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Novel Peptidomimetics of the Antifungal Cyclic Peptide Rhodopeptin: Synthesis of Mimetics and Their Antifungal Activity

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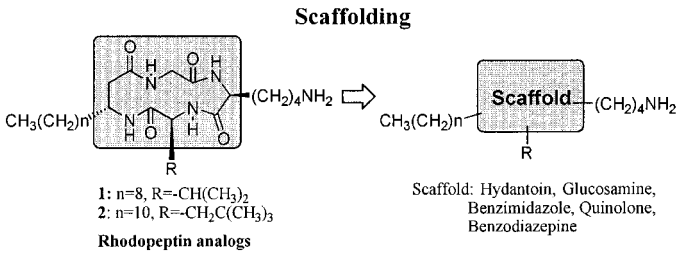
ABSTRACT



Novel peptidomimetics of the antifungal cyclic peptide Rhodopeptin were synthesized. As with the cyclic peptides, the presence of all three Rhodopeptin side chains was found to be indispensable for peptidomimetic activity. We discovered new compounds exhibiting greater antifungal activity and improved physiochemical properties in comparison to the parent compounds.

In the preceding paper,[1] we described our concept and the design of peptidomimetics of Rhodopeptin derivatives utilizing scaffolding methodology. Here, we report the synthesis, the antifungal activity, and the water solubility of those mimetics.

Synthetic preparation of the hydantoin derivatives was accomplished as shown in Scheme 1. Acylation of urea **3** with chloroacetyl chloride, followed by cyclization with neopentylamine, gave compound **4**. Introduction of a protected aminoalkyl moiety at the 3-position and hydrogenolysis furnished compound **5**, which contains two of the three critical side chains. Alternatively, bromination of **4**, Michaelis−Arbuzov reaction with triethyl phosphite, and a Horner−Wadsworth−Emmons-type reaction[2] with *n*-caprinaldehyde provided compound **6** as a mixture of olefin isomers. Subsequent introduction of a protected aminoalkyl moiety at the 3-position and hydrogenolysis furnished trisubstituted hydantoin **7** as a racemate.

The glucosamine derivatives **11** and **14** were synthesized from bromo sugar **8** (Scheme 2). Incorporation of the lipophilic straight chain was accomplished by zinc triflate promoted glycosilation[3] and was followed by deprotection of the 2,2,2-trichloroethoxycarbonyl (Troc) group, introduction of the *tert*-butylacetyl group, and acetate removal to provide **10**. Protection of the 4,6-diol as the corresponding acetonide allowed the 3-hydroxyl group to be acylated and was followed by deprotection of the acetonide and removal of the Cbz group to afford **11**. Alternatively, benzylidene formation on the 4,6-diol of **10**, protection of the 3-hydoxyl group with the Troc group, and $NaBH_3CN$ reduction[4] under

(1) (a) Kawato, H. C.; Inagaki, H.; Ohta, T.; Nakayama, K. *Org. Lett.* **2001**, *3*, 3447.

(2) Meanwell, N. A.; Roth, H. R.; Smith, C. R.; Wedding, D. L.; Wright, J. J. K. *J. Org. Chem.* **1991**, *56*, 6897.

(3) Nakayama, K.; Higashi, K.; Soga, T.; Uoto, K.; Kusama, T. *Chem. Pharm. Bull.* **1992**, *40*, 2909.

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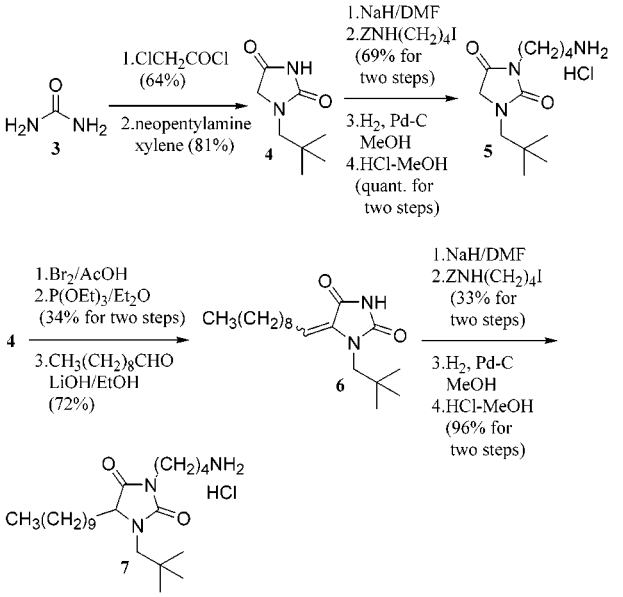
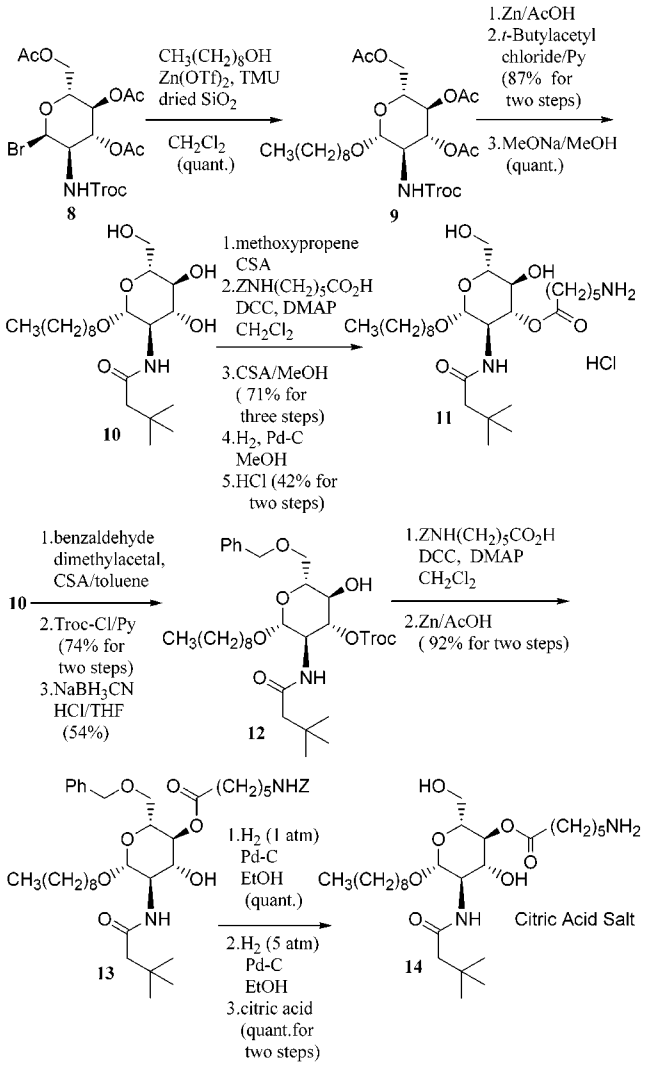
Scheme 1



Scheme 2



acidic conditions provided 3,6-O-protected derivative **12**. Acylation of **12**, followed by stepwise removal of all protecting groups, furnished compound **14**, which bears the basic side chain on the 4-hydroxyl group. It was crystallized as a citric acid salt.

The benzimidazole derivatives were prepared as depicted in Scheme 3. Acylation with isobutyryl chloride on both amino and hydroxyl groups of 2-amino-3-nitrophenol **15**, followed by hydrolysis of the resultant ester, afforded amide **16**. Alkylation with *n*-nonyl bromide, hydrogenolysis, and cyclization under acidic conditions then provided disubstituted benzimidazole **17**. Attempted introduction of the aminoalkyl moiety onto nitrogen gave a mixture of regioisomers **18** and **19**, whose structures were determined by NOE experiments. Isolation of the desired isomer **18** from this unoptimized transformation was followed by hydrogenolysis to furnish **20**.

Synthesis of the quinolone derivative **24**,[5] as depicted in Scheme 4, began with a thermal condensation and ring closure sequence from *m*-nitrophenol **21** to afford **22**. Subsequent alkylation and hydrolysis furnished the *N*-alkylated carboxylic acid **23**. Amide formation, which thus far has only been successfully achieved by a mixed anhydride protocol, followed by deprotection of the Boc group, completed the construction of **24**.

The synthesis of benzodiazepine derivatives **32** and **36**[6] (Scheme 5) commenced with the palladium-mediated cross-coupling reaction[7] between aryl iodide **25** and acetylene derivatives. Selective hydrolysis at the benzylic position with a Na_2S/HCl protocol[8] afforded compounds **28** and **29** in high yields. At this stage, acylation of compound **28** met with some difficulties. The extremely weak nucleophilicity of the amine moiety resulted in initial acylation exclusively at the alcohol residue. Although subsequent acylation of the amine group was unsuccessful under the typical DCC/DMAP conditions, we discovered that dipyridyl carbonate (DPC),[9] along with DMAP, provided a superior coupling protocol to furnish **30** in high yields. We utilized DL-leucine derivatives in these coupling reactions, not only to allow simultaneous biological testing of all stereoisomers but also to avoid any issues associated with possible racemization. From **30**, removal of the Fmoc group and cyclization with acetic acid provided benzodiazepine **31**. Hydrolysis and a Mitsunobu reaction were utilized to introduce the basic side chain, and final alkylation on the nitrogen of the benzodiazepine ring and treatment with hydrazine completed the synthesis of **32**. The other benzodiazepine, **36**, was prepared

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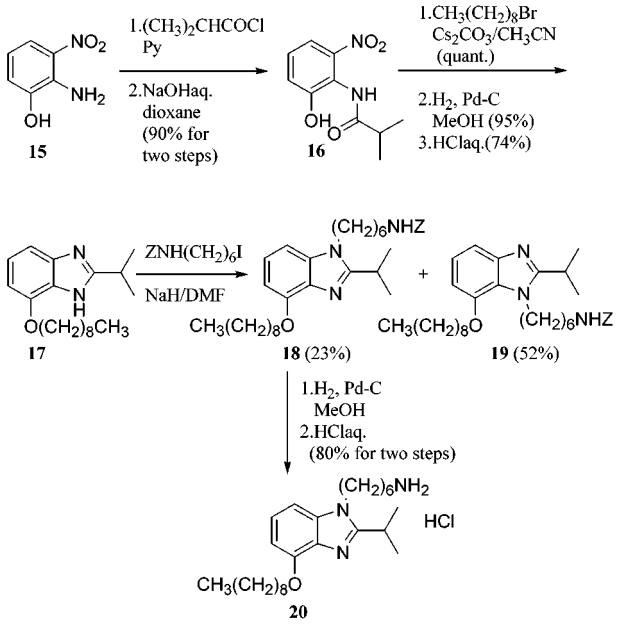
(5) Mozek, I.; Sket, B. *J. Heterocycl. Chem.* **1994**, *31*, 1293.
(6) Wu, C.; Biediger, R. J.; Kogan, T. P. *Synth. Commun.* **1999,** *29*, 3509.
(7) Arcadi, A.; Cacchi, S.; Carnicelli, V.; Marinelli, F. *Tetrahedron* **1994**, *50*, 437.
(8) Chapdelaine, M. J.; Warwick, P. J.; Shaw, A. *J. Org. Chem.* **1989**, *54*, 1218.
(9) Uoto, K.; Ohsuki, S.; Takenoshita, H.; Ishiyama, T.; Iimura, S.; Hirota, Y.; Mitsui, I.; Terasawa, H.; Soga, T. *Chem. Pharm. Bull.* **1997**, *45*, 1793.

Scheme 3

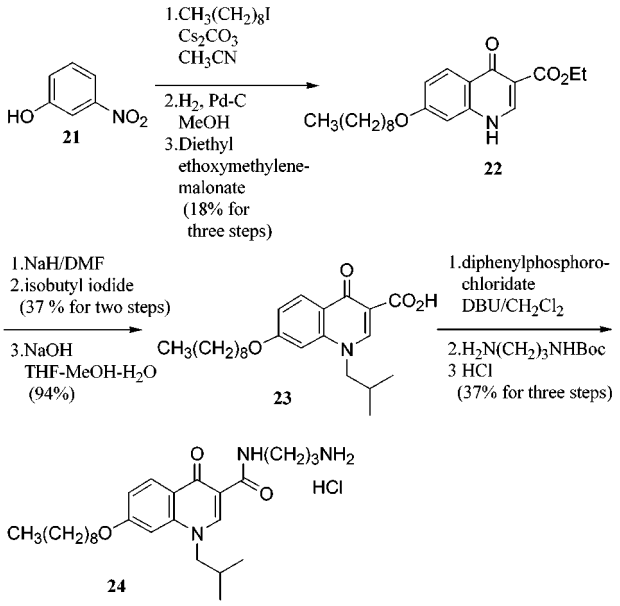


in seven steps in an analogous fashion from compound **29**. It was crystallized as a citric acid salt.

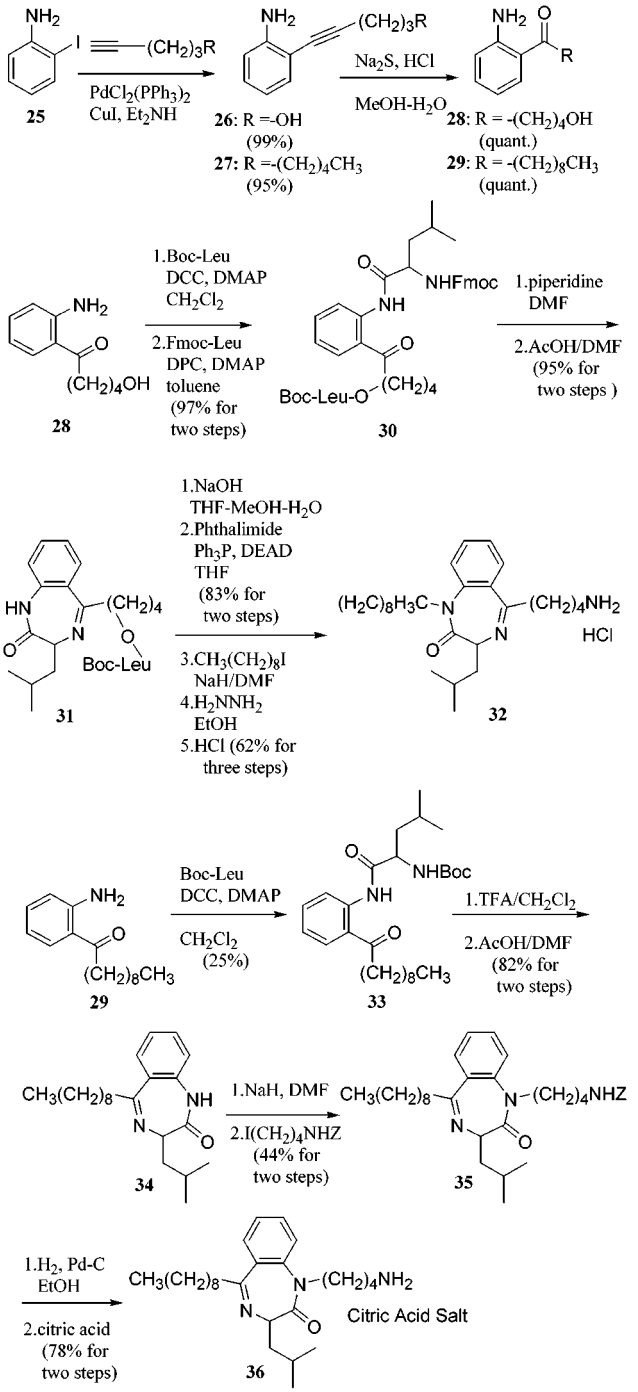
The minimum inhibitory concentration values (MIC, μg/mL) against *Candida albicans* ATCC24433, *Cryptococcus neoformans* IAM12253, and *Aspergillus fumigatus* ATCC-36607 are presented in Table 1,[10] with the activities of AMPH and FLCZ[11] as references. Solubilities in water[12] of selected compounds are also listed.

Compounds **4**, **5**, and **17**, which do not contain all three side-chain moieties, showed no potency or lower activity than the parent peptides **1** and **2**.

Scheme 4



Scheme 5



According to our molecular modeling studies in the preceding paper, each of the designed trisubstituted peptidomimetics displayed reasonably good three-dimensional overlap with the cyclic peptide. However, the location of the bulky hydrophobic side chain of the glucosamine

(10) The in vitro susceptibility testing was done by a microdilution method. Minimum inhibitory concentration (MIC) was defined as the lowest concentration of a compound which gave no visibly detectable fungal growth after incubation at 30 °C for 72 h. Medium: synthetic amino acid medium fungal (SAAMF). Tested concentration of compounds: 2, 10, 50 μg/mL.

(11) Amphotericin B (AMPH; Fungizone, Bristol Myers Squibb, New Brunswick, NJ) and fluconazole (FLCZ; Diflucan, Pfizer, NY).

Table 1. Antifungal Activity and Solubility in Water of Peptidomimetics

compd	MIC (μg/mL) *C. albicans* ATCC24433	*C. neoformans* IAM12253	*A. fumigatus* ATCC36607	solubility in water (mg/mL)
4	>50	>50	>50	
5	>50	>50	>50	
7	50	2	10	>100
11	>50	50	>50	>100
14	>50	50	>50	>100
17	>50	2	>50	
20	10	2	50	>100
24	2	2	10	>100
32	10	10	50	>100
36	10	2	10	>50
1	10	10	>50	0.9
2	10	10	10	2.6
AMPH	2	2	10	
FLCZ	>50	10	>50	

derivatives **11** and **14** deviated slightly from the parent cyclic peptide. Those mimics showed only weak activity against *Cryptococcus neoformans*, suggesting that the position of the bulky side chain is critical. Additionally, it appears that the position of the bulky neutral moiety of mimetics using monocyclic subunits, **7**, **11**, and **14**, contain significant conformation flexibility. In contrast, compound **20**, whose three critical moieties are more constrained in the proper positions, seem to fit the parent peptide better, which might explain its better antifungal activity.

The other peptidomimetics (**20**, **24**, **32**, and **36**) exhibited antifungal activity with equal to or greater magnitude than that of the parent cyclic peptides. Particularly encouraging was the extremely high potency of quinolone analogue **24**. Notably, the location of the basic side chain of this compound was slightly altered relative to the parent compounds and the other mimetics due to hydrogen bonding between the amide proton and the 4-oxo group. This result suggests a correlation between this conformational restriction and a more active conformation. The mechanism of the action of these peptidomimetics and Rhodopeptin itself is now being investigated in our laboratory.

Of equal importance, the peptidomimetics possessed greatly improved solubility in water. Whereas Rhodopeptin analogues typically showed a solubility of only 1−2 mg/mL in water, the peptidomimetics all exhibited solubilities >50 mg/mL. This result demonstrates that the scaffolding methodology is very effective for improving physiochemical properties without losing the desired biological activity.

In conclusion, novel peptidomimetics of the antifungal cyclic peptide Rhodopeptin were designed and synthesized, utilizing a scaffolding methodology. It was observed that the three side chains are indispensable for antifungal activity of the peptidomimetics, which is a similar requirement for the parent cyclic peptides. We have successfully produced potent mimics of Rhodopeptin. Interestingly, the similarity of the three-dimensional structures of the new peptidomimetics relative to Rhodopeptin correlated extremely well with the magnitude of antifungal activity. In addition, we discovered new compounds exhibiting greater antifungal activity and improved physiochemical properties. Further investigations to identify additional mimetics through this scaffolding methodology are ongoing in our laboratory and will be reported in due course.

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Supporting Information Available: Spectroscopic and analytical data for compounds **4**−**7**, **9**−**14**, **16**−**20**, **22**−**24**, **26**−**29**, and **31**−**36**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) For peptidomimetics. About 1 mg of each compound was accurately weighed and transferred to a glass tube. About 10 μL of distilled water (the concentration: 100 mg/mL) was added to the tube. The dissolution of the compound was checked by visual observation after the sample tube was shaken vigorously. When the sample was not completely dissolved, a 2-fold dilution (the concentration: 50 mg/mL) was then checked. For Rhodopeptin analogues. Since Rhodopeptin analogues showed a low solubility, another protocol was employed. Thus, the powder (ca. 1 mg) was combined with 200 μL of distilled water (the concentration: ca. 5 mg/mL). After mixing the sample, followed by centrifugation, the concentration of top clear layer was measured by HPLC and the concentration was calculated using a calibration curve.