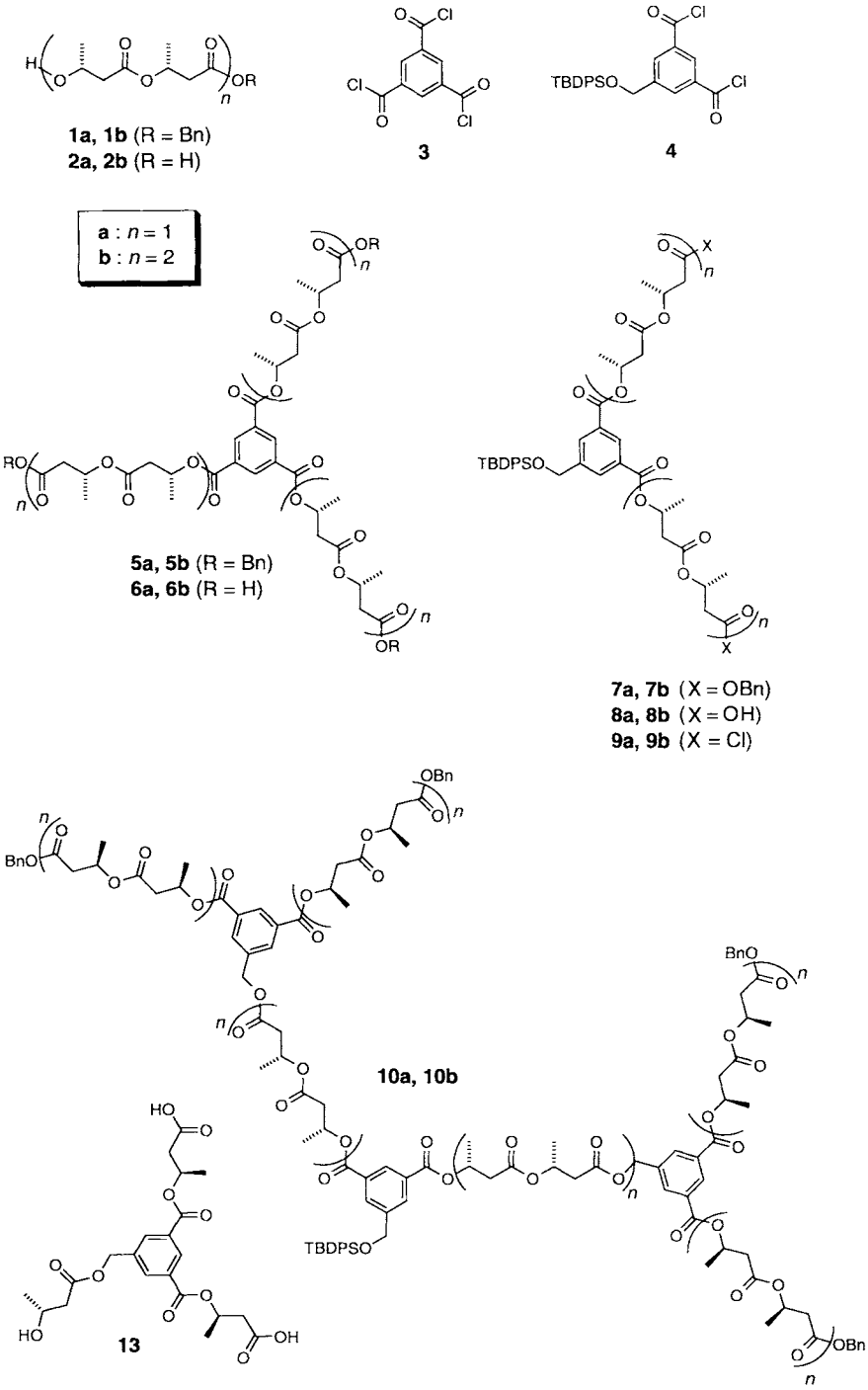
Synthesis and Enzymatic Degradation of Dendrimers from (*R*)-3-Hydroxybutanoic Acid and Trimesic Acid**

Dieter Seebach,* Guido F. Herrmann, Urs D. Lengweiler, Beat M. Bachmann, and Walter Amrein

Dendrimers are attracting growing interest for use in stereoselective synthesis,[1] as a new type of material,[2] and in biological studies, for example as DNA carriers.[3] We now report on the first synthesis of monodisperse, enzymatically degradable, polyanionic dendrimers from (*R*)-3-hydroxybutanoic acid (HB)[4] and trimesic acid.

The convergent synthesis starts with the previously described[4] benzyl esters **1a** and **1b** of the dimer and the tetramer of HB, respectively, which were used as elongation units. For the assembly of the dendritic core and the branching units we employed the commercially available trimesic acid trichloride **3** and the TBDPS-protected 5-hydroxymethyl-1,3-benzenedicarboxylic acid dichloride **4**,[5] which can be easily obtained from the corresponding trimethyl trimesate. Acylation of the HB-oligomers **1** with **3** gave the core structures **5**, and debenzylation yielded the tricarboxylic acids **6**. Branching unit **7** was obtained by acylation of **1** with **4**. The branches **10** for the dendrimers of the second generation were synthesized starting from the silylated ester **7**, followed by debenzylation (→ **8**), then activation (→ **9**),[5] and subsequent acylation of the desilylated derivative of **7** with the acid chloride **9**. The coupling of **6** with the desilylated branches **7** and **10** finally gave the dendrimers of the first (green circle in formula **11**/**12**) and of the second generation (**11**), respectively. The selective hydrogenolysis of the terminal benzyl groups of the dendrimers was achieved by catalytic transfer hydrogenation[6] and gave the polyanionic dendrimers of the first and second generation (**12a**). The dendritic compounds were obtained in 150 mg (**11b**) to 450 mg (**11a**) quantities as viscous oils that were soluble in CH_2Cl_2. All compounds were fully characterized (optical rotation, IR, ^{1}H and ^{13}C NMR spectroscopy, MS, elemental analysis; see experimental part a); Figure 1 shows five MALDI-MS molecular peaks and proves the identity and the monodispersity of the corresponding compounds.



To study the biodegradability[7] of the dendrimers, we tested their stability in the presence of various hydrolases. Figure 2 depicts the degradation of three dendritic compounds **6a**, **6b**, and **11b** with a PHB-depolymerase.[8] The linear tetrameric HB **2b** was used as the standard substrate for the depolymerase. The protected dendrimers with dimeric HB-elongation units were not degraded by the depolymerase, whereas the free acids were moderately good substrates for this enzyme.[9] This observation is surprising as the simple dimeric HB **2a** is not a substrate for the depolymerase. ^{1}H NMR spectroscopic investigations of the degradation products of the deprotected, first-generation dendrimer with dimeric HB-elongation units (green circle in formula **11**/**12**) revealed free HB, the triester of 1,3,5-benzenetricarboxylic acid with HB, and also compound **13**.[10] The enzymatic hydrolysis of the deprotected, first-generation dendrimer, which has dimeric HB-elongation units, was followed by ti-

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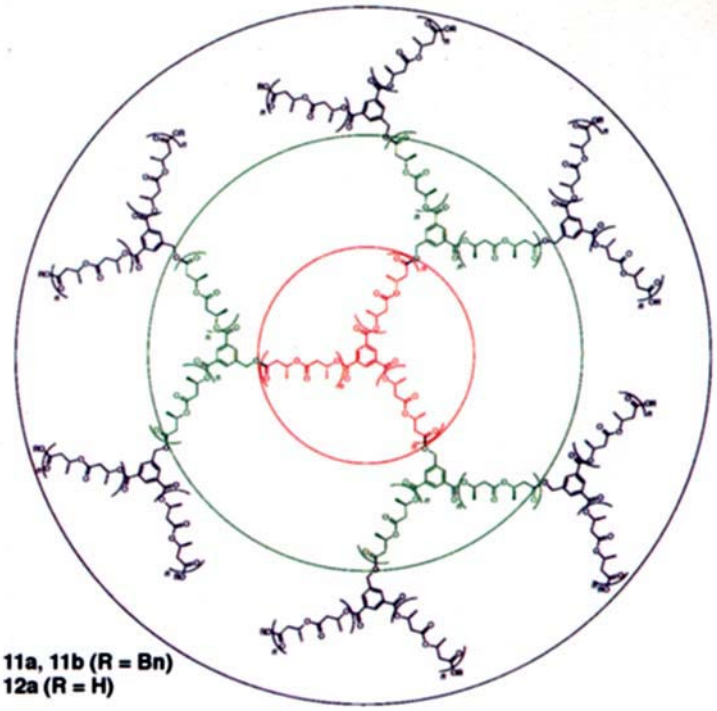

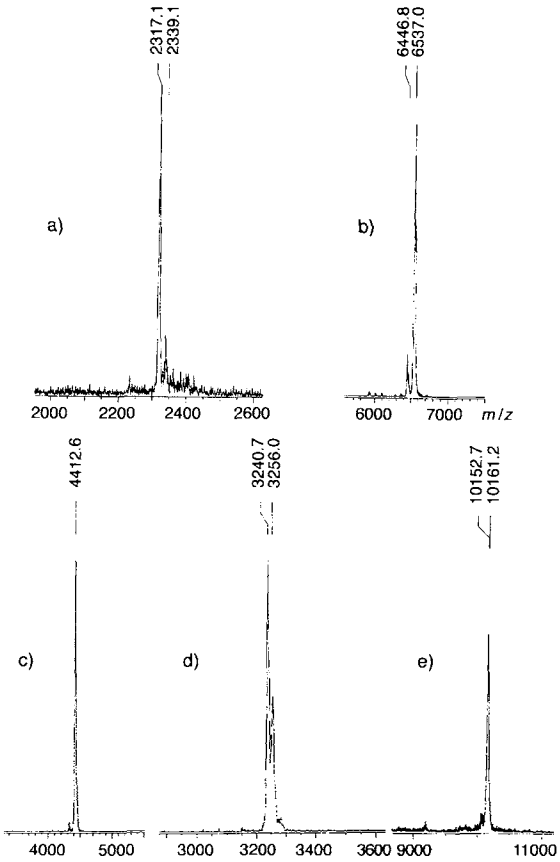

Fig. 1. Characterization of the dendrimers and the building blocks by mass spectrometry (MALDI-MS): a) first generation with dimeric HB-elongation units and free acid groups ($C_{108}H_{132}O_{54}$, 2294.2 g mol^{-1}, 2317 $[M+Na]^+$); b) second generation with dimeric HB-elongation units and R = Bn (**11a**: $C_{342}H_{384}O_{126}$, 6510.7 g mol^{-1}, 6537 $[M+Na]^+$); c) first generation with tetrameric HB-elongation units and protected acid groups ($C_{222}H_{282}O_{90}$, 4390.6 g mol^{-1}, 4412 $[M+Na]^+$); d) protected branch of the second generation with tetrameric HB-elongation units (**10b**: $C_{167}H_{206}O_{61}$, 3217.5 g mol^{-1}, 3240 $[M+Na]^+$, 3256 $[M+K]^+$); e) second generation with tetrameric HB-elongation units and R = Bn (**11b**: $C_{510}H_{640}O_{210}$, 10130.5 g mol^{-1}, 10153 $[M+Na]^+$).

tristatic experiments over 24 hours. More than five equivalents of base were consumed, which corresponds to a conversion of more than 55%. Like **2b**, all dendrimers with tetrameric HB-elongation units are good substrates for the depolymerase (Fig. 2). Zero-order kinetics is observed for the first de-

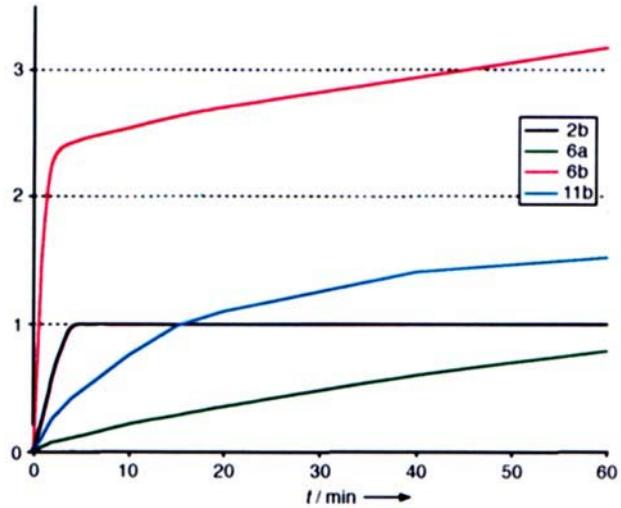

Fig. 2. Enzymatic degradation of the tetrameric HB **2b**, the core unit **6**, and the second-generation dendrimer **11b** with the PHB-depolymerase (0.25 mg mL^{-1} substrate in H_2O, 4.25 U mL^{-1} depolymerase from *Alcaligenes faecalis* [8], pH = 7.5, 30 °C). x = "acid equivalents" formed, in arbitrary units.

gradation step. The reaction is about one hundred times faster than the degradation of dendrimers with dimeric HB-elongation units. In the second degradation step (also zero order), the kinetics is comparable with that of dendrimers with dimeric HB-elongation units. Furthermore, the dendritic compounds were degraded by an esterase, a lipase, and a protease.[11]

In conclusion, we have described the first examples of biodegradable[7] dendrimers, which may have applications in materials science (for example, for the construction of defined nanocavities[2h]) that are similar to those for chemically degradable dendritic and hyperbranched organosilicon compounds.[12] Furthermore, they could possibly be used as drug carriers.[3]

Experimental Procedure

a) Analytical data of two selected compounds [13]:

11a (6510.7 g mol^{-1}): 48% yield (450 mg of material purified on 75 g silica gel, eluent CH_2Cl_2/Et_2O 4/1) for the coupling of **6a** and 3 equiv. desilylated **10a**). $[\alpha]_D = -32.9$ (c = 1.275, CH_2Cl_2); IR ($CHCl_3$, cm^{-1}): $\tilde{\nu}$ = 3035 w, 2986 w, 1735 vs, 1607 w, 1498 w, 1455 m, 1383 m, 1303 s, 1248 s, 1135 s, 1101 m, 1057 s, 973 w; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.75 (s, 3H, arene H), 8.53 (t, J = 1.57 Hz, 9H, arene H), 8.15 (d, J = 1.58 Hz, 6H, arene H), 8.14 (d, J = 1.60 Hz, 12H, arene H), 7.34–7.27 (m, 60H, arene H), 5.55–5.44 (m, 21H, HC), 5.34–5.26 (m, 21H, HC), 5.18–5.10 (m, 18H, H_2C), 5.09–5.05 (m, 24H, H_2C), 2.88–2.50 (m, 84H, H_2C), 1.44–1.38 (m, 63H, H_3C), 1.25–1.21 (m, 63H, H_3C); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 169.83, 169.69, 169.09, 169.00, 164.44, 136.75, 135.67, 133.21, 131.21, 130.35, 128.54, 128.30, 128.28, 68.89, 68.63, 68.57, 67.69, 67.56, 67.52, 66.41, 65.22, 40.98, 40.91, 40.62, 40.36, 19.83, 19.78; MALDI-MS (see Fig. 1b): 6537.0 $[M+3H+Na]^+$; Analysis calcd for $C_{342}H_{384}O_{126}$: C 63.06, H 5.99; found: C 62.99, H 6.20.

10b (3217.48 g mol^{-1}): 69% yield (900 mg of material purified on 80 g silica gel, eluent CH_2Cl_2/Et_2O 8/1) for the coupling of **9b** and 2 equiv. desilylated **7b**). $[\alpha]_D = -16.03$ (c = 1.135, CH_2Cl_2); IR ($CHCl_3$, cm^{-1}): $\tilde{\nu}$ = 3032 m, 2986 m, 2936 m, 2878 w, 1736 vs, 1607 w, 1497 w, 1456 s, 1383 s, 1302 s, 1177 m, 1134 s, 1101 m, 1056 s, 976 m; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.54 (t, J = 1.63 Hz, 2H, arene H), 8.48 (s, 1H, arene H), 8.17 (s, 2H, arene H), 8.15 (d, J = 1.59 Hz, 4H,

arene H), 7.67–7.65 (d, $J = 7.02$ Hz, 4H, arene H), 7.42–7.30 (m, 26H, arene H), 5.54–5.49 (m, 6H, HC), 5.30–5.18 (m, 18H, HC), 5.17 (d, $J = 3.13$ Hz, 4H, H_2C), 5.10 (s, 8H, H_2C), 4.82 (s, 2H, H_2C), 2.81–2.35 (m, 48H, H_2C), 1.42 (d, $J = 6.31$ Hz, 9H, H_3C), 1.41 (d, $J = 6.26$ Hz, 9H, H_3C), 1.28–1.20 (m, 54H, H_3C), 1.11 (s, 9H, $(H_3C)_3C$); ^{13}C NMR (100 MHz, $CDCl_3$): 169.88, 169.71, 169.14, 169.11, 169.07, 164.46, 136.76, 135.69, 135.51, 133.24, 131.22, 130.39, 129.86, 128.57, 128.31, 127.80, 68.61, 68.29, 67.66, 67.63, 67.56, 67.49, 66.44, 65.23, 40.96, 40.75, 40.64, 40.39, 26.81, 19.89, 19.78, 19.75, 19.69; MALDI-MS (see Fig. 1d): 3256.0 $[M + K]^+$; 3240.7 $[M + Na]^+$; Analysis calcd for $C_{167}H_{206}O_{61}Si$: C 61.73, H 6.39; found: C 61.80, H 6.39.

b) Determination of the biodegradability: The substrate (1.0 mg) was dissolved or suspended in 4 mL H_2O. The pH was adjusted to 7.5 after which the hydrolase was added. The pH was kept constant throughout the addition of 5 mM NaOH at 30 °C, and the consumption of base was recorded (1 U = 1 μmol acid formed per min^{-1}). Enzymes used for the experiments: 35 mg of a PHB-depolymerase [8] (from *Alcaligenes faecalis*, 486 U mg^{-1} [14]), 1 mg of an esterase (from pig liver, 220 U mg^{-1} [14]), 1 mg of a lipase (from *Pseudomonas fluorescens*, 42 U mg^{-1} [14]) and 1.25 mg of a protease (trypsin, from bovine pancreas).

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[5] Compound **4** was synthesized by reduction of trimethyl 1,3,5-benzenetricarboxylate with lithium aluminum hydride, chromatographic separation of the singly and doubly reduced compounds, followed by silylation of the hydroxyl group and saponification of the ester group, and subsequent conversion to the acid chloride with $(COCl)_2$ (this reagent was also used for the synthesis of **9** and for the activiation of the tricarboxylic acid **6** for the coupling with desilylated **7**/**10**).

[6] The hydrogenolysis was carried out in DMF with Pd/C as catalyst and 1,4-cyclohexadiene as hydrogen source; a) A. M. Felix, E. P. Heimer, T.-J. Lambros, C. Tzougraki, J. Meienhofer, *J. Org. Chem.* **1978**, *43*, 4194; b) J. S. Bajwa, *Tetrahedron Lett.* **1992**, *33*, 2299; c) G. M. Anantharamaiah, K. Sivanandaiah, *J. Chem. Soc. Perkin Trans. 1* **1977**, 490.

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[8] Isolation and purification of PHB-depolymerase from *A. faecalis*, of which the gene was overproduced in *E. coli*, was carried out as described in T. Saito, K. Suzuki, J. Yamamoto, T. Fukui, K. Miwa, K. Tomita, S. Nakanishi, S. Odani, J.-I. Suzuki, K. Ishikawa, *J. Bacteriol.* **1989**, *171*, 184.

[9] v_{max}^{app} [U mg^{-1}]: **2a**: 0; **2b**: 24.5; **5a**: 0; **5b**: 1.9/0.32; **6a**: 0.5; **6b**: 38.6/0.24; protected, first-generation dendrimer with dimeric HB-elongation units: 0; deprotected, first-generation dendrimer with dimeric HB-elongation units: 0.32; protected, first-generation dendrimer with tetrameric HB-elongation units: 0.84/ < 0.1; deprotected, first-generation dendrimer with tetrameric HB-elongation units (two-stage kinetics): 25.7/0.18; **11a**: 0; **11b**: < 0.1; **12a**: 0.24 (all values are given for the PHB-depolymerase).

[10] The signals of 9 of a total of 18 HB-methine protons in the intact, deprotected, first-generation dendrimer with dimeric HB-elongation units (green circle in formula **11**/**12**; see Fig. 1a; ^1H NMR (500 MHz, $CDCl_3$): 5.19–5.38 (m, 9H, HC)) shifted in a characteristic manner after enzymatic degradation for 72 h (^1H NMR (300 MHz, CD_3OD): 4.23–4.18 (m, 3H, HC); 4.08–4.02 (m, 6H, HC)). The integral of the last signal and its chemical shift correspond to the expected 6 equivalents of free HB.

[11] Deprotected, first-generation dendrimer with dimeric HB-elongation unit: v_{max}^{app} [U mg^{-1}]: 0.3 (PHB-Depolymerase), 10^{-3} (esterase), 10^{-3} (lipase), 0.4 (trypsin); for further details about the enzymes employed, see [8] and experimental part (b).

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[13] The optical rotations of the dendrimers do not exhibit any significant dependence upon their generation or the number of HB-molecules in the elongation units, see experimental part (a).

[14] The activities [U] refer to the standard substrate of the corresponding enzyme.

Synthesis and a Preliminary DNA Binding Study of Hybrids of the Carbohydrate Domain of Calicheamicin γ_1^I and the Aglycone of Daunorubicin: Calichearubicins A and B**

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Many important medicinal agents of natural origin contain a sugar or sugarlike moiety. From a chemical standpoint, such agents can be dissected into aglycone and carbohydrate domains. In most instances, both components in themselves are totally lacking in useful biological activity.[1] Traditionally, considerations about the mechanism of action of the drug have been directed to the aglycone, with the carbohydrate viewed as a necessary appendage for biotransport or for other factors related to drug availability.

While the nature of interplay of the aglycone and carbohydrate components in various drugs presumably varies from case to case, it is now apparent that in some important instances the carbohydrate domain may play a critical role in drug receptor recognition. One example of such a critical role for the carbohydrate sector in receptor contact is that of the enediyne antibiotics[2] such as calicheamicin γ_1^I (**1**)[3] (Fig. 1). Work from our laboratories,[4] as well as from Nicolaou et al.[5] and others[6–8] has established that contacts between the pentacyclic carbohydrate region and the minor groove of DNA provide much of the remarkable sequence selectivity of calicheamicin γ_1^I. Similarly, such interactions also foster the molecular contacts between esperimicin A_1 and its target DNA.[9] Though the aglycones such as calicheamicinone (**2**) retain cytotoxicity and some double-strand cleaving ability, sequence selectivity requires the existence of the carbohydrate domain.[4c] Indeed, moderate sequence selectivity can be realized through co-incubation of non-

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