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RESEARCH ARTICLE

Convenient synthesis of alternatively-bridged tryptophan ketopiperazines and their activities against trypanosomatid parasites

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Abstract: There is an urgent need for the development of new treatments against trypanosomatid parasites; the causative agents of some of the most debilitating diseases in the developing world. This work targets an interesting 6-5-6-6 fused carboline scaffold, accessing a range of substituted derivatives through stereospecific intramolecular Pictet-Spengler condensation. Modification of the cyclisation conditions allowed retention of the carbamate protecting group and gave insight into the reaction mechanism. Compounds' bioactivities were measured against *T. brucei*, *T. cruzi*, *L. major* and HeLa cells. We have identified promising pan-trypanocidal lead compounds based on the core scaffold, and highlight key SAR trends which will be useful for the future development of these compounds as potent trypanocidal agents.

Introduction

The trypanosomatids are a group of biochemically related and exclusively parasitic eukaryotes, responsible for some of the most debilitating neglected tropical diseases in the developing world. The human-infective pathogens Trypanosoma brucei (*T. brucei*), Trypanosoma cruzi (*T. cruzi*), and a range of over twenty Leishmania species are responsible for human African trypanosomiasis (HAT or African sleeping sickness), Chagas' disease and leishmaniasis, respectively.[1] These diseases are endemic in some of the poorest communities in the world, hence their status as neglected tropical diseases, and millions of people live in at-risk areas.[2–4] Current treatments for these diseases are inadequate, with many being prohibitively expensive for the communities in which the diseases are endemic,[4,5] and are accompanied by unacceptable side effects and mounting parasite resistance.[6–8] The drastic need for the development of new affordable and efficacious drugs for the treatment of these diseases has been reported by the WHO.[9] Indole-derived scaffolds, including β-carbolines and diketopiperazines, have a history of activity against the trypanosomatids, with a number of both natural and synthetic examples showing good trypanocidal and leishmanicidal activities. A section in a recently published review is dedicated to the use of indole-derived natural product cores as trypanocidal agents.[10]

The focus of this work, the bridged β-carboline scaffold **1**, bears an sp^3-rich 6-5-6-6 heterocyclic scaffold, and has previously been reported as a useful tryptophan-derived peptidomimetic.[11] Structurally related analogues have been analyzed for their bioactivity against the breast cancer resistance protein efflux transporter (BCRP), with compound **2** showing promising activity.[12] Two independent routes accessing the general scaffold **1** are published within the literature (Figure 1). Route A consists an initial intermolecular Pictet-Spengler condensation of L-tryptophan (L-Trp) and an α-amino aldehyde (**3**) followed by an amide cyclisation. The route suffers from a lack of stereocontrol and leads to formation of a tetracyclic byproduct, meaning yields are poor.[11,13] Route B involves the conversion of a diketopiperazine precursor **5** via an imidoyl chloride intermediate (not shown), but requires hazardous phosgene reagents.[12] The lack of a robust and facile synthetic route that the biological profile of compounds containing this scaffold are largely unexplored. Herein we present a stereospecific synthesis enabling the access of scaffold **1**, which overcomes the synthetic challenges and dangerous reagents of previously reported syntheses. Additionally, we report promising bioactivity against the trypanosomatid parasites; *T. brucei*, *T. cruzi* and *Leishmania* major (*L. major*).

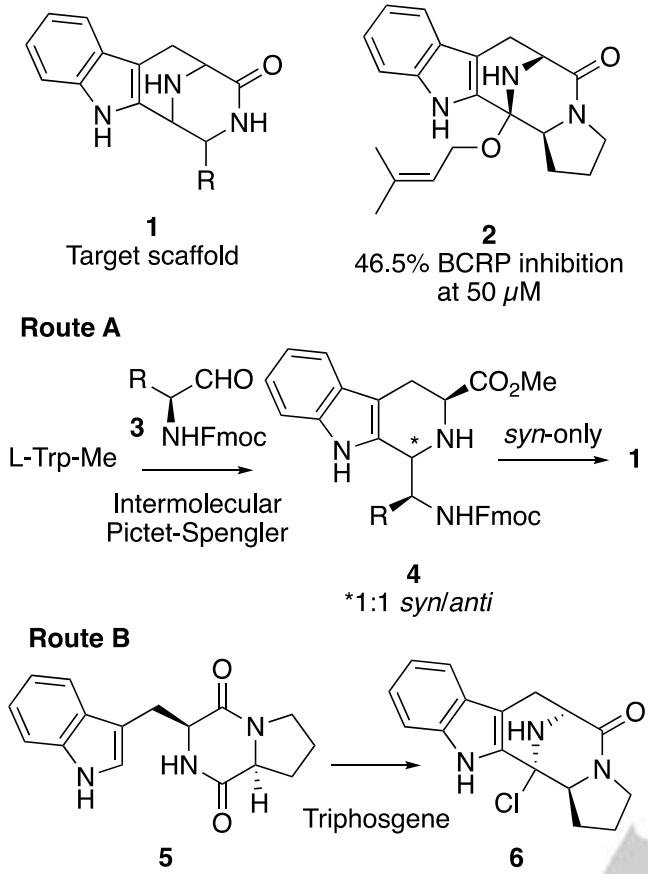


Figure 1. Alternatively bridged tryptophan-derived ketopiperazine scaffold **1** and analogue **2** with reported activity against BCRP. Reported synthetic strategies accessing the target scaffold: Route A – employed by both Tourwé and Greico[11,13]; Route B – reported by Stevens and co-workers.[12]

Results and Discussion

As a diketopiperazine-derived scaffold, we envisioned the construction of the core structure from the dipeptidic aldehyde **7**, which could be assembled from Trp and a second amino acid partner (AA2) using established peptide coupling chemistry. The key step of the synthetic route was an intramolecular acid-catalyzed Pictet-Spengler condensation of aldehyde 7, proceeding through the intermediate imine **8** (Figure 2). While intramolecular Pictet-Spengler condensations have been previously reported,[14–16] they are typically implemented in the construction of comparatively less strained 1,2-bridged β-carboline and isoquinoline structures.

The planned transformation allows for the efficient conversion of the linear indole-bearing precursor **7** to the fused 6-5-6-6 polycyclic scaffold **9**, with the formation of two new rings (Figure 2). Retention of input amino acid stereochemistry, translating to the 2-position (blue) and 5-position (red) of the new cycle, allows tailoring of product stereoconfiguration through readily accessible chiral materials. Furthermore, the newly installed stereochemistry at the 1-position (green) is governed by intramolecular facial attack into the intermediate imine **8**, and is controlled by input tryptophan stereochemistry, with L-Trp and D-Trp enforcing 1*S* and 1*R*, respectively.

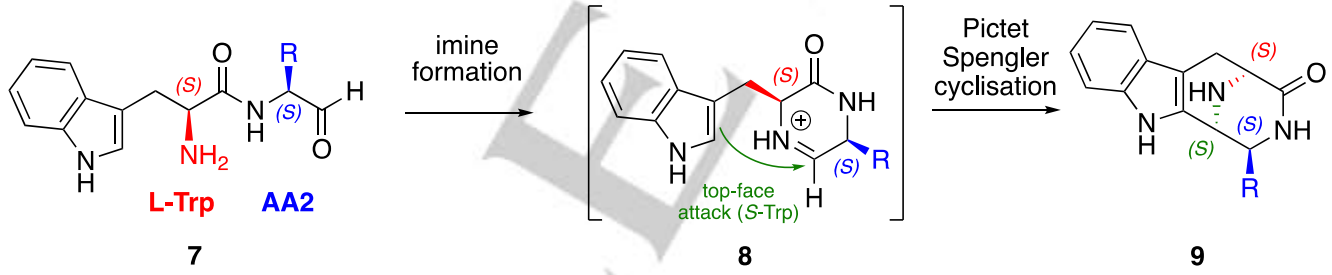
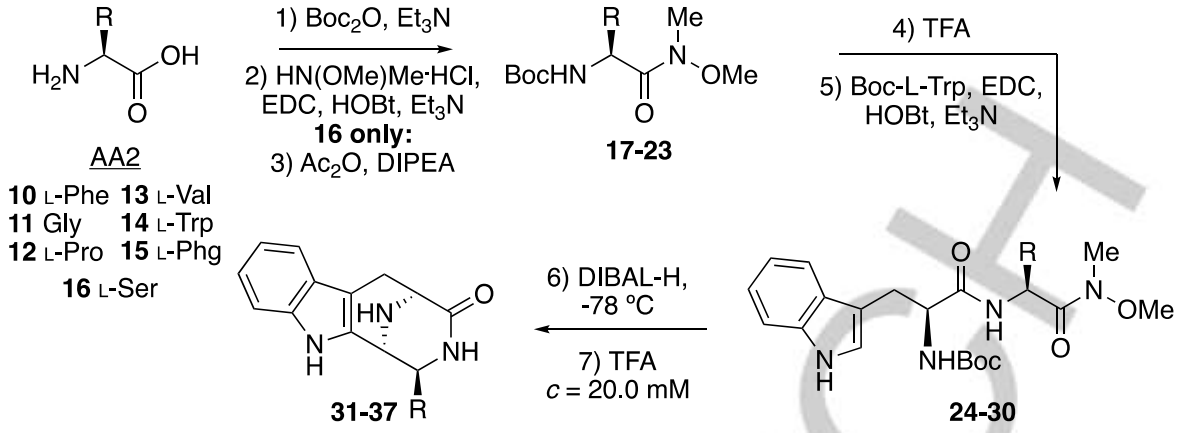


Figure 2. Proposed dipeptidic aldehyde **7** and conversion to the scaffold **9** via an intramolecular acid catalyzed Pictet-Spengler condensation. Stereochemistry of the input amino acids are conserved and define the 5-position (Trp, red) and 2-position (AA2, blue). The newly formed stereocenter at the 1-position (green) is formed by facial attack of the indole which is directed by Trp stereochemistry. Example stereochemistry is shown for L-configured Trp and AA2.

To improve synthetic accessibility of aldehyde, we targeted dipeptide precursors **24**-**30** which carry a Weinreb amide terminus – enabling facile and selective reduction to the corresponding aldehyde while reducing unwanted reactivity during assembly. Secondly, Boc protection of the Trp primary amine was utilized due to its acid lability, giving the opportunity for a one-pot deprotection and Pictet-Spengler cyclisation process. Precursors **24**-**30** were prepared as single diastereomers, with the exception of **29**, which underwent epimerization of the phenyl substituted α-position upon TFA treatment (scheme 1, step 4) to give a 1:1 mixture of *S*,*S*/*S*,*R* diastereomers.

Reduction of dipeptide precursors **24-30** with DIBAL-H provided the desired selective reduction to the intermediate aldehydes. Following aqueous workup aldehydes were taken forward immediately, without concentration or further purification, in order to minimize potential epimerization of the highly reactive α-stereocentre. Subsequent exposure of the intermediate aldehydes to trifluoroacetic acid pleasingly resulted in the proposed one-pot cyclisation and deprotection, giving the desired cyclic products **31-37** in good to moderate yields (Scheme 1 and Table 1).



Scheme 1. Synthesis of dipeptide precursors **24**-**30** from their constituent amino acids and Boc-L-Trp and subsequent reduction and one-pot cyclisation-deprotection.

Table 1. Yields of cycles **31**-**37** from the combined reduction and one-pot cyclisation-deprotection of their respective dipeptide precursors **24**-**30**.

No.	Precursor (AA2)	Yield[a]
31	**24** (L-Phe)	60
32	**25** (Gly)	77
33	**26** (L-Pro)	23
34	**27** (L-Val)	55
35	**28** (L-Trp)	37
36 (38)	**29** (L-Phg)	21 (23)[b]
37	**30** (L-Ser)	26

[a] Isolated two-step combined yield (Scheme 1, step 6/7). [b] Isolated yield of 2*R*-diastereomer **38**.

Cycles **31-35** and **37** were all obtained as single diastereomers, determined by ^{1}H NMR spectroscopy. The connectivity and absolute 1*S*,2*S*,5*S* stereochemical configuration of the phenylalanine derived compound **31** were unambiguously confirmed by X-ray crystallography (Figure 3). The mixed diastereomers of **29** were converted to the two cycles **36** (2*S*) and **38** (2*R*) under the reaction conditions, and pleasingly the two products were separable by flash column chromatography. The stereochemistry of **36** and **38** can be derived from the *S,S* and *S,R* diastereomers of precursor **29**, respectively, but it is likely that the aldehyde intermediate also rapidly epimerizes under TFA treatment.

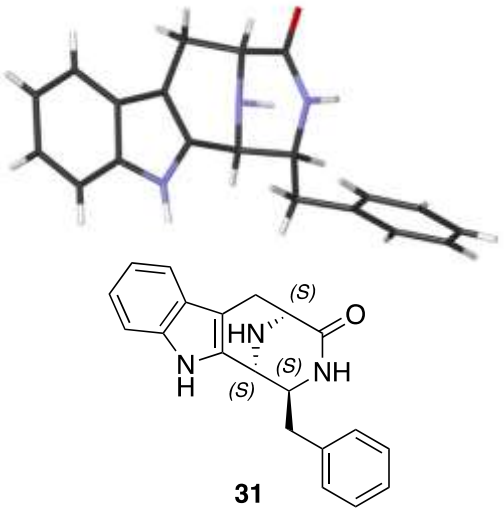
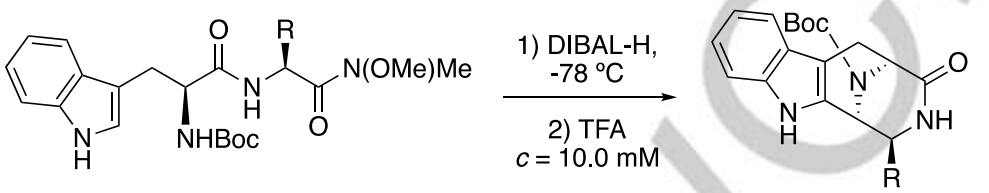


Figure 3. Crystal structure of compound **31** confirming 1*S*, 2*S*, 5*S* stereochemical configuration.

The combined reduction and acid catalyzed intramolecular cyclisation showed good tolerance of various functionalities, with

bulky amino acids valine and phenylglycine tolerated. The proline-derived analogue **33**, which previously received interest for its BCRP bioactivity, showed reduced yield compared to other examples, likely due to increased ring-strain. Pleasingly the reduction-cyclisation of the O-acetylated precursor **30** proceeded with the tandem reductive cleavage of the acetyl protecting group, removing the need for further deprotection steps.

It was found that further dilution of the acidic cyclisation reaction conditions allowed selective conversion of intermediate aldehydes directly to their Boc-protected cycles (Scheme 2). This reactivity is pivotal in suggesting a mechanism of the key cyclisation-deprotection step. The retention of the Boc protecting group indicates that intramolecular cyclisation occurs sequentially before the deprotection step, and suggests that the carbamate group may be important in activating the intermediate imine. The mechanism is presumably similar to the well-reported acyl-iminium Pictet-Spengler condensations often applied in the synthesis of carboline structures.[14,17,18] This modification of reaction conditions would be valuable in cases where further modification of the scaffold is required, removing the need for additional steps to protect the reactive bridging amine.



24, 25, 29, 30 **40, 41, 42** and **43/44**

Scheme 2. Selective synthesis of Boc-protected cycles **40**, **41**, **42** and **43**/**44** through modification of reaction concentration.

Table 2. Selective cyclisation of compounds **24**, **25**, **29** and **30** to the Boc-protected cycles **40**, **41**, **42**, and **43**/**44** respectively.

No.	Precursor (AA2)	Yield[a]
40	**24** (L-Phe)	70
41	**25** (Gly) (12)	94
42	**30** (L-Ser) (17)	33
43/44	**29** (L-Phg) (16)	45 (1.8:1)[b]

[a] Combined isolated two-step yield (Scheme 2, step 1/2). [b] isolated ratio of cycles **43**:**44**.

Dipeptides **24**, **25**, and **30** were subjected to the modified cyclisation conditions, and the resulting Boc-protected scaffolds **40**, **41** and **42** were isolated as single diastereomers (assigned by ^{1}H NMR) in good yields, as shown in Table 2. **29** gave a diastereomeric mixture of the 2*S* cycle (**43**) and 2*R* cycle (**44**) – which is in line with the prior synthesis of the deprotected cycles. Unlike their deprotected counterparts, however, **43** and **44** could not be separated by column chromatography and were analyzed for bioactivity as a 1.8:1 mixture (**43**:**44**).

Compounds were screened against three trypanosomatid parasite cell lines: *T. brucei*, *T. cruzi*, and *Leishmania major* (*L. major*) in addition to HeLa cells. Dipeptide precursors **24**-**30** also showed some interesting trypanocidal and leishmanicidal activity, and their EC_{50} can be found in Supplementary Table 1. Corresponding EC_{50} values and selectivity indexes of cycles **31**-**38** and their *N*-substituted analogues are listed in Table 3. The drugs for neglected diseases initiative (DNDi) have published guideline criteria for the identification of new leads for development against HAT and visceral leishmaniasis. Lead criteria outlined for small molecules include: <10 µM antiparasitic EC_{50}; >10-fold selectivity vs human cell lines; correlation of biochemical and whole-cell activity; and chemical accessibility in <8 steps.[19] We have applied these criteria to the compounds' bioactivity in the following discussion to help identify promising leads.

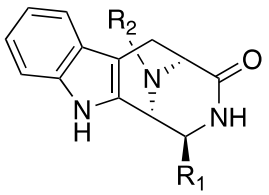
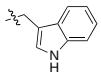


Table 3. Trypanocidal and mammalian activities of unsubstituted cycles **31**-**38** and protected cycles **40**-**42** and **43**/**44**. [a]

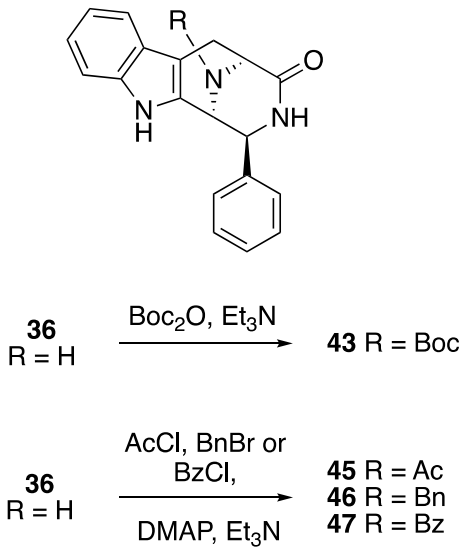
No.	R_1	R_2	*T. brucei* [b] (SI)[e]	*T. cruzi* [c] (SI)[e]	*L.major* [d] (SI)[e]	HeLa (SI)[e]

31		H	52.1 (3.5)	59.0 (3.1)	116.2 (1.2)	181.0
32	H	H	188.8 (>1.3)	>250 (nd)	>250 (nd)	>250
33		H	>250 (nd)	>250 (nd)	>250 (nd)	>250
34		H	189.3 (>1.3)	>250 (nd)	>250 (nd)	>250
35		H	161.1 (1.6)	>250 (nd)	>250 (nd)	>250
36		H	130.8 (1.9)	>250 (nd)	89.1 (2.8)	>250
37		H	70.5 (3.3)	169.3 (1.5)	>250 (nd)	>250
38		H	111.4 (2.2)	>250 (nd)	169.3 (1.5)	249.9
40		Boc	9.2 (2.3)	4.7 (4.4)	8.5 (2.4)	20.7
41	H	Boc	36.1 (3.7)	62.1 (2.1)	57.9 (2.3)	132.0
42		Boc	67.7 (2.4)	167.3 (1.0)	47.8 (3.4)	164.4
43/44		Boc	2.3 (13.0)	9.0 (3.2)	7.1 (4.2)	30.0

[a] Mean EC_{50} (n=4), all activities given in µM. [b] *T. brucei brucei* bloodstream form trypomastigotes. [c] *T. cruzi* epimastigote form. [d] *L. major* promastigote form. [e] Selectivity index (Mammalian EC_{50} / Parasite EC_{50}).

Deprotected cycles **31-38** showed overall poor activity against the three trypanosomatid cell lines, with the benzyl substituted compound **31** showing the most favorable overall bioactivity. Promising bioactivity was observed for Boc-protected cycles **40-43/44,** displaying up to 50-fold increased potency compared to their de-protected counterparts. Benzyl substituted compound **40** and phenyl substituted diasteromeric mixture **43/44** both showed sub-10 µM activity against all three parasite species, in agreement with the potency guidelines outlined by DNDi in their hit selection criteria. The entire suite of compounds also meets the DNDi criteria for chemical accessibility, being prepared in <8 steps. Unfortunately, an increase in mammalian cytotoxicity was also observed in the protected cycles compared to their deprotected counterparts. Nevertheless, the mixture of **43/44** pleasingly surpassed the desired 10-fold selectivity for *T. brucei* compared to mammalian cells and displayed modest accompanying SI values of 3.2 and 4.3 for *T. cruzi* and *L. major*, respectively.

We sought to investigate the clear importance of *N*-substitution upon trypanocidal activity and prepared a range of *N*-substituted compounds, based on the promising pan-trypanocidal phenyl substituted scaffold **43**. *N*-substitutions were selected to sample steric and H-bonding interactions. Compounds **45** (Ac), **46** (Bn) and **47** (Bz) and enantiopure **43** (Boc) were prepared from the single diastereomer **36**, as shown in Scheme 3.



Scheme 3. Synthesis of *N*-substituted compounds **43** and **45**-**47** from compound **36**.

The corresponding EC_{50} values of cycle **36**, along with the newly synthesized N-substituted cycles **43** and **45-47** are shown in Table 3. Analysis of SAR revealed that compound potency increased when the parent cycle **36** was substituted with larger groups: H<Ac<Bn<Bz≈Boc. In *T. cruzi* and HeLa cells, the *tert*-butyl carbamate substituted scaffold **43** gave the highest potency,

whereas in *T. brucei* and *L. major* the benzoylated compound **47** gave improved potency. It is possible that the substituted analogues **43** and **47** gain increased potency through increased passive uptake, which is facilitated by the lipophilic carbamate and amide substituents. While subsequent cleavage by the parasites' cytosolic proteases is possible, the similar increase in potency displayed by the benzylated compound **46** suggests that this is not the case, as it is unlikely that this stable C-N linkage would be rapidly cleaved. We therefore suggest that these substitutions facilitate binding to the target of this scaffold.

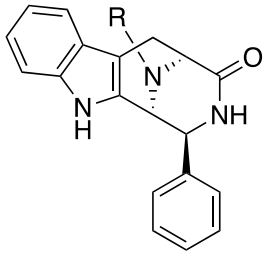
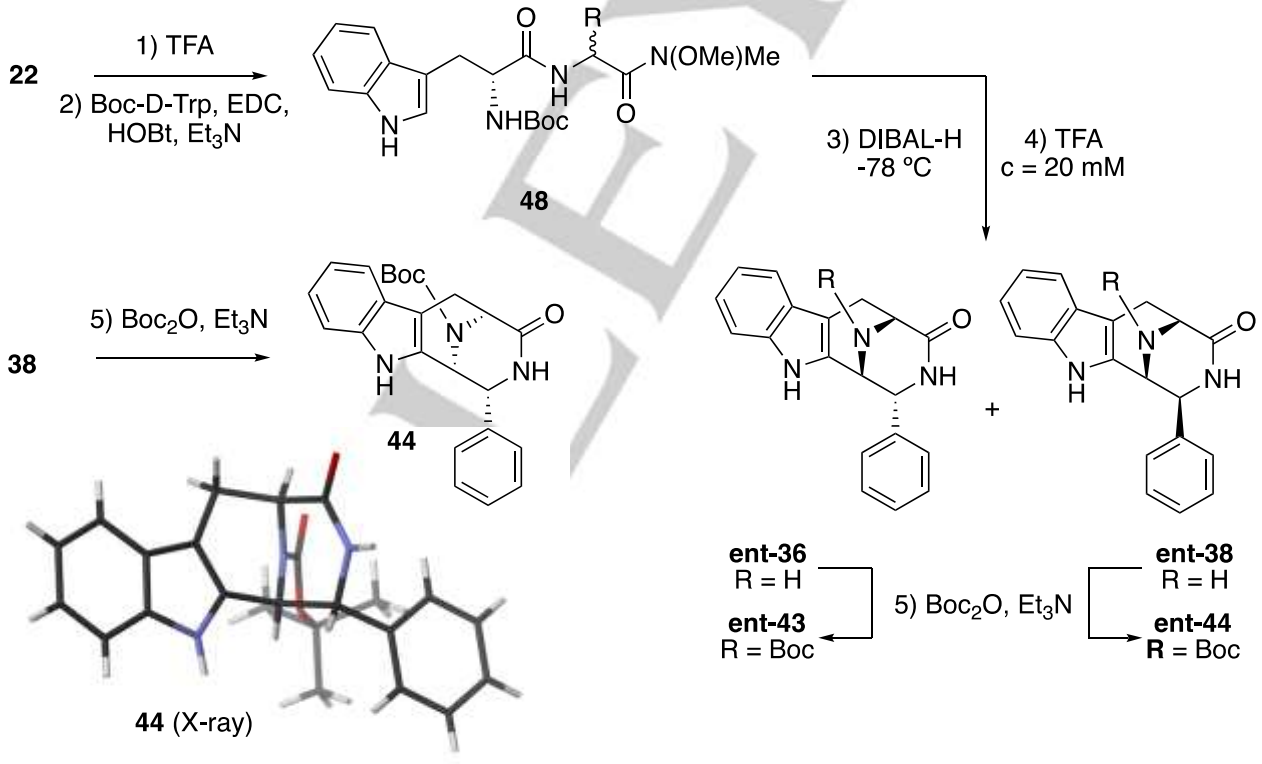


Table 4. Trypanocidal and mammalian activities of *N*-substituted compounds **36**, **43** and **45**-**47**[a]

No.	R=	*T. brucei* [b] (SI)[e]	*T. cruzi* [c] (SI)[e]	*L. major* [d] (SI)[e]	HeLa (SI)[e]
36	H	130.8 (2.1)	>250 (nd)	89.1 (0.7)	>250
43	Boc	30.4 (2.5)	21.0 (3.6)	17.4 (4.3)	75.8
45	Ac	75.1 (3.3)	>250 (nd)	177.7 (1.4)	>250
46	Bn	37.2 (3.0)	47.7 (2.3)	29.0 (3.8)	110.7
47	Bz	15.1 (5.6)	32.8 (2.6)	13.0 (6.5)	84.6

[a] Mean EC_{50} (n=4), all activities given in µM. [b] *T. brucei brucei* bloodstream form trypomastigotes. [c] *T. cruzi* epimastigote form. [d] *L. major* promastigote form. [e] Selectivity index (Mammalian EC_{50} / Parasite EC_{50}).

Notably, the bioactivity of enantiopure **43** did not align with the previously tested **43/44** diastereomeric mixture. We therefore decided to probe the relationship between scaffold stereochemistry and bioactivity, using this promising phenyl substituted scaffold as the basis for the analysis. Preparation of the D-Trp derived dipeptide precursor **48** gave a mixture of diastereomers, in line with the epimerization observed with L-Trp derived precursor **29**. Subsequent exposure to the developed reduction and one-pot cyclisation-deprotection conditions gave access to the two remaining enantiomers **ent-36** and **ent-38**, which were separable by flash column chromatography. With all four possible stereochemical configurations of the N-H structures in hand, the remaining *N*-Boc analogues **44**, **ent-43** and **ent-44** were prepared from their corresponding single diastereomers **38**, **ent-36** and **ent-38** (Scheme 4). X-ray analysis of **44** confirmed the relative 1,2,5 stereochemical configuration, which was assigned as 1*S*, 2*R*, 5*S* based on the use of *S*-tryptophan.



Scheme 4. Synthesis of enantiomers **ent-36** and **ent-38** from precursor **48**, preparation of Boc-substituted cycles **44**, **ent-43** and **ent-44** and X-ray crystal structure of **44** confirming relative stereochemistry (absolute 1*S*, 2*R*, 5*S* configuration assigned from derivative *S*-tryptophan).

The corresponding EC_{50} values of the suite cycles are displayed in Table 5. While *N*-H cycles mostly maintained a low activity, compound **ent-38** showed 20-fold increased activity against *L. major*, compared to its enantiomer **38**, with an EC_{50} of 8.4 µM. This gain in antiparasitic activity, however, did not extend to *T. brucei* nor *T. cruzi*. The compound showed low potency

against HeLa cells providing a promising SI value of 14.8 for *L. major*.

N-Boc cycles followed the previously observed trends, showing 4 to >100-fold increased antiparasitic activity over their unsubstituted counterparts and increased mammalian cytotoxicity. **Ent-43** shows 10-fold higher potency against *T. brucei* than **43**, and a high SI of 29.7. Despite a lack of pan-trypanocidal activity, this molecule may be a promising lead for further development against *T. brucei*.

44 and **ent-44** both displayed promising sub-10 µM activity against all three parasite cell lines. The similar activity of these enantiomers is interesting; however, it is hard to draw any further conclusions about the mechanism behind this activity. Such ambiguity is one drawback of the phenotypic assay employed in this work. Further analysis of the cellular target of these molecules would hopefully illuminate more nuanced SAR trends relating to compounds' stereochemistry.

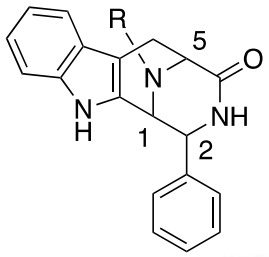


Table 5. Trypanocidal and mammalian activities of *N*-H diastereomers **36**, **38**, **ent-36** and **ent-38** and with *N*-Boc diastereomers **43**, **44**, **ent-43** and **ent-44**.[a]

No.	Stereochemical configuration	*R*=	*T. brucei* [b] (SI)[e]	*T. cruzi* [c] (SI)[e]	*L. major* [d] (SI)[e]	HeLa (SI)[e]
36	1*S*,2*S*,5*S*	H	130.8 (1.9)	>250 (nd)	89.1 (2.8)	>250
38	1*S*,2*R*,5*S*	H	111.4 (2.3)	>250 (nd)	169.3 (1.5)	249.9
ent-36	1*R*,2*R*,5*R*	H	70.9 (3.5)	>250 (nd)	162.9 (1.5)	>250
ent-38	1*R*,2*S*,5*R*	H	56.2 (2.2)	>250 (nd)	8.4 (14.8)	124.6
43	1*S*,2*S*,5*S*	Boc	30.4 (2.5)	21.0 (3.6)	17.3 (4.4)	75.8
44	*1S*,2*R*,5*S*	Boc	9.9 (1.7)	2.0 (8.8)	10.4 (1.7)	17.6
ent-43	1*R*,2*R*,5*R*	Boc	2.8 (29.7)	64.0 (1.3)	29.8 (2.8)	83.2
ent-44	1*R*,2*S*,5*R*	Boc	2.1 (11.2)	8.4 (2.8)	9.1 (2.6)	23.6

[a] Mean EC_{50} (n=4), all activities given in µM. [b] *T. brucei brucei* bloodstream form trypomastigotes. [c] *T. cruzi* epimastigote form. [d] *L. major* promastigote form. [e] Selectivity index (Mammalian EC_{50} / Parasite EC_{50}).

Conclusion

We have developed a convenient synthetic route to the stereochemically rich structures based upon scaffold **1**. The approach utilizes the conversion of an advanced linear dipeptide precursor to the desired bridged scaffold through a scarcely reported 1,3-linked intramolecular Pictet-Spengler condensation. Linear peptide precursors are derived from D or L-tryptophan coupled to a range of α-amino acids, allowing the tailoring of product stereochemistry. The epimerization of the α-position of phenyl-substituted precursors ultimately provided access to two separable diastereomers for biological evaluation. *N*-Boc analogues may be accessed directly through modified reaction conditions, and display significantly higher activities against trypanosomatid parasites. Structure-activity relationships revealed that benzylic or phenyl substituents at the 2-position and substitution of the bridging nitrogen are crucial for trypanocidal activity. Three of the compounds developed (**40**, **44** and **ent-44**) show promising pan-trypanocidal activity surpassing the DNDi activity guideline for leads against HAT and visceral leishmaniasis. These compounds also generally show promising selectivity, with lead compound **52** surpassing the >10-fold guideline for selectivity in *T. brucei* while maintaining sub-10 µM activity against all parasites. These structures could be utilized for the future development of trypanocidal agents, or in the discovery of desperately needed drug targets within *T. brucei*, *T. cruzi* and *Leishmania*.

Experimental Section

General procedure for the synthesis of cyclic β-carbolines **31-48**, and **ent-36**, **ent-38**

A stirred solution of the corresponding dipeptide precursor (2.0 mmol) in dry tetrahydrofuran (20 mL) was cooled to -78 °C. After stirring for 15 minutes, a solution of di-isobutyl aluminum hydride (20 mL, 1.0 M in hexanes, 20 mmol) was added via syringe pump over 1 h. The reaction was stirred for a further 4 h, then diluted with dichloromethane (50 mL). Methanol (5 mL) was added dropwise and the reaction allowed to warm to

room temperature. The mixture was washed with potassium hydrogen sulfate (aq., 1 M, 100 mL), dried (MgSO4) and filtered.

To the resulting organics was added trifluoroacetic acid (8.0 mL, 100 mmol). The reaction was stirred for 16 h, before being quenched with sodium bicarbonate (aq., 1 M, 100 mL), then the organics were separated and the aqueous further extracted with dichloromethane (50 mL). Combined organics were dried (MgSO4), filtered and concentrated under reduced pressure. Crude products were purified via flash column chromatography (5% methanol/DCM) to give cyclic products.

31 – (1*S*,2*S*,5*S*)-2-benzyl-1,2,3,5,6,11-hexahydro-4H-1,5-epiminoazocino[4,5-β]indol-4-one

31 was prepared via the general procedure above using dipeptide precursor **24** (990 mg, 2.0 mmol) to give the product as a pale brown solid (380 mg, 60%). Mp = 280-282 ºC (decomposition); Rf = 0.21 (5% MeOH/DCM); ^{1}H NMR (500 MHz, MeOD) δ 7.47 (dt, *J* = 1.0, 7.9 Hz, 1H), 7.40 – 7.34 (m, 3H), 7.31 – 7.28 (m, 1H), 7.28 – 7.25 (m, 2H), 7.14 (ddd, *J* = 1.2, 7.1, 8.2 Hz, 1H), 7.05 (ddd, *J* = 1.0, 7.1, 8.0 Hz, 1H), 4.42 (d, *J* = 4.1 Hz, 1H), 4.29 (ddd, *J* = 3.4, 4.2, 10.9 Hz, 1H), 3.96 (dd, *J* = 1.3, 6.2 Hz, 1H), 3.34 – 3.27 (m, 1H), 3.19 (ddd, *J* = 0.8, 6.2, 15.9 Hz, 1H), 3.03 (dd, *J* = 1.4, 15.9 Hz, 1H), 2.34 (dd, *J* = 10.9, 13.8 Hz, 1H); ^{13}C NMR (126 MHz, MeOD) δ 174.1, 136.6, 136.5, 129.6, 128.8, 128.8, 126.8, 126.6, 121.5, 118.7, 117.4, 110.8, 107.4, 59.0, 52.6, 49.2, 38.3, 26.0; $[\alpha]_D^{20}$ = –173.3 (c 0.4, MeOH); HRMS (ESI +) Calc. For $[C_{20}H_{20}N_3O]^+$ req. 318.1601 Found 318.1598.

X-ray crystallographic data available.

32 – (1*S*,5*S*)-1,2,3,5,6,11-hexahydro-4H-1,5-epiminoazocino[4,5-β]indol-4-one

32 was prepared via the general procedure above using dipeptide precursor **25** (810 mg, 2.0 mmol) to give the product as an off white solid (349 mg, 77%). Mp = 98-100 ºC; Rf = 0.15 (5% MeOH/DCM); ^{1}H NMR (500 MHz, MeOD) δ 7.50 (d, *J* = 7.9 Hz, 1H), 7.41 (d, *J* = 8.2 Hz, 1H), 7.24 – 7.18 (m, 1H), 7.10 (t, *J* = 7.5 Hz, 1H), 5.18 (d, *J* = 3.9 Hz, 1H), 4.52 (d, *J* = 5.6 Hz, 1H), 4.05 (dd, *J* = 4.2, 13.6 Hz, 1H), 3.66 (d, *J* = 13.5 Hz, 1H), 3.44 (dd, *J* = 5.8, 16.7 Hz, 1H), 3.33 – 3.28 (m, 1H); ^{13}C NMR (126 MHz, MeOD) δ 167.5, 136.9, 126.1, 125.5, 122.7, 119.6, 117.8, 111.3, 106.0, 51.9, 45.2, 44.3, 23.7; $[\alpha]_D^{20}$ = +8.4 (c 1.0, MeOH) Lit. = +7.3; HRMS (ESI +) Calc. For $[C_{13}H_{14}N_3O]^+$ req. 228.1131 Found 228.1133.

33 – (6*S*,13*S*,13a*S*)-1,2,3,6,7,12,13,13a-octahydro-5H-6,13-epiminopyrrolo[1',2':1,2]azocino[4,5-β]indol-5-one

33 was prepared via the general procedure above using dipeptide precursor **26** (890 mg, 2.0 mmol) to give the product as a pale orange solid (124 mg, 23%). Mp = 292-296 ºC (decomposition); Rf = 0.17 (5% MeOH/DCM); ^{1}H NMR (500 MHz, MeOD) δ 7.41 (dt, *J* = 1.0, 7.9 Hz, 1H), 7.32 (dt, *J* = 0.9, 8.2 Hz, 1H), 7.10 (ddd, *J* = 1.2, 7.0, 8.2 Hz, 1H), 7.00 (ddd, *J* = 1.0, 7.0, 8.0 Hz, 1H), 4.45 (d, *J* = 4.1 Hz, 1H), 4.11 (dt, *J* = 4.6, 11.9 Hz, 1H), 3.94 (dd, *J* = 1.5, 5.8 Hz, 1H), 3.42 (ddd, *J* = 2.1, 9.1, 11.7 Hz, 1H), 3.25 (ddd, *J* = 8.0, 10.3, 12.4 Hz, 1H), 3.14 (dd, *J* = 5.8, 15.7 Hz, 1H), 3.04 (dd, *J* = 1.5, 15.7 Hz, 1H), 2.19 (dt, *J* = 5.1, 10.7 Hz, 1H), 1.91 – 1.75 (m, 2H), 1.45 – 1.35 (m, 1H); ^{13}C NMR (126 MHz, MeOD) δ 171.3, 136.3, 129.4, 126.6, 121.3, 118.6, 117.4, 110.6, 107.3, 63.7, 52.3, 48.3, 44.7, 28.7, 26.4, 21.6; $[\alpha]_D^{20}$ = –81.6 (*c* 0.96, MeOH); HRMS (ESI +) Calc. For $[C_{16}H_{17}N_3ONa]^+$ req. 290.1264 Found 290.1261.

34 – (1*S*,2*S*,5*S*)-2-isopropyl-1,2,3,5,6,11-hexahydro-4H-1,5-epiminoazocino[4,5-β]indol-4-one

34 was prepared via the general procedure above using dipeptide precursor **27** (890 mg, 2.0 mmol) to give the product as a pale brown solid (296 mg, 55%). Mp = 241-243 ºC; Rf = 0.17 (5% MeOH/DCM); ^{1}H NMR (500 MHz, MeOD) δ 7.43 (dt, *J* = 1.0, 7.9 Hz, 1H), 7.37 (dd, *J* = 0.9, 8.2 Hz, 1H), 7.11 (ddd, *J* = 1.2, 7.0, 8.2 Hz, 1H), 7.01 (ddd, *J* = 1.0, 7.0, 8.0 Hz, 1H), 4.40 (d, *J* = 4.0 Hz, 1H), 3.95 (dd, *J* = 1.3, 6.3 Hz, 1H), 3.64 (dd, *J* = 4.0, 8.1 Hz, 1H), 3.15 (dd, *J* = 5.8, 15.7 Hz, 1H), 3.01 (dd, *J* = 1.6, 15.7 Hz, 1H), 1.77 (dhept, *J* = 6.6, 8.1 Hz, 1H), 1.22 (d, *J* = 6.7 Hz, 3H), 0.83 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (126 MHz, MeOD) δ 174.5, 136.6, 129.7, 126.5, 121.4, 118.7, 117.2, 110.8, 107.6, 64.3, 52.4), 47.7, 28.8, 26.0, 19.4, 17.1; $[\alpha]_D^{20}$ = –48.5 (c 1.0, MeOH); HRMS (ESI +) Calc. For $[C_{16}H_{20}N_3O]^+$ req. 270.1601 Found 270.1596.

35 – (1*S*,2*S*,5*S*)-2-((1H-indol-3-yl)methyl)-1,2,3,5,6,11-hexahydro-4H-1,5-epiminoazocino[4,5-β]indol-4-one

35 was prepared via the general procedure above using dipeptide precursor **28** (1.06 g, 2.0 mmol) to give the product as a cream solid (363 mg, 51%). Mp = 260-265 ºC (decomposition); Rf = 0.22 (5% MeOH/DCM); ^{1}H NMR (500 MHz, MeOD:$CHCl_3$, 1:1) δ 7.56 (dd, *J* = 1.0, 8.0 Hz, 1H), 7.47 (dt, *J* = 1.0, 7.9 Hz, 1H), 7.39 (dd, *J* = 0.9, 8.2 Hz, 1H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.19-7.10 (m, 2H), 7.10 – 7.02 (m, 2H), 7.02 – 6.97 (m, 1H), 4.45 (t, *J* = 3.4 Hz, 1H), 4.35 (ddt, *J* = 3.3, 5.1, 10.7 Hz, 1H), 3.94 (dd, *J* = 1.3, 6.2 Hz, 1H), 3.38 – 3.29 (m, 2H), 3.17 (dd, *J* = 6.2, 16.0 Hz, 1H), 3.05 (dd, *J* = 1.4, 16.0 Hz, 1H), 2.52 (dd, *J* = 10.9, 14.4 Hz, 1H); ^{13}C NMR (126 MHz, MeOD:$CHCl_3$, 1:1) δ 173.8, 137.1, 136.3, 129.6, 126.7, 126.6, 123.5, 121.8, 121.6, 119.0, 118.9, 117.9, 117.7, 111.5, 111.0, 108.6, 107.5, 57.3, 52.7, 49.1, 28.3, 26.2; $[\alpha]_D^{20}$ = -141.1 (c 1.0 MeOH); HRMS (ESI +) Calc. for $[C_{22}H_{21}N_4O]^+$ req. 357.1710 Found 357.1706.

(1S,5S)-2-phenyl-1,2,3,5,6,11-hexahydro-4H-1,5-epiminoazocino[4,5-β]indol-4-one

36 and **38** were prepared via the general procedure above using dipeptide precursor **29** (960 mg, 2.0 mmol). Products were separated by flash column chromatography to give 36 (130 mg, 21%) and 38 (142 mg, 23%) as off-white solids.

36

Mp = 185-188 ºC; Rf = 0.20 (5% MeOH/DCM); ^{1}H NMR (500 MHz, MeOD) δ 7.45 – 7.41 (m, 1H), 7.34 – 7.29 (m, 1H), 7.25 (t, *J* = 7.4 Hz, 2H), 7.06 – 7.01 (m, 2H), 7.01 – 6.97 (m, 1H), 6.97 – 6.93 (m, 2H), 5.19 (d, *J* = 4.6 Hz, 1H), 4.39 (d, *J* = 4.6 Hz, 1H), 4.02 (dd, *J* = 1.4, 6.3 Hz, 1H), 3.19 (dd, *J* = 6.2, 15.9 Hz, 1H), 3.10 (dd, *J* = 1.5, 15.9 Hz, 1H); ^{13}C NMR (126 MHz, MeOD) δ 174.7, 137.5, 135.9, 129.1, 128.0, 127.9, 126.9, 126.2, 121.1, 118.4, 117.3, 110.6, 107.0, 62.2, 52.3, 51.0, 25.9; $[\alpha]_D^{20}$ = +165.1 (c 1.0, MeOH); HRMS (ESI +) Calc. For $[C_{19}H_{18}N_3O]^+$ req. 304.1444, found 304.1438.

38

Mp = >300 ºC; Rf = 0.22 (5% MeOH/DCM); ^{1}H NMR (500 MHz, MeOD) δ 7.58 (d, *J* = 7.3 Hz, 2H), 7.49 (t, *J* = 7.7 Hz, 2H), 7.45 (dt, *J* = 1.0, 7.9 Hz, 1H), 7.41 – 7.36 (m, 2H), 7.13 (ddd, *J* = 1.2, 7.0, 8.2 Hz, 1H), 7.04 (ddd, *J* = 1.0, 7.0, 7.9 Hz, 1H), 4.81 (s, 1H), 4.26 (s, 1H), 4.04 (dd, *J* = 1.5, 5.8 Hz, 1H), 3.20 – 3.13 (m, 1H), 3.06 (dd, *J* = 1.6, 15.6 Hz, 1H); ^{13}C NMR (126 MHz, MeOD) δ 174.6, 140.8, 136.3, 132.8, 128.4, 127.5, 126.7, 126.3, 121.3, 118.7, 117.4, 110.7, 106.6, 61.4, 52.8, 52.2, 25.9; $[\alpha]_D^{20}$ = +62.4 (c 0.92, MeOH); HRMS (ESI +) Calc. For $[C_{19}H_{18}N_3O]^+$ req. 304.1444, found 304.1435.

37 – (1*S*,2*R*,5*S*)-2-(hydroxymethyl)-1,2,3,5,6,11-hexahydro-4H-1,5-epiminoazocino[4,5-b]indol-4-one

37 was prepared via the general procedure above using dipeptide precursor **30** (950 mg, 2.0 mmol) to give the product as a cream solid (134 mg, 26%). Mp = 262-265 ºC; (decomposition); Rf = 0.15 (5% MeOH/DCM); ^{1}H NMR (500 MHz, MeOD) δ 7.43 (dt, *J* = 1.0, 7.8 Hz, 1H), 7.34 (dt, *J* = 0.9, 8.2 Hz, 1H), 7.11 (ddd, *J* = 1.2, 7.0, 8.2 Hz, 1H), 7.02

(ddd, *J* = 1.0, 7.1, 8.0 Hz, 1H), 4.41 (d, *J* = 4.4 Hz, 1H), 4.09 (ddd, *J* = 4.4, 6.7, 7.8 Hz, 1H), 3.97 (dd, *J* = 1.3, 6.3 Hz, 1H), 3.58 (dd, *J* = 6.7, 10.8 Hz, 1H), 3.39 – 3.35 (m, 1H), 3.16 (dd, *J* = 6.3, 15.9 Hz, 1H), 3.00 (dd, *J* = 1.4, 15.9 Hz, 1H); ^{13}C NMR (126 MHz, MeOD) δ 174.1, 136.3, 129.5, 126.6, 121.4, 118.7, 117.3, 110.7, 107.2, 61.8, 58.9, 52.8, 46.3, 25.8. $[\alpha]_D^{20}$ = +51.6 (c 0.5, MeOH); HRMS (ESI +) Calc. For $[C_{14}H_{16}N_3O_2]^+$ req. 258.1237 found 258.1233.

(1*R*,5*R*)-2-phenyl-1,2,3,5,6,11-hexahydro-4H-1,5-epiminoazocino[4,5-β]indol-4-one

Ent-36 and **ent-38** were prepared via the general procedure above using dipeptide precursor 48 (960 mg, 2.0 mmol). Products were separated by flash column chromatography to give 49 (98 mg, 16%) and 50 (120 mg, 20%) as pale brown solids.

Ent-36

Mp = 198-205 ºC; Rf = 0.20 (5% MeOH/DCM); ^{1}H NMR (500 MHz, MeOD) δ 7.45 – 7.41 (m, 1H), 7.34 – 7.29 (m, 1H), 7.25 (t, *J* = 7.4 Hz, 2H), 7.06 – 7.01 (m, 2H), 7.01 – 6.97 (m, 1H), 6.97 – 6.93 (m, 2H), 5.19 (d, *J* = 4.6 Hz, 1H), 4.39 (d, *J* = 4.6 Hz, 1H), 4.02 (dd, *J* = 1.4, 6.3 Hz, 1H), 3.19 (dd, *J* = 6.2, 15.9 Hz, 1H), 3.10 (dd, *J* = 1.5, 15.9 Hz, 1H); ^{13}C NMR (126 MHz, MeOD) δ 174.7, 137.5, 135.9, 129.1, 128.0, 127.9, 126.9, 126.2, 121.1, 118.4, 117.3, 110.6, 107.0, 62.2, 52.3, 51.0, 25.9; $[\alpha]_D^{20}$ = –173.6 (c 1.0, MeOH); HRMS (ESI +) HRMS (ESI +) Calc. For $[C_{19}H_{18}N_3O]^+$ req. 304.1444, found 304.1437.

Ent-38

Mp = >300 ºC; Rf = 0.22 (5% MeOH/DCM); ^{1}H NMR (500 MHz, MeOD) δ 7.58 (d, *J* = 7.3 Hz, 2H), 7.49 (t, *J* = 7.7 Hz, 2H), 7.45 (dt, *J* = 1.0, 7.9 Hz, 1H), 7.41 – 7.36 (m, 2H), 7.13 (ddd, *J* = 1.2, 7.0, 8.2 Hz, 1H), 7.04 (ddd, *J* = 1.0, 7.0, 7.9 Hz, 1H), 4.81 (s, 1H), 4.26 (s, 1H), 4.04 (dd, *J* = 1.5, 5.8 Hz, 1H), 3.20 – 3.13 (m, 1H), 3.06 (dd, *J* = 1.6, 15.6 Hz, 1H); ^{13}C NMR (126 MHz, MeOD) δ 174.6, 140.8, 136.3, 132.8, 128.4, 127.5, 126.7, 126.3, 121.3, 118.7, 117.4, 110.7, 106.6, 61.4, 52.8, 52.2, 25.9; $[\alpha]_D^{20}$ = –49.7 (c 0.92, MeOH); HRMS (ESI +) Calc. For $[C_{19}H_{18}N_3O]^+$ req. 304.1444, found 304.1435.

General procedure for selective cyclisation toward Boc-protected carbolines **40**-**42** and **43/44**

A stirred solution of the corresponding dipeptide precursor (2.0 mmol) in tetrahydrofuran (20 mL) was cooled to -78 °C. After stirring for 15 minutes, a solution of di-isobutyl aluminium hydride (20 mL, 1.0 M in hexanes, 20 mmol) was added via syringe pump over 1 h. The reaction was stirred for a further 4 h, then was diluted with dichloromethane (50 mL) followed by dropwise addition of methanol (5 mL) and the reaction was allowed to warm to room temperature. The mixture was washed with potassium hydrogen sulfate (100 mL, 1M aq.), dried (MgSO4) and filtered. The resulting organics were further diluted with dichloromethane (100 mL) and trifluoroacetic acid (8.0 mL, 100 mmol) added. The reaction was stirred for 16 h, before being quenched with sodium bicarbonate (100 mL, sat. aq.) then the organics separated and the aqueous further extracted with dichloromethane (50 mL). Combined organics were dried (MgSO4), filtered and concentrated under reduced pressure. Crude products were purified via flash column chromatography (50% ethyl acetate:hexanes) to give Boc-carbolines **40**-**44**.

40 – *tert*-butyl (1*S*,2*S*,5*S*)-2-benzyl-4-oxo-2,3,4,5,6,11-hexahydro-1H-1,5-epiminoazocino[4,5-β]indole-12-carboxylate

40 was prepared via the general procedure above, using **24** (990 mg) to give the product as a white solid (584 mg, 70%). Rf = 0.17 (75% ethyl acetate/hexanes); Mp = 216-221 ºC; observed as 1.5:1 mixture of rotamers via NMR: major rotamer ^{1}H NMR (500 MHz, MeOD) δ 7.47 (dt, *J* = 1.0, 7.9 Hz, 1H), 7.40 (d, *J* = 8.4 Hz, 1H), 7.37 (t, *J* = 7.4 Hz, 2H), 7.33 – 7.25 (m, 3H), 7.16 (ddd, *J* = 1.2, 7.0, 8.2 Hz, 1H), 7.06 (t, *J* = 7.5 Hz, 1H), 5.54 (d, *J* = 4.1 Hz, 1H), 4.93 – 4.88 (m, 1H), 4.34 – 4.23 (m, 1H), 3.37 – 3.30 (m, 1H), 3.23 – 3.15 (m, 1H), 3.08 (dd, *J* = 1.4, 15.8 Hz, 1H), 2.51 – 2.38 (m, 1H), 1.51 (s, 9H); ^{13}C NMR (126 MHz, MeOD) δ 171.7, 153.4, 136.7, 136.7, 136.2, 128.8, 128.8, 126.9, 126.3, 121.8, 119.0, 117.5, 111.0, 107.7, 81.3, 57.9, 53.7, 48.7, 37.8, 25.2; minor rotamer ^{1}H NMR (500 MHz, MeOD) δ 7.47 (dt, *J* = 1.0, 7.9 Hz, 1H), 7.40 (d, *J* = 8.4 Hz, 1H), 7.37 (t, *J* = 7.4 Hz, 2H), 7.33 – 7.25 (m, 3H), 7.16 (ddd, *J* = 1.2, 7.0, 8.2 Hz, 1H), 7.06 (t, *J* = 7.5 Hz, 1H), 5.45 (d, *J* = 4.0 Hz, 1H), 4.98 (d, *J* = 5.9 Hz, 1H), 4.34 – 4.23 (m, 1H), 3.29 – 3.23 (m, 1H), 3.23 – 3.15 (m, 1H), 3.08 (dd, *J* = 1.4, 15.8 Hz, 1H), 2.51 – 2.38 (m, 1H), 1.49 (s, 9H); ^{13}C NMR (126 MHz, MeOD) δ 171.7, 153.4, 136.7, 136.7, 136.2, 128.8, 128.8, 126.9, 126.3, 121.8, 119.0, 117.5, 111.0, 107.7, 81.3, 57.9, 52.2, 50.2, 37.6, 24.8. $[\alpha]_D^{20}$ = –68.2 (c 0.89, MeOH); HRMS (ESI +) Calc. For $[C_{25}H_{27}N_3O_3Na]^+$ req. 440.1945, found 440.1941.

41 – tert-butyl (1*S*,5*S*)-4-oxo-2-phenyl-2,3,4,5,6,11-hexahydro-1H-1,5-epiminoazocino[4,5-β]indole-12-carboxylate

41 was prepared via the general procedure above, using **25** (809 mg) to give the product as an off white solid (613 mg, 94%). Rf = 0.17 (75% ethyl acetate/hexanes); Mp = 224-228 ºC (decomposition); observed as 1.3:1 mixture of rotamers via NMR: major rotamer ^{1}H NMR (500 MHz, MeOD) δ 7.42 (dt, *J* = 1.0, 7.9 Hz, 1H), 7.36 – 7.29 (m, 1H), 7.15 – 7.07 (m, 1H), 7.07 – 6.90 (m, 1H), 5.51 (d, *J* = 4.5 Hz, 1H), 4.89 (d, *J* = 5.7 Hz, 1H), 3.84 (m, 1H), (3.41 m, 1H), 3.17 (dd, *J* = 5.7, 15.7 Hz, 1H), 3.05 (dd, *J* = 1.5, 15.7 Hz, 1H), 1.52 (s, 9H); ^{13}C NMR (126 MHz, MeOD) δ 171.7, 153.6, 136.5, 130.5, 126.4, 121.6, 118.9, 117.4, 110.9, 107.0, 81.2, 54.0, 46.4, 44.7, 27.1; minor rotamer ^{1}H NMR (500 MHz, MeOD) δ 7.42 (dt, *J* = 1.0, 7.9 Hz, 1H), 7.36 – 7.29 (m, 1H), 7.15 – 7.07 (m, 1H), 7.07 – 6.90 (m, 1H), 5.47 (d, *J* = 4.3 Hz, 1H), 4.96 (d, *J* = 5.6 Hz, 1H), 3.84 (m, 1H), 3.41 (m, 1H), 3.17 (dd, *J* = 5.7, 15.7 Hz, 1H), 3.05 (dd, *J* = 1.5, 15.7 Hz, 1H), 1.51 (s, 9H); ^{13}C NMR (126 MHz, MeOD) δ 171.9, 153.4, 136.5, 130.3, 126.4, 121.6, 118.9, 117.5, 110.9, 107.3, 81.2, 52.6, 46.7, 46.2, 27.1. $[\alpha]_D^{20}$ = –26.2 (c 1.0 MeOH); HRMS (ESI –) Calc. For $[C_{22}H_{21}N_4O]^-$ req. 357.1710, found 357.1706.

42 – *tert*-butyl (1*S*,2*R*,5*S*)-2-(hydroxymethyl)-4-oxo-2,3,4,5,6,11-hexahydro-1H-1,5-epiminoazocino[4,5-β]indole-12-carboxylate

42 was prepared via the general procedure above, using **30** (950 mg) to give the product as an off white solid (170 mg, 33%). Rf = 0.22 (50% ethyl acetate/hexanes); Mp = 178-181 ºC; observed as 1.5:1 mixture of rotamers via NMR: major rotamer ^{1}H NMR (500 MHz, MeOD) δ 7.43 (dd, *J* = 1.0, 7.9 Hz, 1H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.15 – 7.11 (m, 1H), 7.05 – 7.01 (m, 1H), 5.57 (d, *J* = 4.4 Hz, 1H), 4.94 (m, 1H), 4.11 – 4.02 (m, 1H), 3.59 – 3.43 (m, 2H), 3.18 (dd, *J* = 6.1, 15.8 Hz, 1H), 3.06 (dd, *J* = 1.4, 15.8 Hz, 1H), 1.49 (s, 9H); ^{13}C NMR (126 MHz, MeOD) δ 171.8, 153.5, 136.5, 127.8, 126.3, 121.7, 119.0, 117.4, 110.9, 107.5, 81.3, 61.2, 57.9, 53.7, 46.3, 27.1, 25.1; minor rotamer ^{1}H NMR (500 MHz, MeOD) δ 7.43 (dd, *J* = 1.0, 7.9 Hz, 1H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.15 – 7.11 (m, 1H), 7.05 – 7.01 (m, 1H), 5.51 (d, *J* = 4.3 Hz, 1H), 5.00 (d, *J* = 5.9 Hz, 1H), 4.11 – 4.02 (m, 1H, H14), 3.59 – 3.43 (m, 2H), 3.18 (dd, *J* = 6.1, 15.8 Hz, 1H), 3.06 (d, *J* = 15.8 Hz, 1H), 1.49 (s, 9H); ^{13}C NMR (126 MHz, MeOD) δ 172.0, 153.3, 136.5, 127.6, 126.3, 121.7, 119.0, 117.5, 110.9, 107.8, 81.3, 61.2, 58.1, 52.3, 48.5, 27.1, 24.7; $[\alpha]_D^{20}$ = –30.7 (c 1.0 MeOH) HRMS (ESI –) Calc. for $[C_{19}H_{22}N_3O_4]^-$ req. 356.1616, found 356.1617.

tert-butyl (1*S*,5*S*)-4-oxo-2-phenyl-2,3,4,5,6,11-hexahydro-1H-1,5-epiminoazocino[4,5-β] indole-12-carboxylate

An inseparable 1.8:1 mixture of diastereomers **43** and **44** was prepared via the general procedure above, using **29** (960 mg). (363 mg, 45%). Rf = 0.22 (50% ethyl acetate/hexanes).

For full characterization data please see the separate syntheses of **43** and **44** below.

General Procedure for the synthesis of Boc-Protected Diastereomers **43**, **44**, **ent-43** and **ent-44**

To a solution of the appropriate carboline cycle in dichloromethane (1.0 mL) at 0 ºC was added di-tert-butyl carbamate (20 mg, 0.09 mmol, 1.2 equiv.) and triethylamine (21 µL, 0.15 mmol, 2.0 equiv.) then the reaction stirred for 16 h. After completion, the reaction mixture was concentrated under reduced pressure and purified via flash column chromatography (50% ethyl acetate/hexanes).

43 – *tert*-butyl (1*S*,2*S*,5*S*)-4-oxo-2-phenyl-2,3,4,5,6,11-hexahydro-1H-1,5-epiminoazocino[4,5-β]indole-12-carboxylate

43 was prepared via the general procedure above using **36** (23 mg, 0.075 mmol) to give the product as an off-white solid (26 mg, 87%) Rf = 0.22 (50% ethyl acetate/hexanes); Mp = 178-184 ºC; observed as 1.5:1 mixture of rotamers via NMR: major rotamer ^{1}H NMR (500 MHz, MeOD) δ 7.45 (d, *J* = 7.7 Hz, 1H), 7.39 – 7.34 (m, 1H), 7.31 (t, *J* = 7.5 Hz, 2H), 7.08 – 7.04 (m, 2H), 7.04 – 6.96 (m, 3H), 5.58 – 5.48 (m, 1H), 5.21 (d, *J* = 4.6 Hz, 1H), 5.00 (d, *J* = 5.8 Hz, 1H), 3.20 (qd, *J* = 3.7, 15.9 Hz, 2H), 1.54 (s, 9H); ^{13}C NMR (126 MHz, MeOD) δ 172.3, 153.5, 136.4, 136.1, 128.3, 128.1, 127.1, 125.9, 121.4, 118.7, 117.4, 110.7, 107.2, 81.4, 60.9, 53.3, 50.4, 27.2, 25.2; minor rotamer ^{1}H NMR (500 MHz, MeOD) δ 7.45 (d, *J* = 7.7 Hz, 1H), 7.39 – 7.34 (m, 1H), 7.31 (t, *J* = 7.5 Hz, 2H), 7.08 – 7.04 (m, 2H), 7.04 – 6.96 (m, 3H), 5.44 (d, *J* = 4.6 Hz, 1H), 5.21 (d, *J* = 4.6 Hz, 1H), 5.07 (d, *J* = 5.8 Hz, 1H), 3.20 (qd, *J* = 3.7, 15.9 Hz, 2H), 1.54 (s, 9H); ^{13}C NMR (126 MHz, MeOD) δ 172.5, 153.3, 136.3, 136.1, 128.3, 128.1, 127.1, 125.9, 121.4, 118.7, 117.4, 110.7, 107.5, 81.4, 61.2, 52.0, 51.9, 27.2, 24.8; $[\alpha]_D^{20}$ = +88.3 (c 1.16 MeOH); HRMS (ESI –) Calc. for $[C_{24}H_{24}N_3O_3]^-$ req. 402.1823, found 402.1812.

44 – *tert*-butyl (1*S*,2*R*,5*S*)-4-oxo-2-phenyl-2,3,4,5,6,11-hexahydro-1H-1,5-epiminoazocino[4,5-β]indole-12-carboxylate

44 was prepared via the general procedure above using **38** (23 mg, 0.075 mmol) to give the product as an off-white solid (29 mg, 96%). Rf = 0.21 (50% ethyl acetate/hexanes); Mp = >300 ºC; observed as 2:1 mixture of rotamers via NMR: major rotamer ^{1}H NMR (500 MHz, MeOD) δ 7.50 – 7.37 (m, 7H), 7.16 (tdd, *J* = 1.2, 3.1, 8.2 Hz, 1H), 7.09 – 7.01 (m, 1H), 5.42 (d, *J* = 1.4 Hz, 1H), 5.12 – 5.10 (m, 1H), 4.85 (d, *J* = 1.4 Hz, 1H), 3.22 – 3.06 (m, 2H), 1.08 (s, 9H); ^{13}C NMR (126 MHz, MeOD) δ 172.2, 152.7, 140.3 (, 136.5, 130.8, 128.5, 127.6, 126.4, 126.1, 121.7, 119.0, 117.6, 110.9, 107.3, 80.6, 61.4, 53.0, 52.1, 26.7, 25.0; minor rotamer ^{1}H NMR (500 MHz, MeOD) δ 7.53 – 7.32 (m, 7H), 7.16 (tdd, *J* = 1.2, 3.1, 8.2 Hz, 1H), 7.09 – 7.01 (m, 1H), 5.41 (s, 1H), 5.05 – 5.02 (m, 1H), 4.83 (d, *J* = 1.3 Hz, 1H), 3.23 – 3.06 (m, 2H), 1.41 (s, 9H); ^{13}C NMR (126 MHz, MeOD) δ 170.0, 153.1, 140.3, 136.4, 130.8, 128.3, 127.7, 126.4, 126.1, 121.7, 118.9, 117.5, 110.9, 107.0, 80.6, 61.1, 53.9, 51.2, 27.1, 25.3; $[\alpha]_D^{20}$ = +64.3 (c 0.76 MeOH); HRMS (ESI –) Calc. for $[C_{24}H_{24}N_3O_3]^-$ req. 402.1823. Found 402.1813.

X-ray crystallograpic data available.

Ent-43 – *tert*-butyl (1*R*,2*R*,5*R*)-4-oxo-2-phenyl-2,3,4,5,6,11-hexahydro-1H-1,5-epiminoazocino[4,5-β]indole-12-carboxylate

Ent-43 was prepared via the general procedure above using **36** (23 mg, 0.075 mmol) to give the product as an off-white solid (17 mg, 56%). Rf = 0.20 (50% ethyl acetate/hexanes); Mp = 241-243 ºC; observed as 1.5:1 mixture of rotamers via NMR: major rotamer ^{1}H NMR (500 MHz, MeOD) δ 7.45 (d, *J* = 7.7 Hz, 1H), 7.39 – 7.34 (m, 1H), 7.31 (t, *J* = 7.5 Hz, 2H), 7.08 – 7.04 (m, 2H), 7.04 – 6.96 (m, 3H), 5.58 – 5.48 (m, 1H), 5.21 (d, *J* = 4.6 Hz, 1H), 5.00 (d, *J* = 5.8 Hz, 1H), 3.20 (qd, *J* = 3.7, 15.9 Hz, 2H), 1.54 (s, 9H); ^{13}C NMR (126 MHz, MeOD) δ 172.3, 153.5, 136.4, 136.1, 128.3, 128.1, 127.1, 125.9, 121.4, 118.7, 117.4, 110.7, 107.2, 81.4, 60.9, 53.3, 50.4, 27.2, 25.2; minor rotamer ^{1}H NMR (500 MHz, MeOD) δ 7.45 (d, *J* = 7.7 Hz, 1H), 7.39 – 7.34 (m, 1H), 7.31 (t, *J* = 7.5 Hz, 2H), 7.08 – 7.04 (m, 2H), 7.04 – 6.96 (m, 3H), 5.44 (d, *J* = 4.6 Hz, 1H), 5.21 (d, *J* = 4.6 Hz, 1H), 5.07 (d, *J* = 5.8 Hz, 1H), 3.20 (qd, *J* = 3.7, 15.9 Hz, 2H), 1.54 (s, 9H); ^{13}C NMR (126 MHz, MeOD) δ 172.5, 153.3, 136.3, 136.1, 128.3, 128.1, 127.1, 125.9, 121.4, 118.7, 117.4, 110.7, 107.5, 81.4, 61.2, 52.0, 51.9, 27.2, 24.8; $[\alpha]_D^{20}$ = –89.2 (c 0.30 MeOH); HRMS (ESI +) Calc. for $[C_{24}H_{26}N_3O_3]$+ req. 404.1969. Found 404.1961.

Ent-44 – *tert*-butyl (1*R*,2*S*,5*R*)-4-oxo-2-phenyl-2,3,4,5,6,11-hexahydro-1H-1,5-epiminoazocino[4,5-β]indole-12-carboxylate

Ent-44 was prepared via the general procedure above using **ent-38** (23 mg, 0.075 mmol) to give the product as an off-white solid (28 mg, 92%). Rf = 0.21 (50% ethyl acetate/hexanes); Mp = 262-267 ºC; observed as 2:1 mixture of rotamers via NMR: major rotamer ^{1}H NMR (500 MHz, MeOD) δ 7.53 – 7.33 (m, 7H), 7.18 – 7.13 (m , 1H), 7.08 – 7.02 (m, 1H), 5.42 (d, *J* = 1.3 Hz, 1H), 5.13 – 5.09 (m, 1H), 4.85 (d, *J* = 1.4 Hz, 1H), 3.21 – 3.09 (m, 2H), 1.08 (s, 9H); ^{13}C NMR (126 MHz, MeOD) δ 172.2, 152.7, 140.3, 136.5, 130.8, 128.5, 127.6, 126.4, 126.1, 121.7, 119.0, 117.6, 110.9, 107.3, 80.6, 61.4, 53.0, 52.1, 26.7, 25.0; minor rotamer ^{1}H NMR (500 MHz, MeOD) δ 7.53 – 7.33 (m, 7H), 7.18 – 7.13 (m , 1H), 7.08 – 7.02 (m, 1H), 5.41 – 5.39 (m, 1H), 5.06 – 5.03 (m, 1H), 4.84 – 4.80 (m, 1H), 3.21 – 3.09 (m, 2H), 1.40 (s, 9H); ^{13}C NMR (126 MHz, MeOD) δ 171.8, 153.1, 140.3, 137.5, 130.8, 128.3, 127.7, 126.4, 126.1, 121.7, 118.9, 117.5, 110.9, 107.3, 81.0, 61.1, 53.9, 51.2, 27.1, 25.3; $[\alpha]_D^{20}$ = –63.7 (c 0.76, MeOH); HRMS (ESI -) Calc. for $[C_{24}H_{24}N_3O_3]^-$ req. 402.1823. Found 402.1812.

General Procedure for Preparation of *N*-Substituted Cycles **45**-**47**

To a solution of carboline cycle **36** (23 mg, 0.075 mmol) in dichloromethane (1.0 mL) at 0 ºC was added DMAP (10 mg, cat.) and triethylamine (0.015 mL, 0.1 mmol, 1.2 equiv.). The reaction was stirred for 5 min before the corresponding alkyl bromide or acid chloride (0.1 mmol, 1.2 equiv.) was added and the reaction warmed to room temperature, then stirred for a further 2 hours. The reaction was diluted with dichloromethane (10 mL) and washed with citric acid (10 mL, 1M). The organics were dried (MgSO4) and filtered, then concentrated in vacuo and purified by flash column chromatography (50% ethyl acetate/hexanes) to give the corresponding *N*-substituted cycle.

45 – (1*S*,2*S*,5*S*)-12-acetyl-2-phenyl-1,2,3,5,6,11-hexahydro-4H-1,5-epiminoazocino[4,5-β]indol-4-one

45 was prepared via the general procedure above using acetyl chloride (5.5 µL) to give the product as an off-white solid (5.4 mg, 21%). Rf = 0.4 (5% MeOH/DCM); Mp = 133-140 ºC; observed as 2:1 mixture of rotamers by NMR: major rotamer ^{1}H NMR (500 MHz, DMSO) δ 9.74 (s, 1H), 8.38 (s, 1H), 7.42 (d, J = 7.4 Hz, 1H), 7.35 – 7.30 (m, 1H), 7.30 – 7.25 (m, 1H), 7.11 – 6.91 (m, 5H), 5.82 (d, J = 4.5 Hz, 1H), 5.00 (d, J = 4.5 Hz, 1H), 4.85 (d, J = 5.7 Hz, 1H), 3.18 (dd, J = 15.9, 6.0 Hz, 1H), 3.11 (s, 1H), 2.16 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 170.0, 169.7, 167.5, 136.9, 135.7, 128.5, 128.1, 128.1, 127.4, 125.7, 121.3, 118.6, 117.8, 111.4, 107.1, 60.5, 60.0, 54.4, 47.8, 26.1, 20.5; minor rotamer ^{1}H NMR (500 MHz, DMSO) δ 9.52 (s, 1H), 8.34 (s, 1H), 7.42 (d, J = 7.4 Hz, 1H), 7.35 – 7.30 (m, 1H), 7.30 – 7.25 (m, 1H), 7.11 – 6.91 (m, 5H), 5.31 (d, J = 4.6 Hz, 1H), 5.27 – 5.20 (m, 2H), 3.11 (s, 1H), 3.09 – 2.95 (m, 1H), 2.24 (s, 3H); ^{13}C NMR (126 MHz, DMSO) δ 169.9, 169.6, 167.5, 136.8, 135.8, 128.2, 128.1, 128.1, 127.4, 125.6, 121.4, 118.6, 117.8, 111.5, 107.5, 60.4, 59.9, 53.3, 49.5, 24.9, 21.1; $[\alpha]_D^{20}$ = +36.6 (c 0.63 MeOH) HRMS (ESI –) Calc. For $[C_{21}H_{18}N_3O_2]^-$ req. 344.1405 Found 344.1405.

46 – (1*S*,2*S*,5*S*)-12-benzyl-2-phenyl-1,2,3,5,6,11-hexahydro-4H-1,5-epiminoazocino[4,5-β]indol-4-one

46 was prepared via the general procedure above using benzyl bromide (12.0 µL) to give the product as an off-white solid (7.0 mg, 23%). Rf = 0.28 (50% ethyl acetate/hexanes); Mp = 220-224 ºC; ^{1}H NMR (500 MHz, MeOD) δ 7.48 (dt, *J* = 1.2, 7.1 Hz, 1H), 7.44 – 7.40 (m, 2H), 7.39 – 7.35 (m, 2H), 7.34 – 7.26 (m, 2H), 7.24 – 7.19 (m, 2H), 7.06 – 6.98 (m, 3H), 6.91 – 6.87 (m, 2H), 5.19 (d, *J* = 4.7 Hz, 1H), 4.16 (d, *J* = 4.7 Hz, 1H), 3.92 – 3.82 (m, 2H), 3.74 (d, *J* = 13.2 Hz, 1H), 3.36 – 3.28 (m, 1H), 2.98 (dd, *J* = 1.1, 16.3 Hz, 1H); ^{13}C NMR (126 MHz, MeOD) δ 174.7, 137.7, 137.6, 136.0, 128.7, 128.2, 127.9, 127.8, 127.4, 127.2, 127.0, 126.2, 121.0, 118.4, 117.3, 110.5, 106.0, 61.2, 57.4, 55.4, 55.1, 21.5; $[\alpha]_D^{20}$ = +58.3 (c 0.49 MeOH); HRMS (ESI +) Calc. For $[C_{26}H_{24}N_3O]^+$ req. 394.1914 Found 394.1906.

47 – (1*S*,2*S*,5*S*)-12-benzoyl-2-phenyl-1,2,3,5,6,11-hexahydro-4H-1,5-epiminoazocino[4,5-β]indol-4-one

47 was prepared via the general procedure above using benzoyl chloride (12.0 µL) to give the product as a yellow oil (25.1 mg, 82%). Rf = 0.25 (50% ethyl acetate/hexanes); Mp = 238-244 ºC; observed as 1.5:1 mixture of rotamers by NMR; major rotamer: ^{1}H NMR (500 MHz, MeOD) δ 7.67-7.51 (m, 5H, H18), 7.49 (d, *J* = 7.8 Hz, 1H), 7.35 (m, 2H), 7.24 (t, *J* = 7.5 Hz, 1H), 7.15 – 6.94 (m, 5H), 6.05 (d, *J* = 4.6 Hz, 1H), 5.37 (d, *J* = 4.5 Hz, 1H), 4.68 (d, *J* = 6.0 Hz, 1H), 3.48 – 3.18 (m, 2H); ^{13}C NMR (126 MHz, MeOD) δ 171.3, 170.2, 136.3, 136.2, 134.1, 130.7, 128.7, 128.4, 128.2, 127.2, 126.8, 125.8, 121.6, 118.8, 117.5, 110.8, 106.9, 60.1, 56.4, 49.5, 26.3; minor rotamer ^{1}H NMR (500 MHz, MeOD) δ 7.67 – 7.51 (m, 5H, H19), 7.49 (d, *J* = 7.8 Hz, 1H), 7.42 – 7.27 (m, 2H), 7.15 – 6.94 (m, 5H), 5.57 (d, *J* = 6.1 Hz, 1H), 5.37 (d, *J* = 4.5 Hz, 1H), 5.15 (d, *J* = 4.6 Hz, 1H), 3.48 – 3.18 (m, 2H); ^{13}C NMR (126 MHz, MeOD) δ 171.7, 170.4, 136.2, 135.8, 134.5, 130.6, 128.4, 128.4, 128.1, 127.0, 126.5, 125.8, 121.7, 118.8, 117.5, 110.7, 107.7, 61.4, 55.2, 51.1, 24.9; $[\alpha]_D^{20}$ = –22.0 (c 1.0 MeOH); HRMS (ESI +) Calc. For $[C_{26}H_{21}N_3O_2Na]$+ req. 430.1526 Found 430.1515.

Dose-response assays

Detailed descriptions can be found in the supporting information. In general: compound was serially diluted in a 96-well plate containing growth media (100 µL), then 100 µL growth media containing mid-log-phase parasite or HeLa cells in were added at the appropriate seeding density. Plates were incubated at 72 h, at the appropriate temperature, after which 10 µM alamar blue (1.1 mg/mL resazurin sodium salt in PBS) was added. Plates were incubated for a further 7 h before recording fluorescence. EC_{50} values were determined using a 4-parameter non-linear logistic regression equation and were normalized with no cell/no inhibitor controls.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: cyclization • polycycles • antiprotazoal agents • biological activity • structure activity relationships

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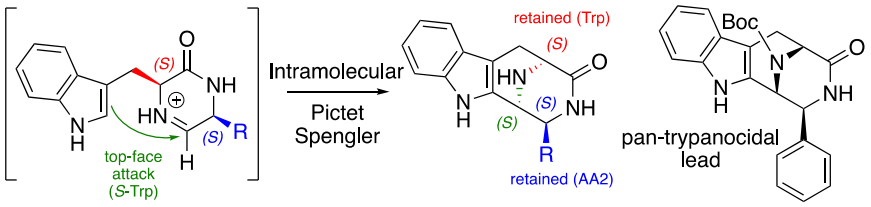

A diastereoselective intramolecular Pictet Spengler condensation was developed to achieve an SP^3-rich 6-5-6-6 ketopiperazine-type scaffold. The cyclisation employs tryptophan-based dipeptide precursors which allow product stereochemistry to be tailored using readily available D and L amino acids. SAR analysis, examining different scaffold substitutions and stereochemical configurations, led to the discovery of lead compounds with activity against *Trypanosoma brucei*, *Trypanosoma cruzi* and *Leishmania major*.