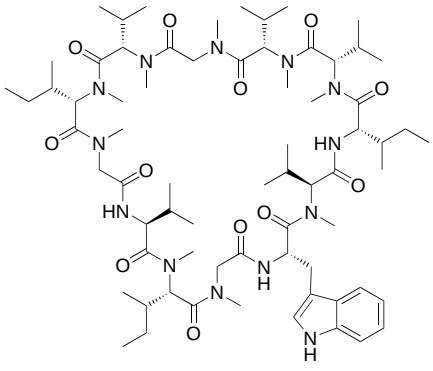
Total Synthesis of the Nematicidal Cyclododecapeptide Omphalotin A by Using Racemization-Free Triphosgene-Mediated Couplings in the Solid Phase**

Bernd Thern, Joachim Rudolph, and Günther Jung*

In memory of Ernst Bayer

The natural product omphalotin A (**1**) belongs to a family of cyclic dodecapeptides from the basidiomycete *Omphalotus olearius*.[1] and shows a selective activity against phytopathogenic nematodes such as *Meloidogyne incognita*.[1] Under



1 2 3 4 5 6 7 8 9 10 11 12
cyclo(Trp - MeVal - Ile - MeVal - MeVal - Sar - MeVal - MeIle - Sar - Val - MeIle - Sar)

1

in vitro conditions, omphalotin A outreaches known nematicides such as ivermectin in potency and selectivity.[2] The high specificity and structure of **1**, which are unusual for a nematicide, lead to the assumption that a hitherto unknown biological target is responsible for the activity of **1**. To elucidate this target, high amounts of the cyclopeptide are required which cannot be produced by fermentation alone.[2]

Structurally, the highly lipophilic omphalotin A is closely related to the immunosuppressive cyclosporin therapeutics; nine of its twelve amino acids are N-methylated. This high degree of methylation results in conformational freedom and complicates chemical syntheses considerably, as the experience from syntheses of cyclosporins illustrates.[3] The main problems are low coupling yields, side reactions such as diketopiperazine formation or racemization,[4] and the lability of N-alkylated peptides towards acids.[5] Despite numerous reports on reagents for the coupling of sterically hindered N-methyl amino acids,[6] a satisfactory solution for this problem has not yet been found.[7]

Here we report on the synthesis of **1** using Fmoc amino acids on a polystyrene support with a trityl linker (TCP resin). This linker allows product cleavage under very mild conditions using hexafluoroisopropanol (HFIP).[8] In view of the acid lability of the products, these mild conditions were crucial.

First, we did model reactions to form all N-methylated peptide bonds in **1** using four different reagents (dicyclohexylcarbodiimide = DCC, triphosgene = BTC, diisopropylcarbodiimide/hydroxyazabenzotriazole = DIC/HOAt, tetramethylfluoroformamidinium hexafluorophosphate = TFFH). As far as coupling efficiency is concerned, the BTC method of Falb et al.[9] turned out to be far superior (Table 1).

Table 1. Each of the eight N-methylated amide bonds of omphalotin A were formed using four different reagents. In the case of complete conversion, a negative chloranil test showed the absence of secondary amines after one (++) or two (+) coupling cycles, respectively. In the case of incomplete conversion (–), the chloranil test was positive even after double coupling cycles. The results were verified by HPLC or HPLC–MS after cleavage from the resin.

N-methylated amide bond[a]	TFFH[b]	DIC/HOAt[c]	DCC[d]	BTC[e]
Fmoc-Trp-OH →H-MeVal-R	–	+	+	++
Fmoc-Ile-OH →H-MeVal-R	–	–	+	++
Fmoc-Sar-OH →H-MeVal-R	–	++	++	++
Fmoc-MeVal-OH →H-MeVal-R	–	–	–	++
Fmoc-MeVal-OH →H-MeIle-R	–	–	–	++
Fmoc-Val-OH →H-MeIle-R	–	–	+	++
Fmoc-MeVal-OH →H-MeGly-R	++	++	++	++
Fmoc-MeIle-OH →H-MeGly-R	++	++	++	++

[a] R = Phe-TCP-resin; [b] Fmoc AA, TFFH: 5 equiv, DIEA: 10 equiv, 1 h in DMF (AA = amino acid); [c] Fmoc AA, HOAt, DIC, DIEA: 3 equiv, 1 h in DMF; [d] Fmoc AA: 6 equiv, DCC: 3 equiv, 1.5 h in DMF; [e] see ref. [9].

HPLC–MS, however, showed considerable amounts of byproducts in the cleavage products from the BTC couplings, as did the HPLC chromatograms published by Falb et al.[9] Furthermore, the BTC coupling turned out to be useless for the synthesis of longer peptides. After only a few such couplings, no product could be isolated from the TCP resin. On Wang resin with the less acid-labile *p*-alkoxybenzyl linker, quantitative diketopiperazine formation resulted upon Fmoc removal from the dipeptidyl resin with piperidine. Therefore, we decided to use the TCP resin, and to adapt the methodology of the BTC coupling to the properties of this resin.[10]

For the first successful synthesis of **1**, the following protocol was developed: The N-Fmoc-deprotected peptidyl–TCP resin is pretreated with diisopropylethylamine (DIEA) and the activation of the Fmoc-N-methyl amino acid is carried out

[*] Prof. Dr. G. Jung, Dipl.-Chem. B. Thern
Institute of Organic Chemistry
University of Tübingen
Auf der Morgenstelle 18, 72076 Tübingen (Germany)
Fax: (+49) 7071-295-560
E-mail: guenther.jung@uni-tuebingen.de
Dr. J. Rudolph
Central Research, Bayer AG
51368 Leverkusen (Germany)
New address: Bayer Corporation
Pharmaceutical Division, Department of Chemistry
400 Morgan Lane, West Haven, CT 06516 (USA)

[**] B.T. received a scholarship from the graduate college "Analytical Chemistry" of the DFG. We are grateful to Dietmar G. Schmid for high-resolution FTICR–MS measurements, to Graeme Nicholson for enantiomeric GC–MS analyses, to Dr. Joachim Wesener for discussions on MS analysis, to Daniel Bischoff for preparative HPLC–MS, and to Heidi Theis and Roman Hanke for experimental assistance.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

  1433-7851/02/4113-2307 $ 20.00+.50/0

at room temperature in THF by addition of BTC and collidine.[11] This procedure enables us to use BTC on acid-labile TCP resin. Premature cleavage from the resin, which was initially observed during syntheses starting from resin-bound sarcosine, was prevented by limiting the pretreatment of the resin with DIEA, as well as the Fmoc deprotection with piperidine, to the shortest possible duration. An elevated temperature during the coupling reaction was unnecessary, as the presence of the strong base DIEA appears to accelerate the coupling reaction.[12] By use of these modifications, the formation of by-products was almost completely eliminated. Furthermore, the amount of Fmoc amino acid needed per coupling reaction could be reduced from 5 to 3.5 equivalents without any loss of coupling efficiency.

Even in the exceedingly difficult coupling of Fmoc-MeVal to resin-bound MeVal, quantitative conversion can be observed when the modified BTC activation is used. The coupling of unmethylated Fmoc amino acids, on the other hand, often gave insufficient coupling yields. In these cases, DIC/HOAt or HATU activation led to better results.

Starting from TCP resin preloaded with Fmoc-sarcosine, and using a combination of BTC, DIC/HOAt, and HATU-couplings, the linear dodecapeptide with C-terminal Sar[6] [OmA(7-6)] was obtained in 84% yield and 90% purity (HPLC, $\lambda = 214$ nm). Only three coupling reactions had to be repeated, whereas the remaining eight were complete after a single coupling cycle (Figure 1). The crude product of the coupling reaction was purified by flash chromatography on silica gel. Diastereomerically pure[13] omphalotin A was obtained in a cyclization yield of 37% and in an overall yield of 31% with respect to the first loading of the resin with Fmoc-sarcosine.

Analogous syntheses starting from resin-bound Sar[9] or Sar[12] also yielded **1** in a diastereomerically pure form. A total of 102 mg of pure **1** was synthesized.

The high-resolution ^{1}H and ^{13}C NMR spectra correspond to the data given in the literature.[1b] The molecular mass determined by ES-FTICR-MS in the ultrahigh-resolution mode corresponds to the theoretical value up to a relative mass error of 2 ppm (Figure 2). Additionally, the identity of the synthetic product was confirmed by co-elution with the natural compound in the analytical HPLC and by comparison of their ES-FTICR-MS fragmentation spectra.

The modified BTC coupling was thus demonstrated to be a highly efficient, experimentally simple and very low-cost method for the coupling of N-methyl amino acids. We showed

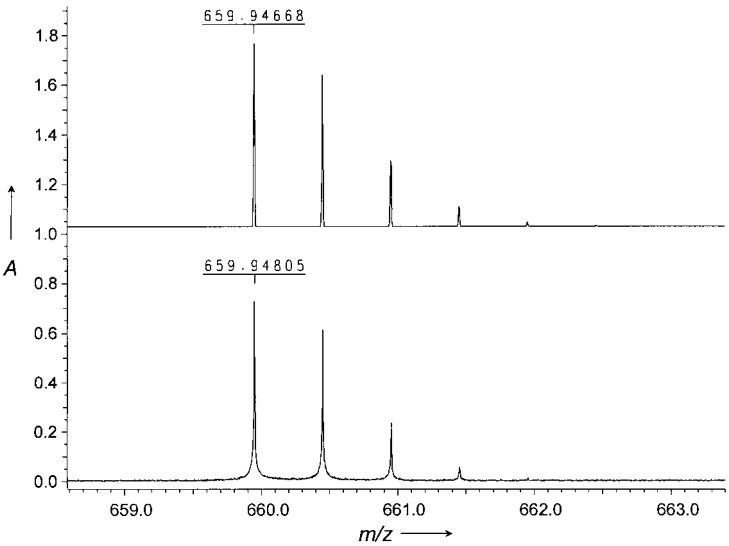


Figure 2. ES-FTICR-mass spectrum of synthetic omphalotin A ($[M+2H]^{2+}$). The upper trace shows the simulation, the lower trace shows the spectrum recorded in the ultrahigh-resolution mode. The relative mass error is 2 ppm.

that the novel BTC activation is racemization free.[13] Using this solid-phase coupling, we developed a methodology which allows the synthesis of omphalotin A in a very short time and in high yields compared to both solid-phase and solution-phase syntheses of the structurally related cyclosporins. This method is expected to facilitate greatly the synthesis of numerous other N-alkylated peptides such as cyclosporins,[14] tentoxins, dolastatins, jaspamides, and didemnines.[7] It has a high potential for automation in the multiple, parallel peptide synthesis[15] which we are currently investigating. Experiments to improve the efficiency of the BTC method for the coupling of nonmethylated amino acids are also currently under way in our laboratory.

Experimental Section

Fmoc-*N*-methyl amino acids were prepared according to Freidinger et al.[16] HOAt and HATU were purchased from Applied Biosystems (Foster City, CA, USA). TFFH was purchased from Advanced ChemTech (Bamberg, Germany). TCP resin was obtained from PepChem (Tübingen, Germany). Preparative HPLC was performed on the "high-throughput purifier" (HTP) coupled to the M-8000 ES-MS (Merck-Hitachi, Darmstadt, Germany) using a C18-RP column (isocratic elution, 62% acetonitrile in H_2O, 0.1% TFA; TFA = trifluoroacetic acid) with MS and UV detection (diode array). ES-FTICR-MS measurements were performed on a Daltonic APEX II spectrometer (Bruker, Bremen, Germany).

The synthesis of **1** starting from Sar[6] was performed on 238 mg of TCP resin preloaded with Fmoc-sarcosine (0.58 mmol g^{-1}; 138 μmol; 1 equiv).

BTC coupling: Fmoc-peptidyl resin was deprotected with 20% piperidine/DMF (2 min + 8 min). After washing, the resin was treated with dry THF (1 mL) for 15 min. Meanwhile, the following Fmoc amino acid (483 μmol; 3.5 equiv) was added to a 68 mM solution of BTC in dry THF (2.4 mL; 1.15 equiv BTC). *Sym*-collidine (180 μL; 10 equiv) was added to the clear solution, upon which a precipitate of

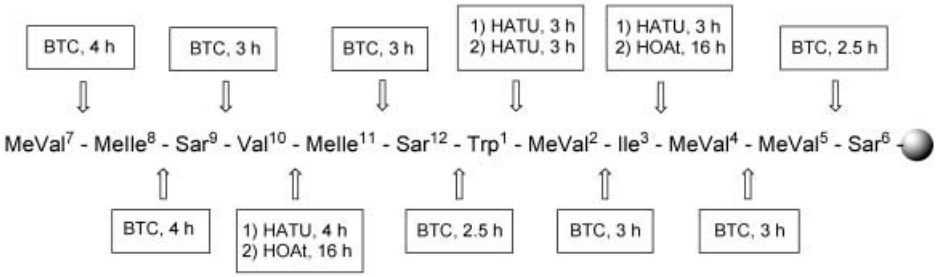


Figure 1. Synthesis outline for the linear dodecapeptide with Sar[6] [omphalotin A(7-6)]. After the times given, the respective couplings were quantitative (chloranil test, Kaiser test, and/or HPLC). Peak area of the Fmoc-deprotected dodecapeptide (crude product): 90% (HPLC, $\lambda = 214$ nm).

  1433-7851/02/4113-2308 $ 20.00+.50/0 *Angew. Chem. Int. Ed.* **2002**, *41*, No. 13

collidinium chloride was formed. DIEA (190 μL, 8 equiv) was added to the resin, immediately followed by addition of the suspension. The mixture was shaken for the reaction times given in Figure 1, filtered, and washed.

DIC/HOAt coupling: Fmoc amino acid (414 μmol; 3 equiv) and HOAt (57 mg; 3 equiv) were dissolved in a small volume of CH_2Cl_2/DMF (1:1). DIC (65 μL; 3 equiv) was added and the mixture was shaken. After 15 min, this solution was added to the Fmoc-deprotected peptidyl resin (swollen in DMF) and shaken for the reaction times given in Figure 1.

HATU coupling: Fmoc amino acid (552 μmol; 4 equiv) and HATU (210 mg; 4 equiv) were dissolved in a small volume of CH_2Cl_2/DMF (1:1). DIEA (190 μL; 8 equiv) was added and the mixture was shaken. After 15 min, this solution was added to the Fmoc-deprotected peptidyl resin (swollen in DMF) and shaken for the reaction times given in Figure 1.

Cleavage and deprotection: Following Fmoc deprotection, the dodecapeptidyl–TCP resin was washed and HFIP/CH_2Cl_2 1:5 (5 mL) was added. The suspension was shaken for 15 min, after which the filtrate was collected and evaporated to dryness under reduced pressure. The cleavage procedure was repeated twice. Yield: 155 mg linear dodecapeptide OmA(7-6) (116 μmol; 84 %), HPLC purity ($\lambda = 214$ nm): 90 %.

This peptide (155 mg; 116 μmol; 1 equiv) was dissolved in CH_2Cl_2 (400 mL). HOAt (32 mg; 2 equiv), 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide (EDCI; 45 mg; 2 equiv) and DIEA (160 μL; 8 equiv) were added successively. After stirring for 16 h at RT, 2/3 of the solvent was evaporated under reduced pressure. The organic phase was washed with saturated $NaHCO_3$, 8 % citric acid, and brine, dried over Na_2SO_4, and evaporated to dryness. The cyclopeptide **1** was purified by flash chromatography (silica gel, ethyl acetate/methanol 95:5). Yield: 57 mg **1** (43 μmol; 31 %).

Analytical data for **1**: ^{1}H and ^{13}C NMR data of the synthetic omphalotin A correspond to the literature data for the natural compound.[1b] HR-MS (ESFTICR-MS): calcd: m/z 659.94668, found: m/z 659.94805 ($[M+2H]^{2+}$) (Figure 2). For further analytical and experimental details, see Supporting Information.

Received: February 22, 2002 [Z18750]

[1] a) A. Mayer, H. Anke, O. Sterner, *Nat. Prod. Lett.* **1997**, *10*, 25–32; b) O. Sterner, W. Etzel, A. Mayer, H. Anke, *Nat. Prod. Lett.* **1997**, *10*, 33–38; c) A. Mayer, H. Anke, O. Sterner, M. Kilian, R. Hain, D. Berg, W. Etzel, H. Gau (Bayer AG), WO 97/20857, **1996**.

[2] A. Mayer, M. Kilian, B. Hoster, O. Sterner, H. Anke, *Pestic. Sci.* **1999**, *55*, 27–30.

[3] S. Y. Koo, R. M. Wenger, *Helv. Chim. Acta* **1997**, *80*, 695–705; b) P. Raman, S. S. Stokes, Y. M. Angell, G. R. Flentke, D. H. Rich, *J. Org. Chem.* **1998**, *63*, 5734–5735.

[4] J. R. McDermott, N. L. Benoiton, *Can. J. Chem.* **1973**, *51*, 2555–2561.

[5] a) J. Urban, T. Vaisar, R. Shen, M. S. Lee, *Int. J. Pept. Protein Res.* **1996**, *37*, 182–189; b) T. Vaisar, J. Urban, *J. Mass Spectrom.* **1998**, *33*, 505–524.

[6] a) P. Li, J. C. Xu, *Tetrahedron Lett.* **1999**, *40*, 8301–8304; b) P. Li, J. C. Xu, *J. Org. Chem.* **2000**, *65*, 2951–2958.

[7] J. M. Humphrey, A. R. Chamberlin, *Chem. Rev.* **1997**, *97*, 2243–2266.

[8] R. Bollhagen, M. Schmiedberger, K. Barlos, E. Grell, *J. Chem. Soc. Chem. Commun.* **1994**, *22*, 2559–2560.

[9] F. Falb, T. Yechezkel, Y. Salitra, C. Gilon, *J. Pept. Res.* **1999**, *53*, 507–517. The amino acid is activated with BTC and collidine in THF and reacted with peptidyl resin for 1 h at 50 °C.

[10] Falb et al.[9] prepared peptide amides on Rink amide resin which does not allow the formation of diketopiperazines.

[11] J. Rudolph, G. Jung, B. Thern (Bayer AG), Patent application pending.

[12] An increase in coupling efficiency by the combination of a weak base for carboxy activation and a stronger base for the coupling reaction has been described for the system DIC/HOAt:L. A. Carpino, A. El-Faham, *Tetrahedron* **1999**, *55*, 6813–6830.

[13] GC–MS analyses of the totally hydrolysed **1** and of intermediate products were performed on chiral phase (W. König, I. Benecke, N. Lucht, E. Schmidt, J. Schulze, S. Sievers, *J. Chromatogr.* **1983**, *279*, 555–562). Only negligible amounts of D-amino acids were found (see Supporting Information).

[14] B. Thern, J. Rudolph, G. Jung, *Tetrahedron Lett.* **2002**, *43*, in press.

[15] a) *Combinatorial Peptide and Nonpeptide Libraries* (Ed.: G. Jung), Wiley-VCH, Weinheim, **1996**; b) *Combinatorial Chemistry—Synthesis, Analysis and Screening* (Ed.: G. Jung), Wiley-VCH, Weinheim, **1999**.

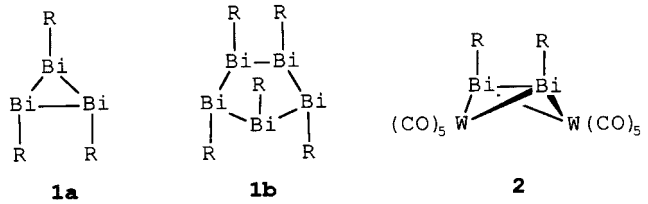
[16] R. M. Freidinger, J. S. Hinkle, D. S. Perlow, B. H. Arison, *J. Org. Chem.* **1983**, *48*, 77–81.

Synthesis of the Dibismuthene Complex $[\{\mu\text{-}\eta^2\text{-}(cis\text{-}Me_3SiCH_2Bi)_2\}\{W(CO)_5\}_2]$ from a Cyclobismuthane and $[W(CO)_5(thf)]$

Lucia Balázs, Hans Joachim Breunig,* and Enno Lork

Owing to relativistic effects it is expected that two valence electron pairs of bismuth should be inert.[1, 2] Consequently, Bi^I compounds should be relatively stable and their reactivity should differ considerably from that of corresponding compounds the lighter homologues. To date there is little evidence for these effects because true Bi^I compounds are rare.[2] The first organometallic examples are *trans*-dibismuthenes, RBi=BiR[3] and two cyclobismuthanes, $(RBi)_n$ ($n = 3,4$),[4a] which are protected by very bulky aryl groups or by the $(Me_3Si)_2CH$ group, respectively. Recently, the ring compounds $(RBi)_4$ ($R = (Me_3Si)_3Si$) and R_6Bi_8 ($R = (Me_3Si)_3Sn$) were described.[4b]

Searching for less hindered Bi^I compounds, for which the specific properties might emerge more clearly, we have studied the bismuth ring system trimethylsilylmethylbismuth(I) (**1**), whose main components are the new cyclobismuthane **1a**, a three-membered ring, and **1b**, the first bismuth five-membered ring. Three- and five-membered rings are well known in the chemistry of P, As, and Sb.[5]



R = Me_3SiCH_2

[*] Prof. Dr. H. J. Breunig, L. Balázs, Dr. E. Lork
Universität Bremen
Institut für Anorganische und Physikalische Chemie
28334 Bremen (Germany)
Fax: (+49) 421-218-4042
E-mail: breunig@chemie.uni-bremen.de