cluster evidently acts as a fluorescence quencher, presumably by accepting the excited electrons.

In conclusion, the reported Pd-catalyzed C–C coupling of iodo-functionalized hexamolybdate building blocks and alkynes thus offers a route to hybrid materials in which POM clusters are covalently bonded with organic conjugated segments.

Experimental Section

A mixture containing $[Bu_4N]_2[Mo_6O_{18}{\equiv}NC_6H_2(CH_3)_2I]$ ($[Bu_4N]_2$-**1**), (2.00 g, 1.26 mmol), 1-ethynyl-3,5-di(*tert*-butyl)benzene or 1-ethynyl-4-methylbenzene (1.90 mmol, 1.5 equiv), $[Pd(PPh_3)_2Cl_2]$ (0.026 g, 0.03 equiv), CuI (0.014 g, 0.06 equiv), and triethylamine (1 g) was stirred in acetonitrile (40 mL) at room temperature for 20 min, which led to a shiny red solution. Dichloromethane (100 mL) was then added. The resulting solution was washed twice with H_2O and brine water, and then concentrated to about 10 mL. After the addition of hexane (200 mL) to the above concentrated solution, it was left standing for 2 h. The top yellowish clear solution was discarded. The oily dark red residue was dried under vacuum to yield the products as dark red solids ($[Bu_4N]_2$-**2a**, 85% yield; $[Bu_4N]_2$-**2b**, 72% yield).

$[Bu_4N]_2$-**2a**: Elemental analysis (%) for $C_{56}H_{101}N_3O_{18}Mo_6$: calcd: C 40.04, H 6.06, N 2.51; found: C 40.99, H 6.07, N 2.55; ^1H NMR (250 MHz, $[D_6]$acetone, 25 °C, TMS): $\delta = 0.97$ (t, $J = 7.25$ Hz, 24H; CH_3), 1.34 (s, 18H; $C(CH_3)_3$), 1.47 (sextet, $J = 7.30$ Hz, 16H; CH_2), 1.83 (quintet, $J = 7.94$ Hz, 16H; CH_2), 2.64 (s, 6H; ArH), 3.45 (t, $J = 8.50$ Hz, 16H; NCH_2), 7.25 (s, 2H; ArH), 7.39 (s, 2H; ArH), 7.52 (s, 2H; ArH).

$[Bu_4N]_2$-**2b**: Elemental analysis (%) for $C_{49}H_{87}N_3O_{18}Mo_6$: calcd: C 37.21, H 5.54, N 2.66; found: C 36.83, H 5.32, N 2.61; ^1H NMR (250 MHz, $[D_6]$acetone, 25 °C, TMS): $\delta = 0.99$ (t, $J = 7.38$ Hz, 24H; CH_3), 1.46 (sextet, $J = 7.3$ Hz, 16H; CH_2), 1.83 (quintet, $J = 7.94$ Hz, 16H; CH_2), 2.37 (s, 3H; ArH), 2.64 (s, 6H; ArH), 3.47 (t, $J = 8.5$ Hz, 16H; NCH_2), 7.23 (s, 2H; ArH), 7.25 (d, $J = 8.5$ Hz, 2H; ArH), 7.42 (d, $J = 7.25$ Hz, 2H; ArH).

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Chemoenzymatic-Chemical Synthesis of a (2-3)-Sialyl T Threonine Building Block and Its Application to the Synthesis of the N-Terminal Sequence of Leukemia-Associated Leukosialin (CD 43)[**]

Nicole Bézay, Gregor Dudziak, Andreas Liese, and Horst Kunz[*]

*Dedicated to Professor Dieter Hoppe
on the occasion of his 60th birthday*

Enzymatic reactions are valuable tools in syntheses of polyfunctional natural products and drugs. This holds true, in particular, for syntheses of oligosaccharides and their glycoconjugates, since numerous protecting-group manipulations can be avoided and glycosidic linkages can be formed regio- and stereoselectively by using glycosyltransferases[1] and glyocosidases.[2] Successful enzymatic chain extensions of saccharides have been reported, in particular, for the formation of oligosaccharides and glycopeptides that contain sialic acid, in the course of which the enzymatic reactions constitute the final steps of the whole synthesis.[3] If, however, enzymatically prepared sialyl glycoconjugates are to be used in continuing chemical syntheses (e.g. of glycopeptides), their numerous functional groups must be blocked by selectively removable protecting groups.

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[*] Prof. Dr. H. Kunz, Dr. N. Bézay
Institut für Organische Chemie
Universität Mainz
Duesbergweg 10–14, 55128 Mainz (Germany)
Fax: (+49) 6131-3924786
E-mail: hokunz@mail.uni-mainz.de

Dr. G. Dudziak, Dr. A. Liese
Forschungszentrum Jülich GmbH
Institut für Biotechnologie, 52425 Jülich (Germany)

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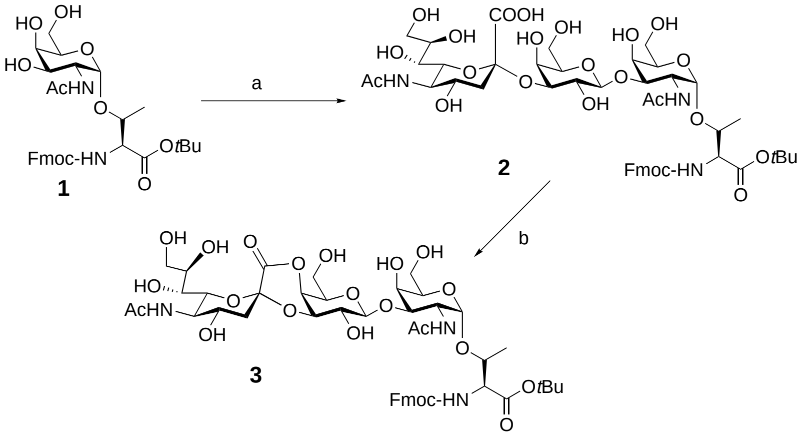
We describe herein a solution to this problem in the synthesis of the N-terminal glycopeptide sequence of leukosialin (CD 43) with a (2-3)-sialyl T antigen saccharide side chain. Leukosialin[4] with the tumor-associated sialyl T antigen (Neu5A(α2 $\rightarrow$3)Gal-(β1 $\rightarrow$3)GalNAc(α1 $\rightarrow$ *O*)-Ser/Thr) is found on leukocytes of patients suffering from acute myeloid leukemia,[5] and with other aberrant carbohydrates on colon carcinoma cells.[6] In addition, the (2-3)-sialyl T antigen has been detected on breast cancer cells.[7] Therefore, glycopeptides with the (2-3)-sialyl T structure as well as the regioisomeric (2-6)-sialyl T antigen analogues[8] and the sialyl T_N antigen glycopeptides[9] are considered candidates for the development of antitumor vaccines.

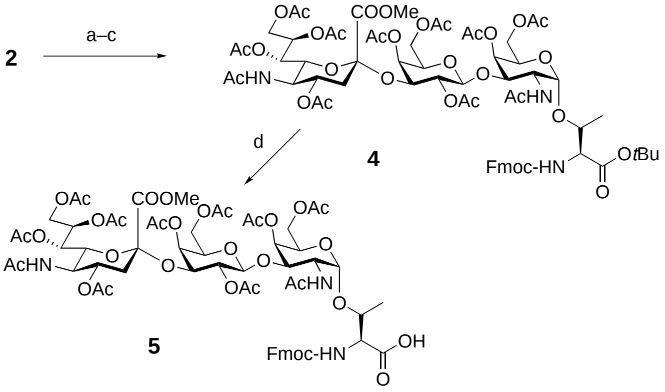
The chemical synthesis of sialyl T antigen threonine conjugates[10] requires numerous protecting-group manipulations and three stereoselective glycosylation reactions of which the sialylation is particularly demanding. The chemoenzymatic synthesis of sialyl T threonine esters can start with *N*-Fmoc-(*O*-*N*-acetylgalactosaminyl)threonine *tert*-butyl ester (**1**), which is readily available in preparative amounts.[11] The sialyl T threonine derivative **2** was obtained in a one-pot reaction cascade consisting of a regio- and stereoselective galactose transfer from lactose, catalyzed by a galactosidase from bovine testes, and a subsequent enzymatic sialylation[12] (Scheme 1). At first, the low solubility of **1** in water appeared to be an insuperable problem, but was overcome by the insertion of the hydrophobic Fmoc moiety into a β-cyclodextrin dimethyl ether (twofold excess relative to **1**), and **2** was obtained in an overall yield of 50 % (100 mg). Because of the reduced stability of the sialyltransferase in weak acids, the reactions were carried out at pH 6.5. To prevent inhibition by the cytidine phosphate, alkaline phosphatase was added.

Prior to the use of **2** as a building block in glycopeptide synthesis, the numerous functional groups in its carbohydrate portion must be blocked by protecting groups. The protection of the sialic acid carboxy group is of particular importance. This protection must be of orthogonal stability to the protecting groups within the peptide portion. Treatment of **2** with acidic ion-exchange resin in dry methanol did not give the methyl ester, but the 4-lactone **3** and, presumably, the corresponding 2-lactone (MALDI-TOF-MS in α-cyanocinnamic acid (cca) matrix *m*/*z* 1076.8, $[M+\mathrm{Na}]^+$, calcd 1077.1).



Scheme 1. a) Lactose, β-galactosidase (bovine testes) and CMP-Neu5Ac, sialyltransferase and calf intestine alkaline phosphatase in the presence of bovine serum albumin, 2,6-di-*O*-methyl-β-cyclodextrin in water, pH 6.5, yield 50 %;[12] b) Dowex 50 W (H^+-form) in methanol. Fmoc = 9-fluorenylmethoxcarbonyl.

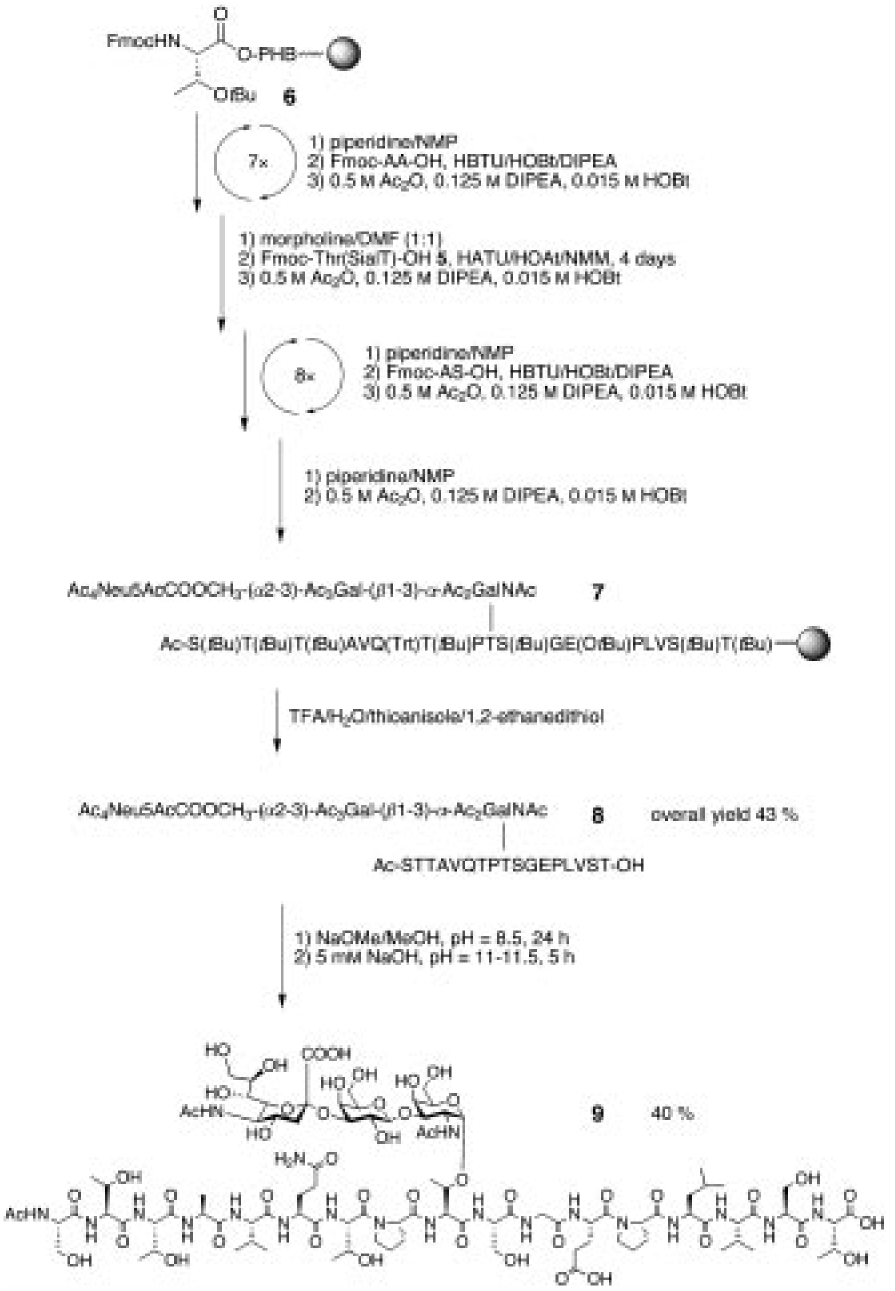
In contrast to the lactones of the chemically synthesized, fully protected sialyl T threonine derivatives[10, 13, 14] and to the 4-lactones of sialyl Lewis^X glycopeptides,[15] the lactone **3** and its isomer are unstable during subsequent reactions. Therefore, an alternative protection of the carbohydrate functional groups of **2** was developed. Treatment of **2** with acetic anhydride/pyridine (1:3) for 20 h at room temperature and subsequent codistillation with toluene (three times) resulted in complete O-acetylation and formation of a mixed anhydride. The latter was solvolyzed by the addition Hünig's base in methanol (*N*-ethyldiisopropylamine, DIPEA, 0.1 %) to yield the methyl ester of the *N*-acetylneuraminic acid. The accompanying de-O-acetylation was corrected by a second treatment with acetic anhydride/pyridine. The fully protected sialyl T threonine building block **4** was isolated in 61 % yield (Scheme 2).



Scheme 2. a) Ac_2O/pyridine; b) MeOH, $i\mathrm{Pr}_2\mathrm{EtN}$; c) Ac_2O/pyridine, 61 % over three steps, d) trifluoroacetic acid (TFA), 60 min, 84 %.

Acidolysis of the *tert*-butyl ester gave the Fmoc-protected (2-3)-sialyl T threonine building block **5**, which was applied to the solid-phase synthesis of the N-terminal glycoheptadecapeptide of leukosialin with a sialyl T antigen side chain.

TentaGel S resin[16] equipped with the acid-labile Wang anchor[17] was loaded with Fmoc threonine-*O*-*tert*-butyl ether to give **6**. The first seven amino acids were coupled according to Fmoc strategy by using a peptide synthesizer (Scheme 3)[18]. For removal of the Fmoc group, the resin was treated three times for 2.5 min with piperidine in *N*-methylpyrrolidone (NMP; 20 %). The coupling reactions were carried out by using a fivefold excess of each Fmoc amino acid, *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU),[19] 1-hydroxybenzotriazole (HOBt), and Hünig's base. Unreacted amino groups were acetylated by using Ac_2O/$i\mathrm{Pr}_2\mathrm{EtN}$/HOBt (4:1:0.12) in NMP. The resin-linked octapeptide was transferred from the



Scheme 3. Solid-phase synthesis on TentaGel S resin[16] equipped with a Wang linker.[17] AA = amino acid, PHB = *para*-hydroxybenzyl, Trt = trityl = triphenylmethyl.

synthesizer into a reaction vessel, and the Fmoc group was cleaved by using morpholine/dimethylformamide (DMF) over 1.5 h. For the coupling of the valuable sialyl T threonine building block **5** (1.1 equiv), 1.2 equivalents each of *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N'*,*N'*-tetramethyluronium hexafluorophosphate (HATU)[20] and 1-hydroxy-7-azabenzotriaazole (HOAt), and 2.4 equivalents of *N*-methylmorpholine (NMM) were applied, and the mixture was vigorously shaken with the resin-linked peptide for four days. The formed polymer-linked glycopeptide was then transferred into the peptide synthesizer. After acetylation with acetic anhydride, the additional eight Fmoc amino acids (40-fold excess) were coupled by activation with HBTU/HOBt/DIPEA. Finally, the Fmoc group was removed by using piperidine/NMP (see above), and the terminal amino group was acetylated.

The completely protected resin-linked sialyl T glycoheptadecapeptide **7** was detached from the polymeric support by using trifluoroacetic acid (TFA)/water/thioanisole/1,2-ethanedithiol (87.5:5:5:2.5). Under these conditions, all *tert*-butyl protecting groups were simultaneously removed from the amino acid residues. The sialyl T glycoheptadecapeptide **8** was isolated after purification by preparative HPLC in an overall yield of 43 % over 35 steps from **6** (27 mg, using 35 mg of **5**; Scheme 3).[21]

The *O*-acetyl groups were removed from the carbohydrate portion of **8** by using catalytic sodium methanolate in methanol (pH 8.2–8.5), and the methyl ester of the *N*-acetylneuraminic acid was hydrolyzed with aqueous NaOH (5 mM, pH 11–11.5).[11] This deprotection must be monitored by analytical HPLC. In addition to the target compound **9**, a second component was isolated, which according to ^{1}H NMR spectroscopic and mass spectrometric analysis still contains an *O*-acetyl group.

The pure glycoheptadecapeptide **9**,[22] which represents the N-terminus of leukosialin (CD 43) with (2-3)-sialyl T antigen structure typical for acute myeloid leukemia, was isolated by preparative HPLC in 40 % yield (10 mg). This synthetic antigen as well as analogous glycopeptides with shorter T_N and T antigen saccharides and the nonglycosylated heptadecapeptide are presently under investigation for immunizations and induction of antibodies.[9]

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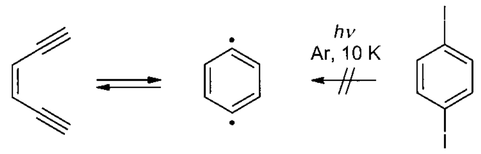
[21] **8**: $[\alpha]^{23}_{\mathrm{D}} = -52.9$ ($c = 0.89$, H_2O); $R_t = 34.6$ min (0.1 % TFA in CH_3CN/H_2O, gradient 1:99 (0–5 min), then 50:50 (45 min), and finally 100:0 (50–55 min)); MALDI-TOF-MS (cca matrix, positive-ion mode): m/z (%): 2806.1 (29) [$M+K^+$], 2790.2 (100) [$M+Na^+$]; ^1H NMR (COSY, 400 MHz, D_2O): $\delta = 5.39$ (s, 3H; H4, H7″, H8″), 4.99–4.84 (m, 2H; H1, H4″), 4.80–4.70 (m, 1H; H1′), 4.70–4.58 (m, 1H; E$^{\alpha}$), 4.58–4.38 (m, 6H; H2′, P$^{\alpha}$ {4.54}, S$^{\alpha}$), 4.38–4.21 (m, 7H; H2 {4.24}, A$^{\alpha}$ {4.31}, L$^{\alpha}$ {4.30}, Q$^{\alpha}$ {4.38}, 3 × T$^{\beta}$), 4.21–3.98 (m, 5H; H3′ {4.05}, V$^{\alpha}$ {4.04, 4.14}, 2 × T$^{\beta}$), 3.98–3.71 (m, 18H; H3, H5″, H9″a, H9″b, G$^{\alpha}$, S$^{\beta}$, 3 × P$^{\delta}$, CH_3O {3.80}), 3.70–3.59 (m, 1H; 1 × P$^{\delta}$), (4.58–4.38 (m, 2H), 4.38–4.21 (m, 6H), 4.21–3.98 (m, 4H), 3.98–3.71 (m, 1H)) (H4′, H5, H5′, H6, H6′, H6″, T$^{\alpha}$), 2.58 (d_b, 1H; H3″$_{eq}$), 2.49–2.38 (m, 2H; E$^{\gamma}$), 2.34–1.76 (m, 16H; E$^{\beta}$ {2.03, 1.88}, P$^{\beta}$, P$^{\gamma}$, Q$^{\beta}$ {2.01, 1.90}, Q$^{\gamma}$ {2.30}, V$^{\beta}$), 2.24, 2.18, 2.10, 2.06, 2.03, 2.00, 1.96, 1.82 (8 × s, 33H; CH_3CO), 1.66–1.46 (m, 4H; H3″$_{ax}$, L$^{\beta}$, L$^{\gamma}$), 1.31 (d, 3H; $J_{A\alpha,A\beta} = 6.2$ Hz; A$^{\beta}$), 1.26, 1.19 (2 × d, 6H; $J_{T\beta,T\gamma} = 4.8$ Hz; T$^{\gamma}$), 1.23–1.08 (m, 12H; T$^{\gamma}$), 0.98–0.78 (m, 18H; L$^{\delta}$, V$^{\gamma}$); ^{13}C NMR (broad-band decoupling, DEPT, 100.6 MHz, D_2O), $\delta = 177.6$, 176.9, 174.5, 174.3, 173.6, 173.3, 172.9, 172.7, 172.1, 171.7, 171.4 (C=O), 100.7 (C1′), 99.2 (C1), 97.0 (C2″), 77.1 (C3), 73.5 (T$^{\beta}$*), 71.5, 71.2, 71.0, 70.2, 69.9, 69.5, 68.1, 67.4, 67.0, 66.8 (C2′, C3′, C4, C4′, C4″, C5, C5′, C6″, C7″, C8″, T$^{\beta}$), 63.1 (C9″), 62.0, 61.5, 61.3, 60.9 (C6, C6′, S$^{\beta}$), 60.2, 59.4, 59.1, 59.0, 58.8, 57.9, 57.1, 57.0, 55.5, 55.2, 52.6, 52.4, 50.7, 49.6, 48.5 (C2, C5″, A$^{\alpha}$, E$^{\alpha}$, L$^{\alpha}$, P$^{\alpha}$, Q$^{\alpha}$, S$^{\alpha}$, T$^{\alpha}$, V$^{\alpha}$), 53.6 (CH_3O), 48.3, 47.8 (P$^{\delta}$), 42.1 (G$^{\alpha}$), 39.5 (L$^{\beta}$), 36.6 (C3″), 30.9 (Q$^{\gamma}$), 30.2, 29.9 (V$^{\beta}$), 29.5, 29.4, 29.2 (E$^{\gamma}$, P$^{\beta}$), 26.8, 25.5, 24.6, 24.2 (E$^{\beta}$, Q$^{\beta}$, P$^{\gamma}$), 24.2, 22.2, 22.0, 21.7, 21.6, 20.8, 20.7, 20.2, 20.1, 19.8, 18.7, 18.6, 18.3, 18.1, 17.7, 17.5 ($\mathit{C}H_3CO$, L$^{\gamma}$, L$^{\delta}$, T$^{\gamma}$, V$^{\gamma}$), 16.3 (A$^{\beta}$).

[22] **9**: ESI-MS (positive-ion mode), m/z (%): 1210.3 (15) [$(M+2Na^+)/2$], 1207.3 (10) [$(M+H^++K^+)/2$], 1199.3 (40) [$(M+H^++Na^+)/2$], 1188.3 (100) [$(M+2H^+)/2$]; ^1H NMR (COSY, 400 MHz, D_2O): $\delta = 4.85$ (d, 1H, $J_{H\text{-}1,H\text{-}2} = 3.0$ Hz; H1), 4.66–4.58 (m, 1H; E$^{\alpha}$), 4.58–4.53 (m, 1H; 1 × P$^{\alpha}$), 4.53–4.47 (m, 2H; 1 × S$^{\alpha}$, 1 × T$^{\alpha}$), 4.46–4.39 (m, 3H, $J_{H\text{-}1',H\text{-}2'} = 7.9$ Hz; H1′, 2 × S$^{\alpha}$), 4.39–4.09 (m, 12H; H4, A$^{\alpha}$ {4.31}, L$^{\alpha}$ {4.29}, 1 × P$^{\alpha}$ {4.36}, Q$^{\alpha}$ {4.36}, 4 × T$^{\alpha}$, 3 × T$^{\beta}$ {4.26}), 4.18–4.09 (m, 3H; H2, 1 × T$^{\beta}$, 1 × V$^{\alpha}$), 4.09–3.89 (m, 6H; H3 {4.00}, H3′ {4.00}, H4′, H9″a, 1 × T$^{\beta}$ {4.05}, 1 × V$^{\alpha}$), 3.88–3.71 (m, 10H; G$^{\alpha}$, S$^{\beta}$, 2 × P$^{\delta}$), (3.88–3.71 (m, 2H), 3.71–3.49 (m, 12H)] (H4″ {3.60}, H5, H5′, H5″, H6a, H6b, H6′a, H6′b, H6″, H7″, H8″, H9″b, 2 × P$^{\delta}$), 3.49–3.41 (m, 1H; H2′), 2.70 (dd, 1H; $J_{H3''eq,H3''ax} = 12.3$, $J_{H3''eq,H4''} = 3.7$ Hz; H3″$_{eq}$), 2.46–2.38 (m, 2H; E$^{\gamma}$), 2.29 (t, 2H, $J_{Q\beta,Q\gamma} = 7.5$ Hz; Q$^{\gamma}$), (2.25–2.12 (m, 1H), 2.08–1.89 (m, 12H)) (E$^{\beta}$a {2.02}, P$^{\beta}$, P$^{\gamma}$, Q$^{\beta}$ {2.02, 1.90}, V$^{\beta}$), 2.00, 1.96, 1.94 (3 × s, 9H; CH_3CO), 1.89–1.78 (m, 1H; E$^{\beta}$b), 1.71 (t, 1H, $J_{H3''eq,H3''ax} = J_{H3''ax,H4''} = 12.0$ Hz; H3″ax), 1.64–1.47 (m, 3H; L$^{\beta}$, L$^{\gamma}$), 1.31 (d, 3H, $J_{A\alpha,A\beta} = 7.0$ Hz; A$^{\beta}$), (1.26 (d, 3H, $J_{T\beta,T\gamma} = 5.9$ Hz), 1.20 (d, 3H, $J_{T\beta,T\gamma} = 6.4$ Hz), 1.18–1.08 (m, 9H)) (T$^{\gamma}$), 0.88 (m, 12H; V$^{\gamma}$), 0.83 (d, 6H, $J_{L\gamma,L\delta} = 5.9$ Hz; L$^{\delta}$); ^{13}C NMR (100.6 MHz, D_2O): $\delta = 177.8$, 177.0, 176.6, 174.6, 173.3, 173.2, 172.7, 171.9, 171.7, 171.4, 170.9, 170.8 (C=O), 104.6 (C1′), 99.0 (C2″), 77.4, 75.8, 74.9, 73.0, 71.8, 71.1, 68.9, 68.8, 68.4, 68.3, 67.3, 67.0, 66.9 (C2′, C3, C3′, C4, C4′, C4″, C5, C5′, C6″, C7″, C8″, T$^{\beta}$), 62.8 (C9″), 61.4, 61.2, 61.0 (C6, C6′, S$^{\beta}$), 60.5, 59.5, 59.3, 59.1, 57.2, 55.7, 55.4, 52.9, 52.6, 51.9, 50.9, 49.8 (C2, C5″, A$^{\alpha}$, E$^{\alpha}$, L$^{\alpha}$, P$^{\alpha}$, Q$^{\alpha}$, S$^{\alpha}$, T$^{\alpha}$, V$^{\alpha}$), 48.5, 48.0 (P$^{\delta}$), 42.3 (G$^{\alpha}$), 39.7 (L$^{\beta}$), 39.5 (C3″), 31.2 (Q$^{\gamma}$), 30.4, 30.2 (V$^{\beta}$), 29.7, 29.4 (E$^{\gamma}$, P$^{\beta}$) 27.0, 25.8, 24.7 (E$^{\beta}$, Q$^{\beta}$, P$^{\gamma}$), 24.5, 22.2, 21.8, 21.1, 18.9, 18.8, 18.5, 18.2, 17.8, 17.7 ($\mathit{C}H_3CO$, L$^{\gamma}$, L$^{\delta}$, T$^{\gamma}$, V$^{\gamma}$), 16.6 (A$^{\beta}$); the signal for C1 could not be assigned.

Matrix Isolation of Perfluorinated *p*-Benzyne**

Hans H. Wenk, Andreas Balster, Wolfram Sander,*
David A. Hrovat, and Weston T. Borden

The thermal cyclization of enediynes **1** to produce *p*-benzyne (*p*-didehydrobenzene) and its derivatives **2** (Bergman cyclization)[1] is the key step for the biological activity of the enediyne antibiotics.[2] During the past few years this reaction and the proposed intermediates have thus been the target of a large number of synthetic,[3–11] spectroscopic,[12–16] and theoretical[17–21] studies.[22] The heat of formation of 1,4-didehydrobenzene (**2a**) was determined by Roth et al. to be 138.0 ± 1.0 kcal mol^{-1}, which results in a reaction enthalpy for the cyclization of **1a** → **2a** of 8.5 ± 1.0 kcal mol^{-1}.[23] The activation barrier for the ring-closure of **1a** was determined to be 28.7 ± 0.5 kcal mol^{-1}. A singlet ground state was predicted for **2a**, and a singlet–triplet splitting ΔE_{ST} of −3.8 ± 0.5 kcal mol^{-1} was measured by Squires et al. using negative-ion photoelectron spectroscopy (NIPES).[14] Recently the matrix isolation and characterization of **2a** by IR spectroscopy was reported from our laboratory.[15]



1a **2a** **3a**

The experimental data available for *p*-didehydrobenzene (**2a**) has contributed to the understanding of the biological activity of calicheamicin-type antitumor drugs. The challenge is now to modify the diradicals **2** in order to control their reactivity.[24] However, so far all attempts to isolate and characterize derivatives of benzyne **2a** failed.

Density functional theory (DFT) and CASSCF calculations indicate that 1,4-didehydro-2,3,5,6-tetrafluorobenzene (**2b**) should be a suitable target for synthesis and isolation in low-temperature matrices. The fluorine substituents at the terminal acetylenic positions result in a large destabilization of 1,3,4,6-tetrafluoro-hex-3-ene-1,5-diyne (**1b**) with respect to *p*-benzyne **2b**, and the cyclization of **1b** is estimated to be exothermic by 7.9 kcal mol^{-1} (UB3LYP/6-311++G(d,p)). The barrier for the ring-opening of **2b** is calculated to be 37.5 kcal mol^{-1}, almost twice that of **2a** (19.8 ± 1 kcal mol^{-1}).[23]

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[*] Prof. Dr. W. Sander, Dipl.-Chem. H. H. Wenk, Dipl.-Chem. A. Balster
Lehrstuhl für Organische Chemie II der Ruhr-Universität Bochum
44780 Bochum (Germany)
Fax: (+49) 234-321-4353
E-mail: sander@xenon.orch.ruhr-uni-bochum.de

D. A. Hrovat, Prof. Dr. W. T. Borden
Department of Chemistry, University of Washington
Seattle, WA 98195-1700 (USA)

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