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Preparation of penta-azole containing cyclopeptides: challenges in macrocyclization

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Abstract—Herein is described the synthesis of several analogs of the natural product IB-01211 from concatenated azoles, via a biomimetic pathway based on cyclization–oxidation of serine containing peptides combined with the Hantzsch synthesis. The macrocyclization of rigid peptide compounds **1** and **2** to give IB-01211 and its epimer **12b** was explored, and the results are compared here to those previously obtained for the macrocyclization of more flexible structures in the syntheses of YM-216391, telomestatin, and IB-01211. Lastly, the preliminary results of anti-tumor activity screening of the synthesized analogs are discussed.

1. Introduction

Directly linked 2,4-azoles are found in natural products that have interesting biological activities and fascinating structures. Numerous bis- and trisoxazoles, as well as a few oxazole–thiazoles, have been isolated from marine organisms, whereas linked thiazole-containing natural products have generally been obtained from microorganisms.[1] Marine organism secondary metabolites such as ulapualides,[2] halichondramides,[3] kabiramides,[3] mycalolides,[4] halishigamides,[5] and jaspisamides,[6] all contain a trisoxazole fragment. These compounds show a broad range of unusual biological activities.[7]

Telomestatin, a potent telomerase inhibitor isolated from *Streptomyces anulatus* 3533-SV4[8] that interacts specifically with the human telomeric intramolecular G-quadruplex without affecting DNA polymerases or reverse transcriptases, contains a novel macrocyclic structure comprised of seven linked oxazoles and one thiazoline unit. A related cyclopeptide, YM-216391, containing only four oxazoles and one thiazole, has been isolated from *Streptomyces nobilis*.[9]

A more recently discovered macrocyclic peptide, IB-01211 (Fig. 1), has been isolated from the marine-derived microorganism strain ES7-008, which is phylogenetically close to *Thermoactinomyces* genus.[10,11] It is strongly cytotoxic against several tumor cell lines,[12] and contains four oxazoles and one thiazole.

Concatenated azoles have been prepared following several strategies,[1c] including cyclodehydration of peptides containing serine, threonine or cysteine followed by oxidation of the azoline to azole; the classical Hantzsch synthesis allowing a one-pot synthesis of the azole ring; Pd(0) catalyzed cross-coupling reaction—despite difficulties in the preparation of several precursors; sequential [3+2] cycloaddition of an appropriate rhodium carbene with nitriles, a new route to polyoxazoles from an ulapualide fragment; sequential Chan-type rearrangements of tertiary amides for the preparation of trisoxazoles; and finally, iterative oxazole assembly via base-promoted cyclization of alkynyl glycine derivatives prepared from the corresponding α-chloroglycinates by reaction with alkynyl dimethyl-aluminum reagents. The total syntheses of YM-216391,[13] telomestatin,[14] and IB-01211[15] have recently been described. The three procedures possess a common feature, a macrocyclization of flexible precursors (Scheme 1).

The key step in the aforementioned synthesis of IB-01211 is macrocyclization by Hantzsch formation of the thiazole ring. Alternatively, the macrocyclization could be envisaged through formation of an amide bond between penta-azole peptides **1** and **2** as the last synthetic step (see Fig. 2). Thus, with the aim of synthesizing IB-01211 and related

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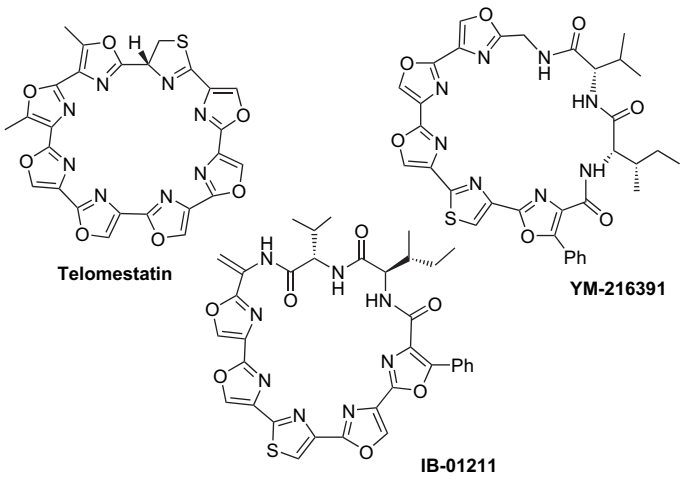
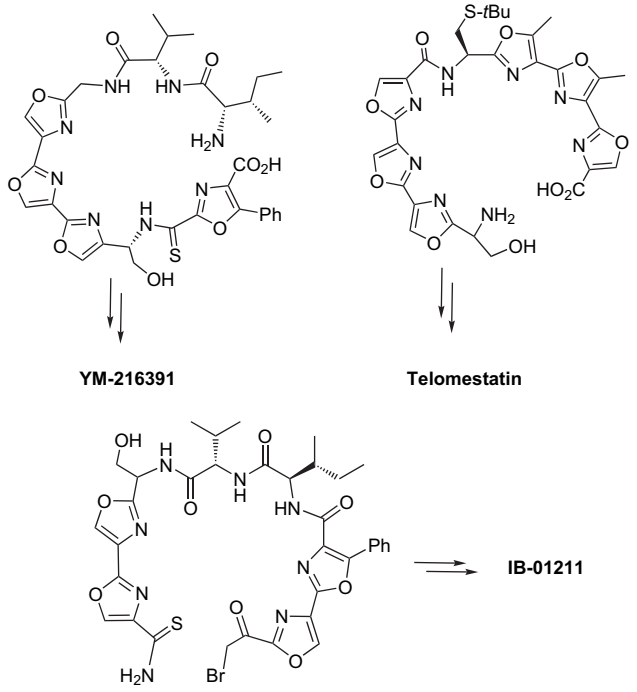


Figure 1. Natural compounds with 2,4-concatenated azoles.



Scheme 1. Macrocyclization in the syntheses of YM-216391, tetomestatin, and IB-01112.

derivatives, we studied the macrocyclization of penta-azole containing peptides. We describe here the preparation of several open chain IB-01211 derivatives, including subsequent macrocyclization studies and anti-tumor activity screening.

2. Results and discussion

The retro-synthetic strategy used for this work is shown in Figure 2. Disconnection of the amide bonds between the D-Val and the aminoethylidene (disconnection a), or between the D-*Allo*-Ile and the phenyloxazolcarbonyl (disconnection b), affords the penta-azole peptides **1** or **2**, respectively. Both peptides possess a *tert*-butyl protected alcohol, which can be readily transformed into the exocyclic methylidene present in the natural compound. The strategy involving intermediate **1** was thought to be more favorable than that with **2**, which involves a coupling reaction of a hindered α-amine and a poorly reactive carboxylic group. A less convergent strategy through amide bond formation between the hindered Ile and Val residues was rejected. The common precursors for both azole peptides are the penta-azole **3** and the dipeptide **4**. Finally, assembly of the middle thiazole present in compound **3** was planned from appropriately functionalized bisoxazoles **5** and **6**.

2.1. Synthesis of penta-azole 3

Preparation of **3** was attempted by transformation of peptide **7a** into thioamide **7b**, followed by cyclization and oxidation (Scheme 2A).[16] Reaction of the acid **5a** and the amine **6a** using the general procedure described in Section 5 for peptide bond formation afforded **7a** in good yield.[17] The bisoxazole derivative **5a** was obtained by cyclization of the proper Ser peptide followed by oxidation of the resulting oxazolines, as previously described by our group.[15] However, reaction of **7a** with the Lawesson reagent to produce the thioamide **7b** gave a complex mixture from which no product could be isolated.

After exploring a broad range of reagents and conditions to afford the thiazole moiety, including the use of a cysteine-containing building block,[18] we took a major shift in strategy: a classical Hantzsch synthesis using a thioamide and an α-bromoketone (Scheme 2B).

Bisoxazole **10**, containing the protected α-bromoketone residue for the Hantzsch synthesis, was obtained by two sequential oxazole-ring formations in order to minimize the amount of by-product resulting from water elimination, and also because formation of a conjugated carbon–carbon double bond is favored by the presence of the phenyl ring. Cyclization and oxidation of dipeptide **8**[19] afforded an oxazole with a protected amino ethanol substituent at position 2 of the ring, which was then deprotected with 95% trifluoroacetic acid (TFA) to give the amino alcohol **9**. Compound **9** was used in the following amide bond formation by reaction with bromopyruvic acid dimethyl acetal. Subsequent ring closure and oxidation then provided **10**. Elimination of the acetal protecting group of **10** by treatment with formic

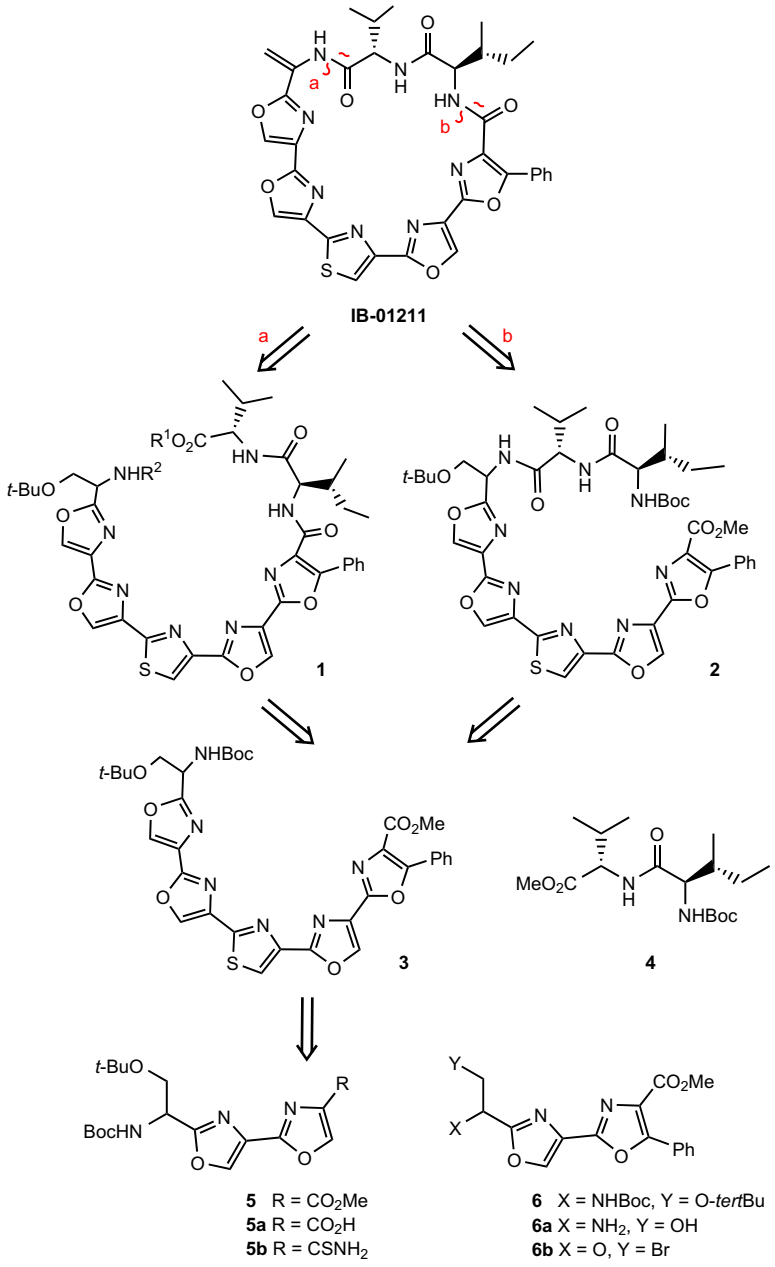


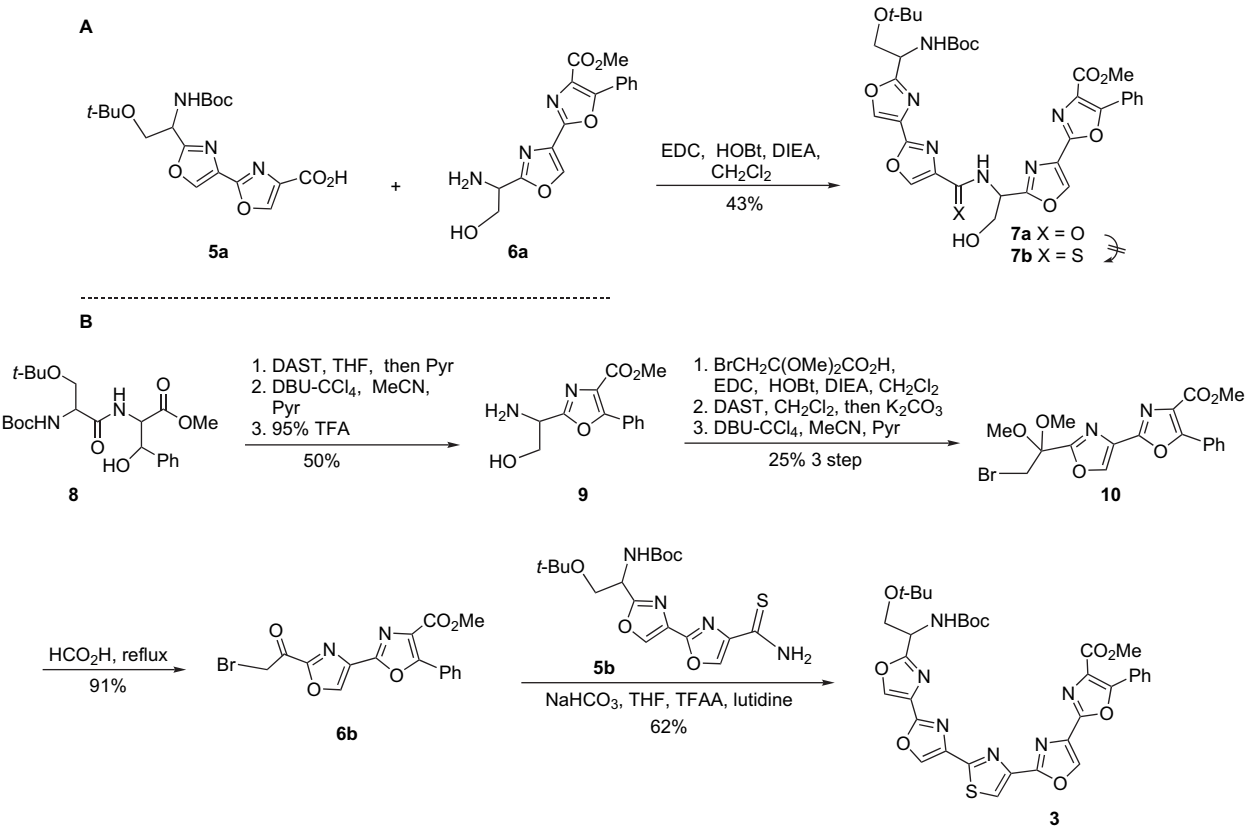
Figure 2. Retro-synthetic analysis of IB-01211.

acid at reflux gave the bromoketone **6b** in quantitative yield. Penta-azole **3** was obtained in 62% yield by Hantzsch thiazole synthesis using the bisoxazolethioamide **5b** and the bis-oxazolyl α-bromomethyl ketone **6b**. It was characterized by ^{1}H NMR, whereby the four singlets in the aromatic region, and the five aromatic protons, were taken as representative signals.

2.2. Macrocyclization reaction

Peptides **4** and **4a** were synthesized in solution from Boc–D-*allo*-Ile–OH and H–Val–OMe, and from Boc–D-Ile–OH and H–Val–OMe, respectively, using EDC·HCl/HOBt and DIEA as coupling reagents. The *N*-deprotected and *C*-deprotected peptides were obtained by TFA treatment and by methyl ester hydrolysis, respectively. With penta-azole **3** and epimeric peptides **4** and **4a** in hand, we considered two options for macrocyclization, both of which implied preparation of peptides **1** and **2** (Scheme 3). The macrocyclization studies were performed with **4a**, as it is the cheaper of the two peptides.

Compound **1a** was prepared in good yield by methyl ester hydrolysis of **3** with LiOH, followed by condensation with *N*-deprotected-**4a** using EDC·HCl and HOBt as activating agents, and *N*,*N*-diisopropylethylamine (DIEA). In parallel, **2a** was also obtained with good yields, by N- and O-deprotection of **3** with 95% TFA, followed by condensation with the acid obtained in the saponification of **4a**, as described above. C- and N-deprotection of the linear precursors **1a** (R^1=Me, R^2=Boc) and **2a** (R^1=Me, R^2=Boc) were obtained in situ by methyl ester hydrolysis with LiOH followed



Scheme 2. Synthesis of penta-azole **3**.

by TFA treatment. Several macrocyclization trials were performed from *C*- and *N*-deprotected **1a** (R^1=R^2=H) using the following activating agents: 1-[bis(dimethylamino)-methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium hexafluorophosphate 3-oxide (HATU)/*N*-methylmorpholine (NMM), HATU/DIEA, (benzotriazol-1-yloxy)tris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP)/HOBt/DIEA, and (7-azabenzotriazol-1-yloxy)tris(pyrrolidino)phosphonium hexafluorophosphate (PyAOP)/DIEA. No traces of cyclized compound were detected by either ^{1}H NMR or MS. Likewise, no sign of **12b** was detected when the same cyclization conditions were applied to the methylidene derivative **11** (R^1=R^2=H), obtained by dehydration of **2a** (R^1=Me, R^2=Boc) with mesyl chloride (MsCl) in Et_3N followed by deprotection. Finally, macrocyclization of **1a** (R^1=R^2=H) was achieved using pentafluorophenol (Pfp-OH) as an activating agent. Thus, methyl ester hydrolysis of **1a** (R^1=Me, R^2=Boc), conversion of the resulting acid into the pentafluorophenyl ester, N-deprotection,[20] and finally, macrocyclization by treatment with DIEA in a highly diluted THF solution gave the cyclic peptide **12a** (Scheme 3).

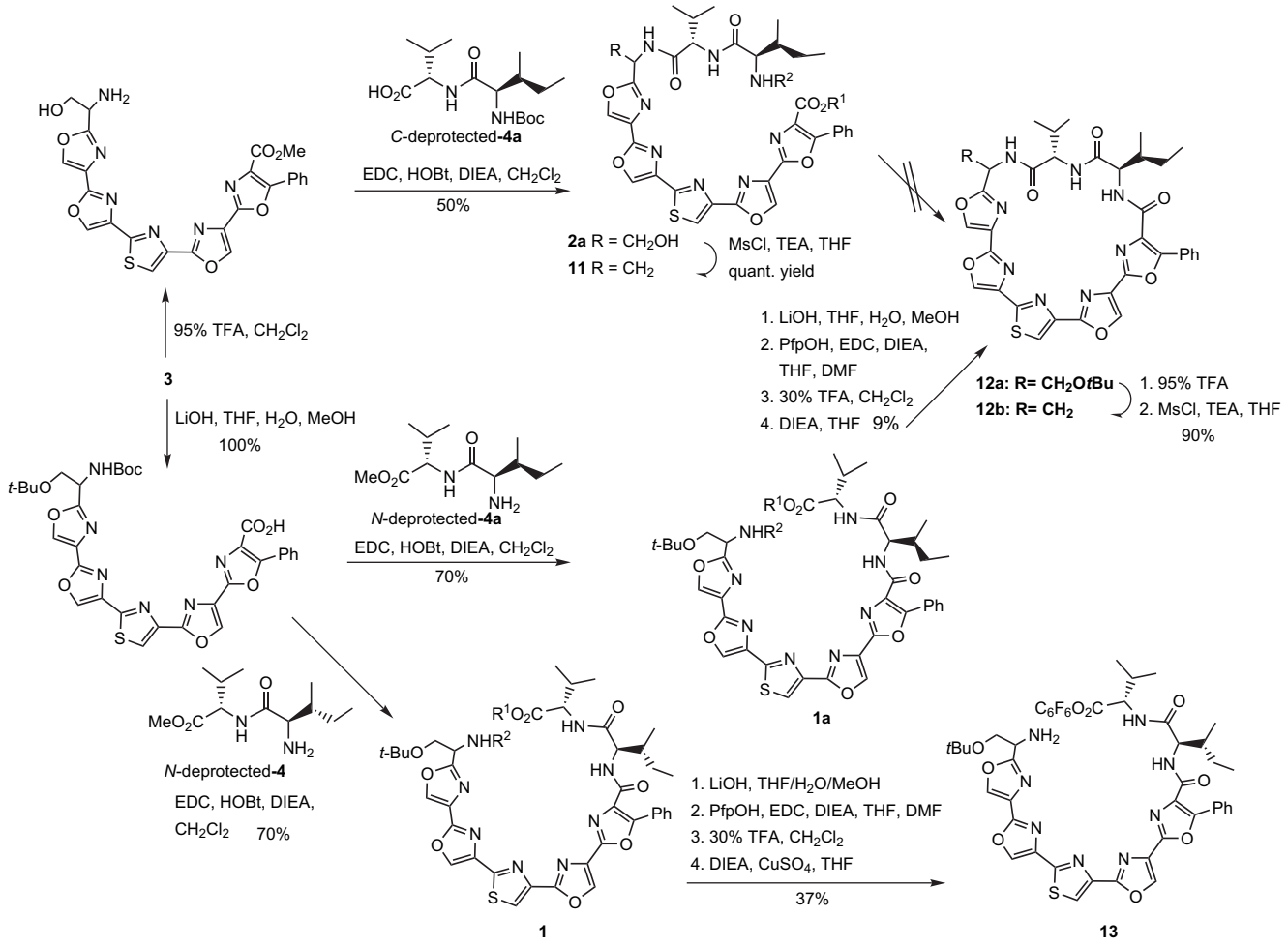
The exocyclic methylidene of **12b** was prepared by O-deprotection and dehydration of **12a** using mesyl chloride as an activating agent and *N*,*N*,*N*-triethylamine (TEA) in THF. It was characterized by ^{1}H NMR, whereby the two methylidene singlets at 6.06 and 6.70 ppm, and the four singlets of the azole rings (7.96, 8.20, 8.27, and 8.30 ppm), were taken as representative signals.[21] As the yield of this macrocyclization was poor (less than 10%), we decided to test cyclization using a copper salt template.

The peptide-heterocycle **1** (R^1=Me, R^2=Boc) was obtained by condensation of the free carboxylic acid of **3** with the free amine of **4**, using the same reaction conditions described above for **1a**. Similar conditions were also used for the methyl ester hydrolysis of **1**, conversion of the resulting acid into the pentafluorophenyl ester followed by selective N-deprotection. Macrocyclization was then attempted using DIEA and $CuSO_4$ in a highly dilute THF solution. Unfortunately, no trace of the cyclized product was found, and only the pentafluorophenyl ester **13** of the open chain peptide-heterocycle was obtained.

3. Biological activity

The cytotoxicity of the concatenated azoles was evaluated against a panel of three human tumor cell lines: A-549 lung carcinoma NSCL, HT-29 colon carcinoma, and MDA-MB-231 breast adenocarcinoma. A conventional colorimetric assay was run to estimate GI_{50} values (i.e., the drug concentration at which 50% of cell growth is inhibited after 72 h of continuous exposure to the test molecule), in which IB-01211 was for comparison.

The results obtained are shown in Table 1. A decrease of activity of compounds **3**, **1**, and **13** in relation with IB-01211 has been observed. Penta-azole **3** possesses activity at micromolar (μM) concentration in the three cell lines, whereas **1** possesses the same activity only in A-549 and **13** is inactive. The natural compound, IB-01211, shows activity in the three lines at nanomolar concentration. None of the peptides with



Scheme 3. Macrocyclization of **1** and **2**.

Table 1. Cell growth inhibition (GI_{50}) of synthetized azoles

Compound	Cytotoxicity (GI_{50}, μM)		
	A-549[a]	HT-29[b]	MDA-MB-231[c]
IB-01211	0.06	0.10	0.069
1[e]	4.92	n.a	n.a
1a[e]	9.84	6.78	n.a
2a[e]	n.a	10.1	n.a
3	5.98	4.55	6.26
12a	n.a[d]	n.a[d]	n.a[d]
12b	n.a[d]	n.a[d]	n.a[d]
13	n.a[d]	n.a[d]	n.a[d]

[a] A-549 lung carcinoma NSCL.
[b] HT-29 colon carcinoma cells.
[c] MDA-MB-231 breast adenocarcinoma.
[d] n.a=not active at 10 μg/mL.
[e] R^1=Me, R^2=Boc.

D-Ile, **2a**, **12a**, and **12b**, has notable activity. These results demonstrate the importance of the configuration of the stereocenter to the activity of these compounds.

4. Conclusions

Herein we have reported preparation of the functionalized polyazoles **6a**, **6b**, **7a**, the penta-azole **3**, and the peptide-heterocycles **1**, **1a**, **2a**, **12a**, **12b**, and **13**. Attempts at macrocyclization using the amino acids from the deprotection of **1** and **2** only led to a small amount of cyclized product, underscoring the need to change the synthetic strategy. Likewise, macrocyclization of *N*- and *C*-deprotected **1** using a copper chelating group also failed. While **1** and **2** possess an almost planar polyheterocyclic system of penta-azoles that separate the reactive groups the shown precursor of IB-01211 possesses bigger conformational freedom, which made the macrocyclization easier.

The open chain polyazoles with the same stereocenter configuration as the natural product inhibit growth of human carcinoma cells (GI_{50}) at micromolar concentrations, whereas the epi-analogs obtained with D-Ile are inactive.

5. Experimental section

5.1. General

Melting points (mp) were determined in a Büchi Melting Point B540 in open capillaries and are uncorrected. Reversed-phase analytical HPLC was performed on a Waters Alliance separation module 2695 using a Waters Xterra MS C_{18} column (150×4.6 mm, 5 μm) and a Waters 996

PDA with a photodiode array detector with MeCN (0.036% TFA) and H_2O (0.045% TFA). Sonication was performed in a Branson ultrasound bath. Polarimetry studies were performed in a Perkin–Elmer 241 polarimeter. ^1H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury 400 MHz and Varian 500 MHz spectrometers. Multiplicity of the carbons was assigned with DEPT and gHSQC experiments. Usual abbreviations for off-resonance decoupling have been used here: (s) singlet, (d) doublet, (t) triplet, and (q) quartet. The same abbreviations have also been used for the multiplicity of signals in ^1H NMR, plus: (m) multiplet, (dd) double doublet, (br s) broad singlet, and (br d) broad doublet. Spectra were referenced to appropriate residual solvent peaks ($CDCl_3$ and DMSO-d_6). CIMS were measured in a Hewlett-Packard model 5890A with ammonia (NH_3). MALDI-TOF and ES-MS were performed in a PerSeptive Biosystems Voyager DE RP, using an ACH matrix, for the former, and a Waters alliance 2795 HPLC equipped with a 2487 UV–vis detector and coupled to a ZQ electrospray mass detector, for the latter. The samples were run with MeCN (0.07% HCO_2H) and H_2O (0.1% HCO_2H). HRMS were performed on a Bruker Autoflex high resolution mass spectrometer by the Mass Spectrometry Service of the University of Santiago de Compostela.

Syntheses of compounds **4** ($[\alpha]_D$ +22.3 (*c* 0.56, $CHCl_3$)), **5a**, **5b**, **8**, **9**, and **10** have previously been reported.[15]

5.2. Peptide bond formation. Sample procedure

5.2.1. D-Boc–Ile–L-Val–OMe (4a). D-Boc–Ile–OH·1/2 H_2O (795 mg, 3.31 mmol), EDC·HCl (698 mg, 3.64 mmol), HOBt (491 mg, 3.64 mmol), and DIEA (1.21 mL, 7.11 mmol) were added to a solution of L-H–Val–OMe·HCl (550 mg, 3.31 mmol) and dry CH_2Cl_2 (28 mL) at 0 °C. The mixture was stirred at room temperature for 20 h. The organic solution was washed with 5% $NaHCO_3$ and NH_4Cl, dried, and concentrated to give the title compound (1.02 g, 90%) as a white solid, mp 105–107 °C. $[\alpha]_D$ +16.6 (*c* 0.7, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz) δ 0.88–0.93 (m, 12H); 1.09–1.21 (m, 1H); 1.42 (s, 9H); 1.49–1.55 (m, 1H); 1.86 (m, 1H); 2.11–2.20 (m, 1H); 3.72 (s, 3H); 3.92–3.95 (m, 1H); 4.51–4.55 (m, 1H); 5.04–5.06 (d, 6.8 Hz, 1H); 6.37–6.39 (d, 6.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.31 (q); 15.5 (q); 17.71 (q); 18.9 (q); 24.78 (t); 28.27 (q); 31.20 (d); 36.91 (d); 52.09 (q); 57.01 (d); 59.37 (d); 79.88 (s); 155.73 (s); 171.55 (s); 172.08 (s). MS (CI): *m/z* 345 (M+1, 100), 289 (M, 29), 245 (M, 43). HRMS *m/z* calcd for $C_{17}H_{32}N_2NaO_5$ (M+Na) 367.2203, found 367.2206.

5.2.2. Hydrolysis of methyl esters. LiOH (2 M, 9 mmol) was added to a solution of methyl ester (3 mmol) in THF–H_2O–MeOH (50:6:0.2, 14 mL), and the reaction mixture was stirred at room temperature for 1 h. The pH was brought to 3 by addition of 1 M HCl, and then the solution was extracted with EtOAc. The organic layer was dried over $MgSO_4$ and concentrated to afford the acid as a white solid.

5.2.3. Deprotection with TFA.

5.2.3.1. Method A. *Elimination of N-Boc and O-t-Bu protecting groups*: 95% TFA (5 mL) was added to a solution of the di-protected (*N*-Boc and *O*-*t*-Bu) compound (2.38 mmol), and the solution was stirred at room temperature for 5 h. The TFA was then removed under reduced pressure, and the crude material was used for subsequent chemistry without further purification.

5.2.3.2. Method B. *Selective elimination of N-Boc protecting group*: a solution of *N*-Boc protected compound (27 μmol) in TFA–CH_2Cl_2 (1 mL, 3:7) was stirred at room temperature for 1 h. The TFA and the solvent were removed, and the crude material was used for subsequent chemistry without further purification.

5.2.4. Peptide 1 (R^1=Me, R^2=Boc). Coupling of the free carboxylic acid of **3** to the free amine of **4** using the general procedure for peptide formation provided **1** (70%) as a white solid, mp (MeCN) 186–188 °C. $[\alpha]_D$ +4.0 (*c* 0.56, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz) δ 0.88–1.08 (m, 12H); 1.12 (s, 9H); 1.22–1.34 (m, 1H); 1.48 (s, 9H); 1.52–1.62 (m, 1H); 2.11–2.25 (m, 2H); 3.70 (s, 3H); 3.74 (dd, *J*=4.4 and 9.2 Hz, 1H); 4.57 (dd, *J*=5.2 and 8.6 Hz, 1H); 4.65 (dd, *J*=5.6 and 8.8 Hz, 1H); 5.06–5.14 (m, 1H); 5.62 (br s, 1H); 6.56 (br s, 1H); 7.42–7.53 (m, 3H); 7.81 (br s, 1H); 8.24 (s, 1H); 8.29 (s, 1H); 8.33–8.38 (m, 2H); 8.42 (s, 1H); 8.47 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.6 (q); 14.7 (q); 17.8 (q); 18.9 (t); 27.3 (q); 28.3 (q); 29.7 (t); 31.2 (d); 37.1 (d); 50.1 (d); 52.1 (q); 57.1 (d); 57.2 (t); 62.9 (t); 73.7 (s); 80.2 (s); 122.0 (d); 126.6 (s); 128.3 (d); 128.5 (d); 129.6 (s); 129.9 (s); 130.2 (d); 131.0 (s); 136.2 (d); 136.3 (s); 139.3 (d); 143.4 (d); 151.9 (s); 153.0 (s); 155.3 (s); 155.6 (s); 158.2 (s); 161.2 (s); 161.3 (s); 164.8 (s); 171.0 (s); 172.0 (s). MS (MALDI): *m/z* 937.33 (M+23, 100). HRMS *m/z* calcd for $C_{45}H_{54}N_8NaO_{11}S$ (M+Na) 937.3525, found 937.3527.

5.2.5. Peptide 1a (R^1=Me, R^2=Boc). Coupling of the free carboxylic acid of **3** to the free amine of **4a** using the general procedure for peptide formation provided **1a** (70%) as a white solid, mp (MeCN) 188–190 °C. $[\alpha]_D$ +3.4 (*c* 0.64, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz) δ 0.87–1.02 (m, 12H); 1.03–1.06 (m, 1H); 1.11 (s, 9H); 1.21–1.33 (m, 1H); 1.47 (s, 9H); 1.60–1.75 (m, 1H); 2.07–2.24 (m, 1H); 3.69 (s, 3H); 3.71–3.76 (m, 1H); 3.84–3.91 (m, 1H); 4.47–4.61 (m, 2H); 5.04–5.14 (m, 1H); 5.60 (br s, 1H); 6.50 (br s, 1H); 7.42–7.53 (m, 3H); 7.82 (br s, 1H); 8.23 (s, 1H); 8.28 (s, 1H); 8.32–8.38 (m, 2H); 8.4 (s, 1H); 8.47 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.4 (q); 15.7 (q); 17.6 (q); 19.0 (q); 25.1 (t); 27.3 (q); 28.3 (q); 31.2 (d); 36.9 (d); 50.1 (d); 52.1 (q); 57.2 (d); 58.1 (d); 62.9 (t); 73.7 (s); 80.2 (s); 122.0 (d); 126.7 (s); 128.4 (d); 128.5 (d); 129.7 (s); 130.0 (s); 130.2 (s); 131.1 (s); 136.2 (d); 136.4 (s); 139.3 (d); 143.4 (d); 151.8 (s); 153.1 (s); 155.3 (s); 155.6 (s); 158.2 (s); 161.1 (s); 161.2 (s); 164.8 (s); 170.7 (s); 171.9 (s). MS (MALDI): *m/z* 937.31 (M+Na, 100). HRMS *m/z* calcd for $C_{45}H_{54}N_8NaO_{11}S$ (M+Na) 937.3525, found 937.3529.

5.2.6. Peptide-heterocycle 2a (R^1=Me, R^2=Boc). The free amino alcohol, obtained by N- and O-deprotection of **3** using 95% TFA (2 mL), was coupled to the acid of **4a** following the general procedure for peptide formation to provide **2a** (50%) as a white solid, mp (MeCN) 224–226 °C. ^1H NMR (DMSO, 400 MHz) δ 0.76–0.88 (m, 12H); 0.95–1.10 (m, 1H); 1.37 (s, 9H); 1.45–1.46 (m, 1H); 1.63–1.72 (m, 1H); 1.94–2.06 (m, 1H); 3.57–3.63 (m, 1H); 3.76–3.84 (m, 1H); 3.86 (s, 3H); 3.97–4.07 (m, 1H); 4.11–4.17 (m, 1H); 5.14–5.21 (m, 1H); 5.30–5.36 (br s, 1H); 6.77 (br s, 1H); 7.55–7.61 (m, 3H);

7.73–7.80 (br s, 1H); 8.06–8.11 (m, 2H); 8.67 (s, 1H); 9.03 (s, 1H); 9.08 (s, 1H); 9.2 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 10.8 (q); 15.3 (q); 17.9 (q); 19.0 (q); 29.0 (t); 29.8 (q); 36.27 (d); 40.6 (d); 50.2 (d); 52.0 (q); 57.7 (d); 58.7 (d); 60.5 (t); 69.7 (s); 78.8 (s); 123.4 (d); 126.1 (s); 127.2 (s); 128.2 (2d); 128.5 (2d); 129.8 (s); 129.9 (s); 130.6 (d); 135.4 (d); 139.1 (s); 141.2 (d); 141.3 (s); 141.5 (s); 142.6 (d); 152.8 (s); 154.3 (s); 155.2 (s); 157.4 (s); 160.4 (s); 161.6 (s); 163.6 (s); 164.1 (s); 172.6 (s). MS (MALDI): *m/z* 881.07 (M+Na, 78), 783 (95), 759 (100). HRMS *m/z* calcd for $C_{41}H_{46}N_8NaO_{11}S$ (M+Na) 881.2899, found 881.2886.

5.2.7. Methyl 2′-{2-[2′-(2-*tert*-butoxy-1-*tert*-butoxycarbonyl-aminoethyl)-[2,4′]bis-oxazol-4-yl]thiazol-4-yl}-5-phenyl[2,4′]bis-oxazolyl-4-carboxylate (3). Bromoketone **6b** (64 mg, 0.16 mmol) was added to a suspension of **5b** (45 mg, 0.11 mmol) and $NaHCO_3$ (29 mg, 0.35 mmol) in THF (2 mL). The mixture was stirred for 8 h at room temperature, at which point it was filtered over alumina and washed with CH_2Cl_2–MeOH (4:1). The organic layer was concentrated to give a crude material, which was dissolved in dry THF (2 mL) and cooled to −10 °C. Lutidine (0.10 mL, 0.87 mmol) and TFA (46 μL, 0.33 mmol) were then added to the solution, and the reaction mixture was stirred at room temperature overnight. Concentration in vacuo gave a brown residue, which was purified by silica gel chromatography. Elution with CH_2Cl_2–EtOAc (9:1) gave **3** (47.8 mg, 62%) as a white solid, mp (MeCN) 216–218 °C. $[\alpha]_D$ −12.2 (*c* 0.51, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.11 (s, 9H); 1.47 (s, 9H); 3.73 (dd, *J*=4.4 and 9.2 Hz, 1H); 3.86–389 (m, 1H); 3.98 (s, 3H); 5.06–5.12 (m, 1H); 5.58–5.60 (d, *J*= 8.4 Hz, 1H); 7.49–7.52 (m, 3H); 8.17–8.20 (m, 2H); 8.22 (s, 1H); 8.28 (s, 1H); 8.46 (s, 1H); 8.49 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 27.29 (q); 28.32 (q); 29.69 (q); 50.14 (s); 52.43 (d); 62.94 (t); 73.75 (s); 121.92 (d); 126.46 (s); 127.68 (s); 128.44 (d); 128.66 (d); 129.95 (s); 130.63 (s); 131.0 (s); 136.17 (d); 136.4 (s); 139.30 (s); 139.37 (d); 143.51 (d); 153.03 (s); 155.61 (s); 158.01 (s); 161.17 (s); 162.35 (s); 164.79 (s). MS (FAB): *m/z* 720.1 (M+18, 65), 589 (M+1, 100). HRMS *m/z* calcd for $C_{34}H_{34}N_6NaO_9S$ (M+Na) 725.200, found 725.1993.

5.2.8. Methyl 2′-(2-*tert*-butoxy-1-*tert*-butoxycarbonyl-amino-ethyl)-5-phenyl[2,4′]bis-oxazolyl-4-carboxylate (6). The free amine of **9** was coupled with Boc–L-Ser(*t*-Bu)–OH following the general procedure for peptide formation. Cyclization using DAST/K_2CO_3 and oxidation with DBU-CCl_4 in Pyr and ACN as solvents gave a crude, which was purified by column chromatography on silica gel. Elution with hexane–EtOAc (3:1) gave **6** (50%) as a solid, mp (MeCN) 66–68 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.1 (s, 9H); 1.47 (s, 9H); 3.72 (dd, *J*=4.0 and 9.2 Hz, 1H); 3.83–3.89 (m, 1H); 3.96 (s, 3H); 5.02–5.12 (m, 1H); 5.6 (br s, 1H); 7.45–7.51 (m, 3H); 8.10–8.15 (m, 2H); 8.33 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 27.24 (q); 28.30 (q); 50.10 (d); 52.32 (q); 62.89 (t); 73.70 (s); 80.13 (s); 126.50 (s); 127.60 (s); 128.40 (2d); 128.56 (2d); 129.80 (s); 130.50 (s); 139.41 (d); 153.31 (s); 155.34 (s); 162.35 (s); 164.50 (s). MS (ES) *m/z* 486.53 (M+1, 100). HRMS *m/z* calcd for $C_{25}H_{32}N_3O_7$ 486.2235, found 486.2222.

5.2.9. Methyl 2′-(2-bromoacetyl)-5-phenyl[2,4′]bis-oxazolyl-4-carboxylate (6b). A mixture of **10** (200 mg, 452 μmol) and formic acid (3.5 mL) was refluxed for 2 h, and then cooled to room temperature. The organic solution was poured into an aqueous solution of $NaHCO_3$ and extracted with CH_2Cl_2. The organic layer was dried over $MgSO_4$ and concentrated to give **6b** (164 mg, 91%) as a yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.98 (s, 3H); 4.68 (s, 2H); 7.51–7.53 (m, 3H); 8.13–8.16 (m, 2H); 8.58 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 30.3 (t); 52.5 (q); 126.1 (s); 127.8 (s); 128.5 (2d); 128.6 (2d); 130.9 (d); 131.7 (s); 142.5 (d); 151.3 (s); 155.9 (s); 156.1 (s); 162.1 (s); 178.7 (s). MS (ES): *m/z* 408.1 (MBr^{79}+18, 61), 410.1 (MBr^{81}+18, 62), 391 (MBr^{79}, 62), 393 (MBr^{81}, 63), 313 (100). HRMS *m/z* calcd for $C_{16}H_{12}BrN_2O_5$ 390.9924, found 390.9911.

5.2.10. Peptide 7a. The free carboxylic acid of **5** (40 mg, 0.10 mmol) was coupled to **6a** (33 mg, 0.10 mmol) using the general procedure for peptide formation to provide **7a** (31 mg, 43%) as a yellow solid, mp (MeCN) 98–100 °C. $[\alpha]_D$ −13.4 (*c* 0.35, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.09 (s, 18H); 1.45 (s, 9H); 3.6–3.70 (m, 1H); 3.79–3.84 (m, 1H); 3.93 (s, 3H); 4.09–4.13 (m, 1H); 4.28–4.32 (m, 1H); 5.01–5.09 (m, 1H); 5.55–5.59 (m, 1H); 7.43–7.48 (m, 4H); 7.95 (d, *J*=8.0 Hz, 1H); 8.07–8.10 (m, 2H); 8.20 (s, 1H); 8.26 (s, 1H); 8.33 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 27.2 (q); 28.2 (q); 48.5 (d); 50.1 (d); 52.4 (q); 62.9 (t); 63.2 (t); 73.7 (s); 80.3 (s); 126.6 (s); 127.8 (s); 128.7 (d); 128.8 (d); 129.9 (s); 130.9 (d); 136.5 (s); 139.5 (d); 140.2 (d); 141.8 (d); 153.0 (s); 153.6 (s); 155.6 (s); 155.8 (s); 155.9 (s); 160.5 (s); 160.8 (s); 163.2 (s); 164.2 (s); 171.3 (s). MS (MALDI-TOF) 745 (M+K); 729.24 (M+Na, 100); 707.64 (60); 674 (60). HRMS *m/z* calcd for $C_{34}H_{38}N_6NaO_{11}$ (M+Na) 729.2491, found 729.2480.

5.2.11. Peptide 12a. A solution of the carboxylic acid (125 mg, 190 μmol) obtained from **1a** in dry THF–DMF (120 and 5 mL) was cooled to 0 °C. EDC·HCl (190 mg, 99 μmol), DIEA (167 μL, 99 μmol), and Pfp-OH (194 mg, 1.05 mmol) were then added, and the mixture was stirred at room temperature for 20 h. The solvents were removed in vacuo, and the residue was diluted with CH_2Cl_2 and washed with 5% aqueous $NaHCO_3$ and aqueous NH_4Cl. The organic solution was dried and concentrated, and the residue was diluted with TFA–CH_2Cl_2 (6 and 14 mL). The solution was stirred for 1 h at room temperature, and then the TFA was removed, and the crude material was dissolved in THF (250 mL). DIEA (479 μL, 2.82 mmol) was added, and the mixture was stirred for 96 h at room temperature. The solvents were removed, and the residue was then washed with MeOH. The residue obtained after removing the solvent was purified by preparative HPLC. A gradient of H_2O (0.045% TFA)–MeCN (0.036% TFA) from 6:4 until 1:9 in 15 min gave a white solid (10 mg, 9%, rt=7.47 min), mp (MeCN) 206–208 °C. $[\alpha]_D$ −48.3 (*c* 0.35, DMSO). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 0.82–0.95 (m, 12H); 1.02–1.06 (m, 2H); 1.22 (s, 9H); 1.49–1.57 (m, 1H); 1.85–1.91 (m, 1H); 3.85–3.94 (m, 1H); 3.97–4.04 (m, 1H); 4.17–4.24 (m, 1H); 4.62–4.71 (m, 1H); 5.16–5.21 (m, 1H); 5.28–5.35 (m, 1H); 7.53–7.58 (m, 3H); 8.01 (br s, 1H); 8.03 (br s, 1H); 8.27–8.37 (m, 2H); 8.49 and 8.62 (2br s, 1H); 8.73 (s, 1H); 9.04 (s, 1H); 9.12 (s, 1H); 9.23 (s, 1H); 10.17 (br s, 1H). ^{13}C NMR (DMSO-d_6, 125 MHz) δ 11.3 (q); 14.2 (q); 15.6 (q); 18.7 (q); 18.8 (q); 24.5 (t); 38.1 (d); 50.5 (d); 56.5 (d); 57.7 (d); 60.9 (t); 67.8 (d); 123.9 (d);

128.4 (2d); 128.9 (2d); 130.5 (d); 138.0 (d); 141.8 (d); 141.9 (d). MS (MALDI): *m/z* 782.19 (M, 40).

5.2.12. Peptide 12b. Peptide **12a** (9 mg, 0.0114 mmol) was deprotected with 95% TFA (1 mL). The crude was dissolved in dry THF (1 mL), and the solution was cooled to 0 °C, TEA (15.82 μL, 0.114 mmol) and MsCl (4.4 μL, 0.057 mmol) were added dropwise. The resulting solution was stirred for 2 h at 0 °C, then washed with NH_4Cl and water, dried, and concentrated. The solvents were removed and the residue was washed with MeCN to give a white solid (7.4 mg, 90%), mp (MeCN) 147–149 °C. $[\alpha]_D$ +12.4 (*c* 0.15, $CHCl_3$). ^1H NMR ($CDCl_3$, 500 MHz) δ 0.79–0.92 (m, 12H); 0.99–1.09 (m, 2H); 1.41–1.45 (m, 1H); 1.52–1.55 (m, 1H); 3.65–3.75 (m, 2H); 3.78–3.89 (m, 1H); 6.06 (s, 1H); 6.70 (s, 1H); 7.43–7.52 (m, 4H); 7.96 (s, 1H); 8.20 (s, 1H); 8.27 (s, 1H); 8.30 (s, 1H); 8.31 (br s, 1H); 8.36–8.37 (m, 2H); 8.47 (br s, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz) δ 11.29 (q); 13.9 (q); 15.2 (q); 18.7 (q); 25.2 (t); 30.2 (d); 30.7 (d); 53.6 (d); 63.2 (d); 111.7 (t); 119.5 (d); 127.9 (d); 128.4 (2d); 130.2 (2d); 137.7 (d); 139.0 (d); 139.4 (d). MS (MALDI): *m/z* 749.3 (M+Na, 100), 765.3 (M+K, 47).

5.2.13. Peptide 13. A solution of carboxylic acid (150 mg, 0.166 mmol) obtained from **1** in dry THF–DMF (10 and 2 mL) was cooled to 0 °C. EDC·HCl (0.22 mg, 1.16 mmol), DIEA (0.19 mL, 1.16 mmol), and Pfp-OH (0.229 mg, 1.24 mmol) were then added, and the reaction mixture was stirred at room temperature for 20 h. The solvents were removed in vacuo, and the residue was diluted with CH_2Cl_2, and then washed with 5% aqueous $NaHCO_3$ and aqueous NH_4Cl. The organic solution was dried and concentrated, and the residue was diluted in TFA–CH_2Cl_2 (1 and 3 mL). The solution was stirred for 1 h at room temperature, and then the TFA was removed. The crude material was dissolved in THF (300 mL). DIEA (0.28 mL, 1.66 mmol) and $CuSO_4$ (132 mg, 0.83 mmol) were added, and the mixture was stirred for 72 h at room temperature. The solvents were removed, and the crude was purified by silica gel chromatography (9:1 CH_2Cl_2–MeOH) to give **13** (58.8 mg, 37%) as a yellow solid, mp (MeCN) 140–142 °C. $[\alpha]_D$ +7.7 (*c* 0.39, $CHCl_3$). ^1H NMR ($CDCl_3$, 400 MHz) δ 0.94–1.09 (m, 12H); 1.13 (s, 9H); 1.20–1.32 (m, 1H); 1.51–1.61 (m, 1H); 2.08–2.26 (m, 1H); 2.32–2.43 (m, 1H); 3.77–3.83 (m, 1H); 3.96–4.01 (m, 1H); 4.55–4.65 (m, 1H); 4.82–4.91 (m, 1H); 5.38–5.45 (m, 1H); 6.67 (d, J=8.4 Hz, 1H); 6.81 (d, J=8.4 Hz, 1H); 7.42–7.47 (m, 3H); 8.24 (s, 1H); 8.29–8.30 (m, 2H); 8.31 (s, 1H); 8.40 (s, 1H); 8.47 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.4 (q); 14.7 (q); 17.6 (q); 18.9 (q); 26.2 (t); 27.3 (q); 30.9 (d); 36.4 (d); 48.9 (d); 57.2 (d); 57.5 (d); 61.8 (t); 74.3 (s); 122.3 (d); 125.1 (s); 126.4 (s); 126.5 (s); 128.3 (2d); 128.5 (2d); 128.7 (s); 129.4 (s); 130.1 (s); 130.3 (d); 130.9 (s); 131.5 (s); 136.3 (s); 136.4 (d); 139.3 (d); 139.8 (d); 143.3 (s); 151.8 (s); 153.2 (s); 155.1 (s); 156.8 (s); 157.2 (s); 158.2 (s); 161.0 (s); 161.5 (s); 162.0 (s); 167.7 (s); 171.5 (s). ^{19}F NMR ($CDCl_3$, 400 MHz) δ 84.0 (s). MS (MALDI): *m/z* 1006.7 (M+K, 100).

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17. **7a** was obtained as unique stereoisomer as indicated in its H and C NMR maintaining the configuration of starting L-Ser used in the preparation of **5a** and **6a**.

18. *S*-trityl cysteine containing peptide was treated with $TiCl_4$ and Ph_3PO/Tf_2O for a concomitant removal of the trityl group and cyclization following the methods developed by Kelly and coworkers: (a) Raman, P.; Razavi, H.; Kelly, J. W. *Org. Lett.* **2000**, *2*, 3289; (b) You, S.-L.; Razavi, H.; Kelly, J. W. *Angew. Chem., Int. Ed.* **2003**, *42*, 83; (c) You, S.-L.; Kelly, J. W. *J. Org. Chem.* **2003**, *68*, 9506.
19. The peptide **8** was prepared as a stereoisomer mixture from *N*–Boc–*O*–*t*-Bu–L-Ser–OH and D,L-PhSer–OMe as it is described in Ref. 15. The three stereocenters of **8** were lost in the bisoxazole **10**.
20. Selective deprotection of the *N*-Boc in front of *O*-*t*-Bu group was afforded by treatment with a solution of TFA in CH_2Cl_2 (30:70) for 1 h at room temperature.
21. Assays of coelution in the HPLC of the natural product and the obtained macrocyclic peptide demonstrated that both compounds were different. A sample of IB-01211 was kindly supplied by PharmaMar S.L.