Synthesis and Biological Evaluation of Photoaffinity Labeled Fusidic Acid Analogues

Ditte Riber,[†] Musturi Venkataramana,[‡] Suparna Sanyal,[‡] and Tore Duvold*,[†]

LEO Pharma, Industriparken 55, 2750 Ballerup, Denmark, and Department of Cell and Molecular Biology, Uppsala University, Box-596, BMC, Uppsala, Sweden

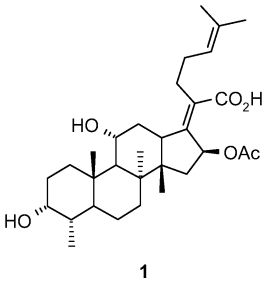
Received June 19, 2005

Abstract: Novel photoaffinity labeled fusidic acid analogues were obtained by a synthetic sequence employing a Wittig reaction between a fusidic acid aldehyde and benzyl bromides in the key step. Three commonly used photoreactive groups, benzophenone, trifluoromethyldiazirine**,** and aryl azide, were used. The photoaffinity labeled fusidic acid analogues demonstrated a potent antibacterial activity (MIC 0.016−4 μg/mL) and therefore represent a potential tool for the elucidation of the interactions between fusidic acid and its receptor EF-G.

Fusidic acid (Figure 1), **1**, is a unique antibiotic with a potent activity against *Staphlococcus aureus*, including strains resistant to other classes of antibiotics. Fusidic acid inhibits the bacterial protein synthesis by interference with elongation factor G (EF-G) in the translocation step,[1] the process by which the ribosome moves relative to mRNA.[2] In the presence of fusidic acid, EF-G remains bound to the ribosome after GTP hydrolysis and translocation, preventing further protein synthesis. Although the overall role of fusidic acid in bacterial protein synthesis is well described, little is known about the details in the interaction with EF-G. A putative binding site has recently been proposed by analyzing a number of fusidic acid resistant bacteria with mutations in EF-G.[3] The location of a mutation cluster conferring resistance to fusidic acid was identified and used to suggest a possible binding site. However, the exact binding mode of fusidic acid to EF-G and the ribosome remains unknown.

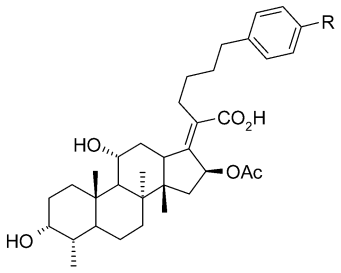
A detailed binding model should represent a useful tool for the rational design of new fusidic acid analogues with improved antibacterial properties and resistance profile. In the absence of a cocrystal structure of fusidic acid and EF-G, photoaffinity labeling studies offer a useful approach in identifying the amino acid residues involved in ligand binding. We herein describe the first example of an efficient synthetic sequence for the preparation of photoaffinity labeled fusidic acid with three different types of photoreactive groups in the side chain.

The structure−activity relationship (SAR) of fusidic acid has been extensively studied, and a large number of analogues have been prepared and tested for antibacterial activity.[4−6] These results demonstrate the crucial importance of the carboxylic acid and the acetate as well as of the hydroxy group at C-3. In a recent paper studying the role of the side chain, we showed that the side chain can only occupy a limited conformational space with regard to both the carboxylic acid group and the lipophilic moiety.[7] However, lipophilic substituents can be linked to the side chain without causing loss of antibacterial potency.[8] The SAR data clearly suggest that the lipophilic part of the side chain represents an ideal location for aromatic photoaffinity moieties (Figure 2), and thus retention of potency and minimum changes in the ligand−receptor interactions could be expected.

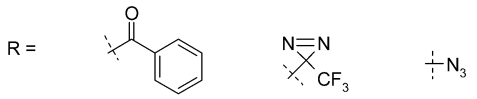


1

Figure 1. The structure of fusidic acid.

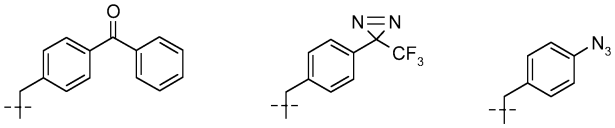


2



a **b** **c**

Figure 2. Photoaffinity labeling the side chain in fusidic acid.



3 **4** **5**

Figure 3. The selected photoaffinity groups.

Three photoreactive groups, benzophenone **3**, trifluoromethyldiazirine **4**, and azide **5**, were selected (Figure 3), as they are considered to be the most reliable and have demonstrated utility in several ligand−receptor studies.[9] The phenyl azide group was chosen instead of the more reactive tetrafluorophenyl azide since we were not able to prepare the necessary triphenylphosphine building block according to the protocol outlined in Scheme 3. The selected photoaffinity groups react with the receptor through different reactive intermediates: a radical (benzophenone), a carbene (trifluoromethyldiazirine), and a nitrene (aromatic azide).[9]

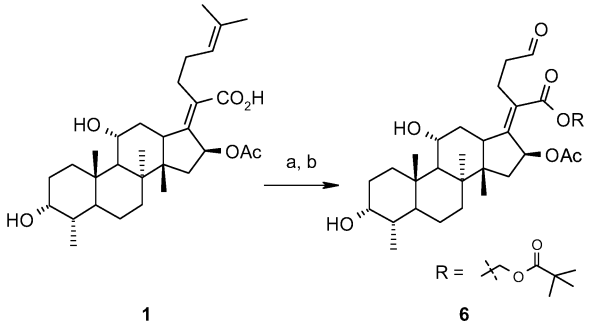
The use of different groups increases the chance of successful cross-linking to the target protein, since it cannot be predicted in advance which group will be most readily attached to the receptor. Upon RNase treatment and protein digestion, the fragments are to be analyzed by MS-MS to determine the peptide sequence to which fusidic acid is bound. Correlating this information to the structural data available, this could subsequently reveal the location of fusidic acid in complex with EF-G and the ribosome.

Aldehyde derivative, **6**, of fusidic acid was synthesized to enable introduction of photoaffinity groups via a Wittig reaction

* Corresponding author. Tel.: +4572262225; Fax: +4544669720; e-mail: tore.duvold@leo-pharma.com.
[†] LEO Pharma.
[‡] Uppsala University.

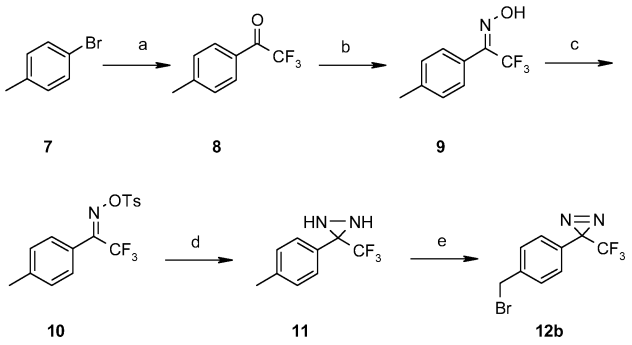
10.1021/jm050583t CCC: $33.50
Published on Web 02/15/2006

Scheme 1. Synthesis of **6**[a]



[a] Reagents: (a) $(CH_3)_3CO_2CH_2Cl$, TEA, DMF, 90%; (b) O_3, CH_2Cl_2, −78 °C, 64%.

Scheme 2. Synthesis of the Diazirine Coupling Compound **12b**[a]



[a] Reagents: (a) *N*-Trifluoroacetylpiperidine, *n*-butyllithium., 83%; (b) hydroxylamine, pyridine, ethanol, 93% (not purified); (c) *p*-toluenesulfonyl chloride, pyridine, 82%; (d) NH_3, 76%; (e) NBS, AIBN, 59%.

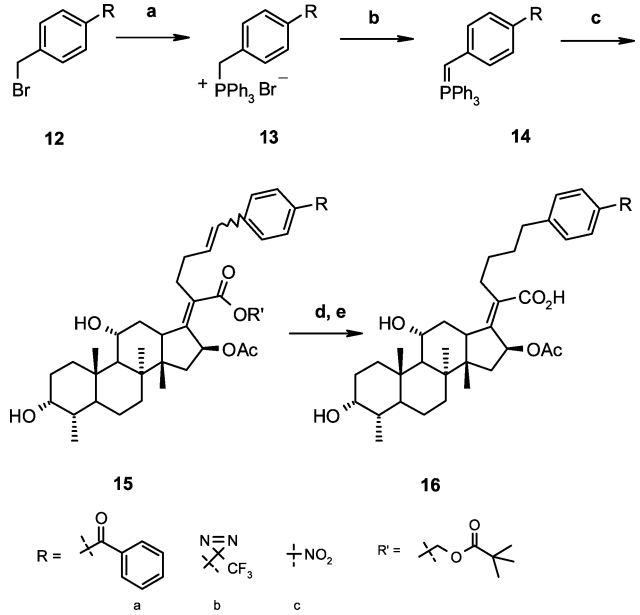
by coupling with benzyl bromides **12a**−**c**. The aldehyde was prepared by protecting the carboxylic acid as a pivaloyloxymethyl ester followed by selective ozonolysis of the ΔC24(25) double bond (Scheme 1).

4-(Bromomethyl)benzophenone, **12a**, and 4-nitrobenzyl bromide, **12c**, were commercially available, whereas bromotrifluoromethyldiazirine, **12b**, was synthesized by slightly modifying a reported procedure (Scheme 2).[10] Starting from readily available 4-bromotoluene, **7**, reaction with *N*-trifluoroacetylpiperidine gave trifluoroacetyltoluene **8**, which was converted to the hydroxyl oxime **9** by condensation with hydroxylamine in pyridine. Tosylation with *p*-toluenesulfonyl chloride gave **10**, which was treated with ammonia under pressure to yield diaziridine **11**. Bromination at the benzylic position with *N*-bromosuccinimide (NBS) using a catalytic amount of 2,2′-azobisisobutyronitrile (AIBN) also promoted formation of the diazirine moiety to give **12b.** The reaction most likely takes place by in situ formation of Br_2 which is capable of oxidizing the diaziridine.

Coupling between fusidic acid aldehyde, **6**, and benzyl bromides, **12a**−**c**, was smoothly achieved via a Wittig reaction (Scheme 3). Synthesis of the benzophenone labeled fusidic acid derivative **16a** was prepared through in situ formation of phosphonium salt **13a** with triphenylphosphine in toluene at reflux, subsequent deprotonation with potassium *tert*-butoxide to give **14a**, and reaction with aldehyde **7** to give coupling product **15a**. Selective hydrogenation of the double bond was possible, but prolonged reaction time led to complete reduction of the carbonyl in the benzophenone moiety as well. Carboxylic acid **16a** was obtained by deprotection with potassium carbonate in methanol.

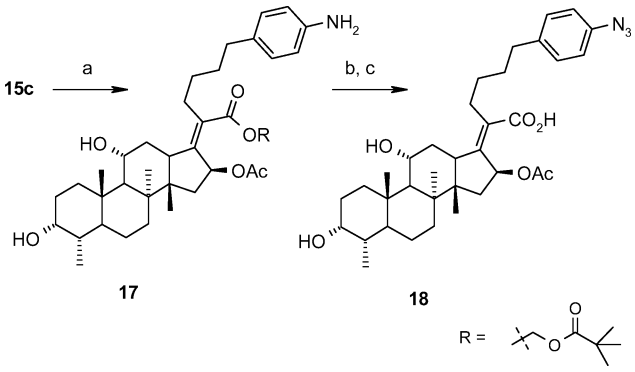
For the preparation of the diazirine photolabeled fusidic acid analogue, phosphonium salt **13b** was synthesized by carefully

Scheme 3. Synthesis of **16a**,**b** + **15c**[a]



[a] Reagents: (a) PPh_3, toluene; (b) *t*-BuOK, toluene; (c) **7**, a: 75%, b: 68%, c: 85% (for three steps); (d) H_2, Pd/C, MeOH, a: 100%, b: 57%; (e) K_2CO_3, MeOH, a: 85%, b: 69%.

Scheme 4. Synthesis of **18**[a]



[a] Reagents: (a) H_2, Pd/C, MeOH, 62%; (b) TfN_3, TEA, $CuSO_4$, H_2O, MeOH, 74% (c) K_2CO_3, MeOH, 86%.

heating a mixture of the diazirine and triphenylphosphine. The reaction was carried out at 80 °C, since the diazirine was found to decompose at temperatures above 90 °C. The phosphonium salt was in this case isolated and purified before treatment with *t*-BuOK to give ylid, **14b**. The coupling product, **15b**, was obtained by condensation with aldehyde **6**, and the double bond was then carefully hydrogenated without reducing the labile diazirine functionality. The desired product **16b** was obtained by deprotection of the acid with potassium carbonate in methanol.

In the case of coupling with 4-nitrobenzyl bromide, **12c**, the Wittig reaction was carried out at room temperature to give **15c**. Hydrogenation of both the double bond and the aromatic nitro group gave **17** (Scheme 4), in which the aromatic amine was converted into an azide by treatment with triflyl azide, triethylamine, and $CuSO_4$ as catalyst.[11] The desired fusidic acid analogue was then obtained by deprotection of the acid with potassium carbonate to give **18** (Scheme 4).

To facilitate the identification of cross-linked fragments after protein digestion, additional radiolabeling was required. The synthesis of the tritium labeled analogues have been successfully obtained by selective tritiation of the Δ24(25) double bond in

Table 1. MIC Values of Photoaffinity Labeled Fusidic Acid Analogues **16a**, **16b**, and **18**

	MIC (μg/mL)[a]			
organism/strain	fusidic acid (**1**)	benzophenone fusidic acid (**16a**)	diazirine fusidic acid (**16b**)	azide fusidic acid (**18**)
S. aureus, CJ 247	0.063	1	-	-
S. aureus, CJ 234 R	<0.063	1−2	1−4	1−2
S. aureus, CJ 200	0.063	4	2−4	1−2
S. aureus, CJ 251	<0.063	1−2	1−4	1−2
S. aureus, CJ 288	<0.063	1−2	1−4	1−2
S. Epidermis, CK 5	0.063	1−4	1−4	4
C. xerosis, FF	0.016	0.016−0.063	0.063−0.125	0.016

[a] The MIC values were obtained from quadruple testing of the labeled fusidic acid analogues and are within the stated intervals.

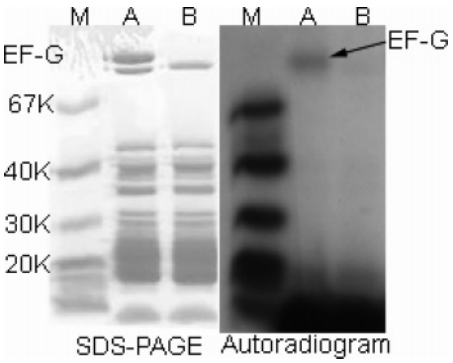


Figure 4. Autoradiogram of the SDS−PAGE of UV-treated 70S ribosome-EF-G-GDP-^{3}H-labeled fusidic acid azide analogue complex reveals cross-linking only on EF-G (lane A), similar treatment with only 70S ribosome shows no cross-linking (lane B). Lane M, ^{14}C-labeled molecular weight markers.

derivatives **15a**, **15b**, and **15b.** The preparation of the tritium labeled analogues will be published elsewhere.

The three compounds revealed potent antibacterial activity with minimum inhibitory concentration (MIC) values ranging from 0.016 to 4 μg/mL (Table 1). The retained antibacterial activity of the photoaffinity labeled fusidic acid analogues suggested that these could also bind to EF-G on the ribosome in the same way as fusidic acid, and they should therefore be suitable for cross-linking experiments.

We have tested their binding to EF-G by nitrocellulose filter-binding assay following the protocol of Bodley et al.[12] The data clearly demonstrate that the three photoaffinity labeled fusidic acid analogues are equally potent in the formation and stabilization of 70S ribosome-EF-G-GDP complex as fusidic acid. Furthermore, UV cross-linking with the ^{3}H-labeled azide analogue to such complex confirms covalent binding only on EF-G when autoradiographed after separation of the complex in a SDS−PAGE (Figure 4). Detection of the cross-linking sites on EF-G is currently ongoing.[13] Successful localization of the fusidic acid binding site should become a valuable tool in the efforts to design new analogues.

In summary, an efficient synthetic route for a series of photoaffinity labeled fusidic acid analogues has been developed employing a Wittig reaction between aldehyde **6** and benzyl bromides **12a−c** in the key step. Antimicrobial evaluation showed that the three analogues retained a potent activity against fusidic acid-sensitive bacteria and that azide fusidic acid **18** was successfully cross-linked to EF-G. Localization of the exact binding site will be a valuable tool in our efforts to design derivatives with improved antibacterial and pharmacokinetic properties.

Acknowledgment. We thank Dr. Hanne Aae Theilgaard and Ms. Merete Henriksen for providing the antimicrobial data. This project was funded by the EU (New Antimicrobials Targeting Translation in Bacteria and Fungi, Contract QLK2-CT-2002-00892) and by Swedish National Research Council (VR) grant to S.S. (621-20002-4747).

Supporting Information Available: General procedure for the Wittig reaction, biological procedure for antimicrobial testing, analytical and spectral chacterization data for compounds **16a**, **16b**, and **18**, and binding of fusidic acid and its photoaffinity labeled analogues to EF-G-GDP on the ribosome. This material is available free of charge via the Internet at http://pubs.acs.org

References

(1) (a) Yamaki, H. Inhibition of protein synthesis by fusidic and helvolinic acids, steroidal antibiotics *J. Antibiot.* **1965**, *18*, 228. (b) Harvey, C. L.; Knight, S. G.; Sih, C. L. On the mode of action of fusidic acid. *Biochemistry* **1966**, *5*, 3320. (c) Tanaka, N.; Yamaki, H.; Lin, Y.; Umezawa, H. Further studies on inhibition of protein synthesis by fusidic and helvolinic acids. *J. Antibiot.* **1967**, *20*, 156−161.

(2) Burns, K.; Cannon, M.; Cundliffe, E. A resolution of conflicting reports concerning the mode of action of fusidic acid. *FEBS Lett.* **1974**, *40*, 219−223.

(3) Laurberg, M.; Kristensen, O.; Martemyanov, K.; Gudkov, A. T.; Nagaev, I.; Hughes, D.; Liljas, A. Structure of a mutant EF-G reveals domain III and possibly the fusidic acid binding site. *J. Mol. Biol.* **2000**, *303*, 593−603.

(4) Godfredsen, W. O.; Albrethsen, C.; von Daehne, W.; Tybring, L.; Vangedal, S. Transformations of fusidic acid and the relationship between structure and antibacterial activity. *Antimicrob. Agents Chemother*. **1965**, 132−137.

(5) von Daehne, W.; Godtfredsen, W. O.; Rasmussen, P. R. Structure activity relationships in fusidic acid-type antibiotics. *Adv. Appl. Microbiol*. **1979**, *25*, 95−146.

(6) Duvold, T.; Sørensen, M. D.; Björkling, F.; Henriksen, A. S.; Rastrup-Andersen, N. Synthesis and conformational analysis of fusidic acid side chain derivatives in relation to antibacterial activity. *J. Med. Chem.* **2001**, *44*, 3125−3131.

(7) Duvold, T.; Jørgensen, A.; Andersen, N. R.; Henriksen, A. S.; Sørensen, g, F. 17*S*,20*S*-Methanofusidic acid, a new potent semi-synthetic fusidane antibiotic. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3569−3572.

(8) Duvold et al. unpublished results.

(9) (a) Kotzyba-Hilbert, F.; Kapfer, I.; Goeldner, M. Recent trends in photoaffinity labelling. *Angew. Chem., Int. Ed. Engl*. **1995**, *34*, 1296−1312. (b) Dormán, G.; Prestwich, G. D. Using photolabile ligands in drug discovery and development. *Trends Biotechnol.* **2000**, *18*, 64−77. (c) Hatanaka, Y.; Sadakane, Y. Photoaffinity labelling in drug discovery and developments: Chemical gateway for entering proteomic frontier. *Curr. Topics Med. Chem.* **2002**, *2*, 271−288. (d) Fleming, S. A. Chemical reagents in photoaffinity labelling. *Tetrahedron* **1995**, *51*, 12479−12520.

(10) Strømgaard, K.; Saito, D. R.; Shindou, H.; Ishii, S.; Shimizu, T.; Nakanishi, K. Ginkgolide derivatives for photolabeling studies: Preparation and pharmacological Evaluation. *J. Med. Chem.* **2002**, *45*, 4038−4046.

(11) Liu, Q.; Tor, Y. Simple conversion of aromatic amines into azides. *Org. Lett*. **2003**, *5*, 2571−2572.

(12) Bodley, J. W.; Zieve, F. J.; Lin, L.; Zieve, S. T. Studies on translocation.3. Conditions necessary for the formation and detection of a stable ribosome-G factor-guanosine diphosphate complex in the presence of fusidic acid. *J. Biol. Chem.* **1970**, *245*, 5656−5661.

(13) These studies are being carried out by Dr. Sanyal at Uppsala University.

JM050583T