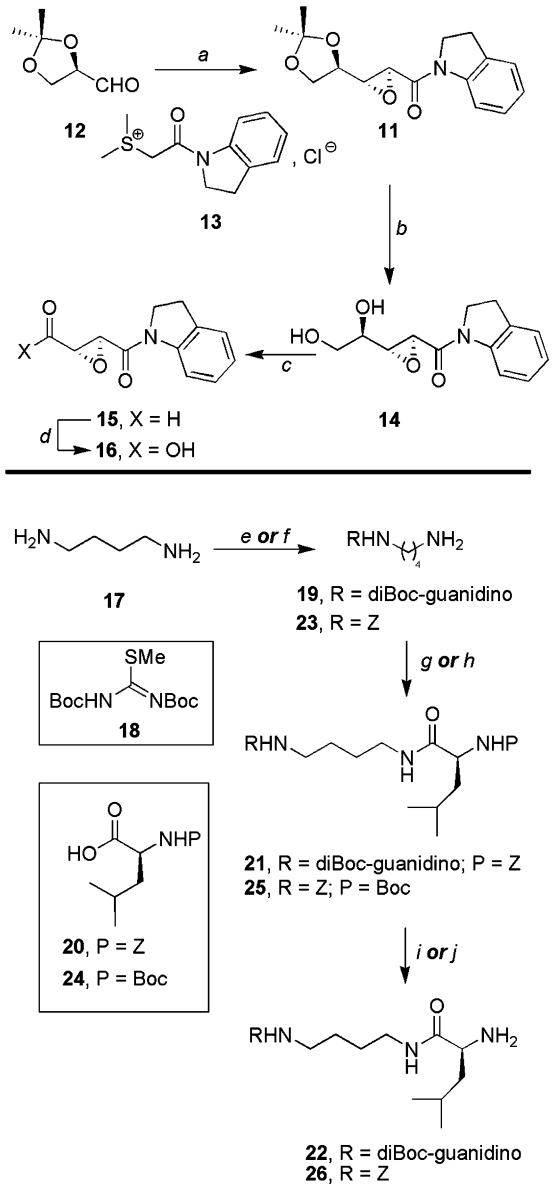
in these molecules. In addition, compound **11** represents an excellent candidate for the synthesis of a large variety of analogues of the targeted epoxy-peptides. The possibility of changing the order of the key steps, acetal hydrolysis and oxidative cleavage of the resulting diol (strategy 1), or indoline oxidation and hydrolysis (strategy 2), would permit the synthesis of these bioactive compound E-64 (*a* and *b*) or CA-074 (*b* and *a*) as well as related compounds (Scheme 1).

The present paper reports the results obtained in pursuit of the synthesis of these interesting compounds utilizing sulfur ylide chemistry for the stereoselective synthesis of the epoxides contained in these cysteine-protease inhibitors. Comparatively, our synthetic strategy offers some advantages with respect to other syntheses described in the literature, many of which are based on the use of (+)- or (−)-diethyl tartrates as starting materials. Thus, our methodology permits a selective construction of both types of cysteine proteases inhibitors from a common precursor, compound **11**, taking the advantage of the asymmetric architecture contained in this compound. In addition, **11** represents an excellent building block for the preparation of a wide variety of potential epoxysuccinyl-type cysteine proteases inhibitors in terms of structural diversity.

2. Results and discussion

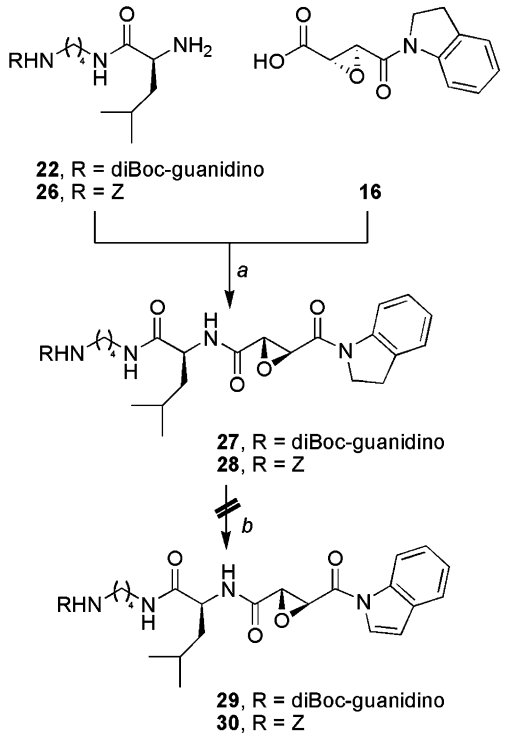
According to the general synthetic scheme, epoxyamide **11** represented the key compound for the preparation of all the targeted epoxy peptides depicted in Figure 1. Thus, **11** was prepared by reaction of aldehyde **12** with the sulfur ylide generated in situ from the sulfonium salt **13** in a 62% yield and high stereoselectivity of 90:10. Following the synthetic route towards E-64 (**1**), epoxyamide **11** was treated with Amberlyst-15, to obtain diol **14**. This compound was then subjected to the action of $NaIO_4/SiO_2$[12] and the resulting aldehyde **15** was treated with Oxone®,[13] to afford acid **16** in a 59% overall yield. The synthesis of the peptidic fragment that contains the guanidino group was achieved by starting from 1,4-butanediamine **17**, which was monoprotected with the di-Boc guanidine moiety **18**[14] to furnish **19**. This derivative was coupled with the Z protected L-leucine derivative **20**, to obtain amide **21**. Deprotection of the *Z* amino group contained in **21** was done by catalytic hydrogenation with ammonium formate,[15] to obtain compound **22**. In a similar way, the *Z*-peptide derivative **26** was prepared starting with the monoprotection of **17** with *Z*–Cl to obtain **23**,[16] coupling with the Boc-derivative of L-leucine **24** and final Boc cleavage with trifluoroacetic acid (Scheme 2).

With the key fragments **16**, **22** and **26** in hand, we proceeded with the coupling as described in Scheme 3. Thus, coupling was achieved by the action of the coupling reagent BOP. Other coupling reagents such as HBTU or the more conventional DCC or EDCI in the presence of HOBt furnished either decomposition or poor yields of the resulting epoxyamides **27** and **28**, in contrast to the above mentioned BOP method,[17] which provided **27** and **28**, in yields of 58% and 62%, respectively. The advanced precursor for E-64 (**1**), compound



Scheme 2. Reagents and conditions: (a) 1.0 equiv **13**, 1.0 equiv NaOH, CH_2Cl_2/H_2O, 0 °C, 2 h, 62%. (b) Amberlyst-15, MeOH, reflux, 1 h, 91%. (c) 1.5 equiv $NaIO_4$ supported on SiO_2, CH_2Cl_2, 25 °C, 2 h, 86%. (d) 1.0 equiv of Oxone, 25 °C, DMF, 1 h, 75%. (e) 0.77 equiv **18**, THF/H_2O, 50 °C, 1 h, 99% for **19**. (f) 1.5 equiv Z–Cl, 2.0 equiv *p*-TsOH, H_2O/EtOH, 25 °C, 1 h, 40% for **23**. (g) 1.6 equiv of Z-Leu-H **20**, 1.4 equiv EDCI, CH_2Cl_2, 25 °C, 8 h, 72% for **21** overall for two steps. (h) 1.5 equiv of **24**, 1.5 equiv EDCI, CH_2Cl_2, 25 °C, 8 h, 65% for **25**. (i) 20.0 equiv NH_4HCO_2, Pd/C, EtOH, 25 °C, 1.5 h, 75% for **22**. (j) 30.0 equiv TFA, CH_2Cl_2, 25 °C, 99%. BOP = (benzotriazol-1-yloxy) tris-(dimethylamino)phosphonium hexafluoro-phosphate. DIPEA = diisopropylethylamine.

27, was then subjected to oxidation with DDQ in order to obtain the readily hydrolyzed indole **29**, which was to be submitted to the sequential treatment of LiOH, followed by TFA, to afford the targeted E-64. However, the oxidation of **27** with DDQ was completely unsuccessful, producing decomposition of the indoline amide **27**. This discouraging result was attributed to the chemical susceptibility of the Boc-groups and oxirane ring to the resulting phenols produced during the oxidation



Scheme 3. Reagents and conditions: (a) 1.5 equiv **22**, 1.0 equiv DIPEA, 1.0 equiv BOP, DMF, 25 °C, 8 h, 58% for **27**; 1.7 equiv **26**, 2.0 equiv DIPEA, 1.2 equiv BOP, DCM, 25 °C, 8 h, 62% for **28**. (b) See text for conditions.

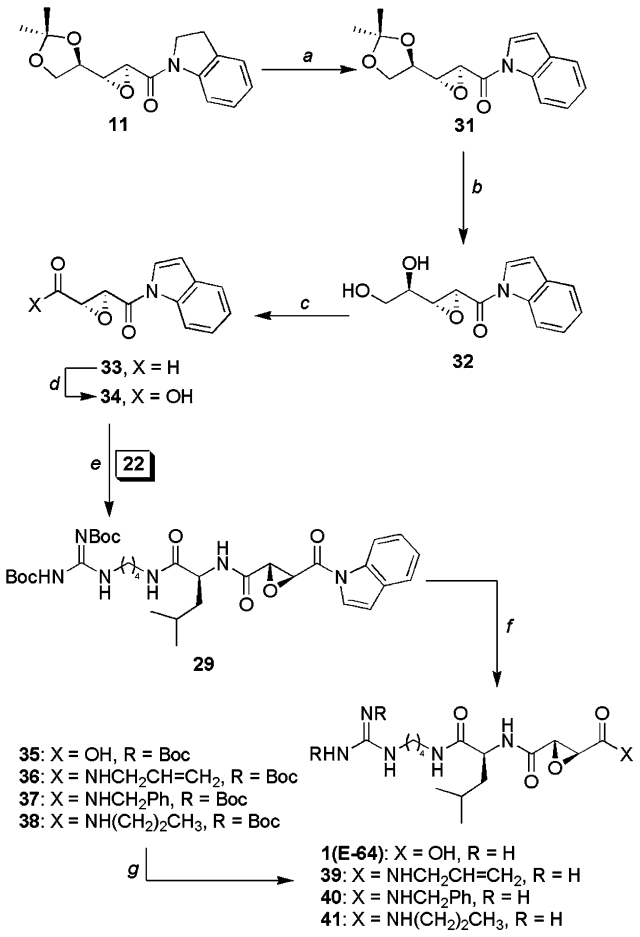
reaction. For this reason, different conditions of temperature (25 °C, reflux), solvents (benzene, toluene, methylene chloride, tetrahydrofuran) and reagents (*p*-chloroanil,[11a] TPAP, TEMPO-Oxone, CAN, ammonium molibdate, among others) were investigated, unfortunately with similar disappointing results or with the recovery of starting material in the cases when mild conditions were used. Therefore, after extensive experimentation with this reaction without obtaining the desired indole **29**, it was clear that the transformation of the indoline amide to the corresponding indole might be undertaken earlier in the synthetic sequence, prior to the incorporation of the guanidino group. However, when **28** was subjected to the oxidation with DDQ in benzene, no reaction occurred, with only starting material being recovered. Similar observations were obtained in methylene chloride, THF and toluene.

In the light of these discouraging results, we decided to achieve this oxidation with the starting epoxyamide **11** to obtain the corresponding indole **31** by treatment with DDQ. We expected that the resulting indole amide would be stable enough to permit the subsequent synthetic steps to be carried out, enabling us to carry this functional group through until the end of the synthesis. Thus, we proceeded with the same synthetic pathway as described in Schemes 2 and 3 for the indoline derivatives to obtain in good yield the desired E-64 derivative **29** through the intermediates diol **32**, aldehyde **33** and acid **34**. Finally, the indole hydrolysis by treatment with lithium hydroxide, followed by Boc cleavage, furnished E-64 (**1**) as the trifluoroacetate salt, whose physical and

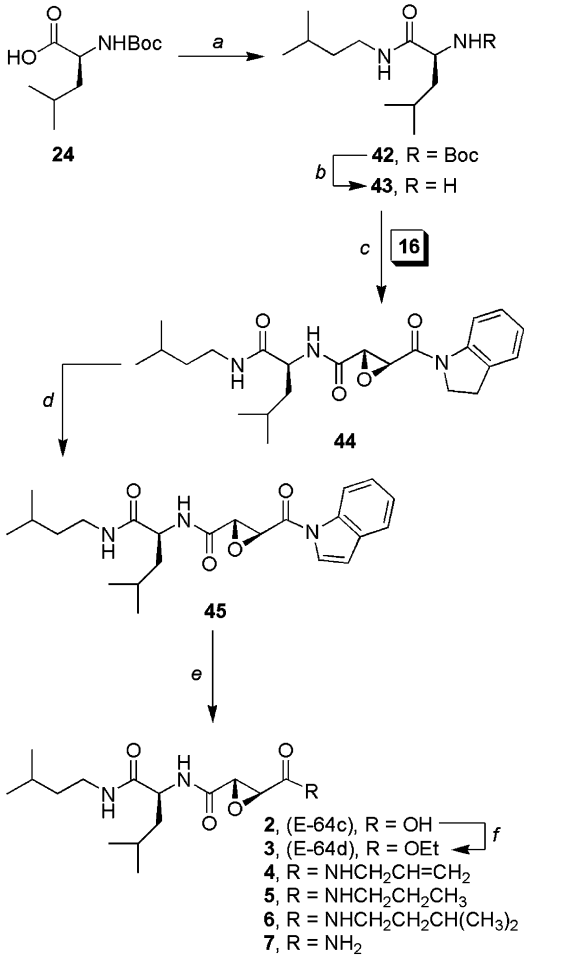
spectroscopic properties were identical to a sample of natural compound.[1,18] In a similar way, a series of E-64 analogues were prepared by reaction of the indole epoxyamide **29** with various amines (allylamine, benzylamine and *n*-propylamine) to obtain the corresponding *N*-allyl, *N*-benzyl and *N*-*n*-propylamides **36**, **37** and **38**, respectively, which were transformed into the trifluoracetate salts **39**, **40** and **41** after the *N*-Boc protecting groups cleavage by treatment with TFA (Scheme 4).

The synthesis of the E-64c related compounds required the preparation of the isoamyl amide of L-leucine, derivative **42**, which after treatment with TFA to yield the deprotected amine derivative **43** was reacted with epoxy-acid **16** in the presence of BOP to obtain the indoline epoxyamide **44** in 60% yield. In this case, the oxidation to the indole **45** was carried out without difficulty by reaction with DDQ in refluxing benzene. The resulting indole **45** represents a key common precursor for the preparation of a family of E-64c related compounds by reaction with different nucleophiles including hydroxyl anion, ethoxide, allylamine, propylamine, isoamylamine or ammonia, to afford the compounds **2**–**7**, respectively, which represent some of the most active cathepsin inhibitors described to date.[2] In addition, the reaction of **45** with more complicated nucleophiles offers the opportunity of constructing a wide variety of novel and more complex cathepsin B inhibitors related to E-64c (**2**) (Scheme 5).

Finally, the preparation of the epoxy-peptide CA-074 (**8**) was accomplished following the synthetic pathway outlined in Scheme 1, using the epoxyamide **11** as the source of the enantiomerically pure oxirane ring contained in all these products. In this series of compounds, however, it was necessary to reverse the order of the two key steps, oxidation of the indoline amide to indole, followed by hydrolysis of the indole to the acid and then coupling to the peptidic residue. Finally, elaboration of the acetal

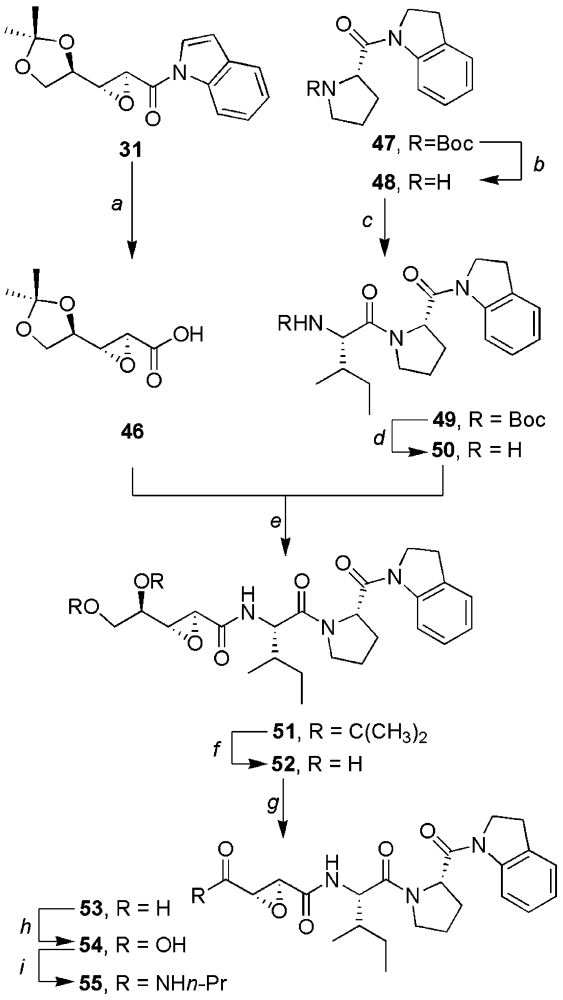


Scheme 4. Reagents and conditions: (a) 2.0 equiv DDQ, C_6H_6, 80 °C, 8 h, 92%. (b) Amberlyst-15, MeOH, reflux, 1 h, 95%. (c) 1.5 equiv $NaIO_4$ supported on SiO_2, CH_2Cl_2, 25 °C, 1 h, 90%. (d) 1.2 equiv of Oxone, 25 °C, DMF, 1 h, 75%. (e) 2.0 equiv **34**, 1.0 equiv **22**, 1.0 equiv DIPEA, 1.0 equiv BOP, DMF, 25 °C, 1 h, 51%. (f) i. 2.0 equiv LiOH, THF/H_2O, 0 °C, 5.0 min, 99% for **35**; ii. 2.2 equiv amine, 0.1 equiv LiCN, CH_2Cl_2, 25 °C, 1 h, 49% for **36**; 76% for **37**; 59% for **38**. (g) 22.0 equiv TFA, neat, 25 °C, 1 h, quantitatives for E-64 (**1**), **39**, **40** and **41**. DDQ = 2,3-dichloro-5,6-dicyanobenzoquinone.



Scheme 5. Reagents and conditions: (a) 1.5 equiv isoamylamine, 1.2 equiv EDCI, CH_2Cl_2, 25 °C, 4 h, 75%. (b) 22.0 equiv TFA, CH_2Cl_2, 25 °C, 40 min, 95%. (c) 1.0 equiv **16**, 1.2 equiv **43**, 1.2 equiv BOP, 1.1 equiv DIPEA, DMF, 25 °C, 1 h, 60%. (d) 2.0 equiv DDQ, C_6H_6, 80 °C, 72 h, 77%. (e) i. 2.0 equiv LiOH, THF/H_2O, 0 °C, 0.5 h, 82% for **2**; ii. 2.2 equiv allylamine, CH_2Cl_2, 25 °C, 1 h, 99% for **4**; iii. 1.1 equiv propylamine, CH_2Cl_2, 25 °C, 1.5 h, 99% for **5**; iv. 1.1 equiv isoamylamine, CH_2Cl_2, 35 °C, 8 h, 99% for **6**; v. 1.1 equiv $NH_{3(aq)}$, CH_2Cl_2, 25 °C, 8 h, 99% for **7**. (f) 1.2 equiv EtOH, 1.5 equiv EDCI, 1.5 equiv 4-DMAP, DMF, 25 °C, 4 h, 30% for **3**.

side chain would lead to the desired compound **8**. Thus, acid **46**[19] was obtained in good yields from the indole **31**. Simultaneously, dipeptide **49** was prepared by coupling of the L-proline derivative **48** with the Boc L-isoleucine derivative, promoted by EDCI, followed by Boc deprotection. The peptidic bond formation between the acid **46** with the dipeptide **50** was achieved by treatment with the coupling reagent BOP, to obtain **51** in a 75% yield. This coupling product was then subjected to the action of *p*-toluenesulfonic acid, followed by $NaIO_4/SiO_2$ treatment to obtain aldehyde **53**, through the diol **52**. After various attempts towards the oxidation of **53** to the acid **54** by methods such as PDC in DMF, the best results were obtained with *m*-CPBA,[20] providing the acid **54** in 73% yield after purification by flash column chromatography. With the acid **54** prepared, the reaction with *n*-propylamine in the presence of BOP provided amide **55**. Finally, the oxidation of indoline amide **55** to the indole **56** with DDQ was achieved, followed by hydrolysis with lithium hydroxide in THF/H_2O to provide the cathepsin B inhibitor CA-074 (**8**)[21] (Schemes 6 and 7). This synthetic strategy could be similarly applied for the synthesis of other CA-074 (**8**) related compounds such as CA-030 (**9**) and CA-028 (**10**).



Scheme 6. Reagents and conditions: (a) 2.0 equiv LiOH, THF/H_2O, 0 °C, 10 min, 75%. (b) 65.0 equiv TFA, CH_2Cl_2, 25 °C, 0.5 h, 85%. (c) 1.0 equiv **48**, 1.5 equiv Boc-Ile-H, 1.5 equiv EDCI, 0.85 equiv HOBt, CH_2Cl_2, 25 °C, 8 h, 75%. (d) 130.0 equiv TFA, CH_2Cl_2, 25 °C, 0.5 h, 94%. (e) 1.0 equiv **46**, 1.1 equiv **50**, 1.2 equiv BOP, 1.0 equiv DIPEA, DMF, 25 °C, 8 h, 75%. (f) 1.0 equiv TsOH, MeOH, 25 °C, 8 h, 75%. (g) 2.6 equiv $NaIO_4$ on SiO_2, CH_2Cl_2, 25 °C, 1 h, 99%. (h) 3.0 equiv *m*-CPBA, THF, 25 °C, 3 days, 73%. (i) 1.2 equiv *n*-$PrNH_2$, 1.2 equiv BOP, 1.2 equiv DIPEA, DMF, 25 °C, 8 h, 90%.

3. Conclusions

The stereoselective synthesis of epoxides and their subsequent ring opening constitute a powerful synthetic tool for the preparation of a diverse set of interesting bioactive compounds. In the present paper we have described the preparation of natural and synthetic cysteine proteases inhibitors, preserving the integrity of the oxirane ring, which was diastereoselectively prepared via the reaction of amide-stabilized sulfur ylides with chiral aldehydes. An interesting contribution is also the use of epoxyamides derived from indoline as precursors to the corresponding indoles, which represents a suitable functional group for (1) the facile transformation to the corresponding acid, and (2) the facile nucleophilic displacement by various nucleophiles with the potential for constructing a library of new and potentially biologically active inhibitors with applications in medicine. Finally, the work serves as a basis for the future preparation of a library using the epoxyamide **11** as a template. The extension of this chemistry to the solid phase and the library design of inhibitors are areas that we are currently investigating.

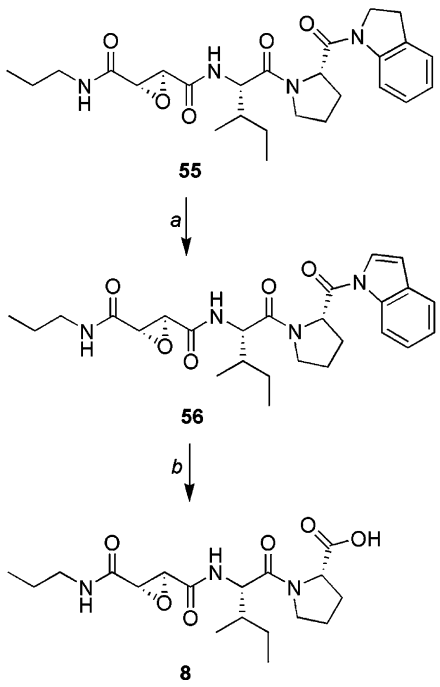
4. Experimental

4.1. General techniques

All reactions were carried out under an argon atmosphere with dry, freshly distilled solvents under anhydrous conditions, unless otherwise noted. Tetrahydrofuran (THF) and ethyl ether (ether) were distilled from sodium-benzophenone, and methylene chloride (CH_2Cl_2), benzene (PhH), and toluene from calcium hydride. Yields refer to chromatographically and spectroscopically (^1H NMR) homogeneous materials, unless otherwise stated. All solutions used in workup procedures were saturated unless otherwise noted. All reagents were purchased at highest commercial quality and used without further purification unless otherwise stated.

All reactions were monitored by thin-layer chromatography carried out on 0.25 mm E. Merck silica gel plates (60F-254) using UV light as visualizing agent and 7% ethanolic phosphomolybdic acid or *p*-anisaldehyde solution and heat as developing agents. E. Merck silica gel (60, particle size 0.040–0.063 mm) was used for flash column chromatography. Preparative thin-layer chromatography (PTLC) separations were carried out on 0.25, 0.50 or 1 mm E. Merck silica gel plates (60F-254).

NMR spectra were recorded on a Bruker Avance-400 instrument and calibrated using residual undeuterated solvent as an internal reference. The following



Scheme 7. Reagents and conditions: (a) 4.0 equiv DDQ, C_6H_6, 80 °C, 16 h, 88%. (b) 4.0 equiv LiOH, THF/H_2O, 0 °C, 2 h, 60%.

abbreviations were used to explain the multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; band, several overlapping signals; b, broad. Optical rotations were recorded on a Perkin–Elmer 241 polarimeter. High resolution mass spectra (HRMS) were recorded on a Kratos MS 80 RFA mass spectrometer under fast atom bombardment (FAB) conditions.

4.2. Sulfonium salt 13. Treatment of indoline 2-chloroacetamide with methyl sulfide

A suspension of indoline 2-chloroacetamide (5.5 g, 28.13 mmol, 1.0 equiv) in dimethyl sulfide (30 mL) was heated at 60 °C for 1 week in a pressure sealed-tube. After that time, the mixture was filtered and the white solid washed with acetone, to obtain sulfonium salt **12** (5.1 g, 75%) as a white solid. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.14 (s, 6H, SMe_2), 3.19 (m, 2H, CH_2CH$_2$N–), 4.07–4.22 (m, 2H, CH$_2$CH_2N–), 5.27 (s, 2H, CH_2SMe$_2$), 6.99–7.08 (m, 1H, Ph), 7.12–7.19 (m, 2H, Ph), 7.97 (d, J = 8.0 Hz, 1H, Ph).

4.3. Epoxyamide 11. Reaction of 2,3-*O*-isopropylidene-D-glyceraldehyde 12 with sulfur ylide derived from sulfonium salt 13

To a solution of 2,3-*O*-isopropylidene-D-glyceraldehyde **12** (1.68 g, 12.9 mmol, 1.0 equiv) and sulfonium salt **13** (3.31 g, 12.9 mmol, 1.0 equiv) in DCM was added an aqueous solution of NaOH (1.29 mL, 40% w/v, 12.9 mmol, 1.0 equiv) at 0 °C. The mixture was vigorously stirred at this temperature, and after 2 h, the reaction mixture was diluted with water and the organic layer separated, washed with water (twice), brine (twice), dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The resulting crude was purified by flash column chromatography (silica gel, 40% AcOEt in hexanes) to obtain pure epoxyamide **11** (2.3 g, 62%) as a yellow solid: R_f = 0.38 (silica gel, 40% AcOEt in hexanes); $[\alpha]_D$ −3.42 (*c* 0.39, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.36 (s, 3H, C(CH$_3$)$_2$), 1.44 (s, 3H, C(CH$_3$)$_2$), 3.23–3.27 (m, 2H, CH_2CH$_2$N–), 3.31 (dd, J = 2.2, 5.9 Hz, 1H, CH(O)CH), 3.61 (d, J = 2.2 Hz, 1H, CH(O)CH), 3.95–4.02 (m, 2H), 4.16–4.32 (m, 3H), 7.02–7.06 (m, 1H, Ph), 7.18–7.21 (m, 2H, Ph), 8.17 (d, J = 8.1 Hz, 1H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 142.4, 130.9, 127.7, 124.6, 124.5, 117.3, 110.2, 75.1, 67.2, 58.1, 52.7, 47.2, 28.2, 26.6, 25.1. MS: 290 (100, M+H$^+$), 289 (73; M$^+$), 232 (93), 190 (31), 146 (53), 119 (60), 101 (37), 59 (95); FAB HRMS *m/e* 289.1310, M$^+$ calcd for $C_{16}H_{19}NO_4$ 289.1314.

4.4. Diol 14. Hydrolysis of epoxyamide 11

A solution of epoxyamide **11** (1.0 g, 3.46 mmol, 1.0 equiv) in MeOH (30 mL) was treated with Amberlyst-15 (3 g) in large excess and refluxed for 1 h until depletion of starting acetal (TLC with AcOEt 100%). Then, the suspension was filtered through a Celite pad and the solvent evaporated, yielding diol **14** (788 mg, 91%) of crude product, as a brown foamy solid, that was used directly in the next step without further purification.

4.5. Aldehyde 15. Treatment of diol 14 with silica supported sodium periodate

To a suspension of silica gel (6.4 g) in DCM (50 mL) was added an aqueous solution of $NaIO_4$ (6.4 mL, 0.65 M, 4.16 mmol, 1.5 equiv) dropwise to form a flaky suspension. Then, a solution of diol **14** (788 mg, 2.72 mmol, 1.0 equiv) in DCM (6.4 mL) was added. The reaction was monitored by TLC (silica gel, AcOEt) and after completion (ca. 1 h), the suspension was filtered through a fritted glass funnel, and the solid was washed twice with DCM. The filtrate was evaporated under reduced pressure and the resulting crude corresponded to aldehyde **15** (500 mg, 86%), practically pure by NMR, not requiring further purification: R_f = 0.50 (silica gel, AcOEt); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.23 (m, 2H, CH_2CH$_2$N–), 3.71 (dd, J = 1.8, 5.8 Hz, 1H, CH(O)CH-CHO), 3.89 (d, J = 1.8 Hz, 1H, CH(O)CHCHO), 4.04–4.33 (m, 2H, CH$_2$CH_2N), 7.03–7.11 (m, 1H, Ph), 7.17–7.23 (m, 2H, Ph), 8.15 (d, J = 8.8 Hz, 1H, Ph), 9.19 (d, J = 6.1 Hz, 1H, CHO).

4.6. Epoxyacid 16. Treatment of aldehyde 15 with Oxone

A solution of aldehyde **15** (500 mg, 2.3 mmol, 1.0 equiv) in DMF (2.3 mL) was treated with Oxone (1.42 g, 2.3 mmol, 1.0 equiv) at room temperature. After 1 h, the reaction was complete and, then, AcOEt (15 mL) was added and the solution was washed with water (3 × 5 mL). The organic layer was separated, dried over anhydrous $MgSO_4$, filtered and the solvent was evaporated to obtain epoxyacid **16** (402 mg, 75%) as a white solid, which did not require further purification:

$R_f = 0.19$ (silica gel, AcOEt); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.22–3.30 (m, 2H, CH_2CH_2N–), 3.85 (d, $J = 1.8$ Hz, 1H, CH(O)CHCO$_2$H), 3.93 (d, $J = 1.8$ Hz, 1H, CH(O)CHCO$_2$H), 4.15–4.40 (m, 2H, CH_2CH_2N–), 7.03–7.11 (m, 1H, Ph), 7.16–7.28 (m, 2H, Ph), 8.15 (d, $J = 7.9$ Hz, 1H, Ph).

4.7. *N*,*N*′-Bis-Boc-agmatine 19. Coupling of *N*,*N*′-bis-Boc-methylisothiourea 18 with 1,4-butanediamine 17

To a solution of 1,4-butanediamine **17** (0.18 mL, 1.79 mmol, 1.0 equiv) in a mixture of THF (2.63 mL) and water (0.12 mL) was added a solution of *N*,*N*′-bis-Boc-methylisothiourea **18** (0.2 g, 0.69 mmol, 0.77 equiv) in THF (1.7 mL) dropwise at 25 °C. After addition, the reaction was heated at 50 °C for 1 h, and then, the solvent was evaporated under vacuum. The resulting crude was partitioned between $CHCl_3$ and saturated aqueous solution of $NaHCO_3$. The organic extracts were dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure, to furnish the agmatine derivative **19**[14] (230 mg, 99%) as a cloudy oil, which was used without further purification in the next step: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.30–1.70 (m, 22H), 3.20 (m, 2H), 2.77 (t, $J = 6.6$ Hz, 2H), 3.38 (t, $J = 6.6$ Hz, 2H), 8.31 (bs, 1H), 11.47 (s, 1H).

4.8. Leucine derivative 21. Coupling of Z-leucine 20 with the agmatine derivative 19

To a solution of Z-Leu-OH **20** (300 mg, 1.13 mmol, 1.0 equiv) in anhydrous DCM (10 mL) was added a solution of di-Boc-Agm **19** (230 mg, 0.69 mmol, 0.63 equiv) in DCM (10 mL) prior to the addition of EDCI (200 mg, 1.04 mmol, 1.4 equiv) at 25 °C. The reaction mixture was stirred overnight and, after that time, the reaction was worked up by successive washings with water, saturated aqueous Na_2CO_3, aqueous solution of citric acid and brine. The organic layer was then separated, dried over anhydrous $MgSO_4$, filtered and the filtrate was concentrated under reduced pressure. The crude was purified by flash column chromatography (silica gel, 40% AcOEt in hexanes), to afford pure coupling product **21** (291 mg, 72% over two steps) as a white solid: $R_f = 0.46$ (silica gel, 40% AcOEt in hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.89–0.94 (m, 6H, CH(CH_3)$_2$), 1.46 (s, 18H, $2 \times C(CH_3)_3$), 1.53–1.47 (m, 6H, $3 \times CH_2$), 1.61–1.64 (m, 1H, CH(CH$_3$)$_2$), 3.21–3.29 (m, 2H, CH_2NHC(O)), 3.29–3.36 (m, 2H, CH_2NHC(=NBoc)), 4.11–4.14 (m, 1H, CHNHZ), 5.06 (m, 2H, OCH$_2$Ph), 5.36 (d, $J = 7.5$ Hz, 1H, NHZ), 6.42 (bs, 1H, NHLeu), 7.31 (m, 5H, Ph), 8.32 (m, 1H, Boc-NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.3, 163.4, 156.2, 153.3, 128.5, 128.2, 128.1, 128.0, 83.2, 79.4, 67.0, 66.9, 53.6, 52.3, 41.6, 41.4, 40.3, 39.1, 28.3, 28.0, 26.6, 26.4, 24.7, 22.9, 22.8, 22.0, 21.8.

4.9. Leucine derivative 22. Z-Cleavage of 21

To a solution of Z-Leu-diBoc-Agm **21** (50 mg, 0.087 mmol, 1.0 equiv) in degassed EtOH (7.4 mL) was added Pd–C (96 mg), and after formation of a homogeneous suspension, a solution of ammonium formate (25% (w/v), 1.1 mL, 4.36 mmol, 20 equiv) was added at 25 °C. After 1.5 h, the reaction was complete as determined by TLC (silica gel, 40% AcOEt in hexanes). The suspension was filtered through a Celite pad and the volatiles were evaporated. The crude mixture was partitioned between AcOEt and brine; the organic layer was separated and washed twice with brine, dried over anhydrous $MgSO_4$, filtered and concentrated, to furnish compound **22** (138 mg, 75%), as a white solid, which did not require further purification.

4.10. Amine 23. Monoprotection of 1,4-butanediamine with CbzCl

1,4-Butanediamine **17** (171 mg, 1.7 mmol, 1.0 equiv) was dissolved in water (0.33 mL) and bromocresol green was added as indicator. *p*-Toluenesulfonic acid (0.586 g in 0.66 mL of water, 3.4 mmol, 2.0 equiv) was added until colour changed (yellow), and a few drops of KOAc buffer were added to reach the equivalence point (yellowish green). Then, the mixture was diluted with EtOH (0.92 mL) and a solution of Z–Cl (255.7 mg, 1.5 mmol, 1.5 equiv) in dimethoxy methane (0.33 mL) was added dropwise until colour of the solution changed (yellow). Then, buffer was carefully added again to reach the equivalence point, but careful not to increase the pH over the range 3.7–3.8 (yellowish green colour); in this case, large amounts of diprotected amine is formed. This procedure was repeated iteratively until all the Z–Cl was added. The pH is maintained at 3.7 for 1 h, and after this time, the volatile components were evaporated and the resulting crude was diluted with water. The diprotected amine precipitated and it was filtered off and washed with water. The mother liquors were washed with benzene and then basified with 40% (w/v) NaOH, followed by further extraction with benzene (2×5 mL). The organic extracts were washed with brine and dried with $MgSO_4$. After evaporating the solvent, a cloudy oil was obtained, which corresponded to the *Z*-monoprotected diamine **23**[16] (187 mg, 40%). This oil was subjected to coupling with Boc-Leu-OH without further purification. ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.30–1.60 (m, 4H, $2 \times CH_2$), 2.59–2.79 (m, 2H, CH_2NH$_2$), 2.97–3.39 (m, 2H, CH_2NHZ), 4.95–5.09 (m, 2H, CH_2Ph), 7.11–7.38 (m, 5H, Ph).

4.11. Leucine derivative 25. Coupling between Boc-Leu 24 and Z-butanediamine 23

Boc-Leu-OH **24** (293 mg, 1.27 mmol, 1.5 equiv) was dissolved in DCM (10 mL) under Ar atmosphere, and a solution of Z-butanediamine **23** (187 mg, 0.84 mmol, 1.0 equiv) in 10 mL of DCM was added. When homogeneous, EDCI (241 mg, 1.26 mmol, 1.5 equiv) was added, and the solution was stirred overnight at room temperature. After that time, the reaction was worked up by washing with water (2×10 mL) and brine (2×10 mL). After drying with $MgSO_4$ and solvent evaporation, the crude was purified by flash column chromatography (silica gel, 30% AcOEt in hexanes), obtaining pure coupling product **25** (209 mg, 65% over two steps) as a white solid: $R_f = 0.47$ (silica gel, 30% AcOEt in hexanes); ^{1}H

NMR ($CDCl_3$, 200 MHz) δ 0.84–0.97 (m, 6H, $CH(CH_3)_2$), 1.39 (s, 9H, $C(CH_3)_3$), 1.39–1.71 (m, 7H, $3\times CH_2$, $CH(CH_3)_2$), 3.07–3.29 (m, 5H), 5.06 (s, 2H, CH_2Ph), 6.50 (bs, 1H, NHZ), 7.31 (m, 5H, Ph).

4.12. Leucine derivative 26. Boc cleavage of 25

Compound **25** (184.3 mg, 0.42 mmol, 1.0 equiv) was dissolved in 6 mL of anhydrous DCM, and TFA (0.98 mL, 30 equiv) was added. When complete (TLC AcOEt–hexane (30:70)), the reaction mixture was treated with saturated aqueous Na_2CO_3 solution until a pH of 9 was achieved. Then, it was extracted with DCM (2×15 mL), the extracts dried with $MgSO_4$ and evaporated, obtaining the leucine derivative **26** (142 mg, 99%) as a colourless oil: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.88–0.95 (m, 6H, $CH(CH_3)_2$), 1.20–1.44 (m, 1H, $CH(CH_3)_2$), 1.44–1.58 (m, 4H, $2\times CH_2$), 1.59–1.75 (m, 2H, CH_2), 3.09–3.31 (m, 4H, $2\times CH_2$), 3.38 (dd, J = 2.4, 9.2 Hz, 1H, $CHNH_2$), 4.89 (bt, J = 5.5 Hz, 1H, NHZ), 5.07 (s, 2H, CH_2Ph), 7.27–7.45 (m, 5H, Ph).

4.13. Epoxyamide 27. Coupling of epoxyacid 16 with amine 22

A solution of epoxyacid **16** (49 mg, 0.21 mmol, 1.0 equiv) in anhydrous DMF (3.0 mL) was subjected to the action of Hünig's base (36.2 μL, 0.21 mmol, 1.0 equiv) at 25 °C, and after 5 min at this temperature, a solution of amino compound **22** (137.5 mg, 0.31 mmol, 1.5 equiv) in anhydrous DMF (3.0 mL) was added at 25 °C. After stirring for 15 min, BOP (88 mg, 2.1 mmol, 1.0 equiv) was added in one portion and the reaction mixture was stirred overnight. After this time, the mixture was diluted with ether, and the resulting solution was washed with saturated aqueous NH_4Cl solution (3×10 mL). The aqueous phase was extracted with ether (2×5 mL) and the combined organic layers were separated, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. Purification by flash column chromatography (silica gel, AcOEt (60 → 80%) in hexanes) afforded the coupling product **27** (82.3 mg, 58%) as a pale brown solid: R_f = 0.76 (silica gel, 70% AcOEt in hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.91–0.93 (m, 6H, $CH(CH_3)_2$), 1.47 (s, 9H, $C(CH_3)_3$), 1.48 (m, 2H, $CH_2CH(CH_3)_2$), 1.48 (s, 9H, $C(CH_3)_3$), 1.54–1.73 (m, 5H, $2\times CH_2$, $CH(CH_3)_2$), 3.25 (m, 3H), 3.32–3.42 (m, 3H), 3.66 (d, J = 1.6 Hz, 1H, CH(O)CH), 3.76 (d, J = 1.6 Hz, CH(O)CH), 4.19–4.25 (m, 2H, CH_2CH_2N–), 4.37–4.43 (m, 1H, CHNH), 6.41–6.43 (bs, 1H, NH), 6.76 (d, J = 8.6 Hz, 1H, NH), 7.03–7.07 (m, 1H, Ph), 7.17–7.20 (m, 2H, Ph), 8.10 (d, J = 8.1 Hz, 1H, Ph), 8.33–8.35 (m, 1H, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.1, 166.4, 163.4, 162.5, 156.2, 153.2, 142.1, 131.1, 127.6, 124.8, 124.6, 117.3, 83.3, 79.4, 54.0, 53.5, 51.6, 47.2, 41.3, 40.3, 39.2, 28.2, 28.0, 26.6, 26.4, 24.9, 22.8, 22.2.

4.14. Oxidation of epoxydiamide 27

A solution of coupling product **27** (83 mg, 0.12 mmol, 1.0 equiv) in dry benzene (10 mL) was treated with DDQ (165 mg, 0.72 mmol, 6.0 equiv) and the mixture was refluxed overnight. After work up, a complex mixture of decomposition products was formed. Under other conditions such as various solvents (THF, DCM and toluene), and oxidizing reagents (MnO_2, Oxone, TPAP/NMO, Pd–C, NBS/$NaHCO_3$ (aq)) and temperatures, the results were similarly unsuccessful, with the decomposition or recovery of **27**.

4.15. Epoxyamide 28. Coupling between epoxyacid 16 and amine 26

Epoxyacid **16** (198 mg, 0.85 mmol, 1.0 equiv) was dissolved in 10 mL of anhydrous DCM. Hünig's base (0.24 mg, 1.87 mmol, 2.0 equiv), and after 5 min, amino compound **26** (500 mg, 1.48 mmol, 1.7 equiv) in 10 mL of anhydrous DCM were added at room temperature. When homogeneous, BOP (455.6 mg, 1.08 mmol, 1.2 equiv) was added and the mixture was stirred overnight. The reaction was worked up by washing with water (3×10 mL), drying with $MgSO_4$ and evaporation under reduced pressure. The resulting crude was washed with ether and filtered, to afford **28** (289.5 mg, 62%) as a white solid: R_f = 0.54 (silica gel, AcOEt); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.83–0.97 (m, 6H, $CH(CH_3)_2$), 1.32–1.71 (m, 7H, $3\times CH_2$, $CH(CH_3)_2$), 3.07–3.32 (m, 6H, $3\times CH_2$), 3.72 (d, J = 1.8 Hz, 1H, CH(O)CH), 3.80 (d, J = 1.8 Hz, 1H, CH(O)CH), 4.14 (t, J = 7.3 Hz, 1H), 4.39 (m, 1H, CHNH), 5.05 (s, 2H, CH_2Ph), 6.57 (bm, 1H, NH), 6.87–7.80 (m, 3H, Ph), 7.28 (m, 5H, Ph), 8.07 (d, J = 7.3 Hz, 1H, Ph).

4.16. Oxidative treatment of 28 with DDQ

Indoline amide **28** (20 mg, 0.036 mmol, 1.0 equiv) was dissolved in 10 mL of dry benzene and DDQ (33 mg, 0.14 mmol, 4.0 equiv) was added. Then, the reaction mixture was heated under reflux overnight. After work up, no oxidation had taken affect. Other solvents tested were THF, toluene and DCM, and no reaction was similarly observed with the recovery of starting indoline amide **28**.

4.17. Indole epoxyamide 31. Oxidation of the indoline amide 11 with DDQ

A solution of epoxyamide **11** (200 mg, 0.69 mmol, 1.0 equiv) in anhydrous benzene (9.0 mL) was treated with DDQ (314.2 mg, 1.38 mmol, 2.0 equiv). The reaction mixture was heated overnight, after which, diethyl ether (20 mL) was added, and the organic layer was washed extensively with saturated aqueous $NaHCO_3$ solution until a constant, pale red colour was achieved. The organic phase was separated, dried ($MgSO_4$), filtered and concentrated, to yield a flaky brown solid that, which after purification by flash column chromatography (silica gel, 20% AcOEt in hexanes), provided pure indole-amide **31** (178 mg, 90%) as a white foamy solid: R_f = 0.33 (silica gel, 20% AcOEt in hexanes); $[\alpha]_D$ +30.1 (c 0.4, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.38 (s, 3H, $C(CH_3)_2$), 1.47 (s, 3H, $C(CH_3)_2$), 3.39 (dd, J = 1.6, 5.4 Hz, 1H, CH(O)CH), 4.01–4.10 (m, 3H, CH(O)CH, –$OCHCH_2O$–), 4.22 (dd, J = 2.2, 5.9 Hz,

1H, –OCHCH_2O–), 6.70 (d, J = 3.8 Hz, 1H, CH=CHN), 7.27–7.37 (m, 1H, Ph), 7.34–7.37 (m, 1H, Ph), 7.56 (d, J = 8.1 Hz, 1H, CH=CHN), 7.66 (d, J = 3.8 Hz, 1H, Ph), 8.40 (d, J = 8.6 Hz, 1H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 164.9, 135.5, 130.2, 125.5, 124.4, 123.7, 121.0, 116.5, 110.64, 74.8, 67.0, 58.5, 52.8, 26.6, 24.9; MS: 288 (34; M+H$^+$), 287 (22; M$^+$), 230 (68), 188 (43), 144 (27), 118 (77), 101 (33), 59 (100); FAB HRMS *m/e* 287.1154, calcd for $C_{16}H_{17}NO_4$ 287.1157.

4.18. Diol 32. Hydrolysis of epoxyamide 31

A solution of epoxyamide **31** (500 mg, 1.74 mmol, 1.0 equiv) in MeOH (10 mL) was treated with Amberlyst-15 (500 mg) according to the procedure described above for **11** to obtain diol **32** (18 mg, 95%) of crude product, as a brown foamy solid, that was used directly in the next step without further purification [R_f = 0.46 (silica gel, AcOEt)].

4.19. Aldehyde 33. Treatment of diol 32 with silica supported sodium periodate

Aldehyde **33** (16 mg, 90%) was prepared from diol **32** (18 mg, 0.073 mmol) by treatment with $NaIO_4$ (0.15 mL, 0.65 M, 0.09 mmol, 1.23 equiv) supported on silica gel (146 mg) according to the same procedure described above for the preparation of **15** [R_f = 0.68 (silica gel, AcOEt)].

4.20. Epoxyacid 34. Treatment of aldehyde 33 with Oxone

The oxidation of aldehyde **33** (16 mg, 0.074 mmol, 1.0 equiv) was carried out exactly as described for **15** above, to yield epoxyacid **34** (17 mg, 75%) as a brown solid, which did not require further purification: R_f = 0.54 (silica gel, AcOEt); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.86 (d, J = 1.8 Hz, 1H, CH(O)CHCO$_2$H), 4.31 (d, J = 1.8 Hz, 1H, CH(O)CHCO$_2$H), 6.71 (d, J = 3.7 Hz, 1H, CH=CHN), 7.27–7.37 (m, 3H, Ph), 7.53–7.64 (m, 2H, Ph, CH=CHN), 8.40 (d, J = 7.3 Hz, 1H, Ph).

4.21. Epoxyamide 29. Coupling of epoxyacid 34 with amine 22

A solution of epoxyacid **34** (186 mg, 0.81 mmol, 2.0 equiv) in anhydrous DMF (2.0 mL) was reacted with amine **22** (160.0 mg, 0.36 mmol, 1.0 equiv) according to the same procedure as described above for the coupling of **16** and **22**, to afford, after similar processing, pure coupling product **29** (305.7 mg, 51%) as a pale yellow solid: R_f = 0.53 (silica gel, 50% AcOEt in hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.86–1.00 (m, 6H, CH(CH_3)$_2$), 1.46 (s, 9H, C(CH$_3$)$_3$), 1.48 (s, 9H, C(CH$_3$)$_3$), 1.48–1.88 (m, 7H, 3 × CH$_2$, CH(CH$_3$)$_2$), 3.20–3.47 (m, 4H, 2 × CH_2NH), 3.88 (d, J = 1.8 Hz, 1H, CH(O)CH), 4.13 (d, J = 1.8 Hz, CH(O)CH), 4.37–4.54 (m, 1H, CHNH), 6.52 (bt, J = 6.1 Hz, 1H, NH), 6.71 (d, J = 3.7 Hz, 1H, CH=CHN), 6.93 (bd, J = 9.2 Hz, 1H, NH), 7.27–7.37 (m, 2H, Ph), 7.53–7.64 (m, 2H, CH=CHN, Ph), 8.40 (d, J = 6.7 Hz, 1H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.9, 165.6, 163.1, 158.1, 156.3, 152.4, 130.2, 125.7, 124.7, 123.4, 121.1, 116.5, 111.2, 83.2, 79.5, 54.4, 53.7, 51.6, 41.4, 40.3, 39.3, 28.3, 28.0, 26.7, 26.2, 24.9, 22.8, 22.3.

4.22. E-64 (1). Hydrolysis of 29

Indole-amide **29** (50.0 mg, 0.076 mmol, 1.0 equiv) was dissolved in THF (2 mL), and LiOH (1.52 mL, 0.1 M, 2.0 equiv) was added dropwise at 0 °C. After addition, TLC (silica gel, 40% AcOEt in hexanes) revealed that the reaction was complete in 5 min. The work up was carried out by extracting the aqueous phase with AcOEt (2 × 3 mL), the aqueous extracts were acidified with saturated aqueous citric acid solution until pH 3 and reextracted with AcOEt (3 × 5 mL). Combined organic layers were dried with $MgSO_4$, filtered and concentrated, to obtain crude acid **35**, which was subjected to the action of TFA at 25 °C. After 1 h, the crude mixture was concentrated under reduced pressure to obtain the trifluoracetate salt of E-64 (**1**) (36 mg, 99%) whose spectroscopic and physical properties were identical to an authentic sample of natural E-64 (**1**)[1]: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.84 (d, J = 6.5 Hz, 3H, CH(CH_3)$_2$), 0.88 (d, J = 6.5 Hz, 3H, CH(CH_3)$_2$), 1.37–1.59 (m, 7H, 3 × CH$_2$, CH(CH$_3$)$_2$), 3.00–3.12 (m, 4H, 2 × CH_2NH), 3.45 (d, J = 2.2 Hz, 1H, CH(O)CH), 3.66 (d, J = 2.2 Hz, 1H, CH(O)CH), 4.23–4.32 (m, 1H, CHNH), 7.56 (bs, 1H, NH), 8.14 (bt, J = 5.7 Hz, 1H, NH), 8.60 (bd, J = 8.1 Hz, 1H, NH); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 171.3, 168.9, 165.0, 156.7, 51.3, 41.1, 40.4, 38.1, 26.3, 25.9, 24.3, 22.9, 21.7.

4.23. Reaction of indole epoxyamide 29 with amines. General procedure

Indole-amide **29** (17 mg, 0.026 mmol, 1.0 equiv) was dissolved in DCM (0.5 mL), and amine (2.0 equiv) was added at 25 °C, followed by the addition of LiCN (0.1 equiv). After completion of the reaction (ca. 1 h), the crude mixture was diluted with EtOAc and washes with water and brine. The organic layer was separated, dried ($MgSO_4$) and concentrated under reduced pressure. The resulting crude was purified by flash column chromatography (silica gel, 50 → 80% AcOEt in hexanes), providing pure epoxyamides **36** (7.6 mg, 49%), **37** (12.7 mg, 76%) and **38** (9.1 mg, 59%), as colourless oils. [**36**]: R_f = 0.48 (silica gel, AcOEt); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.87–0.98 (m, 6H, CH(CH_3)$_2$), 1.48 (s, 18H, 2 × C(CH$_3$)$_3$), 1.48–1.64 (m, 7H, 3 × CH$_2$, CH(CH$_3$)$_2$), 3.21–3.45 (m, 4H, 2 × CH_2NH), 3.49 (d, J = 1.8 Hz, 1H, CH(O)CH), 3.51 (d, J = 1.8 Hz, CH(O)CH), 3.81–3.92 (m, 2H, NHCH_2CH=CH$_2$), 4.32–4.54 (m, 1H, CHNH), 5.09–5.21 (m, 2H, NHCH$_2$CH=CH_2), 5.67–5.92 (m, 1H, NHCH$_2$CH= CH$_2$), 6.17–6.31 (bs, 1H, NH), 6.40–6.54 (bs, 1H, NH), 6.69 (bd, J = 8.6 Hz, 1H, NH), 8.27–8.41 (m, 1H, NH), 11.46 (m, 1H, Boc*NH*); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 171.1, 165.6, 165.5, 163.4, 161.1, 156.3, 153.3, 133.1, 117.2, 83.2, 79.5, 54.8, 54.7, 51.3, 41.4, 40.3, 39.3, 28.3, 28.1, 26.7, 26.3, 24.8, 22.9, 22.1. [**37**]: R_f = 0.52 (silica gel, 50% AcOEt in hexanes); ^{1}H NMR ($CDCl_3$, 100 MHz) δ 0.87–0.96 (m, 6H, CH(CH_3)$_2$), 1.47 (s, 18H, 2 × C(CH$_3$)$_3$), 1.47–1.72

(m, 7H, $3\times CH_2$, $CH(CH_3)_2$), 3.19–3.41 (m, 4H, $2\times CH_2NH$), 3.50 (d, $J = 1.8$ Hz, 1H, CH(O)CH), 3.54 (d, $J = 1.8$ Hz, CH(O)CH), 4.32–4.48 (m, 3H, CHNH, CH_2Ph), 6.30–6.56 (bs, 1H, NH), 6.61–6.75 (bs, 1H, NH), 7.17–7.32 (m, 5H, Ph), 8.33 (bs, 1H, NH), 11.45 (m, 1H, BocNH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 171.1, 165.7, 159.7, 159.1, 156.3, 153.3, 137.2, 128.8, 127.9, 127.8, 83.2, 79.5, 54.8, 54.7, 52.4, 41.3, 41.0, 40.3, 39.2, 28.3, 28.1, 26.7, 26.3, 24.7, 22.8, 22.1. [**38**]: $R_f = 0.42$ (silica gel, 70% AcOEt in hexanes); ^1H NMR ($CDCl_3$, 100 MHz) δ 0.82–0.96 (m, 9H, $CH(CH_3)_2CH_3$), 1.48 (s, 18H, $2\times C(CH_3)_3$), 1.48–1.73 (m, 9H, $4\times CH_2$, $CH(CH_3)_2$), 3.12–3.44 (m, 6H, $3\times CH_2NH$), 3.45 (d, $J = 2.4$ Hz, 1H, CH(O)CH), 3.48 (d, $J = 2.4$ Hz, CH(O)CH), 4.31–4.53 (m, 1H, CHNH), 6.06–6.31 (bs, 1H, NH), 6.38–6.56 (bs, 1H, NH), 6.57–6.69 (bs, 1H, NH), 8.28–8.41 (bs, 1H, NH), 11.45 (m, 1H, BocNH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 171.2, 165.7, 163.4, 161.1, 156.2, 153.2, 83.2, 79.4, 54.8, 54.7, 41.3, 40.7, 40.3, 39.2, 28.2, 28.0, 26.7, 26.3, 24.7, 22.8, 22.6, 22.1.

4.24. Treatment of epoxyamides 36–38 with TFA. General procedure

Indole-amide (0.03 mmol, 1.0 equiv) was treated with TFA neat at 25 °C for 1 h. After that time, the crude mixture was concentrated under reduced pressure to give the corresponding trifluoroacetate salt in quantitative yield.

4.25. Leucine derivative 42. Coupling between isoamylamine and Boc-Leu-OH 24

Compound **24** (613 mg, 2.64 mmol, 1.0 equiv) was dissolved in DCM (20 mL) under an Ar atmosphere, and isoamyl-amine (0.46 mL, 3.96 mmol, 1.5 equiv) was added. After homogeneization, EDCI (606 mg, 3.17 mmol, 1.2 equiv) was added, and the solution was stirred at 25 °C for 4 h. After that time, water (10 mL) was added, and the organic layer separated, washed with water (2×10 mL) and aqueous citric acid (10 mL, 1 M). The combined organic layers were dried ($MgSO_4$) and the solvent evaporated, to furnish **42** (597 mg, 75%) as a white solid, which was used without further purification: $[\alpha]_D$ −31.3 (*c* 0.62, CH_2Cl_2); $[\alpha]_D$ −24.8 (*c* 0.57, MeOH); ^1H NMR ($CDCl_3$, 400 MHz) δ 0.86 (d, $J = 6.5$ Hz, 6H, isoamyl $CH(CH_3)_2$), 0.87–0.89 (m, 6H, Leu $CH(CH_3)_2$), 1.31–1.36 (m, 2H, CH_2), 1.39 (s, 9H, $C(CH_3)_3$), 1.41–1.47 (m, 1H), 1.53–1.61 (m, 3H), 3.17–3.22 (m, 2H, CH_2NH–), 4.03 (m, 1H, CHNH–), 5.00 (bm, 1H, NHBoc), 6.34 (bm, 1H, NH); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.5, 155.8, 53.0, 41.3, 38.3, 37.7, 28.3, 25.7, 24.7, 22.8, 22.4, 22.3.

4.26. Leucine derivative 43. Cleavage of the Boc group in 42

Boc-leucine derivative **42** (0.597 g, 1.98 mmol, 1.0 equiv) was dissolved in DCM (2.2 mL), and TFA (3.3 mL, wide excess) was added. After 40 min, the reaction mixture was treated with saturated aqueous Na_2CO_3 solution until a pH of 9 was achieved with vigorous stirring for 20 min. Then, the aqueous phase was extracted with DCM (2×15 mL), the extracts combined, dried ($MgSO_4$), filtered and evaporated, obtaining compound **43** (378.2 mg, 95%) as a yellowish oil: ^1H NMR ($CDCl_3$, 200 MHz) δ 0.85–0.98 (m, 12H, $2\times CH(CH_3)_2$), 1.27–1.46 (m, 2H, CH_2), 1.49–1.77 (m, 4H, CH_2, $2\times CH(CH_3)_2$), 3.15–3.30 (m, 2H, CH_2NH–), 3.35 (dd, $J = 3.7$, 9.8 Hz, 1H, CHNH–), 7.17 (bs, 1H, NH).

4.27. Epoxyamide 44. Coupling between epoxyacid 16 and amine 43

Epoxyacid **16** (61.6 mg, 0.26 mmol, 1.0 equiv) was dissolved in DMF (2.4 mL); Hünig's base (49 μL, 0.28 mmol, 1.1 equiv) and, after 5 min, **43** (63.4 mg, 1.71 mL of a 0.185 M solution in DMF, 1.2 equiv) were sequentially added. When the solution was homogeneous, BOP (128.6 mg, 0.3 mmol, 1.2 equiv) was added, and the reaction stirred at room temperature. After 1 h, analysis by TLC (silica gel, 5% MeOH in AcOEt) indicated the depletion of starting material. The crude mixture was diluted with ether (10 mL) and washed with saturated aqueous NH_4Cl solution (3×10 mL). The aqueous phase was separated and extracted with ether (2×5 mL) and the organic extracts were combined, dried ($MgSO_4$), filtered and the solvents evaporated. Purification by flash column chromatography (silica gel, 60% AcOEt in hexanes), provided epoxyamide **44** (65.4 mg, 60%) as a yellowish solid: $R_f = 0.28$ (silica gel, 40% AcOEt in hexanes); $[\alpha]_D$ +7.54 (*c* 0.17, CH_2Cl_2); ^1H NMR ($CDCl_3$, 400 MHz) δ 0.89 (d, $J = 6.5$ Hz, 6H, $CH(CH_3)_2$), 0.90–0.93 (m, 6H, $CH(CH_3)_2$), 1.35–1.40 (m, 2H, CH_2), 1.49–1.61 (m, 4H, CH_2, $2\times CH(CH_3)_2$), 3.15–3.29 (m, 4H), 3.68 (d, $J = 1.6$ Hz, 1H, CH(O)CH), 3.78 (d, $J = 1.6$ Hz, 1H, CH(O)CH), 4.19–4.25 (m, 2H, indoline CH_2N–), 4.36–4.41 (m, 1H, CHNH–), 6.08 (bm, 1H, CHNH–), 6.87–6.89 (bm, 1H, CH_2NH–), 7.03–7.08 (m, 1H, Ph), 7.16–7.19 (m, 2H, Ph), 8.12 (d, $J = 8.6$ Hz, 1H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.8, 166.5, 142.1, 128.9, 127.7, 124.9, 124.7, 117.4, 54.1, 53.6, 51.6, 47.2, 41.1, 38.2, 38.0, 28.1, 25.8, 24.9, 22.7, 22.4, 22.3; MS: 416 (74; $M+H^+$), 227 (85), 190 (98), 186 (28), 146 (34), 120 (100), 119 (80), 118 (40), 88 (50), 86 (40); FAB HRMS *m/e* 416.2544, M^+ calcd for $C_{23}H_{34}N_3O_4$ 416.2549.

4.28. Indol epoxyamide 45. Oxidation of epoxyamide 44 with DDQ

A solution of indoline epoxyamide **44** (59.7 mg, 0.14 mmol, 1.0 equiv) in benzene (2 mL) was treated with DDQ (65.3 mg, 0.29 mmol, 2.0 equiv) under reflux conditions for 72 h. After that time, the mixture was diluted with ether and washed with saturated aqueous $NaHCO_3$ solution extensively until constant colour of the organic layer. This phase was dried with $MgSO_4$, filtered and the solvent evaporated to yield compound **45** (45.6 mg, 77%) as a slightly yellow solid, which was used without purification in the next step: $R_f = 0.60$ (silica gel, 40% AcOEt in hexanes); $[\alpha]_D$ +46.4 (*c* 0.075, CH_2Cl_2); ^1H NMR ($CDCl_3$, 400 MHz) δ 0.89 (d, $J = 6.5$ Hz, 6H, $CH(CH_3)_2$), 0.93–0.95 (m, 6H, $CH(CH_3)_2$), 1.38 (m, 2H, CH_2), 1.52–1.71 (m, 4H, CH_2, $2\times CH(CH_3)_2$), 3.21–3.29 (m, 2H, CH_2NH–), 3.87 (d, $J = 2.2$ Hz, 1H,

CH(O)C*H*), 4.12 (d, $J = 2.2$ Hz, 1H, C*H*(O)CH), 4.38–4.44 (m, 1H, C*H*NH–), 5.96 (bs, 1H, CHN*H*), 6.71 (d, $J = 3.7$ Hz, 1H, C*H*=CHN), 6.87 (bs, 1H, CH_2N*H*), 7.29 (m, 1H, Ph), 7.35 (m, 1H, Ph), 7.55 (m, 2H, Ph, CH=C*H*N), 8.35 (d, $J = 7.5$ Hz, 1H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.7, 165.9, 165.7, 130.2, 127.9, 125.7, 124.7, 123.4, 121.1, 111.3, 54.4, 53.7, 51.6, 41.3, 38.2, 38.1, 25.8, 24.9, 22.7, 22.4, 22.3; MS: 414 (78; $M+H^+$), 299 (18), 227 (82), 188 (36), 170 (38), 118 (72), 117 (100), 88 (30), 86 (45); FAB HRMS *m/e* 414.2397, M^+ calcd for $C_{23}H_{32}N_3O_4$ 414.2392.

4.29. E-64c (2). Hydrolysis of 45

Indole-amide **45** (43.6 mg, 0.1 mmol, 1.0 equiv) was dissolved in THF (2 mL), and LiOH (2.17 mL, 0.1 M, 2.0 equiv) was added dropwise at 0 °C. After addition, TLC (silica gel, 40% AcOEt in hexanes) revealed that the reaction was complete. The work up was carried out by extracting the aqueous phase with AcOEt (2×3 mL), the aqueous extracts were acidified with Amberlyst-15 until pH 5 and reextracted with AcOEt (3×5 mL). Combined organic layers were dried with $MgSO_4$, filtered and concentrated, to obtain pure acid **2** (27 mg, 82%) as a white solid and whose spectroscopic and physical properties were identical to those reported in the literature: ^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.80–0.96 (m, 12H, $2 \times CH(CH_3)_2$), 1.27 (q, J=7.0 Hz, 2H, CH_2), 1.43–1.63 (m, 4H, CH_2, $2 \times CH(CH_3)_2$), 3.05 (m, 2H, CH_2NH–), 3.45 (d, $J = 1.7$ Hz, 1H, CH(O)C*H*), 3.66 (d, $J = 1.7$ Hz, 1H, C*H*(O)CH), 4.29 (m, 1H, C*H*NH–), 8.02 (t, $J = 5.4$ Hz, 1H, CH_2N*H*), 8.56 (d, $J = 8.4$ Hz, 1H, CHN*H*); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 170.9, 168.7, 164.8, 52.6, 51.2, 41.1, 37.9, 36.7, 25.1, 24.3, 22.8, 22.3, 21.7.

4.30. Loxistatine (3). Ethanolysis of 45

Indole-amide **45** (54.2 mg, 0.13 mmol, 1.0 equiv) was dissolved in freshly distilled dry EtOH (2 mL), and LiOEt (1.31 mL, 0.1 M in EtOH, prepared by treatment of dry EtOH with BuLi at 0 °C) was added. After 10 min, the reaction was diluted with AcOEt (5 mL) and washed with water (2×3 mL). This procedure caused the hydrolysis of the ester (75% of the acid **2** recovered). To achieve the synthesis of this compound, free acid **2** (31 mg, 0.099 mmol, 1.0 equiv) was dissolved in DMF (2 mL), and 4-DMAP (18.1 mg, 0.15 mmol, 1.5 equiv) and EDCI (28.0 mg, 0.146 mmol, 1.5 equiv) were sequentially added. When the mixture was homogeneous, dry EtOH (6.85 μL, 0.12 mmol, 1.2 equiv) was added; the reaction was stirred at room temperature for 4 h; then, it was worked up by dilution with AcOEt (10 mL) and washing with water (2×5 mL) and saturated aqueous $NaHCO_3$ solution (5 mL). The organic extracts were dried with $MgSO_4$, filtered and the solvents evaporated, affording pure loxistatine (**3**) (10 mg, 30%), as a colourless oil, whose spectroscopic and physical properties were identical to those reported in the literature: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.90–0.95 (m, 12H, $2 \times CH(CH_3)_2$), 1.32 (t, $J = 7.1$ Hz, 3H, OCH_2CH_3), 1.39 (q, $J = 7.3$ Hz, 2H, CH_2), 1.47–1.72 (m, 4H, CH_2, $2 \times CH(CH_3)_2$), 3.24 (m, 2H, CH_2NH–), 3.46 (d, $J = 1.8$ Hz, 1H, CH(O)C*H*), 3.68 (d, $J = 1.8$Hz, 1H, C*H*(O)CH), 4.22–4.40 (m, 3H, C*H*NH, OCH_2CH_3), 5.92 (m, 1H, CH_2N*H*), 6.56 (d, $J = 8.6$ Hz, 1H, CHN*H*); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.7, 166.4, 166.0, 62.3, 53.8, 53.0, 51.3, 41.2, 38.3, 38.0, 25.8, 24.8, 22.8, 22.4, 22.3, 22.2, 14.1.

4.31. Allyl epoxyamide 4. Reaction of indol epoxyamide 45 with allyl-amine

Indole-amide **45** (19.1 mg, 0.046 mmol, 1.0 equiv) was dissolved in DCM (0.5 mL), and allyl-amine (0.38 mL of a 0.254 M solution in DCM, 2.2 equiv) was added at 25 °C. The mixture was stirred overnight at this temperature, and, after that, the crude mixture was concentrated under reduced pressure and dried under high vacuum. The transamidation reaction occurred in quantitative yield, providing pure epoxyamide **4** (20 mg, 99%) as a colourless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.87–0.90 (m, 12H, $2 \times CH(CH_3)_2$), 1.33–1.39 (m, 2H, CH_2), 1.50–1.59 (m, 4H, CH_2, $2 \times CH(CH_3)_2$), 3.11–3.30 (m, 2H, CH_2NH–), 3.54–3.57 (m, 2H, C*H*(O)C*H*), 3.83–3.86 (m, 2H, $CH_2CH=CH_2$), 4.50–4.47 (m, 1H, C*H*NH), 5.11–5.17 (m, 2H, CH=CH_2), 5.72–5.82 (m, 1H, C*H*=CH_2), 6.49 (bs, 1H, CH_2N*H*), 7.22 (bs, 1H, CHN*H*); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.2, 165.9, 165.8, 133.1, 117.1, 54.6, 54.5, 51.5, 41.5, 41.3, 38.1, 37.9, 25.8, 25.7, 22.8, 22.4, 22.3, 22.2.

4.32. *n*-Propyl epoxyamide 5. Treatment of indol epoxyamide 45 with *n*-propylamine

Indole-amide **45** (21 mg, 0.051 mmol, 1.0 equiv) was dissolved in DCM (0.5 mL), and *n*-propylamine (4.5 μL, 0.056 mmol, 1.1 equiv) in DCM was added at 25 °C. After stirring for 1.5 h at this temperature, the solvent was evaporated and the crude product was dried under high vacuum. The transamidation reaction occurred in quantitative yield as seen by NMR, without affecting the oxirane moiety, to afford epoxyamide **5** (20 mg, 99%) as a colourless oil: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.81–0.98 (m, 15H, CH_3, $2 \times CH(CH_3)_2$), 1.30–1.65 (m, 8H, $3 \times CH_2$, $2 \times CH(CH_3)_2$), 3.13–3.29 (m, 4H, $2 \times CH_2NH$–), 3.46 (d, $J = 2.4$ Hz, 1H, CH(O)C*H*), 3.47 (d, $J = 2.4$ Hz, 1H, C*H*(O)CH), 4.26–4.44 (m, 1H, C*H*NH), 5.98–6.23 (bs, 2H, $2 \times NH$), 6.71–6.76 (bd, $J = 8.6$ Hz, 1H, NH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 171.0, 165.8, 165.6, 54.9, 54.7, 51.4, 42.3, 40.8, 38.2, 38.0, 25.8, 24.8, 22.8, 22.6, 22.4, 22.3, 22.2, 11.2; FAB HRMS *m/e* 356.2538, M^++1 calcd for $C_{18}H_{33}N_3O_4$ 356.2544.

4.33. Isoamyl epoxyamide 6. Treatment of 45 with isoamyl amine

Indole-amide **45** (18 mg, 0.043 mmol, 1.0 equiv) was dissolved in DCM (0.5 mL), and isoamyl amine (5.45 μL, 0.047 mmol, 1.1 equiv) in DCM was added at room temperature. The reaction mixture was stirred overnight at 35 °C, and was worked up by evaporating the solvent and drying at high vacuum. The transamidation reaction occurred in quantitative yield as seen by NMR, to obtain epoxyamide **6** (19 mg, 99%) as a colourless oil:

^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.82–0.96 (m, 18H, $3\times CH(CH_3)_2$), 1.37 (q, J = 7.3 Hz, 4H, $2\times CH_2$), 1.46–1.74 (m, 5H, CH_2, $3\times CH(CH_3)_2$), 3.12–3.36 (m, 4H, $2\times CH_2NH$), 3.46 (d, J = 1.8 Hz, 1H, CH(O)CH), 3.47 (d, J = 1.8 Hz, 1H, CH(O)CH), 4.22–4.49 (m, 1H, CHNH), 6.03–6.10 (m, 2H, $2\times CH_2NH$), 6.72 (d, J = 7.9 Hz, 1H, CHNH); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 171.0, 165.8, 165.5, 54.9, 54.7, 51.4, 41.3, 38.2, 38.1, 37.4, 25.8, 25.7, 24.8, 22.8, 22.4, 22.3, 22.2; FAB HRMS *m/e* 384.2873, M^++1 calcd for $C_{20}H_{37}N_3O_4$ 384.2857.

4.34. Epoxyamide 7. Reaction of 45 with aqueous ammonia

Indole-amide **45** (19 mg, 0.045 mmol, 1.0 equiv) was dissolved in DCM (0.5 mL), and 30% aqueous ammonia (2.81 μL, 0.049 mmol, 1.1 equiv) was added at 25 °C. The mixture was stirred overnight at this temperature, and was worked up by evaporating the solvent and drying at high vacuum. The obtained crude corresponded to epoxyamide **7** (18 mg, 99%), which did not require further purification: ^{1}H NMR (DMSO-d_6, 200 MHz) δ 0.85–0.93 (m, 12H, $2\times CH(CH_3)_2$), 1.27 (q, J = 7.1 Hz, 2H, CH_2), 1.40–1.63 (m, 4H, CH_2, $2\times CH(CH_3)_2$), 3.10 (m, 2H, CH_2NH), 3.44 (d, J = 1.8 Hz, 1H, CH(O)CH), 3.61 (d, J = 1.8 Hz, 1H, CH(O)CH), 4.40–4.55 (m, 1H, CHNH), 7.50 (bs, 1H, NH), 7.77 (bs, 1H, NH), 8.05 (t, J = 5.3 Hz, 1H, NH), 8.56 (d, J = 7.9 Hz, 1H, NH); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 171.0, 168.0, 165.4, 52.6, 52.5, 41.1, 37.9, 36.7, 25.1, 24.3, 22.8, 22.4, 22.3, 21.6.

4.35. Epoxyacid 46. Hydrolysis of indol amide 31

Indole-amide **31** (182 mg, 0.63 mmol, 1.0 equiv) was dissolved in THF (5 mL), and LiOH (5.57 mL, 0.1 M in H_2O) was added dropwise at 0 °C. After TLC analysis (silica gel, 20% AcOEt in hexanes) indicated no presence of starting material (ca. 5 min), AcOEt (10 mL) was added and the aqueous phase was treated with Amberlyst-15 until a pH of 5 was achieved, and, then, extracted with AcOEt (3×15 mL). The organic extracts were combined, dried with $MgSO_4$, filtered and concentrated under reduced pressure, to afford epoxyacid **46** (90 mg, 75%) as a colourless oil: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.34 (s, 3H, $C(CH_3)_2$), 1.45 (s, 3H, $C(CH_3)_2$), 3.30 (dd, J = 1.8, 3.5 Hz, 1H, CH(O)CHC(=O)), 3.42 (d, J = 1.8 Hz, 1H, CH(O)CHC(=O)), 3.87–4.05 (m, 2H), 4.10–4.25 (m, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 173.2, 110.1, 74.4, 65.8, 58.1, 51.2, 26.7, 25.0.

4.36. Boc-proline derivative 47. Coupling between Boc-Pro-H and indoline

To a solution of Boc-Pro-H (0.5 g, 2.32 mmol, 1.0 equiv) in anhydrous DCM (25 mL) was added indoline (0.23 g, 1.92 mmol, 0.83 equiv) and EDCI (0.67 g, 3.4 mmol, 1.5 equiv) at 25 °C. The reaction mixture was kept at this temperature until the reaction was complete as judged by TLC (ca. 4 h). Then, the crude mixture was diluted with DCM (20 mL) and the resulting organic solution was washed with saturated aqueous $NaHCO_3$ solution (2×15 mL) and saturated aqueous Na_2CO_3 solution (2×15 mL). After separation of the phases, the organic layer was dried with $MgSO_4$, filtered and evaporated. The resulting solid was suspended in a minimum volume of ether and filtered through a fritted glass funnel. This procedure was repeated until no solid remained in the mother liquor, thus obtaining **47** (0.51 g, 82%) as a white solid: R_f = 0.58 (silica gel, 20% AcOEt in hexanes); $[\alpha]_D$ −56.9 (c 0.15, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.31 and 1.43 (2s, 9H, $C(CH_3)_3$), 1.73–2.32 (m, 4H, Prol $2\times CH_2$), 3.12–3.25 (m, 2H, indoline CH_2), 3.38–3.54 (m, 1H, Prol CH_2N), 3.57–3.72 (m, 1H, Prol CH_2N), 3.98–4.09 (m, 1H, indoline CH_2N), 4.11–4.23 and 4.32–4.41 (m, 1H, indoline CH_2N), 4.44 (dd, J = 4.7, 8.1 Hz, 1H, NCHCO), 4.59 (dd, J = 3.4, 8.0 Hz, 1H, NCHCO), 6.95–7.04 (m, 1H, Ph), 7.13 (m, 2H, Ph), 8.18–8.24 (m, 1H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 127.6, 127.36, 124.5, 124.3, 123.7, 123.6, 117.3, 117.1, 58.7, 58.4, 47.5, 46.8, 46.7, 30.4, 29.5, 28.5, 28.2, 28.1, 24.18, 23.7.

4.37. L-Proline derivative 48. Cleavage of the Boc in 47

Compound **47** (0.508 g, 1.6 mmol, 1.0 equiv) was dissolved in DCM (10 mL), and TFA (8 mL, wide excess) was added. After 30 min, analysis by TLC (40% AcOEt in hexanes) revealed completion of the reaction, so it was treated with saturated aqueous Na_2CO_3 solution under vigorous stirring for 20 min until pH reached **9**. After that time, the mixture was extracted with $CHCl_3$ (2×15 mL), the extracts were dried with $MgSO_4$, filtered and evaporated, to obtain **48** (290 mg, 85%) as a yellowish oil, which was used in the next step without further purification: $[\alpha]_D$ −79.4 (c 0.17, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.73–1.94 (m, 3H, $2\times CH_2$), 2.16–2.32 (m, 1H, CH_2), 2.97 (m, 1H, Prol CH_2N), 3.18 (m, 2H, indoline $PhCH_2$), 3.25 (m, 1H, Prol CH_2N), 3.98–4.07 (m, 2H, indoline CH_2N), 4.15 (m, 1H, NCHCO), 7.02 (m, 1H, Ph), 7.18 (m, 2H, Ph), 8.17 (d, J = 8.1 Hz, 1H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 142.5, 127.6, 124.7, 117.2, 47.4, 30.3, 28.1, 26.2.

4.38. Dipeptide 49. Coupling between of L-proline derivative 48 with Boc-Ile-OH

A solution of **48** (290 mg, 1.33 mmol, 1.0 equiv) in DCM (25 mL) was treated with HOBt (150 mg, 1.1 mmol, 0.85 equiv), prior to the addition of Boc-Ile-H (483.2 mg, 2.0 mmol, 1.5 equiv), followed of EDCI (385.5 mg, 2.0 mmol, 1.5 equiv) at 25 °C. The reaction mixture was stirred at this temperature overnight. After this time, the crude mixture was washed with saturated aqueous Na_2CO_3 solution (2×15 mL) and water (15 mL). The organic phase was separated, dried with $MgSO_4$, filtered and evaporated. Purification by flash column chromatography (silica gel, 30% AcOEt in hexanes) furnished pure **49** (430 mg, 75%) as a white solid: R_f = 0.52 (silica gel, 30% AcOEt in hexanes); $[\alpha]_D$ −87.0 (c 0.2, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.88 (t, J = 7.5 Hz, 3H, CH_2CH_3), 1.00 (d, J = 7.0 Hz, 3H, $CHCH_3$), 1.04–1.15 (m, 1H, Ile CH_2), 1.49–1.61 (m, 1H, Ile CH_2), 1.40 (s, 9H, $C(CH_3)_3$), 1.70–1.80 (m, 1H, Ile CH), 1.96–2.07 (m, 2H, Prol CH_2), 2.16–2.28 (m,

2H, Prol CH_2), 3.13–3.28 (m, 2H, $PhCH_2$), 3.70–3.78 (m, 1H, Prol CH_2N), 3.85–3.90 (m, 1H, Pro CH_2N), 4.01–4.09 (m, 1H, indoline CH_2N), 4.41–4.48 (m, 1H, indoline CH_2N), 4.29 (dd, $J = 8.6$, 9.7 Hz, 1H, Ile C*H*NH), 4.76 (dd, $J = 4.8$, 7.5 Hz, 1H, Pro C*H*N), 5.10 (d, $J = 9.7$ Hz, 1H, NHBoc), 6.97 (m, 1H, Ph), 7.14 (m, 2H, Ph), 8.15 (d, $J = 8.6$ Hz, 1H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.2, 169.7, 155.6, 142.8, 131.0, 127.2, 124.3, 123.6, 117.1, 58.5, 56.1, 47.6, 37.7, 28.6, 28.2, 27.9, 24.9, 24.1, 15.2, 10.9; MS: 430 ($M+H^+$) (32), 374 (12), 356 (14), 331 (23), 330 (96), 312 (19), 311 (53), 255 (62), 237 (14), 217 (25), 211 (51), 130 (23), 120 (36), 119 (34), 118 (20), 86 (23), 70 (100), 57 (35); FAB HRMS *m/e* 430.2694, M^+ calcd for $C_{24}H_{36}N_3O_4$ 430.2705.

4.39. Dipeptide 50. Cleavage of the Boc group in 49

Compound **49** (430 mg, 1.0 mmol, 1.0 equiv) was dissolved in DCM (10 mL), and TFA (10 mL, wide excess) was added. After 30 min, analysis by TLC revealed completion of the reaction, so it was treated with saturated aqueous Na_2CO_3 solution until a pH of 9 was achieved. The mixture was extracted with DCM (2×15 mL), the extracts were combined, dried with $MgSO_4$, filtered and evaporated, to obtain deprotected dipeptide **50** (307.9 mg, 94%) as a white solid: ^1H NMR ($CDCl_3$, 400 MHz) δ 0.86 (t, $J = 7.5$ Hz, 3H, CH_2CH_3), 0.97 (d, $J = 7.0$ Hz, 3H, $CHCH_3$), 1.02–1.13 (m, 1H, Ile CH_2), 1.48–1.60 (m, 1H, Ile CH_2), 1.64–1.74 (m, 1H, $CHCH_3$), 1.91–2.02 (m, 2H, Pro CH_2), 2.14–2.24 (m, 2H, Pro CH_2), 3.11–3.27 (m, 2H, $PhCH_2$), 3.39 (d, $J = 6.5$ Hz, 1H, Ile C*H*NH), 3.63–3.72 (m, 2H, Pro CH_2N), 3.98–4.07 (m, 1H, indoline CH_2N), 4.39–4.45 (m, 1H, indoline CH_2N), 4.74 (dd, $J = 4.8$, 7.5 Hz, 1H, Pro CHN), 6.96 (m, 1H, Ph), 7.09–7.13 (m, 2H, Ph), 8.09 (d, $J = 8.1$ Hz, 1H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 127.3, 124.4, 123.8, 117.2, 58.8, 57.2, 47.7, 47.4, 38.6, 28.0, 25.0, 23.7, 15.6, 11.0.

4.40. Epoxypeptide 51. Coupling of epoxyacid 46 and dipeptide 50

Epoxyacid **46** (166 mg, 0.88 mmol, 1.0 equiv) was dissolved in DMF (3 mL) and treated with Hünig's base (110 mg, 0.88 mmol, 1.0 equiv) at room temperature. After 5 min, a solution of dipeptide **50** (320 mg, 0.97 mmol, 1.1 equiv) in DMF (3 mL) was added to the reaction mixture, followed by addition of BOP (507 mg, 1.2 mmol, 1.4 equiv). The resulting reaction mixture was stirred overnight at this temperature. After that time, ether (15 mL) was added and the organic solution was washed with saturated aqueous NH_4Cl solution (3×10 mL), and water (10 mL). The combined organic layers were dried with $MgSO_4$, filtered and concentrated under vacuum. Purification by flash column chromatography (silica gel, 75% AcOEt in hexanes), provided epoxypeptide **51** (330 mg, 75%) as a white solid: $R_f = 0.26$ (silica gel, 75% AcOEt in hexanes); $[\alpha]_D$ −67.5 (*c* 0.14, CH_2Cl_2); ^1H NMR ($CDCl_3$, 400 MHz) δ 0.86 (t, $J = 7.5$ Hz, 3H, CH_2CH_3), 0.99 (d, $J = 6.5$ Hz, 3H, $CHCH_3$), 1.05 (m, 1H, Ile CH_2), 1.34 (s, 3H, $C(CH_3)_2$), 1.42 (s, 3H, $C(CH_3)_2$), 1.42–1.49 (m, 1H, Ile CH_2), 1.79–1.85 (m, 1H, $CHCH_3$), 1.95–2.04 (m, 2H, Pro CH_2), 2.17–2.26 (m, 2H, Pro CH_2), 3.09 (dd, $J = 1.6$, 4.8 Hz, 1H, CH(O)CHC(=O)), 3.17–3.24 (m, 2H, $PhCH_2$), 3.32 (d, $J = 1.6$ Hz, 1H, CH(O)CHC(=O)), 3.74–3.92 (m, 3H, Pro CH_2N, $-OCH_2CH$), 4.01–4.09 (m, 3H, indoline CH_2N, $-OCH_2CH$), 4.39–4.46 (m, 1H, indoline CH_2N), 4.59 (dd, $J = 8.1$, 9.1 Hz, 1H, Ile C*H*NH), 4.75 (dd, $J = 4.8$, 7.0 Hz, 1H, Pro C*H*N), 6.65 (d, $J = 9.1$ Hz, 1H, Ile CHN*H*), 6.98 (m, 1H, Ph), 7.14 (m, 2H, Ph), 8.15 (d, $J = 8.1$ Hz, 1H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.9, 167.3, 127.4, 124.5, 123.8, 117.3, 105.4, 74.3, 65.9, 58.8, 58.6, 54.2, 53.2, 47.7, 37.7, 28.8, 28.1, 26.4, 25.2, 25.0, 24.4, 15.3, 11.0; MS: 500 (78; $M+H^+$), 484 (13), 381 (87), 217 (100), 120 (33), 70 (79), 59 (33); FAB HRMS *m/e* 500.2754, M^+ calcd for $C_{27}H_{38}N_3O_6$ 500.2760.

4.41. Diol 52. Acidic treatment of acetal 51

Epoxypeptide **51** (257 mg, 0.51 mmol, 1.0 equiv) was dissolved in MeOH (25 mL), and of *p*-toluenesulfonic acid (98 mg, 0.51 mmol, 1.0 equiv) was added. After stirring overnight at 25 °C, the reaction mixture was washed with saturated aqueous $NaHCO_3$ solution (10 mL) and the aqueous phase extracted with AcOEt (2×20 mL). The combined organic layers were dried ($MgSO_4$), filtered and concentrated, to furnish diol **52** (151.3 mg, 75%) as a white solid: $R_f = 0.34$ (silica gel, 5% MeOH in AcOEt); $[\alpha]_D$ −81.7 (*c* 0.23, CH_2Cl_2); ^1H NMR (CD_3OD, 400 MHz) δ 0.92 (t, $J = 7.5$ Hz, 3H, CH_2CH_3), 1.05 (d, $J = 7.0$ Hz, 3H, $CHCH_3$), 1.13–1.22 (m, 1H, Ile CH_2), 1.56–1.63 (m, 1H, Ile CH_2), 1.86–1.93 (m, 1H, $CHCH_3$), 1.97–2.07 (m, 2H, Pro CH_2), 2.16–2.22 (m, 1H, Pro CH_2), 2.33–2.39 (m, 1H, Pro CH_2), 3.17 (dd, $J = 2.2$, 4.3 Hz, 1H, CH(O)CHC(=O)), 3.24 (m, 2H, $PhCH_2$), 3.52 (d, $J = 2.2$ Hz, 1H, CH(O)CHC(=O)), 3.58–3.67 (m, 3H, $HOCH_2CH$), 3.74–3.79 (m, 1H, Pro CH_2N), 3.99–4.05 (m, 1H, Pro CH_2N), 4.14–4.20 (m, 1H, indoline CH_2N), 4.36–4.43 (m, 1H, indoline CH_2N), 4.57 (d, $J = 8.6$ Hz, 1H, Ile C*H*NH), 4.78 (dd, $J = 5.6$, 7.8 Hz, 1H, Pro C*H*N), 7.03 (m, 1H, Ph), 7.14 (m, 1H, Ph), 7.23 (d, $J = 7.0$ Hz, 1H, Ph), 8.08 (d, $J = 8.1$ Hz, 1H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.1, 171.7, 170.8, 144.1, 133.4, 128.2, 125.9, 125.3, 118.2, 71.2, 64.6, 60.8, 60.4, 59.9, 56.7, 56.5, 53.0, 49.1, 38.1, 30.0, 29.1, 26.2, 25.8, 15.2, 11.2; MS: 460 (9; $M+H^+$), 341 (25), 281 (17), 217 (90), 119 (25), 86 (12), 70 (100); FAB HRMS *m/e* 460.2440, M^+ calcd for $C_{24}H_{34}N_3O_6$ 460.2447.

4.42. Epoxyaldehyde 53. Oxidative cleavage of diol 52

SiO_2 (700 mg) was suspended in DCM (6 mL), and $NaIO_4$ (0.7 mL, 0.65 M in water, 0.45 mmol) was added dropwise to form a flaky suspension. Then, a solution of diol **52** (80 mg, 0.17 mmol, 1.0 equiv) in DCM (6 mL) was added dropwise at 25 °C. After 1 h at this temperature, the crude mixture was filtered through a fritted glass funnel, removing the silica, and the solid was washed twice with DCM. The liquid phase was concentrated until dryness to obtain a crude that corresponded to aldehyde **53** (74 mg, 99%), as a white solid, practically

pure by NMR, not requiring further purification: $R_f = 0.56$ (silica gel, 5% MeOH in AcOEt); $[\alpha]_D$ −99.9 (*c* 0.11, CH_2Cl_2); ^1H NMR ($CDCl_3$, 400 MHz) δ 0.87 (t, $J = 7.0$ Hz, 3H, CH_2CH_3), 0.98–1.07 (m, 1H, Ile CH_2), 1.00 (d, $J = 6.5$ Hz, 3H, $CHCH_3$), 1.40–1.50 (m, 1H, Ile CH_2), 1.81–1.89 (m, 1H, $CHCH_3$), 1.97–2.06 (m, 2H, Pro CH_2), 2.20–2.28 (m, 2H, Pro CH_2), 3.16–3.27 (m, 2H, $PhCH_2$), 3.40 (dd, $J = 2.2$, 5.9 Hz, 1H, $CH(O)CHC(=O)$), 3.69 (d, $J = 2.2$ Hz, 1H, $CH(O)CHC(=O)$), 3.75–3.90 (m, 2H, Pro CH_2N), 4.02–4.08 (m, 1H, indoline CH_2N), 4.38–4.45 (m, 1H, indoline CH_2N), 4.63 (dd, $J = 7.0$, 9.1 Hz, 1H, Ile $CHNH$), 4.75 (dd, $J = 4.8$, 7.5 Hz, 1H, Pro CHN), 6.72 (d, $J = 9.1$ Hz, 1H, Ile $CHNH$), 6.97–7.01 (m, 1H, Ph), 7.15 (m, 2H, Ph), 8.15 (d, $J = 8.1$ Hz, 1H, Ph), 8.99 (d, $J = 5.9$ Hz, 1H, CHO); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 194.5, 169.7, 169.5, 165.2, 142.8, 131.1, 127.5, 124.5, 123.9, 117.3, 58.8, 58.3, 54.5, 52.6, 47.8, 47.7, 37.8, 28.8, 28.1, 25.0, 24.3, 15.4, 10.9; MS: 428 (100; $M+H^+$), 356 (20), 309 (19), 239 (21), 237 (26), 217 (28), 196 (33), 120 (37), 119 (20), 118 (18), 73 (100), 70 (64); FAB HRMS *m/e* 428.2173, M^+ calcd for $C_{23}H_{30}N_3O_5$ 429.2185.

4.43. Epoxyacid 54. Oxidation of the aldehyde 53

Epoxyaldehyde **53** (74 mg, 0.174 mmol, 1.0 equiv) was dissolved in THF (5 mL), and *m*-CPBA (136 mg, 0.79 mmol, 3.0 equiv) was added. The reaction was stirred at room temperature for 3 days, after which, the solvent was evaporated and the crude was subjected to purification by flash column chromatography (silica gel, 30% MeOH in AcOEt → MeOH) to obtain epoxyacid **54** (56 mg, 73%) as a colourless oil: ^1H NMR (CD_3OD, 400 MHz) δ 0.89 (t, $J = 7.5$ Hz, 3H, CH_2CH_3), 1.02 (d, $J = 7.0$ Hz, 3H, $CHCH_3$), 1.10–1.19 (m, 1H, Ile CH_2), 1.53–1.64 (m, 1H, Ile CH_2), 1.91 (m, 1H, $CHCH_3$), 2.00 (m, 2H, Pro CH_2), 2.13–2.20 (m, 1H, Pro CH_2), 2.31–2.38 (m, 1H, Pro CH_2), 3.21–3.25 (m, 2H, $PhCH_2$), 3.38 (d, $J = 1.6$ Hz, 1H, $CH(O)CH$), 3.54 (d, $J = 1.6$ Hz, 1H, $CH(O)CH$), 3.73–3.78 (m, 1H, Pro CH_2N), 3.98–4.05 (m, 1H, Pro CH_2N), 4.13–4.19 (m, 1H, indoline CH_2N), 4.36–4.43 (m, 1H, indoline CH_2N), 4.58 (d, $J = 9.1$ Hz, 1H, Ile $CHNH$), 4.77 (dd, $J = 4.8$, 7.0 Hz, 1H, Pro CHN), 7.02 (m, 1H, Ph), 7.14 (m, 1H, Ph), 7.22 (d, $J = 7.0$ Hz, 1H, Ph), 8.08 (d, $J = 8.1$ Hz, 1H, Ph); ^{13}C NMR (CD_3OD, 100 MHz) δ 172.4, 172.2, 170.2, 144.5, 133.8, 128.6, 126.3, 125.7, 118.6, 61.6, 57.0, 56.1, 54.5, 38.4, 30.38, 29.5, 26.6, 26.3, 16.1, 11.5.

4.44. Epoxypeptide 55. Coupling of epoxyacid 54 with *n*-propylamine

To a solution of **54** (54 mg, 0.12 mmol, 1.0 equiv) in DCM (3 mL) was added Hünig's base (24 μL, 0.14 mmol, 1.2 equiv), *n*-propylamine (12 μL, 0.14 mmol, 1.2 equiv) and BOP (64.3 mg, 0.15 mmol, 1.2 equiv) at 25 °C. After stirring overnight at this temperature, the reaction was worked up by diluting with DCM and washing with water and a solution of citric acid. The organic extracts were dried with $MgSO_4$ and evaporated. Purification by flash column chromatography (silica gel, AcOEt) afforded pure amide **55** (55.2 mg, 90%) as a pale brown solid: $R_f = 0.39$ (silica gel, AcOEt); ^1H NMR (CD_3OD, 400 MHz) δ 0.92 (t, $J = 7.4$ Hz, 3H, Ile CH_2CH_3), 0.93 (t, $J = 7.0$ Hz, 3H, $CH_2CH_2CH_3$), 1.05 (d, $J = 6.8$ Hz, 3H, $CHCH_3$), 1.15–1.24 (m, 1H, Ile CH_2), 1.46–1.58 (sext, $J = 7.0$ Hz, 2H, $CH_2CH_2CH_3$), 1.56–1.65 (m, 1H, Ile CH_2), 1.84–1.96 (m, 1H, $CHCH_3$), 1.95–2.07 (m, 2H, Pro CH_2), 2.12–2.24 (m, 1H, Pro CH_2), 2.30–2.43 (m, 1H, Pro CH_2), 3.17 (t, $J = 7.0$ Hz, 2H, $CH_2CH_2CH_3$), 3.21–3.26 (m, 2H, $PhCH_2$), 3.52 (d, $J = 1.6$ Hz, 1H, $CH(O)CH$), 3.62 (d, $J = 1.6$ Hz, 1H, $CH(O)CH$), 3.71–3.79 (m, 1H, Pro CH_2N), 3.98–4.05 (m, 1H, Pro CH_2N), 4.13–4.20 (m, 1H, indoline CH_2N), 4.35–4.43 (m, 1H, indoline CH_2N), 4.57 (d, $J = 8.7$ Hz, 1H, Ile $CHNH$), 4.76 (dd, $J = 5.3$, 7.8 Hz, 1H, Pro CHN), 7.02 (m, 1H, Ph), 7.14 (m, 1H, Ph), 7.23 (d, $J = 7.3$ Hz, 1H, Ph), 8.08 (d, $J = 8.1$ Hz, 1H, Ph); ^{13}C NMR (CD_3OD, 100 MHz) δ 172.3, 172.2, 169.1, 169.0, 144.5, 133.8, 128.6, 126.3, 125.7, 118.6, 61.2, 57.2, 55.2, 54.8, 42.6, 38.4, 30.4, 29.5, 26.6, 26.3, 24.0, 16.0, 12.1, 11.5.

4.45. Indole epoxypeptide 56. Oxidation of indoline epoxypeptide 55 with DDQ

To a solution of epoxypeptide **55** (114 mg, 0.23 mmol, 1.0 equiv) in benzene (10 mL) was added DDQ (214 mg, 0.94 mmol, 4.0 equiv), and the solution was heated at reflux for 16 h under Ar atmosphere. After that time, ether (15 mL) was added and the organic phase was washed extensively with saturated aqueous $NaHCO_3$ solution until no colour changes of the organic layer were apparent. The organic extracts were dried with $MgSO_4$ and the solvent evaporated, to afford indole amide **56** (100 mg, 88%) as a brown solid, which was used in the next step without further purification: $R_f = 0.55$ (silica gel, 30% AcOEt in hexanes); ^1H NMR ($CDCl_3$, 400 MHz) δ 0.85–0.89 (m, 6H, $2 \times CH_3$), 1.05 (d, $J = 7.0$ Hz, 1H, $CHCH_3$), 1.45–1.51 (m, 2H, $CH_2CH_2CH_3$), 1.81–1.89 (m, 1H, $CHCH_3$), 2.05–2.22 (m, 3H, Pro CH_2CH_2), 2.34–2.40 (m, 1H, Pro CH_2), 3.15–3.20 (m, 2H, $CH_2CH_2CH_3$), 3.42 (d, $J = 2.2$ Hz, 1H, $CH(O)CH$), 3.49 (d, $J = 2.2$ Hz, 1H, $CH(O)CH$), 3.79–3.85 (m, 1H, Pro CH_2N), 3.90–3.96 (m, 1H, Pro CH_2N), 4.64 (dd, $J = 7.5$, 9.1 Hz, 1H, Ile $CHNH$), 5.29 (dd, $J = 4.3$, 8.6 Hz, 1H, Pro CHN), 6.09 (t, $J = 5.6$ Hz, 1H, N*H*n-Pr), 6.66 (d, $J = 3.7$ Hz, 1H, $CH=CHN$), 6.71 (d, $J = 9.1$ Hz, 1H, Ile $CHNH$), 7.25–7.27 (m, 1H, Ph), 7.30–7.34 (m, 1H, Ph), 7.51–7.55 (m, 2H, Ph, $CH=CHN$), 8.40 (d, $J = 8.1$ Hz, 1H, Ph); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.4, 169.7, 169.6, 165.8, 135.8, 130.2, 125.1, 124.0, 123.9, 120.8, 116.7, 109.8, 59.1, 54.8, 54.7, 54.5, 47.6, 40.7, 37.5, 29.5, 24.9, 24.4, 22.5, 15.2, 11.1, 10.8.

4.46. CA-074 (8). Hydrolysis of 56

Indole-amide **56** (21 mg, 0.044 mmol, 1.0 equiv) was dissolved in THF (1 mL), and LiOH (0.871 mL, 0.1 M in H_2O, 2.0 equiv) was added dropwise at 0 °C. After 30 min, the aqueous solution was extracted with AcOEt and the aqueous phase was treated with Amberlyst-15 until a pH of 5 was achieved, and then extracted with

AcOEt (3×5 mL). After drying with $MgSO_4$ and solvent evaporation, epoxyacid **8** (9.65 mg, 60%) was obtained as a white solid.[21]

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