pound was purified by flash chromatography, and its structure was assigned by COSY and HMBC experiments. This compound could be quantitatively converted into the metal-free carbocycle **5e** by exposure to air and sunlight.

Most of the compounds **5** are unstable in solution leading in a few hours to complex and unidentifiable mixtures of products. However, compound **5e** gave a single diastereoisomer of polycyclic system **9**, in 90% yield, upon standing in $CHCl_3$ for ten days (Scheme 4). Longer reaction times led to the release of the trimethylsilyl (TMS) group, and thus to the formation of **10** (92% yield). These products are evidently derived from the six-electron electrocyclization of the starting trienyl system **5e** and the hydrolysis of the resulting enol ether. The single diastereoisomer of **9** can best be explained as arising from epimerization of the α-carbonyl stereocenter in **5e** and the stereoselective cyclization of one of the epimeric ketones.

In conclusion, we have reported the first Dötz-like reaction between conjugated dienyl carbene chromium complexes and terminal alkynes to afford eight-membered carbocycles. We have also studied the ring closure of one of the mentioned systems to yield new polycyclic compounds.

Experimental Section

A solution of dienyl carbene **4** (1 mmol) and of the alkyne (3 mmol) in THF (15 mL) was refluxed, under a nitrogen atmosphere, until analysis by thin-layer chromatography revealed total consumption of the starting complex. The reaction mixture was diluted with hexane (20 mL) and exposed to sunlight and air in order to oxidize the metallic species to the corresponding organic compounds. Filtration through a pad of Celite and flash chromatography provided carbocycles **5**.

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Combinatorial Solid-Phase Synthesis of Multivalent Cyclic Neoglycopeptides**

Valentin Wittmann* and Sonja Seeberger

Dedicated to Professor Horst Kessler

The molecular recognition of carbohydrates by carbohydrate-binding proteins (lectins) is the basis of numerous intercellular recognition processes.[1] High-affinity lectin ligands are of considerable medicinal interest in the diagnosis and manipulation of such processes.[2] Individual carbohydrate epitopes (normally mono- to pentasaccharides) are, however, mostly bound by lectins with only low affinity (dissociation constants in the milli- to micromolar range) and in part broad specificity.[3] Since many membrane-associated lectins have several binding sites or occur in oligomeric or clustered form,[4] the creation of multivalent carbohydrate derivatives is a promising approach to arrive at effective lectin ligands.[5, 6]

Several strategies have been described to achieve the formation of a sufficient number of individual interactions necessary for high avidity of a multivalent ligand. Glycopolymers,[7] for example, are able to cover large areas of cell surfaces and bridge several membrane-located lectins ("statistical" multivalency). Small oligovalent carbohydrate derivatives (miniclusters)[6] on the other hand bind preferentially to several binding sites of a single (oligomeric) lectin proximate

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Supporting information for this article is available on the WWW under http://www.wiley-vch.de/home/angewandte/ or from the author.

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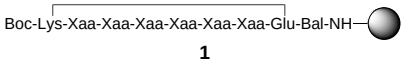
in space and may be tailored to lectins with known three-dimensional (3D) structure ("directed" multivalency).[8] With the carbohydrates connected by an inflexible scaffold, particularly affine ligands are obtained—provided that the sugars are oriented in a way required for multidentate binding.[5] Moreover, conformationally restricted miniclusters are in principle able to differentiate between various multivalent lectins with the same carbohydrate specificity but varying orientation of their binding sites. If the 3D structure of the targeted lectin is unknown and especially if conformationally restricted linkers are used, many potential ligands have to be synthesized and screened in order to "hit" the required orientation of the sugar residues.

We now report a synthetic concept to generate libraries of conformationally restricted miniclusters, comprising the following steps: a) "split–mix" synthesis[9] of a library of scaffold molecules with side chain amino groups in varying amounts and spatial orientation, b) attachment of several identical copies of a carbohydrate ligand to the amino groups.

As scaffolds for the multivalent presentation of carbohydrate ligands, we have chosen cyclic peptides of general type **1** (Bal = β-alanine) in which the residues Xaa are varied



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combinatorially.[10] Using commercially available D- and L-configurated amino acids, a high degree of conformationally diversity may be easily generated.[11] Diamino acids with Ddv-protected[12] side chains of varying length serve as sugar attachment points. To this end, a new urethane-type linker based on the Aloc protecting group[13] has been developed. In contrast to glycosylation reactions employing solid-phase-bound peptides,[14] the formation of an urethane bond proceeds in virtually quantitative yield. Furthermore, after the binding assay the carbohydrates may be cleaved off again under Pd catalysis regenerating the unmodified side chain cyclized peptides. Thus, the analysis of compounds bound to single beads which has to be carried out when using the split–mix method is confined to an automated microsequencing (Edman degradation) under standard conditions.

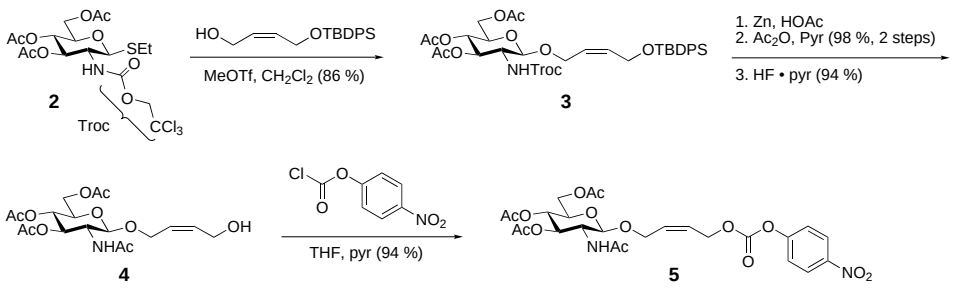
Scheme 1 illustrates the synthesis of carbohydrate-linker derivative **5** activated as *p*-nitrophenyl carbonate which was applied to attach *N*-acetylglucosamine residues to the cyclopeptides. Reaction of literature-known glycosyl donor **2**[15] with mono TBDPS-protected *cis*-but-2-ene-1,4-diol under methyl triflate activation gave β-configurated O-glycoside **3** in high yield. Substitution of the Troc protecting group with an acetyl group followed by removal of the TBDPS group with HF · pyridine complex led to *N*-acetylglucosamine derivative **4**. Condensation with 4-nitrophenyl chloroformate finally gave active carbonate **5** as a crystalline substance that could be stored at room temperature (Table 1).

Table 1. Selected physical data of compounds **5** and **12**.

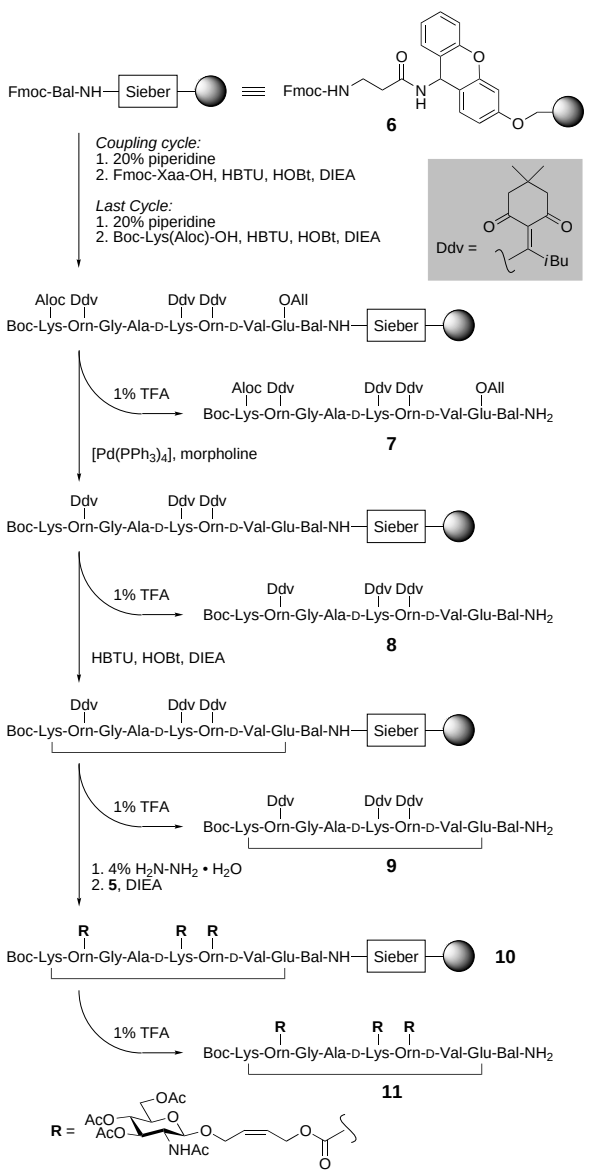
5: $R_f = 0.43$ (silica, EtOAc); m.p. 133 °C (EtOAc/*n*-hexane); ^{1}H NMR (400 MHz, $CDCl_3$, 300 K, TMS): $\delta = 8.29-8.25$ (m, 2H; arenes), 7.40–7.36 (m, 2H; arenes), 5.85–5.75 (m, 2H; vinyl-H), 5.64 (d, $J = 8.7$ Hz, 1H; NH), 5.28 (dd, $J = 9.3$, 10.6 Hz, 1H; H-3), 5.05 (dd, $J = 9.3$, 10.0 Hz, 1H; H-4), 4.90–4.79 (m, 2H; allyl-H), 4.75 (d, $J = 8.3$ Hz, 1H; H-1), 4.44–4.29 (m, 2H; allyl-H), 4.22 (dd, $J = 4.7$, 12.3 Hz, 1H; H-6a), 4.13 (dd, $J = 2.5$, 12.3 Hz, 1H; H-6b), 4.83 (ddd, $J = 8.3$, 8.7, 10.6 Hz, 1H; H-2), 3.69 (ddd, $J = 2.5$, 4.7, 10.0 Hz, 1H; H-5), 2.06 (s, 3H; $C(O)CH_3$), 2.01 (s, 3H; $C(O)CH_3$), 2.00 (s, 3H; $C(O)CH_3$), 1.93 (s, 3H; $C(O)CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$, 300 K, TMS): $\delta = 170.8$, 170.6, 170.2, 169.3, 155.4, 152.4, 145.4, 131.0 and 125.8 (olefins), 125.3 and 121.8 (arene CH), 99.5 (C-1), 72.2 (C-3), 71.8 (C-5), 68.6 (C-4), 64.6 and 64.2 (CH_2^{allyl}), 62.0 (C-6), 54.7 (C-2), 23.3 (CH_3), 20.67 (CH_3), 20.61 (CH_3), 20.55 (CH_3); elemental analysis calcd for $C_{25}H_{30}N_2O_{14}$ (%): C 51.55, H 5.19, N 4.81; found: C 51.52, H 5.28, N 4.79.

12: ^{1}H NMR (600 MHz, H_2O/D_2O 9:1, 298 K): Boc-Lys^1: $\delta = 6.767$ (αNH), 3.917 (αH), 1.565 (βH_2), 1.27–1.17 (γH_2), 1.379 (δH_2), 3.083 and 3.003 (εH_2), 7.688 (εNH), 1.304 (Boc); Orn: $\delta = 8.262$ (αNH), 4.254 (αH), 1.722 and 1.633 (βH_2), 1.47–1.38 (γH_2), 3.023 (δH_2), 6.791 (δNH); Gly^3: 8.201 (NH), 3.855 and 3.788 (αH_2); Ala^4: 7.999 (NH), 4.203 (αH), 1.277 (βH_3); D-Lys^5: 8.148 (αNH), 4.185 (αH), 1.690 and 1.615 (βH_2), 1.248 and 1.215 (γH_2), 1.382 (δH_2), 2.998 (εH_2), 6.733 (εNH); Orn: $\delta = 8.316$ (αNH), 4.254 (αH), 1.731 and 1.633 (βH_2), 1.478 and 1.420 (γH_2), 3.032 (δH_2), 6.767 (δNH); D-Val^7: $\delta = 8.136$ (NH), 3.982 (αH), 2.041 (βH), 0.820 and 0.786 (2 γH_3); Glu^8: $\delta = 8.201$ (NH), 4.107 (αH), 1.831 and 1.999 (βH_2), 2.201 and 2.136 (γH_2); Bal^9: $\delta = 8.049$ (NH), 2.41–2.33 (αH_2), 3.40–3.30 (βH_2); 3 equivalent GlcNAc: $\delta = 8.103$ (NH), 4.427 (H-1), 3.588 (H-2), 3.429 (H-3), 3.38–3.31 (H-4, H-5), 3.810 and 3.651 (2 H-6), 1.927 (Ac); 5.72–5.60 (6 vinyl-H), 4.52–4.48 and 4.30–4.18 (each 6 allyl-H); ESI-MS [$M+H^+$]: calcd. 1963.0, found 1963.7.

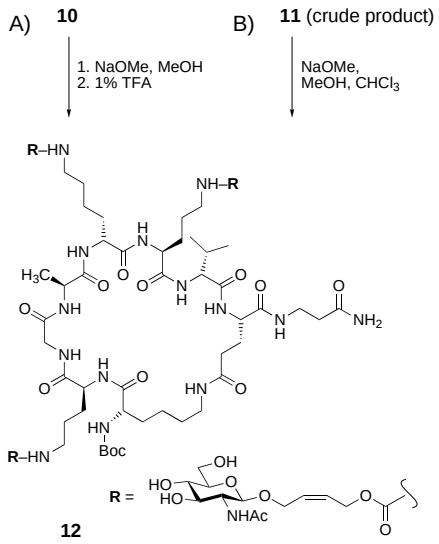
The assembly of the cyclic neoglycopeptides was optimized by using model compound **12** as an example (Schemes 2 and 3, Table 1). For attachment of the first amino acid to the solid support (TentaGel) the Sieber linker was applied.[16] Thus it was possible to release the product of each reaction step by treatment with a 1% solution of TFA in dichloromethane (→**7**–**9**, **11**, **12**) and monitor the course of the reaction by analytical HPLC (Figure 1) and subsequent ESI-MS. Synthesis of the linear peptide followed the Fmoc strategy;[17] solely in the last coupling step an N^{α}-Boc-protected amino acid was applied. For protection of the ω-NH_2 groups of lysine and ornithine we preferred the Ddv group[12] which distinguishes itself from the frequently used Dde group[18] by a higher stability under the conditions of Fmoc cleavage. Preparatory for the



Scheme 1. Synthesis of carbohydrate derivative **5** activated as its *p*-nitrophenyl carbonate.



Scheme 2. Solid-phase synthesis of cyclic neoglycopeptide **11**.



Scheme 3. Synthesis of deprotected neoglycopeptide **12**.

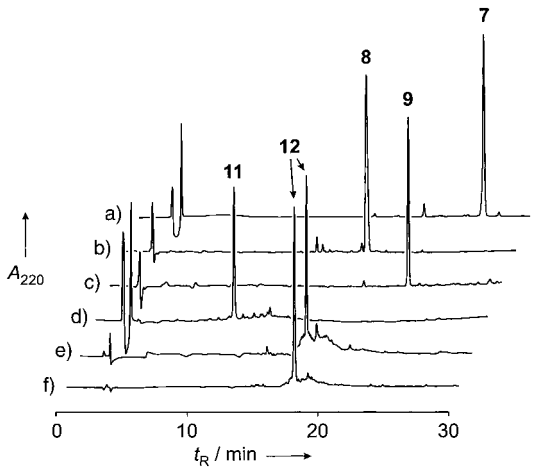


Figure 1. RP-HPLC analysis of the crude peptides **7**–**9**, **11**, and **12** obtained according to Scheme 2 (a–d), Scheme 3, route A (e), and Scheme 3, route B (f).

cyclization step the allyl ester and the Aloc group were simultaneously removed by palladium(0)-catalyzed allyl transfer to morpholine.[13]

Addition of a mixture of HBTU, HOBt, and Hünig's base (DIEA) led to the desired cyclopeptide which after cleavage from the resin was obtained in excellent purity (Figure 1c). Side products due to reaction of coupling reagent with the free amino group[19] (guanylation) was not observed. Other cyclization methods (*N*,*N*′-diisopropyl carbodiimide/HOBt or activation of the carboxyl group as a Pfp ester[20]) were clearly less effective. Subsequently, the Ddv groups were removed by batchwise treatment with a 4% solution of hydrazine hydrate which turned out to be advantageous over the literature-recommended[12] 2% solution.[21]

Attachment of the sugars was effected by addition of excess (5 equiv per free NH_2 group) of **5** in the presence of DIEA. After complete reaction, the absence of free amino groups was verified by Kaiser test[22] and by addition of bromophenol blue.[23] In order to deacetylate the sugars, **10** was treated with a solution of sodium methoxide in methanol (Scheme 3, route A). After cleavage from the resin, the HPL-chromatogram shown in Figure 1e was obtained in which beside the desired glycopeptide **12** noticeable amounts of side products are visible. Since their appearance is possibly due to the acid lability of **12**[24] and therefore the chromatogram might not reflect the realities on the solid support, we carried out the deacetylation after cleavage from the resin for comparison (Scheme 3, route B). The obtained chromatogram of the crude product (Figure 1f) now indicates only slight amounts of side products demonstrating the high efficiency of the whole synthesis.

In order to verify that the described reaction conditions, especially those of the critical cyclization step, are transferable to other peptide sequences, we synthesized the neoglycopeptide library **15** comprising 18 compounds by using the split–mix method (Scheme 4). Again, the course of the synthesis was monitored by withdrawal of small resin samples and analysis of the cleavage products by HPLC in combination with ESI-MS (Figure 2, Table 2). The expected

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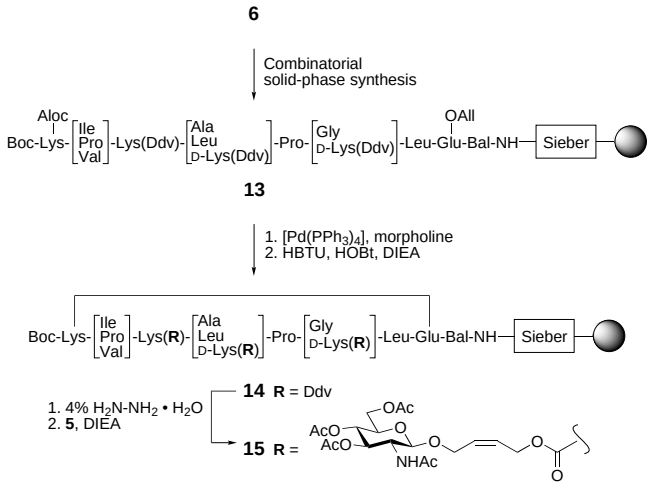


Scheme 4. Split–mix synthesis of neoglycopeptide library **15**. At the positions denoted by square brackets the resin was distributed at two and three reaction vessels, respectively, and each coupled with one of the given amino acids.

18 products were identified in all chromatograms. It turned out that all peptides underwent cyclization without forming noteworthy amounts of side products. Particularly, after mass spectrometric analysis of **14** we were not able to detect any starting materials or linear or cyclic peptide dimers.

The introduced synthetic strategy is suitable for efficient construction of large libraries of neoglycopeptides presenting any desired carbohydrate ligands in variable quantity and varying distances to each other. With the convergent approach it is easily possible to apply a once prepared cyclopeptide library in studying different lectins by simply attaching appropriate carbohydrate ligands to it.

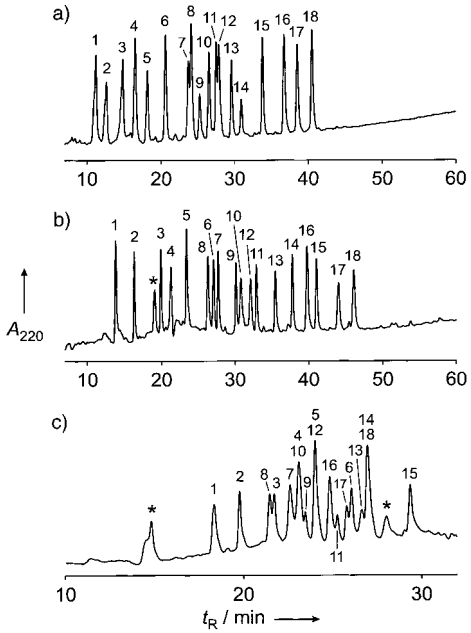


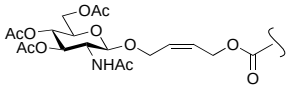
Figure 2. RP-HPLC analysis of the compound libraries obtained from treatment of a) **13**, b) **14**, and c) **15** with a 1% solution of TFA. The assignment of peaks resulted from mass spectrometric analysis and is given in Table 2. Peaks of corresponding peptide derivatives are marked with identical numbers. Those marked with asterisks stem from non-peptide impurities.

Experimental Section

The solid-phase peptide synthesis was carried out on NovaSyn TG Sieber resin (Novabiochem) (abbreviation: Sieber-TG) following standard protocols[17] (loading after immobilization of the first amino acid: 0.17 mmol g^{-1}). Couplings were performed in NMP. For cleavage solid-phase aliquots (ca. 5 mg) were repeatedly treated with TFA/*i*Pr_3SiH/CH_2Cl_2 (1:1:98). Products

Table 2. Calculated and experimentally found masses of the library of cyclic neoglycopeptides obtained by treatment of **15** with a 1% solution of TFA (peak numbers correspond to the assignment given in Figure 2c).

Peak	Compound[a]	$[M+H]^+$	
		calcd	found
1	*cyclo*[Boc-Lys-Pro-Lys(**R**)-Ala-Pro-Gly-Leu-Glu]-Bal-NH_2	1434.7	1435.4
2	*cyclo*[Boc-Lys-Val-Lys(**R**)-Ala-Pro-Gly-Leu-Glu]-Bal-NH_2	1436.7	1437.3
8	*cyclo*[Boc-Lys-Pro-Lys(**R**)-D-Lys(**R**)-Pro-Gly-Leu-Glu]-Bal-NH_2	1934.9	1935.7
3	*cyclo*[Boc-Lys-Ile-Lys(**R**)-Ala-Pro-Gly-Leu-Glu]-Bal-NH_2	1450.8	1451.2
7	*cyclo*[Boc-Lys-Pro-Lys(**R**)-Ala-Pro-D-Lys(**R**)-Leu-Glu]-Bal-NH_2	1949.0	1950.0
4	*cyclo*[Boc-Lys-Pro-Lys(**R**)-Leu-Pro-Gly-Leu-Glu]-Bal-NH_2	1476.8	1477.4
10	*cyclo*[Boc-Lys-Val-Lys(**R**)-D-Lys(**R**)-Pro-Gly-Leu-Glu]-Bal-NH_2	1937.0	1938.0
9	*cyclo*[Boc-Lys-Val-Lys(**R**)-Ala-Pro-D-Lys(**R**)-Leu-Glu]-Bal-NH_2 or *cyclo*[Boc-Lys-Ile-Lys(**R**)-D-Lys(**R**)-Pro-Gly-Leu-Glu]-Bal-NH_2	1951.0	1952.0
5	*cyclo*[Boc-Lys-Val-Lys(**R**)-Leu-Pro-Gly-Leu-Glu]-Bal-NH_2	1478.8	1479.4
12	*cyclo*[Boc-Lys-Ile-Lys(**R**)-D-Lys(**R**)-Pro-Gly-Leu-Glu]-Bal-NH_2 or *cyclo*[Boc-Lys-Val-Lys(**R**)-Ala-Pro-D-Lys(**R**)-Leu-Glu]-Bal-NH_2	1951.0	1952.0
16	*cyclo*[Boc-Lys-Pro-Lys(**R**)-D-Lys(**R**)-Pro-D-Lys(**R**)-Leu-Glu]-Bal-NH_2	2449.2	2450.1
11	*cyclo*[Boc-Lys-Ile-Lys(**R**)-Ala-Pro-D-Lys(**R**)-Leu-Glu]-Bal-NH_2	1965.0	1966.2
17	*cyclo*[Boc-Lys-Val-Lys(**R**)-D-Lys(**R**)-Pro-D-Lys(**R**)-Leu-Glu]-Bal-NH_2	2451.2	2452.8
6	*cyclo*[Boc-Lys-Ile-Lys(**R**)-Leu-Pro-Gly-Leu-Glu]-Bal-NH_2	1492.8	1493.3
13	*cyclo*[Boc-Lys-Pro-Lys(**R**)-Leu-Pro-D-Lys(**R**)-Leu-Glu]-Bal-NH_2	1991.0	1991.8
14	*cyclo*[Boc-Lys-Val-Lys(**R**)-Leu-Pro-D-Lys(**R**)-Leu-Glu]-Bal-NH_2	1993.0	1993.6
18	*cyclo*[Boc-Lys-Ile-Lys(**R**)-D-Lys(**R**)-Pro-D-Lys(**R**)-Leu-Glu]-Bal-NH_2	2465.2	2465.9
15	*cyclo*[Boc-Lys-Ile-Lys(**R**)-Leu-Pro-D-Lys(**R**)-Leu-Glu]-Bal-NH_2	2007.0	2008.0

[a] **R** = 

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that were hardly soluble in the cleavage cocktail were washed off the solid support with a suitable solvent. Before concentration cleavage solutions were neutralized with pyridine. Analytical HPLC was carried out on C18 reversed-phase columns (250×4 mm) with linear gradients of acetonitrile in water/0.1% TFA and a flow of $1\,mL\,min^{-1}$. Product peaks were characterized by ESI-MS. Experimental details for the synthesis of **8**, **9**, **11**, and **12** are found in the Supporting Information.

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Wound-Activated Chemical Defense in Unicellular Planktonic Algae**

Georg Pohnert*

Diatoms are highly successful unicellular algae occurring in ocean and fresh water phytoplankton, as well as in biofilms on solid substrates. They are exceedingly abundant and are among the most important primary sources sustaining the marine food chain. Despite this, little is known about the chemical defense of these micro algae. Two of the few reported examples are the aldehydes decadienal **5** and decatrienal **6** (see Scheme 1) from the diatom *Thalassiosira rotula*, which reduce the hatching success from eggs of copepods (zooplankton grazers).[1] This observed activity explains the paradox that herbivorous copepods are less successful feeding on diatoms, although these algae are considered as high-quality food.

Here I provide biosynthetic and kinetic data on the formation of fatty acid derived metabolites in planktonic diatoms, demonstrating that the release of $\alpha,\beta,\gamma,\delta$-unsaturated dienals is widespread among this class of algae. The enzymatic mechanism to produce these metabolites is efficiently activated seconds after cell disruption and leads to high local concentrations of the defensive metabolites **5** and **6** or of structurally related potentially active aldehydes like **9**.

The simultaneous production of C_{11} hydrocarbons and 9-oxonona-5*Z*,7*E*-dienoic acid from C_{20} fatty acids was demonstrated with the benthic diatom *Gomphonema parvulum*.[2, 3] The polar dienoic acid contains the same aldehydic

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