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Tetraethylene Glycol-Derived Spacer for Oligonucleotide Synthesis

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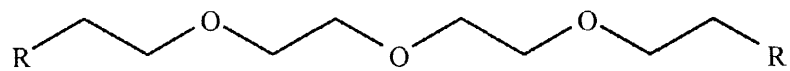
Abstract: 3,6,9-Trioxaundecane-1,11-diisocyanate (**6**) was synthesised from tetraethylene glycol in 5 steps and 48 % overall yield. Spacer **6** was monofunctionalised with the fully protected adenosyl-3'-O-succinate derivative **7** and linked to aminomethyl polystyrene (50 % crosslinked with divinylbenzene) affording a solid support suitable for oligoribonucleotide synthesis (loading: ~20 μmol/g). The HPLC analysis of a crude oligoribonucleotide synthesis and the isolated yield of purified oligomer show that this spacer compares well to hexamethylene diamine.

The utility of hydrophilic spacers for the linkage of (bio)molecules to some solid support has gained importance with the wide-spread application of combinatorial chemistry. In this context polyethylene glycol-derived substances were found to have ideal properties: desirable stability, polarity, and a preference for an extended (all-*gauche*) conformation in solution.[1] For the solid support synthesis of oligonucleotides a tetraethylene glycol spacer proved to be superior in terms of, both, coupling yields and homogeneity of the final product, when compared to several other tested spacer molecules of up to double its length.[2] At present there is a considerable interest in finding reaction conditions for spacer molecules such as α,ω-diols, -diazides, and -diamines that allow for an efficient monofunctionalisation.[3] *Kumar* et al. found that, in contrast to diols and diamines, diisocyanates such as hexamethylene or toluene-2,4-diisocyanate can readily be monofunctionalised and subsequently linked to solid supports in good yields.[4] We envisaged combining the desirable properties of tetraethylene glycol-derived spacers, the optimal reactivity of α,ω-diisocyanates, and the superiority of highly crosslinked aminomethyl polystyrene (H_2NCH_2-PS) over alkylamino controlled-pore glass,[5] and synthesised 3,6,9-trioxaundecane-1,11-diisocyanate (**6**) from tetraethylene glycol (Scheme 1), coupled it to the fully protected adenosyl-3'-O-succinate derivative **7** and linked it to H_2NCH_2-PS (Scheme 2).



$$R = OH\ (\mathbf{1}) \xrightarrow{a)} Br\ (\mathbf{2}) \xrightarrow{b)} N_3\ (\mathbf{3}) \xrightarrow{c)} NH_2\ (\mathbf{4}) \xrightarrow{d)} NH_{1\text{-}0}(SiMe_3)_{1\text{-}2}\ (\mathbf{5}) \xrightarrow{e)} N{=}C{=}O\ (\mathbf{6})$$

Scheme 1: a) Ph_3P, CBr_4, CH_3CN, 40°/18 h, MPLC: 85 %; b) NaN_3, DMF, 100°/100 h, MPLC: 93 %; c) H_2/Pd-$BaSO_4$, EtOH/H_2O 26:1, 1 atm/30 h, distill.: 93 %; d) Me_3SiNEt_2, $(NH_4)_2SO_4$, 85°/1.5 h→100°/24 h→ 150°/40 h; e) $(Cl_3CO)_2CO$, MgO, CH_2Cl_2, 25°/43 h, distill.: 65.4 % (**4**→**6**).

Tetraethylene glycol (**1**) was first converted to dibromide **2** with triphenylphosphine and tetrabromomethane at elevated temperatures.[6] Since **2** would not react with NaCNO neither without nor with phase transfer catalysts, the compound was submitted to an azide substitution in DMF,[7] and diazide **3** was reduced to diamine **4**.[8] We attempted to directly convert this compound to the diisocyanate **6** using phosgene (immediate poly-

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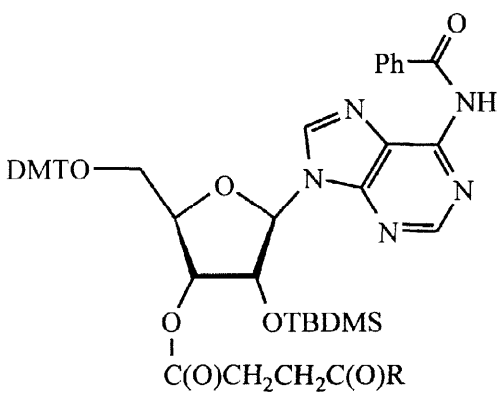
merisation) and triphosgene ($Cl_3COC(O)OCCl_3$)/Et_3N (29 % yield: rapid polymerisation of **6**). According to *Mironov* et al., amines can be smoothly converted to isocyanates (with phosgene at 0°) after *N*-trimethylsilylation.[9] Trimethylsilylation of **4** using neat refluxing hexamethyldisilazane (HMDS) with catalytic amounts of $(NH_4)_2SO_4$ or trimethylsilyl acetamide (TMSA) proved too inefficient (low degree of silylation) and, upon evaporation of the reagents and treatment of the crude or distilled silylated product with phosgene/Et_3N or triphosgene/Et_3N, resulted in low yields of rapidly polymerising **6** (max. 24 %). The presence of residual amounts of, both, primary amino groups and Et_3N are detrimental to the stability of **6**. According to ^1H NMR we obtained a high degree of silylation in **5** (ca. 70-75 % of amino protons were substituted by TMS groups; 70-83 % of the amino groups were disilylated) using *N,N*-diethyl-(trimethylsilyl)amine (TMSDEA) and shifting the reaction equilibrium with a flow of argon gas (evaporation of Et_2NH).[10] This material underwent a clean phosgenation to **6** using triphosgene and MgO as the base.[11] MgO has the advantage of being readily removable by filtration; a high degree of silylation of the starting material was however necessary, or else considerable loss of material owing to irreversible adsorption to MgO was observed. A $CDCl_3$-solution of diisocyanate **6** synthesised that way was stable at room temperature over a week and neat **6** showed no tendency to polymerise at all when stored for months at 4°.



DMT: dimethoxytrityl
TBDMS: *tert*-butyldimethylsilyl
PS: polystyrene (50 % DVB-crosslinked)

R = OH (**7**)
a) ↓ $-CO_2$
R = $NHCH_2CH_2O(CH_2CH_2O)_2CH_2CH_2NCO$ (**9**)
R = $NH(CH_2)_6NCO$ (**11**)
b) c) d) ↓
R = $NH(CH_2)_2O(CH_2CH_2O)_2(CH_2)_2NHC(O)NHCH_2$–PS (**10**)
R = $NH(CH_2)_6NHC(O)NHCH_2$–PS (**12**)

Scheme 2: a) DMAP (1.0 equiv.), **6** or $OCN(CH_2)_6NCO$ (**8**) (1.0 equiv.), CH_2Cl_2, 15 min.; b) H_2NCH_2-PS (5 g/mmol 7), Et(*i*-prop)$_2$N (1.0 equiv.), 48 h/CH_2Cl_2 (5 ml/g H_2NCH_2-PS), Et_2O (wash); c) H_2O/C_5H_5N (2:8), 2 h; d) Ac_2O/ Et_3N/N-methylimidazole/CH_2Cl_2 (1:1:0.3:6), 0.5 h.

6-N-Benzoyl-3'-O-*tert*-butyldimethylsilyl-5'-O-dimethoxytrityladenosine (*Peninsula Lab.*) was succinylated with succinic anhydride, 4-(dimethylamino)pyridine (DMAP) and Et_3N in 1,2-dichloroethane to give succinate **7** in quantitative yield.[4] One portion was derivatised with **6** and the other with hexamethylene diisocyanate (**8**, *Fluka*), and the resulting isocyanates **9** and **11**, respectively (Scheme 2), were coupled to H_2NCH_2-PS (*ABI*, 33 µmol (NH_2)/g, prod. # 360865C).[4] The loading of the resins **10** and **12** was measured by UV absorption at 504 nm (= λ_{max}) of the DMT cations released in 3 % trichloroacetic acid (TCA)/CH_2Cl_2: 20.3 and 18.6 µmol/g, respectively.[12]

In a separate test reaction **7** → **9** the reaction mixture was quenched after 15 min. with an excess of anhydrous methanol (**9** → R = $NH(CH_2)_2O(CH_2CH_2O)_2(CH_2)_2NHC(O)OCH_3$), evaporated and analysed by ^1H-, ^{13}C-NMR and ESI mass spectroscopy. We were unable to find any doubly coupled trioxaundecanedinucleosidyl disuccinate derivative in the crude mixture. Finally, both resins **10** and **12** were tested on the RNA synthesiser using commercial phosphoramidites and reagents (Fig. 1).

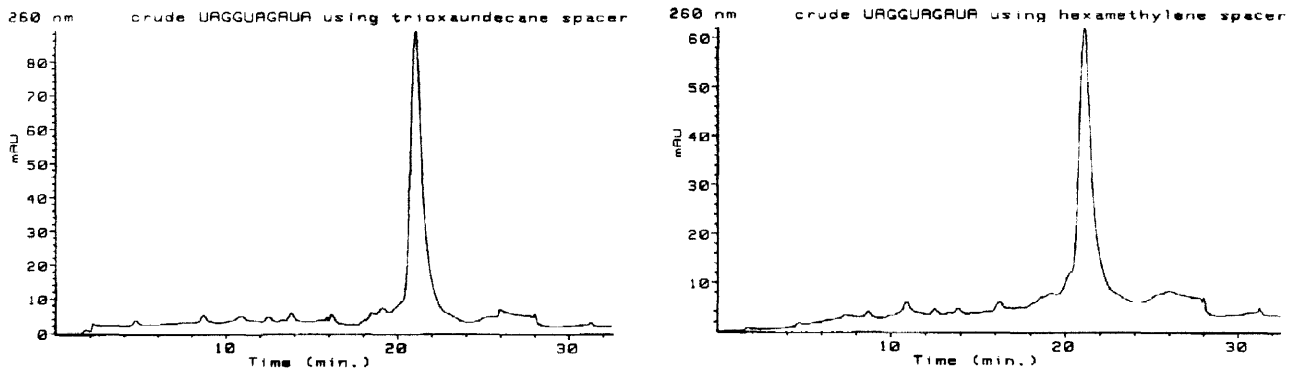


Figure 1: Anion exchange HPLC of crude RNA decamer UAGGUAGAUA synthesised on resin **10** (left), and on resin **12** (right).[13]

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6. To a suspension at –10° of Ph_3P (22.16 g, 84.4 mmol) in CH_3CN (110 ml) containing **1** (7.76 g, 40.0 mmol, *Fluka* puriss.) neat CBr_4 (28.0 g, 84.4 mmol, *Fluka* purum) was added dropwise during 120 min. The clear solution was slowly warmed up to 40° (during 3-4 h) and stirred for another 18 h. After evaporation of the solvent, the oily-crystalline yellowish crude mixture was vigorously stirred and refluxed in H_2O/*n*-hexane (200 ml each) during 30 min., to precipitate $Ph_3P{=}O$. After cooling down to ambient temp. the biphasic suspension was filtered, the precipitate was extensively washed with H_2O (200 ml in several portions), and *n*-hexane was evaporated under reduced pressure from the pooled filtrates. The aqueous phase was extracted in a *Ludwig* extractor with CH_2Cl_2 for 3 h. Evaporation of CH_2Cl_2 furnished 14.8 g of a pale-yellow oil that was further purified by MPLC (LiChroprep® Si 60, 15-25 µm, 36 x 460 mm; EtOAc-gradient in hexane, 50 ml fract.; detection: tlc, EtOAc/*n*-hexane 3:7, I_2 vap.). 10.89 g (34 mmol, 85 %) **2** were obtained as an oil. Bp.: 114°/0.06 mbar. **^{1}H NMR** (300 MHz, $CDCl_3$, $SiMe_4$): 3.48 (*t*, 4 H, ^3J = 6.3, $H_2C(1,11)$); 3.82 (*t*, 4 H, ^3J = 6.3, $H_2C(2,10)$); 3.68 (*s*, 8 H, $H_2C(4,5,7,8)$). **^{13}C NMR** (75 MHz, $CDCl_3$, $SiMe_4$): 30.27 (C(1,11)); 70.50 (2 C); 70.61 (2 C); 71.17 (C(2,10)). **MS** (FAB, – nitrobenzyl alc.): *m/z* = 323 (16.4, M^+, 2 x ^{81}Br); 321 (36.9, M^+, ^{79}Br + ^{81}Br); 319 (22.1, M^+, 2 x ^{79}Br); 153 (36.6); 151 (45.2); 109 (100); 107 (92.5). **IR** (cm^{-1}): 2869 (CH st); 1116 (COC st). **Microanalysis** for $C_8H_{16}Br_2O_3$ (320.02): calc. C 30.03, H 5.04, O 15.00; found C 29.93/30.05, H 4.90/4.97, O 15.06/15.05.
7. **2** (5.14 g, 16.1 mmol) and NaN_3 (2.68 g, 41.2 mmol, CAUTION: causes toxic HN_3 gas upon acidification!) in DMF (25 ml, *Fluka* puriss., over molec. sieves 4 Å) were heated at 100° under Ar for 100 h. After evaporation of the solvent at 40-45° and 0.1-0.15 mbar, the residue was treated with a small amount of H_2O and extracted with CH_2Cl_2 (3 x 100 ml). After drying (Na_2SO_4 anhydr.) and evap., 4.04 g of crude product were separated by MPLC (LiChroprep® Si 60, 40-63 µm, 49 x 460 mm; hexane, then hexane/EtOAc 12 and 25 %; detection: 260 nm and tlc, EtOAc/*n*-hexane 3:7, I_2 vap.). 3.341 g (14.90 mmol, 93 %) **3** were isolated as a colorless liquid. **^{1}H NMR** (300 MHz, $CDCl_3$, $SiMe_4$): 3.39 (*t*, 4 H, ^3J = 5.1, $H_2C(1,11)$); 3.67 (*s*, 8 H, $H_2C(4,5,7,8)$); 3.68 (*t*, 4 H, ^3J = 5.1, $H_2C(2,10)$). **^{13}C NMR** (75 MHz, $CDCl_3$, $SiMe_4$): 50.60 (C(1,11)); 69.92 (2 C); 70.60 (4 C). **MS** (FAB, – nitrobenzyl alc.): *m/z* = 245 (75.5, MH^+); 86 (18.7); 56 (42.9); 42 (100). **IR** (cm^{-1}): 2869 (CH st); 2108 (N_3 st as.); 1302 (N_3 st sym.); 1125 (COC st as.).

8. A soln. of **3** (2884.8 mg, 11.8 mmol) in EtOH (65 ml, >99.8 %) and H_2O (2.5 ml) containing a catalytic amount of Pd (10 %) on $BaSO_4$ (*Fluka*) was hydrogenised for 30 h at 1 atm. During the first 10 h the N_2-containing gas volume was replaced with fresh H_2 every 2-3 h. After completion of the reaction (tlc: 25 % EtOAc/hexane; I_2 vap., or 5 % $H_3Mo_{12}O_{40}P$ in 10 % H_2SO_4), the mixture was filtered over HY-FLO®, and the solvent was evaporated under reduced pressure at 50°. 2.465 g of a crude colorless oil were distilled at 0.08 mbar to furnish 2.099 g **4** (10.92 mmol, 93 %) as a highly hygroscopic oil (234 mg of a brown polymeric residue remained undistilled). Bp.: ca. 130°/0.08 mbar. **^{1}H NMR** (300 MHz, $CDCl_3$, $SiMe_4$): 1.32 (br. *s*, 4 H, 2 x H_2N); 2.86 (*t*, 4 H, ^3J = 5.2, $H_2C(1,11)$); 3.51 (*t*, 4 H, ^3J = 5.2, $H_2C(2,10)$); 3.65 (*m*, 8 H, $H_2C(4,5,7,8)$). **^{13}C NMR** (75 MHz, $CDCl_3$, $SiMe_4$): 41.67 (C(1,11)); 70.13 (2 C); 70.42 (2 C); 73.37 (C(2,10)). **MS** (ESI, MeOH): *m/z* = 194.2 (100, MH^+). **IR** (cm^{-1}): 3372/3290 (NH_2 st); 2865 (CH st); 1596/1458 (NH_2 δ); 1120 (COC st as.).
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10. A soln. of freshly distilled **4** (192.3 mg, 1.0 mmol) in TMSDEA (2.0 g, 13.8 mol, *Fluka* purum) containing $(NH_4)_2SO_4$ (100 mg) was stirred for 1.5 h at 85° under a gentle Ar stream. After liquidification of the catalyst the mixture was kept at 100° for 24 h, and under reflux (bath temp.: 150°) for another 40 h. The mixture was cooled, decanted and TMSDEA was evaporated at 30-35° and 0.07 mbar. The lightly brown sirup, **5** (464.6 mg), was analysed and directly used for the next reaction. Bp.: ca. 120°/ 0.06 mbar. **^{1}H NMR** (300 MHz, $CDCl_3$, $SiMe_4$): $N(TMS)_2/N(TMS)_{0\text{-}1}$: 0.09/0.00 (2 *s*, $(H_3C)_3Si$); 2.97/2.86 (2 *t*, ^3J = 7.6/5.2, (0.7-0.83·2 H/0.3-0.17·2 H), $H_2C(1,11)$); 3.30/3.50 (2 *t*, ^3J = 7.6/5.2, (0.7-0.83·2 H/0.3-0.17·2 H), $H_2C(2,10)$); 3.54-3.66 (*m*, 8 H, $H_2C(4,5,7,8)$); (total H_3C:9)/(total H_2C:16) = mol Si/mol spacer = 70-75 % (integral ratios calculated from 4 batches). **^{13}C NMR** (75 MHz, $CDCl_3$, $SiMe_4$): $N(TMS)_2/N(TMS)_{0\text{-}1}$: 2.04/1.37 ($(CH_3)_3Si$); 44.09/41.82 (C(1,11)); 70.45/70.33 (2 C); 70.65 (2 C); 73.99/73.49 (C(2,10)). **IR** (cm^{-1}): 3382 (NH st); 2953/2873 (CH st); 1252 (Si-CH_3 δ symm.); 1086/915/840.
11. To a stirred soln. of $CO(OCCl_3)_2$ (215.4 mg, 0.726 mmol, *Merck*) in anhydr. CH_2Cl_2 (20 ml) containing anhydr. MgO (605 mg, 15 mmol) was added dropwise a soln. of crude **5** (404.7 mg) in anhydr. CH_2Cl_2 (10 ml) during 3 h at ambient temp. After another 40 h under Ar, the mixture was filtered, the filtrate evaporated, and the residual colorless oil was distilled at 0.08 mbar to give 139.4 mg **6** (0.57 mmol, 64.4 % from **4**), which was stable for months (no polymerisation). Bp.: 140°/0.08 mbar. **^{1}H NMR** (300 MHz, $CDCl_3$, $SiMe_4$): 3.41 (*t*, 4 H, ^3J = 5.2, $H_2C(1,11)$); 3.64 (*t*, 4 H, ^3J = 5.2, $H_2C(2,10)$); 3.69 (*s*, 8 H, $H_2C(4,5,7,8)$). **^{13}C NMR** (75 MHz, $CDCl_3$, $SiMe_4$): 43.10 (C(1,11)); 70.34 (2 C); 70.48 (2 C); 70.62 (C(2,10)); 125.07 (NCO). **MS** (FAB, – nitrobenzyl alc.): *m/z* = 245 (9.0, MH^+); 70 (100); (FAB, – nitrobenzyl alc. + KCl): *m/z* = 283 (31.3, M + K^+); 245 (10.2, MH^+); 70 (100); (CI, NH_3): *m/z* = 262 (100, M + NH_4^+); 245 (16.7, MH^+). **IR** (cm^{-1}): 2871 (CH st); 2264/2222 (NCO, st as.); 1349 (NCO st symm. (weak)); 1125 (COC st). **Microanalysis** for $C_{10}H_{16}N_2O_5$ (244.25): calc. C 49.17, H 6.60, N 11.47, O 32.75; found C 49.28/49.24, H 6.75/6.69, N 11.51/11.49, O 32.51/32.51.
12. ε_{504}(DMT$^+$, 3 % TCA/CH_2Cl_2 *w/w*) = 72'678 M^{-1}, as determined from a linear regression of A_{504} *versus* conc. of a dilution series (22-2.2 μM) of 5'-O-DMT-2'-deoxyadenosine in 3 % TCA/CH_2Cl_2.
13. Monomers (*Glen Research*): 5'-O-DMT-2'-O-TBDMS-3'-O-(O-(2-cyano)ethyl-*N,N*-diisopropyl)-phosphoramidites of uridine, 6-N-phenoxyacetyladenosine, and 2-N-(*N,N*-dimethyl)formamidinoguanosine. Reagents (*ABI*): detrityl.: 3 % TCA/CH_2Cl_2; coupling: 0.25 M 5-(ethylthio)tetrazole/CH_3CN; capping: Ac_2O ('Cap A')/N-MeIm ('Cap B'); oxid.: 0.02 M I_2/THF/C_5H_5N/H_2O. Two small solid support reactor columns containing a) 49.3 mg (1.0 μmol) **10** and b) 53.7 mg (1.0 μmol) **12**, resp.; modified 0.1 μmol RNA cycle on an *ABI* 392 (two-column) DNA/RNA synthesiser; 'WAIT' time periods for: coupling: 300 sec; 1st capping: 5 sec; CH_3CN wash; oxidation: 45 sec; CH_3CN wash; 2nd capping: 5 sec. CH_3CN wash; detrityl. Deprotection: ~3 ml 35 % NH_3/EtOH 3:1 each, 25°/2 h (modified *ABI* 'END RNA' procedure) → 55°/2 h; evap.; 270 μl Et_3N·3 HF, 90 μl DMF, 55°/1 h; quench with 30 μl H_2O; add 3 ml *n*-BuOH, precip. at –20°/0.5 h, spin down; dissolve each pellet in 600 μl HPLC buffer A; yields (crude): a) 52 OU, b) 39 OU. HPLC: column: Nucleogen® DEAE 500-7, 8 x 125 mm (*Macherey-Nagel*); buffer A: 20 mM Na/K phosphate, pH 7.0, buffer B: 0.6 M NaCl in buffer A; flow rate: 2 ml/min; gradient: 0 % B for 5 min., linear 0-80 % B within 15 min., isocratic 80 % B during 5 min., short wash-out at 100 % B; detection: 260 nm. Injection volume for depicted chromatograms: 5.0 μl each. Preparative separation on the same column using non-linear gradient; main product eluted under isocratic conditions at 67 % B. Isolated yields of full-length product: a) (**10**→) 33.2 OU, b) (**12**→) 23.5 OU.