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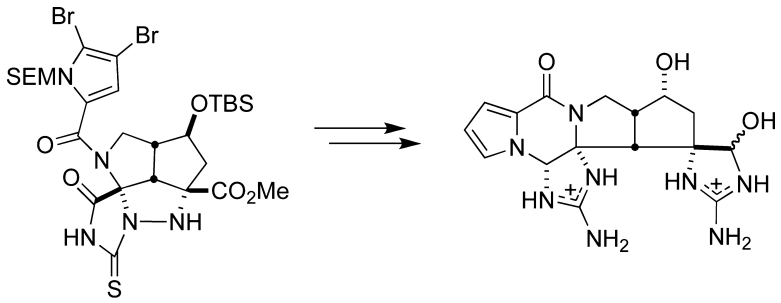

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On the Structure of Palau'amine: Evidence for the Revised Relative Configuration from Chemical Synthesis

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Abstract: Hexacyclic congeners **3** and **4** of palau'amine, which incorporate both guanidine functional groups and have the cis configuration of the azabicyclo[3.3.0]octane core, are prepared in 14 steps from cycloadduct **6**. Synthetic access to these analogues allows the first direct comparison of NMR data for hexacyclic diguanidine structures having the originally proposed *cis*-azabicyclo[3.3.0]octane fragment with data for natural alkaloids of the palau'amine family. This comparison provides convincing evidence in favor of the recently proposed structural revision of these marine alkaloids, fully supporting the trans configuration of the central azabicyclo[3.3.0]octane ring system of palau'amine and congeners.

Introduction

The stunning degree of structural diversity in the pyrrole−imidazole family of alkaloids provides an excellent example of biosynthetic efficiency.[1] This large family of marine natural products is believed to be based on one simple metabolite, oroidin, and includes complex molecular architectures such as the hexacyclic oroidin dimer palau'amine (**1**, Figure 1) and alkaloids such as stylissadines A and B containing four oroidin subunits. Since its structural elucidation by mass spectral and NMR investigations in 1993,[2] palau'amine has received much attention from the synthetic community because of its promising immunomodulatory activity, challenging molecular architecture, and density of functionality.[3] However, despite considerable efforts, no report of a successful total synthesis has emerged to date.[1]

Recently, the isolation and structural elucidation of additional palau'amine congeners has challenged the initial assignment of the relative configuration of palau'amine, leading to the proposal that stereogenic centers C12 and C17 should be inverted (**2**, Figure 1).[4] Most notably, the revised structure **2** contains a highly strained *trans*-azabicyclo[3.3.0]octane fragment. Herein,

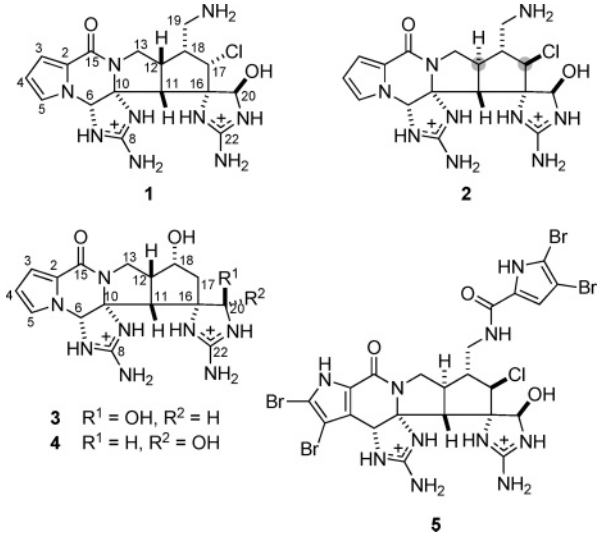

Figure 1. Originally proposed structure of palau'amine (**1**), revised structure of palau'amine (**2**), synthetic derivatives **3** and **4**, and structure of tetrabromostyloguanidine (**5**). Stereogenic centers with revised relative configurations have been highlighted with gray circles.

we describe the synthesis of hexacyclic palau'amine derivatives **3** and **4** possessing the originally proposed *cis*-azabicyclo[3.3.0]-octane central unit. Comparison of these compounds to palau'amine and tetrabromostyloguanidine (**5**)[4c,5] using NMR spectroscopic and computational methods provides additional evidence for the revised relative configuration **2** of palau'amine.

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(1) For recent reviews on the structural diversity of pyrrole-imidazole alkaloids and synthetic approaches toward some of the family members, see: (a) Hoffmann, H.; Lindel, T. *Synthesis* **2003**, 1753−1783. (b) Jacquot, D. E. N.; Lindel, T. *Curr. Org. Chem.* **2005**, *9*, 1551−1565. (c) Köck, M.; Grube, A.; Seiple, I. B.; Baran, P. S. *Angew. Chem., Int. Ed.* **2007**, *46*, 6586−6594.

(2) (a) Kinnel, R. B.; Gehrken, H.-P.; Scheuer, P. J. *J. Am. Chem. Soc.* **1993**, *115*, 3376−3377. (b) Kinnel, R. B.; Gehrken, H.-P.; Swali, R.; Skoropowski, G.; Scheuer, P. J. *J. Org. Chem.* **1998**, *63*, 3281−3286. Additional congeners have been isolated and characterized: (c) Kato, T.; Shizuri, Y.; Izumida, H.; Yokoyama, A.; Endo, M. *Tetrahedron Lett.* **1995**, *36*, 2133−2136. (d) Kobayashi, J.; Suzuki, M.; Tsuda, M. *Tetrahedron* **1997**, *53*, 15681−15684.

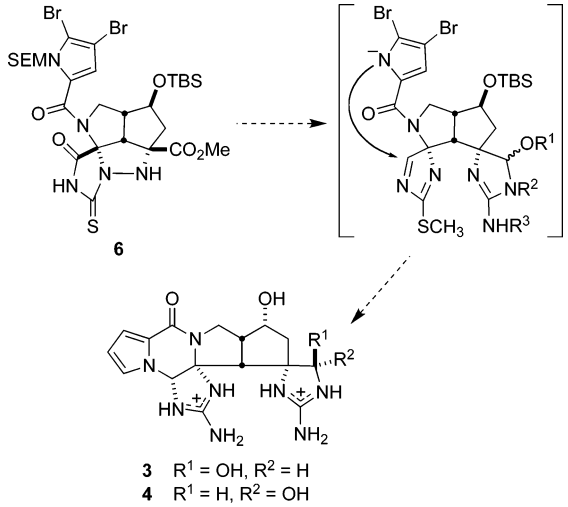
(3) Publications not covered in ref 1a,b include: (a) Dransfield, P. J.; Dilley, A. S.; Wang, S.; Romo, D. *Tetrahedron* **2006**, *62*, 5223−5247. (b) Wang, S.; Dilley, A. S.; Poullennec, K. G.; Romo, D. *Tetrahedron* **2006**, *62*, 7155−7161. (c) Nakadai, M.; Harran, P. G. *Tetrahedron Lett.* **2006**, *47*, 3933−3935. (d) Tan, X.; Chen, C. *Angew. Chem., Int. Ed.* **2006**, *45*, 4345−4348.

(4) (a) Kobayashi, H.; Kitamura, K.; Nagai, K.; Nakao, Y.; Fusetani, N.; van Soest, R. W. M.; Matsunaga, S. *Tetrahedron Lett.* **2007**, *48*, 2127−2129. (b) Buchanan, M. S.; Carroll, A. R.; Addepalli, R.; Avery, V. M.; Hooper, J. N. A.; Quinn, R. J. *J. Org. Chem.* **2007**, *72*, 2309−2317. (c) Grube, A.; Köck, M. *Angew. Chem., Int. Ed.* **2007**, *46*, 2320−2324. The relative configuration of the C20 stereogenic center was assigned as shown for **1** in the initial isolation report (ref 2a), then corrected in the following publication (ref 2b). All congeners of palau'amine were assigned the initial relative configuration at C20 (refs 2c,d, 4a−c). (d) Grube, A.; Immel, S.; Baran, P. S.; Köck, M. *Angew. Chem., Int. Ed.* **2007**, *46*, 6721−6724.

(5) This natural product was also isolated from another marine sponge and named carteramine A (see ref 4a).

10.1021/ja074939x CCC: $37.00

Scheme 1. Plan for the Conversion of Intermediate **6** to Palau'amine Derivatives **3** and **4**



Results and Discussion

In our approach toward the putative original structure **1** of palau'amine, an intramolecular azomethine imine 1,3-dipolar cycloaddition forms the *cis*-azabicyclo[3.3.0]octane subunit having the correct relative configuration at the fully substituted carbons 10 and 16.[6] Starting from this intermediate, elaboration to diguanidine hexacycles **3**/**4** requires installation of the two spiroguanidine units and formation of the ketopiperazine ring. Building on precedent from Büchi's pioneering synthesis of dibromophakellin,[7] ketopiperazine ring closure was projected to occur by attack of the nitrogen of a tethered bromopyrrole on a 2-methylsulfanyl-4*H*-imidazole generated in situ (Scheme 1). The second (right-hand) spirocycle was envisaged to arise in turn from an *S*-methyl thiohydantoin intermediate or by guanylation of the amino functional group of a suitable precursor, followed by spirocyclization.

An optimized synthetic route for the conversion of triazatriquinane **6** into hexacyclic palau'amine deriviatives **3**/**4**, which have the same relative configuration at their stereogenic centers as the originally proposed structure **1** of palau'amine, is outlined in Scheme 2. This sequence began with SmI_2-mediated reduction of the N−N bond of **6**, followed by selective *S*-methylation of the resulting thiohydantoin **7** to furnish the corresponding *S*-methyl isothiourea **8** (76% overall). Subsequent selective Teoc protection of the isothiourea provided intermediate **9** in quantitative yield. Many attempts to elaborate aminoester **9** into a hexacyclic diguanidine via the intermediacy of bis(*S*-methylthiohydantoin) spirocycles established the viability of the synthetic strategy for ketopiperazine ring closure; however, the diguanidine functional group array could not be formed from these intermediates. Alternative strategies involving delayed installation of the second spirocycle suffered from unproductive intramolecular rearrangements, originating from nucleophilic attack of the primary amine onto the adjacent isothiourea. Alternatively, direct guanylation of the α-amino ester functionality of intermediates **8** and **9** to afford carbamate-protected glycocyamidine intermediates proved to be capricious and low-yielding. Therefore, a two-step procedure for glycocyamidine formation was developed, wherein aminoester **9** was first converted in high yield to Cbz-protected thiourea **10** by reaction with benzyloxycarbonyl isothiocyanate (**11**).[8] Incubation of **10** with EDCI and *o*-nitrobenzylamine led to efficient installation of the protected guanidine functionality, presumably via the intermediacy of a carbamoylcarbodiimide, followed by in situ spirocyclization to afford glycocyamidine **12** in 93% overall yield.[9,10]

Glycocyamidine **12** was elaborated to palau'amine derivatives **3**/**4** in the following way. Chemoselective removal of the SEM protecting group in the presence of the secondary TBS ether was first accomplished by reaction of **12** with 10% TFA−CH_2Cl_2 at room temperature, followed by basic aqueous workup to provide the free pyrrole **13**. Reintroduction of the Teoc group furnished bromopyrrole bis(spirocycle) **14** in 85% overall yield. Reduction of the carbonyl groups of the spirocyclic fragments was achieved by exposure of bis(spirocycle) **14** to an excess of $NaBH_4$ in MeOH/THF, providing a mixture of two inseparable bis(hemiaminal) diastereoisomers. In this transformation, the Teoc and *o*-nitrobenzyl groups impart sufficient electronic activation to the spirocycles to allow for smooth reduction of the carbonyl groups under mild conditions. The hemiaminal hydroxyl groups were then acetylated under standard conditions to give diastereomeric diacetates **15** and **16** in 80% combined yield and a ratio of 1.8:1.[11] Although these diastereoisomers could be separated by standard chromatography, short exposure of either diastereoisomer, or the mixture, to TBAF/THF promoted cyclization to form the ketopiperazine ring to give an inseparable mixture of hemiaminal epimers **17** (ratio 1.5:1) in excellent yield (95%).[12,13] The configuration of the secondary alcohol was next inverted by a two-step sequence. Chemoselective oxidation of alcohol **17** with IBX provided ketone **18** in high yield (96%) as an inseparable mixture of hemiaminal epimers (1.8:1). As expected, exposure of this ketone to $NaBH_4$/MeOH resulted in hydride delivery from the convex face to give alcohol **19**, again as an inseparable mixture of epimers (1:1, 94%). Treatment of this mixture with three equiv of purified *m*-chloroperoxybenzoic acid,[14] followed by incubation of the sulfone products with ammonia provided diguanidine **20**

(6) (a) Overman, L. E.; Rogers, B. N.; Tellew, J. E.; Trenkle, W. C. *J. Am. Chem. Soc.* **1997**, *119*, 7159−7160. (b) Bélanger, G.; Hong, F.-T.; Overman, L. E.; Rogers, B. N.; Tellew, J. E.; Trenkle, W. C. *J. Org. Chem.* **2002**, *67*, 7880−7883. (c) Katz, J. D.; Overman, L. E. *Tetrahedron* **2004**, *60*, 9559−9568.

(7) Foley, L. H.; Büchi, G. *J. Am. Chem. Soc.* **1982**, *104*, 1776−1777.

(8) Prepared from potassium thiocyanate and benzyl chloroformate according to: Wang, S. S.; Magliocco, L. G. American Cyanamid Company. US Patent 5194673, 1993 (see the Supporting Information).

(9) Treatment of **10** with EDCI and an excess of hexamethyldisilazane (HMDS) under similar conditions provided the corresponding (monoprotected) guanidine, which could be converted to the glycocyamidine spirocycle by incubation with diisopropylethylamine in toluene at 80 °C for 6 h (see ref 10d). However, carbamate protection of the endocyclic glycocyamidine nitrogen provided derivatives that were labile, and, therefore, inferior to intermediates masked with alkyl groups.

(10) (a) Linton, B. R.; Carr, A. J.; Orner, B. P.; Hamilton, A. D. *J. Org. Chem.* **2000**, *65*, 1566−1568. (b) Manimala, J. C.; Anslyn, E. V. *Tetrahedron Lett.* **2002**, *43*, 565−567. (c) Guisado, O.; Martinez, S.; Pastor, J. *Tetrahedron Lett.* **2002**, *43*, 7105−7109. (d) Shinada, T.; Umezawa, T.; Ando, T.; Kozuma, H.; Ohfune, Y. *Tetrahedron Lett.* **2006**, *47*, 1945−1947.

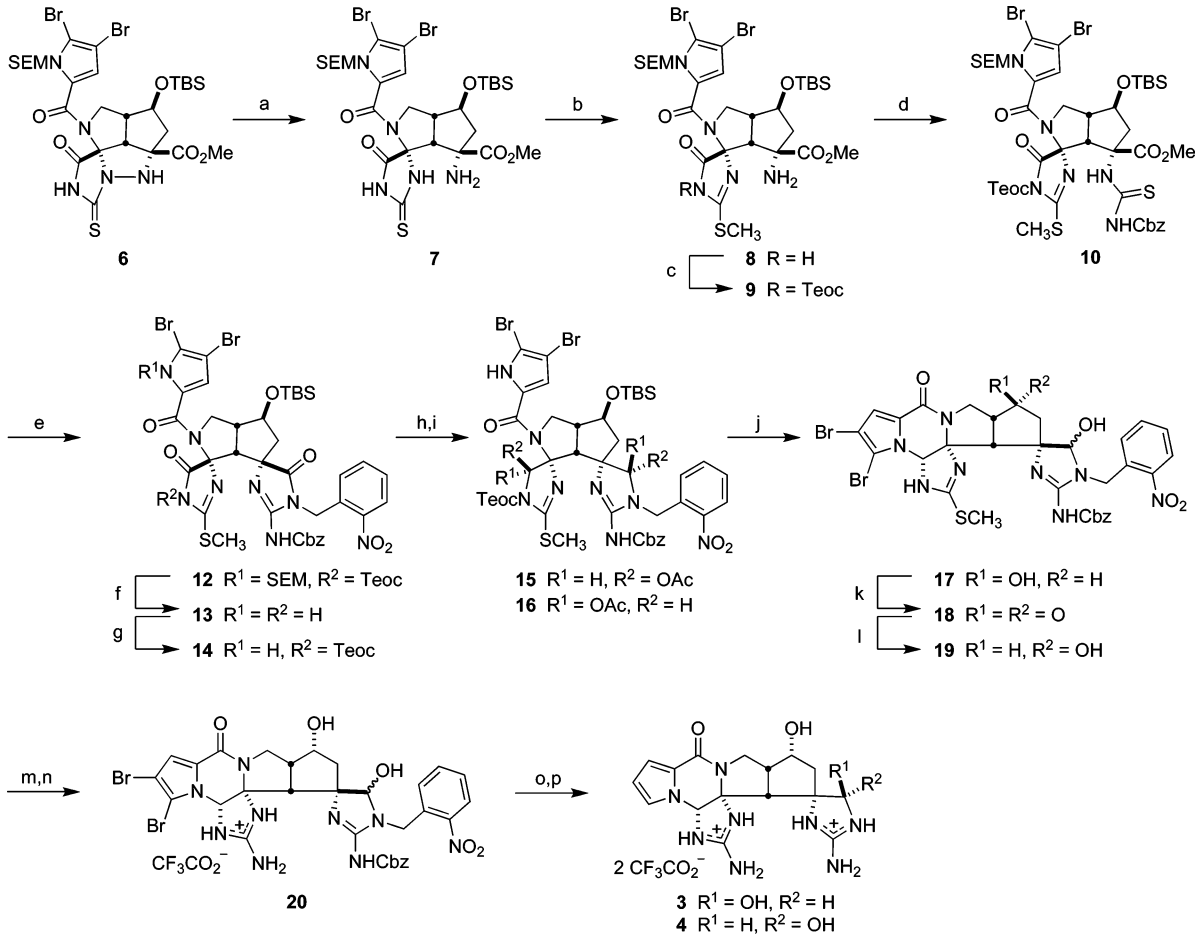
(11) The configuration of the hemiaminal stereocenters was elucidated by 2D NMR experiments (see the Supporting Information).

(12) The inability to separate the hemiaminal epimers of this product and later intermediates likely results from ready interconversion of the diastereomers on SiO_2 and in protic solvents.

(13) The simultaneous removal of the TBS ether is undoubtedly facilitated by the hemiaminal hydroxyl group, because desilylation does not occur in cyclizations of related substrates in which C21 is at the carbonyl oxidation state.

(14) Commercial *m*-chloroperoxybenzoic acid was purified by washing a CH_2Cl_2 solution of the peroxy acid with saturated aqueous $NaHCO_3$ solution, drying the organic phase over Na_2SO_4, and carefully concentrating the dried solution in vacuo.

Scheme 2. Conversion of Intermediate **6** into Diastereomeric Palau'amine Derivatives **3** and **4**[a]



[a] Conditions: (a) SmI_2, THF/MeOH, 23 °C, 15 min, 79%; (b) MeI, *i*-Pr_2EtN, DMAP, CH_2Cl_2, 23 °C, 2 h, 96%; (c) Teoc-Cl, *i*-Pr_2EtN, CH_2Cl_2, 23 °C, 30 min, quant; (d) benzyloxycarbonyl isothiocyanate (**11**), CH_2Cl_2, 40 °C, 1.5 h, 92%; (e) EDCI, 2-nitrobenzylamine hydrochloride, *i*-Pr_2EtN, CH_2Cl_2, 40 °C, 2.5 h, 93%; (f) TFA, CH_2Cl_2, 23 °C, 1 h, then saturated aq Na_2CO_3, 94%; (g) Teoc-Cl, Pr_2EtN, CH_2Cl_2, 23 °C, 30 min, 90%; (h) $NaBH_4$, MeOH/THF (2:1), 0 °C, 40 min; (i) Ac_2O, pyridine, DMAP, CH_2Cl_2, 23 °C, 4 h, 51% (**15**), 29% (**16**) (two steps); (j) TBAF, THF, 23 °C, 8 min, 95%; (k) IBX, DMSO, 23 °C, 4 h, 96%; (l) $NaBH_4$, MeOH, 0 °C, 30 min, 94%; (m) *m*-CPBA, CH_2Cl_2, 0 → 23 °C, 40 min; (n) NH_3, CH_2Cl_2, −78 → 23 °C, 14 h, 79% (two steps); (o) hν, dioxane, 23 °C, 2.5 h; (p) H_2, Pd/C, dioxane/H_2O (0.1% TFA), 43% (**3**), 22% (**4**) (two steps). DMAP = *N*,*N*-dimethylaminopyridine, Teoc-Cl = 2-trimethylsilylethyl chloroformate, EDCI = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, TFA = trifluoroacetic acid, TBAF = tetra(*n*-butyl)ammonium fluoride, IBX = *o*-iodoxybenzoic acid, *m*-CPBA = *m*-chloroperoxybenzoic acid.

in good overall yield (79% after HPLC purification) as a 5:1 mixture of inseparable hemiaminal epimers. To complete the synthesis of **3** and **4**, the *o*-nitrobenzyl and Cbz protecting groups were cleaved by photolysis and hydrogenolysis, respectively. In the course of the latter reaction, the bromine substituents of the pyrrole were also removed to provide a 2:1 mixture of hemiaminal epimers **3** and **4** in 65% combined overall yield. Although this mixture of hemiaminal epimers could be separated by HPLC, interconversion of the two diastereoisomers in H_2O/MeCN/TFA (89:11:0.1) and D_2O thwarted their isolation as single stereoisomers.

Gratifyingly, most of the NMR resonances of diastereoisomers **3** and **4** in D_2O were well separated and allowed for full assignment of ^1H and ^{13}C NMR spectra (Table 1). Comparison of ^1H and ^{13}C chemical shifts of **3** and **4** with those published for palau'amine[2b] reveals a surprisingly close overall match considering the dissimilarities in substituents at C17 and C18. An important difference, however, is the value of the $^1H,^1H$ coupling constant for the angular protons of the azabicyclo-[3.3.0]octane moiety, which are 12.0 and 10.7 Hz for **3** and **4**, respectively. This vicinal coupling constant is greater than 14 Hz for palau'amine and all palau'amine congeners, with the large value of this coupling constant for the natural alkaloids being an important piece of evidence in support of the revised trans fusion of the azabicyclo[3.3.0]octane unit.[4c,d] In addition, qualitative interpretation of the NOESY spectra of **3** and **4** revealed a strong correlation between the two protons at the ring junction (H11/H12), and the absence or weak correlations for the 1,3-related pairs of protons H11/H18 and H11/H13β, respectively. This latter data again stands in sharp contrast to data published for palau'amine and its congeners, in which a NOE correlation between H11 and H12 was often not mentioned[15] and correlations for H11/H13β and H11/H18 were reported. As an additional point of divergence, correlations between H13α and H18 were observed for **3** and **4**, whereas H18 was correlated to H13β in the natural products. No correlation between H13β and H18 was observed for **3** or **4**.

For further corroboration of the qualitative interpretation of NOE correlations, a quantitative NOESY analysis was carried out to experimentally determine important interproton dis-

(15) Among the publications on palau'amine and related structures, an H11/H12 NOE correlation was only mentioned for konbu'acidin A and tetrabromostyloguanidine (refs 2d and 4c).

Table 1. ^{1}H and ^{13}C NMR Data for Palau'amine,[2b] **3**, and **4** in D_2O

	palau'amine		**3**		**4**	
carbon	^{13}C, ppm	^{1}H, ppm (mult, *J* [Hz])	^{13}C, ppm	^{1}H, ppm (mult, *J* [Hz])	^{13}C, ppm	^{1}H, ppm (mult, *J* [Hz])
2	122.5		120.5		120.3	
3	115.6	6.85, (dd, *J* = 3.9, 1.5)	115.0	6.97, (dd, *J* = 4.0, 1.3)	115.0	6.97, (dd, *J* = 4.0, 1.3)
4	113.8	6.35, (dd, *J* = 3.9, 2.8)	113.0	6.47, (dd, *J* = 4.0, 2.7)	113.2	6.47, (dd, *J* = 4.0, 2.7)
5	125.2	6.99, (dd, *J* = 2.8, 1.5)	124.7	7.13, (dd, *J* = 2.7, 1.3)	124.5	7.10, (dd, *J* = 2.7, 1.3)
6	69.0	6.33, (s)	68.1	6.36, (s)	68.7	6.24, (s)
8	157.8		156.9		156.7	
10	80.8		82.0		81.8	
11	56.3	3.08, (d, *J* = 14.1)	53.1	3.82, (d, *J* = 12.0)	59.9	3.35, (d, *J* = 10.7)
12	41.8	2.52, (dddd)	45.3	3.07, (dddd, *J* = 12.0, 10.1, 6.1, 4.1)	44.2	3.15, (dddd, *J* = 10.7, 9.9, 5.1, 4.6)
13	46.1	3.96, (dd, *J* = 10.4, 7.3) 3.28, (dd, *J* = 10.3, 10.4)	42.2	4.16, (*β*) (dd, *J* = 12.3, 10.1) 3.65, (α) (dd, *J* = 12.3, 6.1)	42.7	4.07, (*β*) (dd, *J* = 12.3, 9.9) 3.72, (α) (dd, *J* = 12.3, 4.6)
15	159.5		157.4		157.4	
16	72.1		71.9		70.7	
17	74.0	4.35, (d, *J* = 7.9)	49.9	2.35, (*β*) (dd, *J* = 14.3, 3.7) 2.16, (α) (d, *J* = 14.3)	41.2	2.79, (*β*) (dd, *J* = 15.4, 5.1) 2.02, (α) (dd, *J* = 15.4, 2.5)
18	48.6	2.47, (dddd)	70.1	4.34, (m)	69.2	4.34, (m)
19	41.9	3.32, (dd, *J* = 13.2, 7.0) 3.24, (dd, *J* = 13.2, 7.0)				
20	83.7	5.96, (s)	85.8	5.34, (s)	87.9	5.19, (s)
22	157.9		158.7		158.7	

Table 2. Selected Experimental and Calculated Interproton Distances[a]

protons	**3**[b]	**3**[c]	**4**[b]	**4**[c]	**1**[c]	*trans*–**3**[c,d]	*trans*–**4**[c,d]	**5**[e]	**5**[c]	**2**[c]
11/12	221	221	228	224	226	304	304		304	303
11/13*β*	335	342	334	343	407	268	267	250	270	263
11/18	n.o.	405	n.o.	407	327	253	258	233	253	277
11/20	270	317	208	221	311	322	264	271	332	334
12/18	249	240	227	240	221	305	305		303	303
13α/18	313	299	292	297	381	346	345		343	334
13*β*/18	n.o.	349	n.o.	348	359	251	249	260	247	252

[a] For a more comprehensive dataset see the Supporting Information. Distances given in pm. [b] Average distances from NOESY spectra recorded at 100, 150, and 200 ms mixing times; n.o. = not observed. [c] Distances obtained from molecular modeling using the software package Maestro 5.0.019 with the AMBER* forcefield including solvation (H_2O). Global minima identified by conformational searches were evaluated. [d] *trans*-**3** and *trans*-**4** denote structures corresponding to **3** and **4** having trans-configured azabicyclo[3.3.0]octane ring systems. [e] Data from ref 4c.

tances.[16] Data gathered from these experiments were evaluated by comparison to the corresponding distances obtained from in silico geometry optimization and to values published for tetrabromostyloguanidine (**5**).[4c] Geometry optimizations performed using the molecular modeling packages Maestro 5.0.019 with the AMBER* forcefield including solvation (H_2O) and Spartan 04 (B3LYP 6-31G*) returned consistent results.[17]

A selected set of diagnostic interproton distances obtained from NOESY experiments and the corresponding calculated values are given in Table 2 (for further details see the Supporting Information).

Overall, experimentally determined interproton distances were in good agreement with results obtained from molecular modeling. The similar distances between H11 and H20 for **3** and tetrabromostyloguanidine (**5**) (270 and 271 pm) and the respective shorter distance seen in hemiaminal epimer **4** (208 pm), support a relative configuration at C20 of palau'amine identical to that of analogue **3** and tetrabromostyloguanidine (**5**). As expected for the cis-fusion of the five-membered rings, short distances were obtained between the angular protons H11 and H12 (221 and 228 pm for **3** and **4**, respectively). The corresponding distance in the trans-fused ring system, which is predicted by molecular modeling to be considerably larger (304 pm), unfortunately could not be quantified in the investigation of tetrabromostyloguanidine (**5**).[4c] More diagnostic information was obtained from the examination of 1,3-interrelated proton pairs H11/H13*β*, H11/H18, and H13*β*/18, situated on the same side of the molecular plane. In accordance with the molecular model of cis-configured structure **1**, all of these distances were found to be large (≥334 pm) for **3** and **4**, whereas significantly shorter interproton distances (≤260 pm) were reported for tetrabromostyloguanidine (**5**) and calculated for **2** and hypothetical trans-configured diastereoisomers *trans*-**3** and *trans*-**4**. In particular, the distance between H11 and H13*β*, which could be experimentally quantified in all cases, was found to be 85 pm larger for cis-fused **3** and **4** (335 and 334 pm) than for tetrabromostyloguanidine (**5**) (250 pm). No NOE correlations were observed for proton pairs H11/H18 and H13*β*/18 in the case of **3** and **4**, whereas the corresponding distances for **5** were experimentally determined to be short (233 and 260 pm, respectively). In addition, the close proximity of H12 and H18 (249 and 227 pm) and a NOE correlation between H13α and H18 (313 and 292 pm), observed in the case of **3** and **4**, were not mentioned in the quantitative analysis of tetrabromostyloguanidine (**5**). As highlighted by Grube and Köck,[4c] this discrepancy is best explained by assuming a trans fusion of the central azabicyclic fragment of palau'amine and congeners.

Conclusions

In summary, the first synthesis of hexacyclic palau'amine congeners that incorporate both guanidine functional groups has been accomplished. These palau'amine analogues, **3** and **4**, have the cis configuration of their azabicyclo[3.3.0]octane cores. Synthetic access to these analogues allowed the first direct comparison of NMR data for hexacyclic diguanidine structures having the originally proposed *cis*-azabicyclo[3.3.0]octane frag-

(16) Interproton distances were obtained from NOESY spectra with different mixing times (100, 150, and 200 ms). Intensity data from volume integration of NOESY crosspeaks were calibrated using the geminal proton pair at C13 (178 pm). Each NOESY spectrum was analyzed separately (linear approximation, verified by a linear relationship between volume integrals for different mixing times).

(17) In all cases conformational searches were carried out to determine global minima. Only minima lacking intramolecular H-bonds were considered for calculations using Spartan 04. These results are given in the Supporting Information.

ment with data for natural alkaloids of the palau'amine family. This comparison provides additional strong evidence in favor of the recently proposed structural revision of these compounds, fully supporting the trans configuration of the central azabicyclo-[3.3.0]octane ring system of palau'amine and congeners.

Experimental Section[18]

Intermediate 17. *n*-Tetrabutylammonium fluoride (155 μL of a 1 M solution in THF, 0.155 mmol) was added at room temperature to a solution of bis(spirocycle) **15** (37 mg, 0.031 mmol) in THF (2 mL), and the yellowish solution was stirred at room temperature for 8 min and then partitioned between saturated aqueous NH_4Cl solution and EtOAc. The organic phase was dried over Na_2SO_4 and concentrated. The residue was purified by column chromatography (SiO_2; EtOAc/MeOH 14:1) to afford 24.5 mg (95%) of an inseparable mixture (1.5:1) of hemiaminal diastereoisomers **17** as a colorless, amorphous solid. ^{1}H NMR (500 MHz, CD_3OD): δ 8.04 (d, J = 7.9 Hz, 1 H), 7.90 (d, J = 7.8 Hz, 0.66 H), 7.66 (t, J = 7.9 Hz, 1 H), 7.53−7.45 (m, 3.98 H), 7.36−7.23 (m, 8.3 H), 6.95 (s, 0.66 H), 6.94 (s, 1 H), 6.22 (s, 1 H), 5.81 (s, 0.66 H), 5.06−5.01 (m, 5.64 H), 4.78 (d, J = 17.4 Hz, 1 H), 4.63 (m, 1.66 H), 4.44−4.37 (m, 2.66 H), 3.46 (m, 1.66 H), 3.37 (m, 0.66 H), 2.75−2.59 (m, 3.32 H), 2.52−2.45 (m, 5.64 H), 2.02 (bd, J = 10.3 Hz, 1 H). ^{13}C NMR (125 MHz, CD_3OD): δ 169.9, 149.4, 165.3, 165.2, 163.7, 162.8, 155.3, 150.3, 149.9, 138.8, 138.6, 134.8, 134.5, 134.2, 133.5, 131.8, 130.4, 130.0, 129.61, 129.59, 129.5, 129.14, 129.07, 129.0, 126.2, 126.1, 124.7, 124.4, 117.3, 117.2, 109.3, 109.0, 93.0, 92.5, 89.3, 88.5, 76.8, 76.5, 71.8, 70.5, 69.9, 68.5, 68.3, 60.4, 56.1, 50.7, 50.4, 44.1, 43.3, 40.8, 30.9, 14.6, 13.9. FTIR (film): 3371, 3263, 2933, 1636, 1590, 1524, 1497, 1443, 1426, 1383, 1337, 1287, 1266, 1119, 1063, 982, 799, 735, 700 cm^{-1}. HRMS (ESI): m/z calcd for $C_{32}H_{30}Br_2N_8NaO_7S$ (M+Na), 851.0223; found, 851.0219.

Intermediate 20. A solution of *m*-chloroperoxybenzoic acid (washed with saturated aqueous $NaHCO_3$ solution; 5.4 mg, 0.031 mmol) in CH_2Cl_2 (1 mL) was added at 0 °C to a solution of hemiaminal diastereoisomers **19** (8.6 mg, 0.01 mmol) in CH_2Cl_2 (2 mL) in a sealable high-pressure reaction tube. The resulting clear solution was stirred at 0 °C for 10 min, then allowed to warm to room temperature and stirred for another 30 min. This solution was cooled to −78 °C, and NH_3 (ca. 3 mL) was condensed into the solution. The reaction tube was sealed, and the resulting cloudy solution was allowed to warm to room temperature (clear solution after ca. 10 min) and kept at this temperature for 14 h. The solution was then recooled to −78 °C, the tube was unsealed, and excess NH_3 was allowed to evaporate with warming to room temperature. The resulting suspension was concentrated, and the residue was purified by RP-HPLC (Phenomenex C18, 5 μm, 250 × 21.2 mm, H_2O (with 0.1% TFA)/MeOH linear gradient 50:50 → 10:90 in 20 min, flow rate 10 mL/min, t_R = 14.8 min) to provide 7.5 mg (79%) of an inseparable mixture (5:1) of bis(guanidine) diastereoisomers **20** (as the trifluoroacetate salts) as a colorless, glasslike solid. ^{1}H NMR (500 MHz, CD_3OD): δ 8.17 (d, J = 8.1 Hz, 1 H), 8.09 (d, J = 8.1 Hz, 0.2 H), 7.78 (t, J = 7.6 Hz, 1 H), 7.71 (t, J = 7.7 Hz, 0.2 H), 7.61 (t, J = 7.7 Hz, 1.2 H), 7.55 (d, J = 7.7 Hz, 1 H), 7.41−7.31 (m, 6.2 H), 7.07 (s, 0.2 H), 7.06 (s, 1 H), 6.57 (s, 1 H), 6.25 (s, 0.2 H), 5.31 (d, J = 17.4 Hz, 1 H), 5.29 (d, J = 12.2 Hz, 0.2 H), 5.26 (d, J = 12.2 Hz, 0.2 H), 5.23 (s, 2 H), 4.97 (d, J = 17.4 Hz, 1 H), 4.84 (d, J = 16.6 Hz, 0.2 H), 4.31 (m, 1.2 H), 4.24 (dd, J = 9.9, 12.4 Hz, 1 H), 4.05 (dd, J = 8.9, 12.4 Hz, 0.2 H), 3.86 (d, J = 12.2 Hz, 1 H), 3.77 (dd, J = 3.8, 12.4 Hz, 0.2 H), 3.67 (dd, J = 6.5, 12.4 Hz, 1 H), 3.11 (dd, J = 6.4, 15.5 Hz, 0.2 H), 3.04 (m, 1.2 H), 2.32 (d, J = 13.8 Hz, 1 H), 2.28 (d, J = 13.8 Hz, 1 H), 2.16 (dd, J = 3.1, 15.5 Hz, 0.2 H). ^{13}C NMR (125 MHz, CD_3OD): δ 158.95, 158.93, 158.88, 158.6, 155.8, 155.4, 150.1, 149.7, 137.1, 136.9, 135.4, 135.3, 134.0, 132.2, 132.1, 131.4, 131.3, 130.9, 130.7, 130.5, 130.3, 129.8, 129.6, 129.4, 129.2, 126.8, 126.7, 124.7, 124.5, 118.6, 118.2, 109.3, 109.0, 104.9, 104.5, 90.8, 89.3, 83.5, 83.2, 72.2, 71.8, 70.4, 70.1, 70.0, 69.9, 69.4, 69.0, 54.8, 52.8, 47.2, 45.4, 45.1, 44.8, 44.1, 43.0, 42.5. FTIR (film): 3344, 3261, 2916, 2873, 2831, 1766, 1702, 1671, 1594, 1528, 1439, 1345, 1272, 1202, 1135, 801, 724 cm^{-1}. HRMS (ESI): m/z calcd for $C_{31}H_{29}Br_2N_9O_7$ (M+H), 798.0635; found, 798.0668.

Products 3 and 4. A solution of bis(guanidine) diastereoisomers **20** (25 mg, 0.027 mmol) in dioxane (5 mL) was irradiated at room temperature for 2.5 h using a sunlamp (275 W). Pd/C (10%, 10 mg) was added to the resulting yellow solution, and the suspension was stirred under a H_2 atmosphere for 4 h. The suspension was filtered through Celite, and the Celite was washed with dioxane (1 mL) and H_2O/0.1%TFA (1 mL). Pd/C (10%, 20 mg) was added to the yellowish filtrate, and the suspension was stirred under a H_2 atmosphere for 24 h, then filtered (45 μm nylon filter). The filtrate was concentrated, and the residue was redissolved in H_2O/0.1% TFA and purified by RP-HPLC (Phenomenex C18, 5 μm, 250 × 21.2 mm, H_2O (with 0.1% TFA)/CH_3CN linear gradient 95:5 → 80:20 in 20 min, flow rate 10 mL/min) to afford 8 mg (43%) and 4.1 mg (22%) of bis(guanidine) hemiaminal diastereoisomers **3** and **4**, respectively, (enriched, as the bis(trifluoroacetate) salts) as colorless, glasslike solids.[19] Data for **3**: t_R = 14.9 min. ^{1}H NMR (500 MHz, D_2O): δ 7.13 (d, J = 1.3, 2.7 Hz, 1 H), 6.97 (dd, J = 1.3, 4.0 Hz, 1 H), 6.47 (dd, J = 2.7, 4.0 Hz, 1 H), 6.36 (s, 1 H), 5.34 (s, 1 H), 4.34 (m, 1 H), 4.16 (dd, J = 10.1, 12.3 Hz, 1 H), 3.82 (d, J = 12.0 Hz, 1 H), 3.65 (dd, J = 6.1, 12.3 Hz, 1 H), 3.07 (dddd, J = 4.1, 6.1, 10.1, 12.0 Hz, 1 H), 2.35 (dd, J = 3.7, 14.3 Hz, 1 H), 2.16 (d, J = 14.3 Hz, 1 H). ^{13}C NMR (125 MHz, D_2O): δ 158.7, 157.4, 156.9, 124.7, 120.5, 115.0, 113.0, 85.8, 82.0, 71.9, 70.1, 68.1, 53.1, 49.9, 45.3, 42.2. FTIR (film): 3188, 2836, 1671, 1578, 1557, 1472, 1432, 1389, 1326, 1191, 1135, 1092, 1038, 843, 801, 724 cm^{-1}. HRMS (ESI): m/z calcd for $C_{16}H_{20}N_8O_3$ (M+H), 373.1737; found, 373.1736. Data for **4**: t_R = 13.9 min. ^{1}H NMR (500 MHz, D_2O): δ 7.10 (d, J = 1.3, 2.7 Hz, 1 H), 6.97 (dd, J = 1.3, 4.0 Hz, 1 H), 6.47 (dd, J = 2.7, 4.0 Hz, 1 H), 6.24 (s, 1 H), 5.19 (s, 1 H), 4.34 (m, 1 H), 4.07 (dd, J = 9.9, 12.3 Hz, 1 H), 3.72 (dd, J = 4.6, 12.3 Hz, 1 H), 3.35 (d, J = 10.7 Hz, 1 H), 3.15 (dddd, J = 4.6, 5.1, 9.9, 10.7 Hz, 1 H), 2.79 (dd, J = 5.1, 15.4 Hz, 1 H), 2.08 (dd, J = 2.5, 15.4 Hz, 1 H). ^{13}C NMR (125 MHz, D_2O): δ 158.7, 157.4, 156.7, 124.5, 120.3, 115.0, 113.2, 87.9, 81.8, 70.7, 69.2, 68.7, 59.9, 44.2, 42.7, 41.2.

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Supporting Information Available: Experimental procedures and characterization data for new compounds, comprehensive set of experimentally determined and calculated interproton distances for **3** and **4**, and molecular models of computed lowest-energy conformers. This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) General experimental details are provided in the Supporting Information.

(19) Hemiaminal diastereoisomers **3** and **4** interconvert at room temperature in H_2O and therefore could not be obtained in diastereomerically pure form (i.e., diastereoisomer **3** could only be enriched to ca. 90% (^{1}H-NMR)).