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Diversity-oriented synthesis and cytotoxic activity evaluation of biaryl-containing macrocycles†

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Synthesis of biaryl-containing macrocycles has been carried out through a four-step approach comprising two Ugi four component reactions and a Suzuki–Miyaura macrocyclization. This protocol allowed the synthesis of 12- and 14-membered macrocycles. Cytotoxic activity evaluation showed that some of the molecules were effective against leukemia, glioblastoma and lung cancer cell lines (IC_{50} = 4.0, 5.9 and 7.6, respectively).

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Introduction

Macrocycles are relevant compounds with recognized applications in various fields of contemporary chemistry.[1] Owing to their remarkable biological activities such as antibiotic,[2] antifungal,[3] antiparasitic,[4] and anticancer activities,[5] and as immunosuppressant[6] agents, many natural macrocycles are highly significant in medicine.[7] Particularly, peptide macrocycles having an *endo* aryl–aryl bond (Fig. 1) constitute an attractive family of complex natural products, which includes biphenomycin A and B (**1**, **2**),[8] arylomycin A2 and B2 (**3**, **4**),[9] and bicyclic RP 66453 (**5**)[10] as well as vancomycin-type glycopeptide antibiotics.[11] For the crucial macrocyclization process in their preparation, two strategies have been implemented: (i) construction of the biaryl unit followed by a proper macrocyclization,[12] and (ii) ring closure by the formation of the aryl–aryl bond on a linear preformed peptide backbone.[13] Since the construction of the peptide fragment has typically been carried out through slow and multi-step sequences, the design of practical methodologies that give access to biaryl-containing peptidic macrocycles is of special relevance in drug discovery. Thus, as part of our ongoing program to synthesize pharmacologically relevant macrocyclic scaffolds,[7] we envisioned the combination of a Ugi four component reaction (Ugi-4CR)[14] with a Suzuki–Miyaura cross-coupling for rapid access to macrocycles containing both a peptoid moiety and an *endo* biaryl portion (Scheme 1). Notably, when a Ugi reaction is sequentially combined with a C–C bond-forming process, the resultant synthetic sequence provides access to molecules that generally exhibit amplified molecular complexity and diversity.[15,16] As far as we know, although different processes have been considered using a Ugi-4CR to construct macrocycles,[8,17] the combination with a Suzuki–Miyaura cross-coupling reaction towards biaryl-containing macrocycles has not yet been reported.

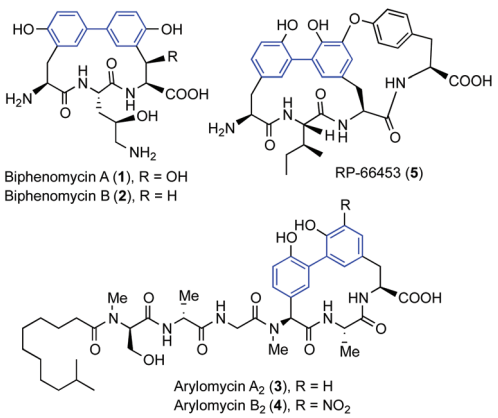

Fig. 1 Structure of some relevant natural occurring biaryl-containing macrocycles.

Thus, the proposed synthetic strategy comprised four steps (Scheme 1): (i) a first Ugi-4CR using two bifunctional building blocks (*i.e.*, mono-Boc protected diamine and iodine-containing carboxylic acid); (ii) Boc cleavage; (iii) a second Ugi-4CR using a boron-containing carboxylic acid and (iv) a Suzuki–

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†Electronic supplementary information (ESI) available: Experimental procedures, NMR spectra, characterization for all new materials and biological evaluation. CCDC 1530930. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c6ob02726a

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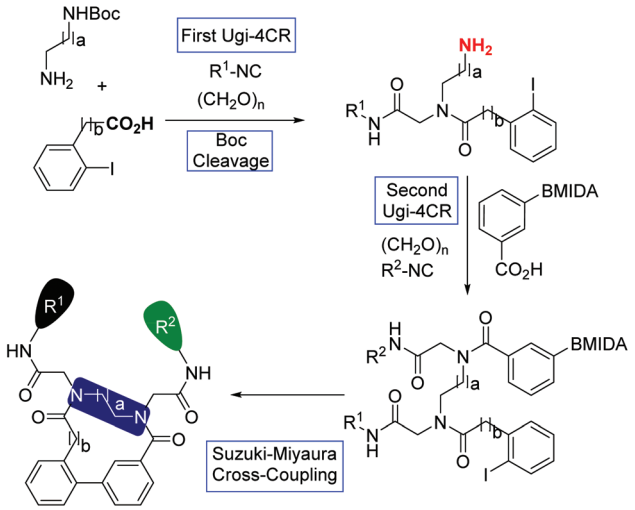
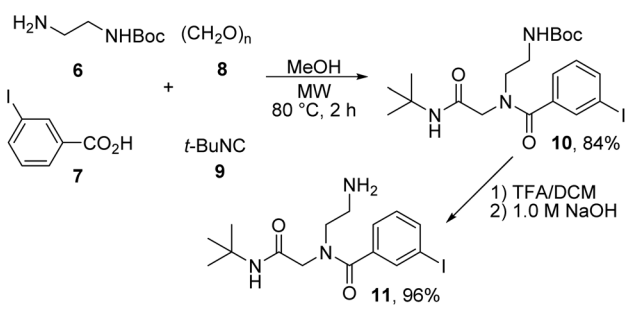


Scheme 1 Designed synthetic strategy for the preparation of biaryl-containing macrocycles.

Miyaura cross-coupling-based macrocyclization. Accordingly, the macrocycle size might be modulated by using aliphatic mono-Boc protected diamines with different chain lengths, as well as dissimilar iodine-containing carboxylic acids (Scheme 1). Furthermore, the utilization of diverse isocyanides in both Ugi-4CRs, would be another element for structural diversity.

Results and discussion

To implement the synthetic strategy, two mono-Boc protected diamines were prepared as reported in the literature, using equimolar amounts of TFA and Boc_2O with iodine as the catalyst.[18] At the outset, *N*-Boc-1,2-diaminoethane (**6**) and 3-iodobenzoic acid (**7**) were chosen as model reagents to examine the designed synthetic strategy (Scheme 2). To avoid the formation of diastereomers, paraformaldehyde (**8**) was used as the carbonyl source in both multicomponent steps. Thus, the first Ugi-4CR proceeded without any difficulty under microwave irradiation at 80 °C for 2 h, using MeOH as the solvent to furnish the desired α-acylaminocarboxamide **10** in 84% yield. *N*-Boc-cleavage with 20% TFA solution in DCM and subsequent treatment with 1.0 M aqueous NaOH solution generated the corresponding amino compound (**11**) in excellent yield (96%).

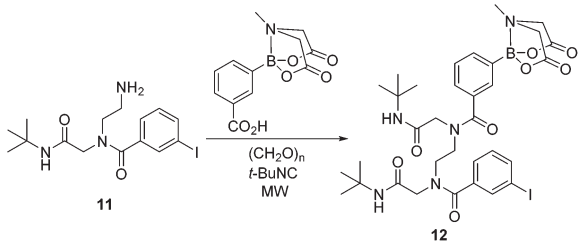


Scheme 2 Synthesis of amine **11** through Ugi-4CR and Boc-cleavage.

Then, due to the well-known stability of MIDA boronates to both column purification and storage,[19] the bifunctional 3-carboxyphenylboronic acid MIDA ester was selected as the acid component for the second Ugi 4-CR. As shown in Table 1, the reaction did not occur in MeOH (entry 1), but proceeded in 2,2,2-trifluoroethanol (TFE, entry 2). Though, MIDA boronates are stable in a wide range of solvents, prolonged exposure to alcoholic solvents may lead to hydrolysis of the MIDA ligand.[19a] Accordingly, the less nucleophilic TFE allowed the preparation of **12** in moderate yield (37%, entry 2). A short reaction time (90 min) resulted in an improved yield (45%, entry 3). Further reduction in the reaction time (60 min) resulted in an incomplete process (entry 4). Finally, the best reaction conditions were observed when a more diluted system was used (0.06 M, instead of 0.1 M, entry 6).

We then examined the intramolecular Suzuki–Miyaura coupling. As shown in Table 2, different catalysts, bases, reaction times and temperatures were evaluated to find the best conditions for this coupling process. All reactions were carried out under highly diluted conditions (MeCN : THF : H_2O/ 1 : 1 : 0.16 solution (5 μM)). Only low yields were observed for the macrocycle **8** when $Pd(PPh_3)_4$ was used as the catalyst (26% and 36% yields, respectively, entries 1 and 2). Moreover, the improvement in yield using $Pd_2(dba)_3$/SPhos *versus* $Pd(PPh_3)_4$ led us to choose the first set of conditions as the most convenient system for the transformation. With regard to the base, K_3PO_4 appeared to be more effective than Na_2CO_3 (entries 1–4). Microwave irradiation significantly shortened the reaction time (from 16 to 1.5 h) and improved the product yield (from 52 to 65%, entries 4 and 7). Lastly, a decrease in yield was observed when a lower temperature was used (entries 5 and 7). In summary, the best conditions for the Suzuki–Miyaura reaction were those used in entry 6. Minimizing column chromatography purification steps[20] is always a desir-

Table 1 Optimization study of the second Ugi reaction



Entry	Solvent	Temp. (°C)	Time (min)	Conc. (M)	**12** (%)
1	MeOH	50	120	0.1	0
2	TFE	50	120	0.1	37
3	TFE	50	90	0.1	45
4	TFE	50	60	0.1	28
5	TFE	60	60	0.1	53
6	TFE	60	60	0.06	62

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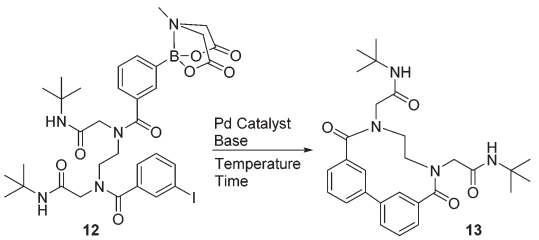
Table 2 Optimization studies of Suzuki–Miyaura reaction-based macrocyclization



12 13

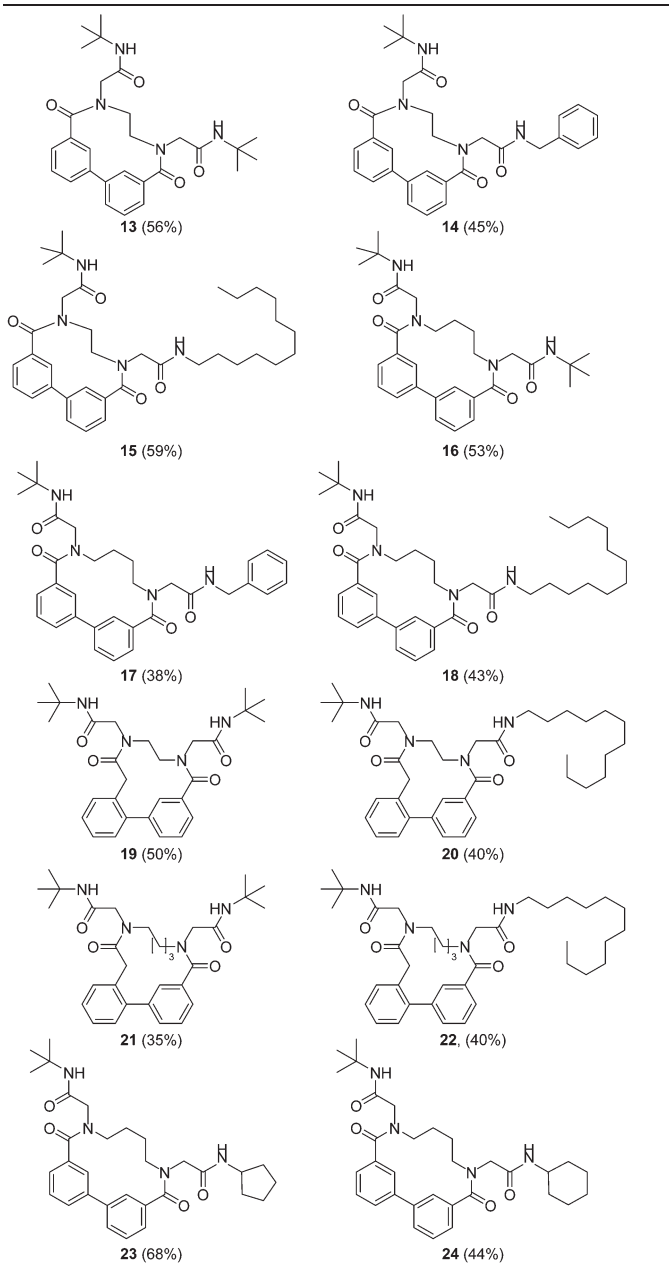
Entry	Catalyst	Base	Time (h)	Temp. (°C)	**13** (%)
1	$Pd(PPh_3)_4$	Na_2CO_3	16	70	26
2	$Pd(PPh_3)_4$	K_3PO_4	16	70	36
3	$Pd_2(dba)_3$/SPhos	Na_2CO_3	16	70	41
4	$Pd_2(dba)_3$/SPhos	K_3PO_4	16	70	52
5	$Pd_2(dba)_3$/SPhos	K_3PO_4	16	40	21
6[a]	$Pd_2(dba)_3$/SPhos	K_3PO_4	1.5	70	65
7[a]	$Pd_2(dba)_3$/SPhos	K_3PO_4	1.5	40	39

[a] Reaction mixtures were heated by microwave irradiation. 0.1 eq. of catalyst, 0.2 eq. of ligand (if necessary) and 7.5 eq. of base were employed.

able goal for the optimization of any synthetic methodology. For this reason, we implemented a protocol in which only the products of the final cross-coupling process were purified by column chromatography. Under this purification program, macrocycle **13** was obtained in 56% overall yield, compared with the 32% overall yield when all steps were subjected to chromatographic purification.

With the optimized conditions, for the synthesis of macrocycle **13**, in hand, we then studied the scope of this approach in the synthesis of a small collection of biaryl-containing macrocycles (Table 3). In all cases, *tert*-butyl isocyanide was used as a component in the first Ugi-4CR. For the second multicomponent process, isocyanides such as benzyl (**14**), *n*-dodecyl (**15**), cyclopentyl (**23**) and cyclohexyl (**24**) isocyanides were employed. These scaffolds were considered because lipid chains are biologically important structural units frequently found in naturally occurring macrocycles, such as arylomycin A_2 and B_2. To obtain larger macrocycles (**16–18**), *N*-Boc-1,4-diaminobutane was used as the amino component without any significant differences in the yield. Moreover, to expand the diversity of this methodology, 2-iodophenylacetic acid was used as the acid component in the first Ugi-4CR. As in the previous examples, both *N*-Boc protected amines and different isocyanides were utilized obtaining macrocycles **19–22** in yields ranging from 35 to 50% (overall yields). The structures of compounds were established by their spectroscopic and spectrometric data. Additionally, the structure of compound **23** was further corroborated by single-crystal X-ray analysis (Fig. 2).[21] Although, the bis-aryl derivatives were isolated in moderate yields, the outcomes were remarkable because of the great structural complexity that was achieved from simple and commercially available starting substrates, in a four-step protocol.

Table 3 Synthesis of biaryl-containing macrocycles using the optimized conditions. The figure displays the overall yield



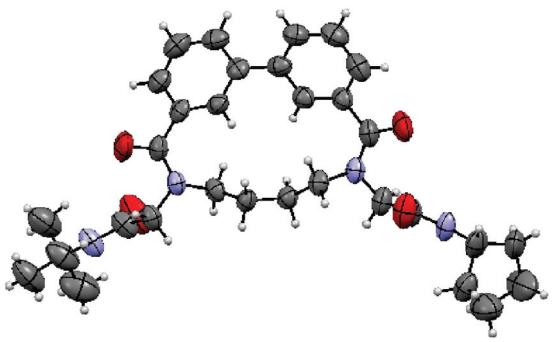


Fig. 2 Single-crystal X-ray analysis of macrocycle **23**.[21]

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Cytotoxic activity

Based on the well-documented anticancer activity of several macrocyclic structures, the cytotoxicity of scaffolds **13–24** was evaluated in a panel of eight human cancer cell lines (U251, human glioblastoma; PC-3, human prostatic adenocarcinoma; K562, human chronic myelogenous leukemia; MCF-7, human mammary adenocarcinoma; SKLU-1, human lung adenocarcinoma; HCT-15, human colorectal adenocarcinoma; MDA-MB231, mammary gland breast cancer and 786-O, renal cell adenocarcinoma) and one noncancerous cell line (HGF, human gingival fibroblasts). The results of the primary screening are shown in Table 4. Unfortunately, the symmetric *tert*-butyl-containing derivative **13**, as well as its analogs **16**, **19** and **21** resulted to be not pharmacologically relevant. The last finding revealed that two bulky *tert*-butyl groups might not contribute to the biological effect.

Furthermore, the replacement of the *tert*-butyl group by benzyl (**14** and **17**) and cyclic alkanes (cyclopentyl, **23** and cyclohexyl, **24**) did not show an enhancement in cell growth inhibition.

A well-documented disadvantage of peptidic macrocycles is their low permeability, and long alkyl chains have been used elsewhere to improve it.[22] Accordingly, a dodecyl chain was introduced instead of the *tert*-butyl group (example **15**), however without significant modification in cytotoxicity. Notably, when the size of the macrocycle **15** was enlarged, or phenylacetic acid was used instead of benzoic acid in the four-component set, the activity was significantly increased. Indeed, macrocycles **18**, **20** and **22**, were the most active among compounds, with inhibition percentages close to 100% in nearly all cell lines assayed. Even though their cytotoxic effects were lower against normal PBPC, these compounds were also the most active against non-transformed cells, thus showing a lack of selectivity against cancerous cells. Although compound **15** also had a 12-membered ring and a lipid chain, it was not as cytotoxic as **20**; macrocycle **20** has higher flexibility due to the incorporation of a methylene group which may contribute to an improved affinity towards the potential active site of its receptor. Hence, further investigation is required to confirm this, and thus a plausible mechanism of action is proposed.

Table 5 IC_{50} values for the most active compounds

Compound	U251	K562	SKLU-1
18	5.9 ± 0.3	4.0 ± 0.9	7.6 ± 0.7
20	13.6 ± 1.8	22.5 ± 2.2	17.1 ± 1.0
22	28.2 ± 0.1	9.6 ± 0.9	26.7 ± 2.7
Etoposide[a]	2.0 ± 0.2	11.3 ± 2.5	4.1 ± 0.4

[a] Reference cytotoxic anticancer drug.

The IC_{50} values for the most active macrocycles are depicted in Table 5. Etoposide, a glycoside of podophyllotoxin, was used as the reference drug. Undoubtedly, macrocycles **18**, **20** and **22** are excellent candidates for further structural optimization due to their micromolar IC_{50} values, specifically macrocycle **18** with an IC_{50} value of less than 10 μM against U251, K562 and SKLU-1 cell lines.

Conclusions

A four-step approach to biaryl-containing macrocycles has been developed. In this strategy, two programmed Ugi-4CRs using bifunctional building blocks allowed the introduction of functionalities for the final cross-coupling reaction-based macrocyclization step. The evaluation of the cytotoxic activity of the final compounds against eight human cancer cell lines showed that the most active were those containing a lipid chain. These very preliminary biological results also suggest that the macrocycle size and flexibility are important structural features that might influence their cytotoxic effects as well. The high reproducibility of the synthetic methodology described herein will likely allow the production of larger collections of biaryl-containing macrocycles, which will advance

Table 4 Preliminary cytotoxicity screening of compounds **13–24** at 50 μM

	Cell growth inhibition percentage								
Comp	U251	PC-3	K562	HCT-15	MCF7	SKLU-1	MDA-MB231	786-O	PBPC
13	6.05	10.4	40.9	32.2	13.7	75.7	NC	0.7	7.6
14	8.85	8.3	55.1	38.4	1.89	63.7	NC	6.1	19
15	16.9	33.9	NC	12.4	11.4	47.4	NC	23.2	NC
16	11.0	5.68	85.5	27.4	10.7	54.7	NC	5.8	7.6
17	11.1	26.2	79.3	44.2	21.4	58.1	NC	27.1	26.7
18	100	100	100	81.8	100	100	100	100	44.6
19	NC	9.97	55.1	22.1	21.8	33.2	NC	3.5	9.8
20	90.3	100	77.5	94.5	100	93.0	100	100	43.8
21	NC	NC	NC	4.81	2.9	18.7	NC	10.8	27.2
22	100	100	3.2	97.2	100	100	100	100	44.8
23	11.3	NC	37.5	47.4	34.3	28	NE	NE	NC
24	6.83	NC	19.3	8.2	12.5	18.5	NE	NE	NC

NC: non-cytotoxic. NE: not evaluated.

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our understanding of the structure–activity relationships of these promising compounds.

Experimental section

General methods

^{1}H NMR and ^{13}C NMR spectra were recorded at 300 MHz for ^{1}H and 75 MHz for ^{13}C, respectively. Chemical shifts (δ) are reported in parts per million relative to the residual solvent signals, and coupling constants (J) are reported in Hertz (Hz). High-resolution mass spectra were recorded with an AccuTOF LC equipped with an ionSense DART controller ionization source. IR spectra were obtained on a Universal diamond ATR top-plate (solids) or in $CHCl_3$ solution (oils). Microwave-assisted Ugi-four component and Suzuki–Miyaura cross-coupling reactions were performed in a Biotage Microwave. Flash column chromatography was carried out using silica gel 60 (230–400 mesh) and analytical thin layer chromatography (TLC) was performed using silica gel aluminum sheets. All commercially available chemicals were used without further purification.

Synthesis of mono-Boc protected diamines

***N*-Boc-1,2-diaminoethane (8).** A solution of trifluoroacetic acid (3.9 mL, 50 mmol) in MeOH (250 mL) was added dropwise to a stirred solution of 1,2-diaminoethane (3 g, 50 mmol) in MeOH (100 mL) at 0–5 °C. The mixture was stirred for 15 min and after addition of H_2O (100 mL) the stirring was continued for 30 min at room temperature. A solution of Boc_2O (10.9 g, 50 mmol) and I_2 (1.3 g, 5 mmol) in MeOH (250 mL) was added dropwise over 10 min and the reaction progress was monitored by TLC ($CHCl_3$/MeOH 6 : 1). After 3 h the volatiles were removed under reduced pressure and 5% aqueous $Na_2S_2O_3$ (100 mL) was added to the residue followed by extraction with Et_2O (2 × 100 mL). 20% aqueous NaOH was added to the aqueous phase until pH was set to 10–12 and then it was extracted with chloroform (3 × 100 mL). The combined organic phases were dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to obtain the crude product that was purified by flash column chromatography ($CHCl_3$/MeOH 6 : 1) to afford the mono-Boc protected diamino compound **8** (6.2 g, 78%) as a colorless oil. R_f = 0.29 ($CHCl_3$/MeOH 6 : 1). IR (KBr, cm^{-1}): 3348, 3304, 2975, 2932, 2872, 1685, 1518, 1274, 1249, 1199; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.22 (s, 9H), 1.31 (s, 2H), 2.56 (t, J = 5.9 Hz, 2H), 2.93 (q, J = 5.9 Hz, 2H), 5.50 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 28.13, 41.56, 43.13, 78.57, 156.13. HRMS m/z $[M + H]^+$ cal for $C_7H_{17}N_2O_2$ 161.1290, found 161.1291.

***N*-Boc-1,4-diaminobutane (9).** A solution of 1,4-diaminobutane (3 g, 34 mmol) in MeOH (65 mL) was treated with trifluoroacetic acid (2.6 mL, 34 mmol), Boc_2O (9.6 g, 44.2 mmol) and I_2 (863 mg, 3.4 mmol) according to the protection procedure described for the synthesis of *N*-Boc-1,2-diaminoethane (**8**). Flash column chromatography purification ($CHCl_3$/MeOH 8 : 1) afforded the mono-Boc protected diamino compound **9** (4.6 g, 72%) as a pale yellow oil. R_f = 0.26 ($CHCl_3$/MeOH 8 : 1). IR (KBr, cm^{-1}): 3354, 2976, 2933, 2866, 1694, 1528, 1276, 1251, 1174; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.44–1.53 (m, 15H), 2.71 (t, J = 6.6 Hz, 2H), 3.11 (m, 2H), 4.94 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 27.46, 28.42, 30.75, 40.39, 41.74, 78.95, 156.09. HRMS m/z $[M + H]^+$ cal for $C_9H_{21}N_2O_2$ 189.1603, found 189.1598.

General procedure for the four-step synthesis of biaryl-containing macrocycles

2,2′-(3,8-Dioxo-4,7-diaza-1,2(1,3)-dibenzenacyclooctaphane-4,7-diyl)bis(*N*-(*tert*-butyl)acetamide) (13)

First step: Ugi-4CR. A mixture of *N*-Boc-1,2-diaminoethane **8** (16 mg, 0.1 mmol) and paraformaldehyde (3 mg, 0.1 mmol) in MeOH (1.5 mL) was stirred in a 2 mL microwave reaction vial for 30 min at room temperature. 3-Iodobenzoic acid (25 mg, 0.1 mmol) and *tert*-butyl-isocyanide (11 μL, 0.1 mmol) were then added and the vial was sealed. The reaction mixture was stirred at 80 °C for 2 h under microwave irradiation and the solvent was removed under reduced pressure to dryness.

Second step: deprotection (N-Boc cleavage). The crude product was dissolved in DCM (2 mL) and after cooling to 0 °C, TFA (0.5 mL) was added dropwise. The reaction was allowed to warm to room temperature, and the stirring was continued for 2 h. The volatiles were then removed under reduced pressure to dryness and the resulting syrup was co-evaporated with toluene (3 × 2 mL). The crude product was dissolved in DCM (5 mL) and washed with 1 M aqueous NaOH (2 × 2 mL) and H_2O (2 × 2 mL). The organic phase was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to dryness.

Third step: Ugi-4CR. The resulting impure primary amine was transferred to a 2 mL microwave tube using TFE (1.5 mL) as the solvent and paraformaldehyde (3 mg, 0.1 mmol) was added. After stirring for 30 min, 3-carboxyphenylboronic acid MIDA ester (28 mg, 0.1 mmol) and *tert*-butyl-isocyanide (11 μL, 0.1 mmol) were successively added. The vial was sealed and the reaction mixture was stirred at 60 °C for 1 h under microwave irradiation. The solvent was removed under reduced pressure to dryness and the crude Ugi adduct was filtered through a silica gel pad to afford the impure MIDA boronate-containing compound.

Fourth step: Suzuki–Miyaura cross-coupling reaction. The impure compound was transferred to a 20 mL microwave tube using a mixture of MeCN/THF (1 : 1, 20 mL), and the tube was sealed with a septum. The solution was degassed by bubbling argon for 30 min, and $Pd_2(dba)_3$ (9.2 mg, 0.01 mmol) and SPhos (8.2 mg, 0.02 mmol) were successively added. After stirring for 15 min under argon a degassed solution of K_3PO_4 (159 mg, 0.75 mmol) in H_2O (1.6 mL) was added. The septum was removed and the tube was sealed with the corresponding cap and placed into the microwave cavity. The reaction mixture was stirred at 70 °C for 90 min under microwave irradiation. The solvents were removed under reduced pressure to dryness and the residue was dissolved in DCM (5 mL) and washed with H_2O (2 × 2 mL). The organic phase was dried over anhydrous Na_2SO_4 and evaporated under reduced pressure to dryness.

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The crude product was purified by flash column chromatography (EtOAc/hexanes/MeOH 2 : 1 : 0.1) to afford macrocycle **13** (27 mg, 56%) as a pale yellow amorphous solid. R_f = 0.28 (EtOAc/*n*-hexane/MeOH 2 : 1 : 0.1). IR (KBr, cm^{-1}): 3311, 3069, 2967, 2929, 1628, 1599, 1546, 1454, 1391, 1364, 1218, 730; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.31, 1.33 (2 × s, 2 × 9H), 3.62–3.73, 3.86–4.01 (2 × m, 8H), 6.15 (s, 2H), 7.04 (m, 2H), 7.48 (t, J = 7.6 Hz), 7.60–7.64 (m, 2H), 7.70–7.73 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 28.81, 50.77, 51.54, 52.60, 126.33, 127.25, 128.26, 131.32, 137.82, 142.58, 168.03, 173.15; HRMS m/z $[M + H]^+$ cal for $C_{28}H_{37}N_4O_4$ 493.28148, found 493.28156.

***N*-Benzyl-2-(7-(2-(*tert*-butylamino)-2-oxoethyl)-3,8-dioxo-4,7-diaza-1,2(1,3)-dibenzenacyclooctaphane-4-yl)acetamide (14).** *N*-Boc-1,2-diaminoethane **8** (16 mg, 0.1 mmol), paraformaldehyde (3 mg, 0.1 mmol), 3-iodobenzoic acid (25 mg, 0.1 mmol), and *tert*-butyl-isocyanide (11 μL, 0.1 mmol) were reacted in MeOH (1.5 mL) according to the first step of the general procedure. After the subsequent three steps, the crude product was purified by flash column chromatography (*n*-hexane/EtOAc/MeOH 1 : 2 : 0.1) to afford macrocycle **14** (24 mg, 45%) as a white amorphous solid: R_f = 0.26 (*n*-hexane/EtOAc/MeOH 1 : 2 : 0.1). IR (KBr, cm^{-1}): 3308, 3063, 2967, 2926, 1628, 1599, 1542, 1454, 1392, 1365, 1218, 744, 699; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.33 (s, 9H), 3.64–4.14 (m, 8H), 4.35–4.47 (m, 2H), 6.20 (s, 1H), 6.88 (m, 1H), 7.00 (m, 2H), 7.22–7.30 (m, 5H), 7.49 (td, J = 2.5/7.6 Hz, 2H), 7.61 (m, 2H), 7.73 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 28.83, 43.66, 50.63, 50.77, 51.61, 51.66, 52.49, 126.37, 127.27, 127.36, 127.60, 127.71, 128.82, 128.94, 131.10, 131.29, 137.82, 138.03, 142.60, 168.11, 168.89, 173.21, 173.46; HRMS m/z $[M + H]^+$ cal for $C_{31}H_{35}N_4O_4$ 527.26583, found 527.26437.

***N*-(*tert*-Butyl)-2-(7-(2-(dodecylamino)-2-oxoethyl)-3,8-dioxo-4,7-diaza-1,2(1,3)-dibenzenacyclooctaphane-4-yl)acetamide (15).** *N*-Boc-1,2-diaminoethane **8** (16 mg, 0.1 mmol), paraformaldehyde (3 mg, 0.1 mmol), 3-iodobenzoic acid (25 mg, 0.1 mmol), and *tert*-butyl-isocyanide (11 μL, 0.1 mmol) were reacted in MeOH (1.5 mL) according to the first step of the general procedure. After the subsequent three steps, the crude product was purified by flash column chromatography (*n*-hexane/EtOAc/MeOH 1 : 3 : 0.2) to afford macrocycle **15** (36 mg, 59%) as a white amorphous solid: R_f = 0.30 (*n*-hexane/EtOAc 1 : 3 : 0.2). IR (KBr, cm^{-1}): 3316, 3071, 2923, 2852, 1657, 1631, 1551, 1460, 1368, 1250, 1220, 1157, 1110, 755, 733; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.87 (m, 3H), 1.23 (br.s, 18H), 1.31 (s, 9H), 1.47 (m, 2H), 3.20 (m, 2H), 3.65–3.76 (m, 2H), 3.90–4.10 (m, 6H), 6.12 (s, 1H), 6.47 (t, J = 5.7 Hz, 1H), 7.03–7.05 (m, 2H), 7.49 (td, J = 1.1/7.6 Hz, 2H), 7.60–7.64 (m, 2H), 7.70–7.74 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.24, 22.80, 27.01, 28.83, 29.37, 29.47, 29.55, 29.66, 29.70, 29.75, 32.03, 39.75, 50.72, 50.75, 51.59, 51.81, 52.49, 126.34, 126.39, 127.21, 127.35, 128.89, 131.15, 131.37, 137.83, 142.56, 142.62, 168.00, 168.76, 173.12, 173.41; HRMS m/z $[M + H]^+$ cal for $C_{36}H_{53}N_4O_4$ 605.40668, found 605.40527.

2,2′-(3,10-Dioxo-4,9-diaza-1,2(1,3)-dibenzenacyclodecaphane-4,9-diyl)bis(*N*-(*tert*-butyl)acetamide) (16). *N*-Boc-1,4-diaminobutane **9** (19 mg, 0.1 mmol), paraformaldehyde (3 mg, 0.1 mmol), 3-iodobenzoic acid (25 mg, 0.1 mmol), and *tert*-butyl-isocyanide (11 μL, 0.1 mmol) were reacted in MeOH (1.5 mL) according to the first step of the general procedure. After the subsequent three steps, the crude product was purified by flash column chromatography (*n*-hexane/EtOAc/MeOH 1 : 3 : 0.1) to afford macrocycle **16** (28 mg, 53%) as a white amorphous solid: R_f = 0.28 (*n*-hexane/EtOAc/MeOH 1 : 3 : 0.1). IR (KBr, cm^{-1}): 3332, 2966, 2927, 1666, 1619, 1578, 1544, 1436, 1406, 1364, 1311, 1260, 1212, 740; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.32 (s, 18H), 2.02 (m, 4H), 3.71 (br.s, 4H), 3.98 (s, 4H), 6.40 (s, 2H), 7.54–7.59 (m, 4H), 7.65–7.68 (m, 2H), 7.79–7.82 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 27.17, 28.82, 51.34, 52.54, 53.04, 128.72, 129.08, 129.32, 129.93, 133.87, 140.58, 168.32, 170.70; HRMS m/z $[M + H]^+$ calcd for $C_{30}H_{41}N_4O_4$ 521.31278, found 521.31234.

***N*-Benzyl-2-(9-(2-(*tert*-butylamino)-2-oxoethyl)-3,10-dioxo-4,9-diaza-1,2(1,3)-dibenzenacyclodecaphane-4-yl)acetamide (17).** *N*-Boc-1,4-diaminobutane **9** (19 mg, 0.1 mmol), paraformaldehyde (3 mg, 0.1 mmol), 3-iodobenzoic acid (25 mg, 0.1 mmol), and *tert*-butyl-isocyanide (11 μL, 0.1 mmol) were reacted in MeOH (1.5 mL) according to the first step of the general procedure. After the subsequent three steps, the crude product was purified by flash column chromatography (*n*-hexane/EtOAc/MeOH 1 : 2 : 0.2) to afford macrocycle **17** (21 mg, 38%) as a white amorphous solid: R_f = 0.29 (*n*-hexane/EtOAc/MeOH 1 : 2 : 0.2). IR (KBr, cm^{-1}): 3305, 3078, 2924, 2874, 1666, 1619, 1541, 1448, 1431, 1366, 1257, 1211, 740, 697; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.33 (s, 9H), 1.72 (s, 2H), 1.97 (br.s, 2H), 3.71 (m, 4H), 3.99 (s, 2H), 4.13 (s, 2H), 4.43 (d, J = 5.9 Hz, 2H), 6.38 (s, 1H), 7.01 (t, J = 5.7 Hz, 1H), 7.13–7.42 (m, 5H), 7.52–7.61 (m, 4H), 7.66–7.70 (m, 2H), 7.76–7.83 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 27.18; 28.84, 43.55, 51.40, 51.59, 52.60, 53.09, 127.52, 127.67, 128.73, 128.80, 129.11, 129.20, 129.35, 129.97, 133.68, 133.89, 138.27, 140.59, 168.34, 169.07, 170.75, 170.99. HRMS m/z $[M + H]^+$ cal for $C_{33}H_{39}N_4O_4$ 555.29713, found 555.29604.

***N*-(*tert*-Butyl)-2-(9-(2-(dodecylamino)-2-oxoethyl)-3,10-dioxo-4,9-diaza-1,2(1,3)-dibenzenacyclodecaphane-4-yl)acetamide (18).** *N*-Boc-1,4-diaminobutane 9 (19 mg, 0.1 mmol), paraformaldehyde (3 mg, 0.1 mmol), 3-iodobenzoic acid (25 mg, 0.1 mmol), and *tert*-butyl-isocyanide (11 μL, 0.1 mmol) were reacted in MeOH (1.5 mL) according to the first step of the general procedure. After the subsequent three steps, the crude product was purified by flash column chromatography (*n*-hexane/EtOAc/MeOH 1 : 2 : 0.2) to afford macrocycle **18** (27 mg, 43%) as a white amorphous solid: R_f = 0.29 (*n*-hexane/EtOAc/MeOH 1 : 2 : 0.2). IR (KBr, cm^{-1}): 3300, 3062, 2927, 2856, 1626, 1540, 1446, 1368, 1259, 1212, 737, 693; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.86 (m, 3H), 1.23 (br.s, 18H), 1.33 (s, 9H), 1.47 (m, 2H), 2.01 (br.s, 2H), 3.21 (m, 2H), 3.72 (br.s, 4H), 3.98, 4.06 (2 × s, 4H), 6.38 (s, 1H), 6.68 (m, 1H), 7.54–7.60 (m, 4H), 7.65–7.69 (m, 2H), 7.79–7.83 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.23, 22.79, 27.00, 27.17, 28.82, 29.38, 29.45, 29.57, 29.67, 29.74, 32.02, 39.64, 51.35, 51.64; 52.52, 53.03, 128.70, 128.74, 129.06, 129.16, 129.36, 129.93, 133.72, 133.89,

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140.52, 140.58, 168.29, 169.00, 170.67, 170.86. HRMS *m*/*z* [M + H]$^+$ cal for $C_{38}H_{57}N_4O_4$ 633.4380, found 633.4377.

2,2′-(3,8-Dioxo-4,7-diaza-1(1,2),2(1,3)-dibenzenacyclononaphane-4,7-diyl)bis(*N*-(*tert*-butyl)acetamide) (19). *N*-Boc-1,2-diaminobutane **8** (16 mg, 0.1 mmol), paraformaldehyde (3 mg, 0.1 mmol), 2-iodophenylacetic acid (26 mg, 0.1 mmol), and *tert*-butyl-isocyanide (11 μL, 0.1 mmol) were reacted in MeOH (1.5 mL) according to the first step of the general procedure. After the subsequent three steps, the crude product was purified by flash column chromatography (*n*-hexane/EtOAc/MeOH 1 : 2 : 0.1) to afford macrocycle **19** (25 mg, 50%) as a white amorphous solid: R_f = 0.26 (*n*-hexane/EtOAc/MeOH 1 : 2 : 0.1). IR (KBr, cm^{-1}): 3305, 3071, 2965, 2924, 1613, 1547, 1545, 1434, 1409, 1362, 1222, 756; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.26, 1.35 (2 × s, 2 × 9H), 3.26–4.48 (m, 10H), 6.36 (s, 2H), 7.20–7.57 (m, 8H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 28.78, 28.85, 38.97, 46.08, 48.61, 49.65, 51.14, 51.16, 55.05, 126.30, 127.82, 127.96, 128.17, 129.23, 130.18, 131.06, 132.94, 133.96, 134.08, 139.12, 139.60; 167.39, 168.08, 173.60, 173.97. HRMS *m*/*z* [M + H]$^+$ cal for $C_{29}H_{39}N_4O_4$ 507.29713, found 507.29665.

***N*-(*tert*-Butyl)-2-(4-(2-(dodecylamino)-2-oxoethyl)-3,8-dioxo-4,7-diaza-1(1,2),2(1,3)-dibenzenacyclononaphane-7-yl)acetamide (20).** *N*-Boc-1,2-diaminobutane **8** (16 mg, 0.1 mmol), paraformaldehyde (3 mg, 0.1 mmol), 2-iodophenylacetic acid (26 mg, 0.1 mmol), and *n*-dodecyl-isocyanide (20 mg, 0.1 mmol) were reacted in MeOH (1.5 mL) according to the first step of the general procedure. After the subsequent three steps, the crude product was purified by flash column chromatography (*n*-hexane/EtOAc/MeOH 1 : 2 : 0.1) to afford macrocycle **20** (25 mg, 40%) as a white amorphous solid: R_f = 0.29 (*n*-hexane/EtOAc/MeOH 1 : 2 : 0.1). IR (KBr, cm^{-1}): 3306, 2928, 2855, 1640, 1535, 1404, 1312, 1219; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.88 (m, 3H), 1.21–1.36 (m, 27H), 1.50 (m, 2H); 3.22–4.58 (m, 12H), 6.33 (s, 1H), 6.62 (t, *J* = 5.8 Hz, 1H), 7.22–7.56 (m, 8H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.21, 22.77, 27.01, 28.78, 29.38, 29.44, 29.58, 29.67, 29.73, 32.00, 38.99, 39.72, 46.20, 48.62, 48.95, 51.44, 55.27, 126.39, 127.87, 128.00, 128.22, 129.26, 130.18, 131.17; 132.90, 133.82, 134.09, 139.08, 139.62, 168.09, 168.18, 173.72, 174.05. HRMS *m*/*z* [M + H]$^+$ cal for $C_{37}H_{55}N_4O_4$ 619.42233, found 619.42264.

2,2′-(3,10-Dioxo-4,9-diaza-1(1,2),2(1,3)-dibenzenacycloundecaphane-4,9-diyl)bis(*N*-(*tert*-butyl)acetamide) (21). *N*-Boc-1,4-diaminobutane **9** (19 mg, 0.1 mmol), paraformaldehyde (3 mg, 0.1 mmol), 2-iodophenylacetic acid (26 mg, 0.1 mmol), and *tert*-butyl-isocyanide (11 μL, 0.1 mmol) were reacted in MeOH (1.5 mL) according to the first step of the general procedure. After the subsequent three steps, the crude product was purified by flash column chromatography (*n*-hexane/EtOAc/MeOH 1 : 3 : 0.2) to afford macrocycle **21** (19 mg, 35%) as a white amorphous solid: R_f = 0.33 (*n*-hexane/EtOAc/MeOH 1 : 3 : 0.2). IR (KBr, cm^{-1}): 3299, 2965, 2926, 1678, 1620, 1549, 1450, 1362, 1263, 1220, 754. Assigned signals in NMR belong to a mixture of conformers. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.21–1.57 (m, 22H), 3.06 (t, *J* = 7.8 Hz, 2H), 3.26 (t, *J* = 7.7 Hz, 2H), 3.61, 3.71 (2 × s, 4H), 3.99 (s, 2H), 5.39, 6.16 (2 × s, 1H), 6.33, 6.49 (2 × s, 1H), 7.19–7.55 (m, 8H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 23.57, 24.16, 24.43, 25.15; 28.74, 28.82, 38.41, 39.38, 43.74, 48.09, 50.04, 50.75, 50.85, 51.22; 51.41, 51.95, 53.36, 125.51, 125.91, 126.02, 127.12, 127.42, 128.17, 128.44, 128.64, 129.51, 129.65, 129.79, 130.98, 131.28, 131.87, 133.10, 135.86, 136.03, 140.80, 141.42, 141.92; 166.60, 168.01, 168.23, 168.59, 171.81, 171.88, 172.17, 172.48. HRMS *m*/*z* [M + H]$^+$ cal for $C_{31}H_{43}N_4O_4$ 535.3284, found 535.3278.

***N*-(*tert*-Butyl)-2-(4-(2-(dodecylamino)-2-oxoethyl)-3,10-dioxo-4,9-diaza-1(1,2),2(1,3)-dibenzenacycloundecaphane-9-yl)acetamide (22).** *N*-Boc-1,4-diaminobutane **9** (19 mg, 0.1 mmol), paraformaldehyde (3 mg, 0.1 mmol), 2-iodophenylacetic acid (26 mg, 0.1 mmol), and *n*-dodecyl-isocyanide (20 mg, 0.1 mmol) were reacted in MeOH (1.5 mL) according to the first step of the general procedure. After the subsequent three steps, the crude product was purified by flash column chromatography (*n*-hexane/EtOAc/MeOH 1 : 2 : 0.1) to afford macrocycle **22** (24 mg, 40%) as a white amorphous solid: R_f = 0.31 (*n*-hexane/EtOAc/MeOH 1 : 2 : 0.1). IR (KBr, cm^{-1}): 3307, 3072, 2925, 2854, 1664, 1629, 1550, 1454, 1437, 1225, 757, 711. Assigned signals in NMR belong to a mixture of conformers. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.88 (m, 3H), 1.24–1.55 (m, 31H), 3.04–4.07 (m, 12H), 6.16 (s, 1H), 6.57 (m, 1H), 7.17–7.55 (m, 8H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 14.24; 22.80, 23.59, 24.10, 24.46, 25.19, 27.04, 28.78, 28.90, 29.40, 29.46, 29.65, 29.75, 32.03, 38.50, 39.69, 48.13, 50.01; 50.67, 50.79, 50.92, 51.26, 51.50, 52.01; 125.58, 126.01, 126.15, 127.18, 127.46, 128.19, 128.50, 128.70, 129.53, 129.69, 129.82, 131.03, 131.39, 131.99, 133.11, 135.78, 135.98, 140.80, 141.45, 141.92, 166.59, 168.24, 168.82, 169.32, 171.82, 171.93, 172.36. HRMS *m*/*z* [M + H]$^+$ cal for $C_{39}H_{59}N_4O_4$ 647.4536, found 647.4542.

***N*-(*tert*-Butyl)-2-(9-(2-(cyclopentylamino)-2-oxoethyl)-3,10-dioxo-4,9-diaza-1,2(1,3)-dibenzenacyclodecaphane-4-yl)acetamide (23).** *N*-Boc-1,4-diaminobutane **9** (19 mg, 0.1 mmol), paraformaldehyde (3 mg, 0.1 mmol), 3-iodobenzoic acid (25 mg, 0.1 mmol), and *tert*-butyl-isocyanide (11 μL, 0.1 mmol) were reacted in MeOH (1.5 mL) according to the first step of the general procedure. After the subsequent three steps, the crude product was purified by flash column chromatography (*n*-hexane/EtOAc/MeOH 1 : 2 : 0.2) to afford macrocycle **18** (36 mg, 68%) as a white amorphous solid: R_f = 0.52 (*n*-hexane/EtOAc/MeOH 1 : 2 : 0.3). IR (KBr, cm^{-1}): 3324, 2931, 2870, 1676, 1615, 1542, 1440, 1212, 733; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.31 (s, 9H), 1.34–1.42 (m, 2H), 1.17–1.49 (m, 6H), 1.86–1.97 (m, 4H), 3.70 (br s, 4H), 4.02–3.97 (2 × s, 4H), 4.12–4.05 (m, 1H), 6.40 (s, 1H), 6.65 (d, *J* = 7.5 Hz, 1H), 7.57–7.52 (m, 4H), 7.66–7.63 (m, 2H), 7.80–7.74 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.03, 24.60, 25.47, 27.04, 28.69, 32.80, 48.14, 51.22, 51.57, 52.33, 52.92, 60.37, 128.56, 128.59, 128.94, 128.98, 129.18, 129.21, 129.80, 133.70, 133.75, 140.40, 140.45, 167.99, 168.17, 170.54, 170.6. HRMS *m*/*z* [M + H]$^+$ cal for $C_{31}H_{41}N_4O_4$ 533.31278, found 533.31295.

***N*-(*tert*-Butyl)-2-(9-(2-(cyclohexylamino)-2-oxoethyl)-3,10-dioxo-4,9-diaza-1,2(1,3)-dibenzenacyclodecaphane-4-yl)acetamide (24).** *N*-Boc-1,4-diaminobutane **9** (19 mg, 0.1 mmol), paraformaldehyde (3 mg, 0.1 mmol), 3-iodobenzoic acid (25 mg, 0.1 mmol), and *tert*-butyl-isocyanide (11 μL, 0.1 mmol) were reacted in

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MeOH (1.5 mL) according to first step for the general procedure. After the subsequent three steps, the crude product was purified by flash column chromatography (*n*-hexane/EtOAc/MeOH 1 : 2 : 0.5) to afford macrocycle **18** (24 mg, 44%) as a white amorphous solid: R_f = 0.31 (*n*-hexane/EtOAc/MeOH 1 : 2 : 0.2). IR (KBr, cm^{-1}): 3300, 3062, 2927, 2856, 1626, 1540, 1446, 1368, 1259, 1212, 737, 693; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.28–1.15 (m, 7H), 1.34 (s, 9H), 1.70–1.57 (m, 4H), 1.89–1.79 (m, 3H), 3.73 (br.s, 4H), 4.07–4.00 (2 × s, 4H), 4.16–4.08 (m, 1H), 6.43 (s, 1H), 6.59 (d, J = 8.1 Hz, 1H), 7.61–7.57 (m, 4H), 7.70–7.67 (m, 2H), 7.83–7.81 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 21.03, 24.60, 25.47, 27.04, 28.69, 32.80, 48.14, 51.22, 51.57, 52.33, 52.92, 60.37, 128.54, 128.59, 128.94, 128.98, 129.18, 129.21, 129.80, 133.70, 133.75, 140.40, 140.45, 167.99, 168.17, 170.54, 170.68. HRMS m/z $[M + H]^+$ cal for $C_{32}H_{43}N_4O_4$ 547.32843, found 547.32871.

Cytotoxicity assays

The biaryl-containing macrocycles were screened *in vitro* against eight human cancer cell lines: colorectal adenocarcinoma (HCT-15), mammary adenocarcinoma (MCF-7), chronic myelogenous leukemia (K562), glioblastoma (U251), human prostatic adenocarcinoma (PC-3), human lung adenocarcinoma (SKLU-1), mammary gland breast (MDA-MB231) and renal cell adenocarcinoma (786-O). Cell lines were supplied by the National Cancer Institute, USA. The cytotoxicity of the synthetic compounds was determined using the protein-binding dye sulforhodamine B (SRB) in the microculture assay to measure cell growth, as described in the protocols established by NCI.[23] The cell lines were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum, 2 μM L-glutamine, 10% antibiotic-antimycotic (Gibco) and 10% non-essential amino acids (Gibco). The cultures were maintained at 37 °C in a 5% CO_2 humidified atmosphere. As determined with trypan blue, the viability of the cells used in the experiments exceeded 95%.

The cells were removed from the tissue culture flasks by treatment with trypsin and diluted with fresh media. 100 μL aliquots of cell suspension containing 5000–10 000 cells per well, were transferred into 96 well microtiter plates (Costar) and incubated at 37 °C for 24 h in a 5% CO_2 atmosphere.

Stock solutions of the tested compounds in DMSO (20 μM) were initially prepared and further diluted in medium to produce the desired concentrations. 100 μL aliquots of diluted solutions of the tested compounds were added to each well. Cell cultures were exposed to the compounds under analysis at concentrations 1, 0.5, 0.25 and 0.125 μM for 48 h. After the incubation period, the cells were fixed to the plastic substratum by the addition of 50 μL of cold 50% aqueous trichloroacetic acid. The plates were incubated at 4 °C for 1 h, washed with tap H_2O and dried in air. The trichloroacetic acid-fixed cells were stained by the addition of 0.4% SRB. Free SRB solution was then removed by washing with 1% aqueous acetic acid. The plates were dried in air, and the bound dye was solubilized by the addition of unbuffered Tris base (100 μL, 10 μM). The plates were placed on a shaker for 5 min prior analysis. Optical densities were determined using an Ultra Microplated Reader (Elx 808, BIO-TEX Instruments, Inc.) at λ = 515 nm.

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