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One Pot Synthesis of Unsymmetrically Functionalized Polyamines by a Solid Phase Strategy Starting from their Symmetrical Polyamine-Counterparts.

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Abstract:. We have developed a solid phase methodology which allows a quick and easy access to a high number of mono-functionalized geometrically varied polyamines. The feasibility of the method was demonstrated by the unsymmetrical introduction of a single carboxylic acid functional group into differing symmetrical polyamines. © 1997 Elsevier Science Ltd.

The synthesis of unsymmetrically substituted polyamines commonly involves tedious multistep reaction synthesis[1]. In the context of our gene therapy program, we were challenged to synthezise novel and varied lipopolyamines as non-viral vectors for DNA delivery. Thus, we have developed a solid phase methodology which allows a quick and easy access to a high number of mono-functionalyzed polyamines. In this approach, the alkylating reagent is covalently attached to the polymeric support through esterification. The principle of the method is the use of solid phase synthesis[2] to rise a "high dilution effect" at the proximity of the alkylating reagent. These dilution effect will prevent poly-alkylation of the polyamine once the first alkylation took place. The symmetrical polyamine reacts with the alkylating reagent on the solid phase to yield the unsymmetrically mono-functionalized polyamine attached to the support. The free amines of the product can be protected on the solid phase with groups such as Boc (see products **1-2**, **5-7**) or orthogonally protected with DdeOH[3] for the primary amines from one side and Boc for the secondary amines on the other side (see products **3-4**). In the absence of primary amines, secondary amines will react to give the unsymmetrically functionalized polyamine as assessed by the synthesis of a derivative of the cryptand 1,4,8,11-tetraazacyclotetradecane (see product **7**). When polyamines contain primary as well as secondary symmetrical polyamines, some of the secondary amino-functionalized derivatives will be obtained (see products **2** and **4**). The secondary-functionalized polyamines are easily separated from their primary counterparts after protection. Finally, we report a procedure[4] suitable for non acidic cleavage of products from Cl-Trityl chloride solid phase support using CH_2Cl_2/trifluoroethanol to obtain protected products in the absence of acid, and allowing further modifications by classical solution methodologies.

The combination of the unsymmetrical functionalization of polyamines and orthogonal amine protection with DdeOH[5-6], implies a significant extension of solid phase chemistry of polyamines, allowing novel applications such as the use of polyamines for the construction of polyamine-combinatorial libraries[7], synthesis of novel analogs of polyamine-containing toxins[8], introduction of substituted polyamines into peptides[9], synthesis of functionalized metal-complexing cryptands[10,11] suitable for introduction into peptides, or synthesis of novel cationic lipids for gene delivery which is currently being investigated in our laboratory.

The starting polyamines may be commercially available spermine, tris(2-aminoethyl)amine, 1,4,8,11-tetraazacyclotetradecane, or synthesized by classical methods[12] through exhaustive cyanoethylation of

commercially available diamino propane, tris(2-aminoethyl)amine, or spermine followed by hydrogenation in the presence of Raney-nickel catalyst to give branched polyamines. The alkylating function was bromoacetyl linked to a polymeric support through ester bond. The polymer support was the acid sensitive resin for solid phase peptide synthesis O-chlorotrityl chloride.

We have adapted the classical Kaiser[13] test to dicriminate between primary and secondary amines on the solid support by performing it at room temperature. Indeed, we have found that free primary amines give positive Kaiser test after 1 min at room temperature, whereas free secondary polyamines give a negative Kaiser test under the same conditions. Therefore, the progression of the orthogonal protection of the primary amines with DdeOH (see product 3), could be monitored using the cold Kaiser test, which became negative (during 5 min) after completed reaction. At this point the classical Kaiser test (110°C) was positive after 15 sec. indicating the presence of free secondary amines.

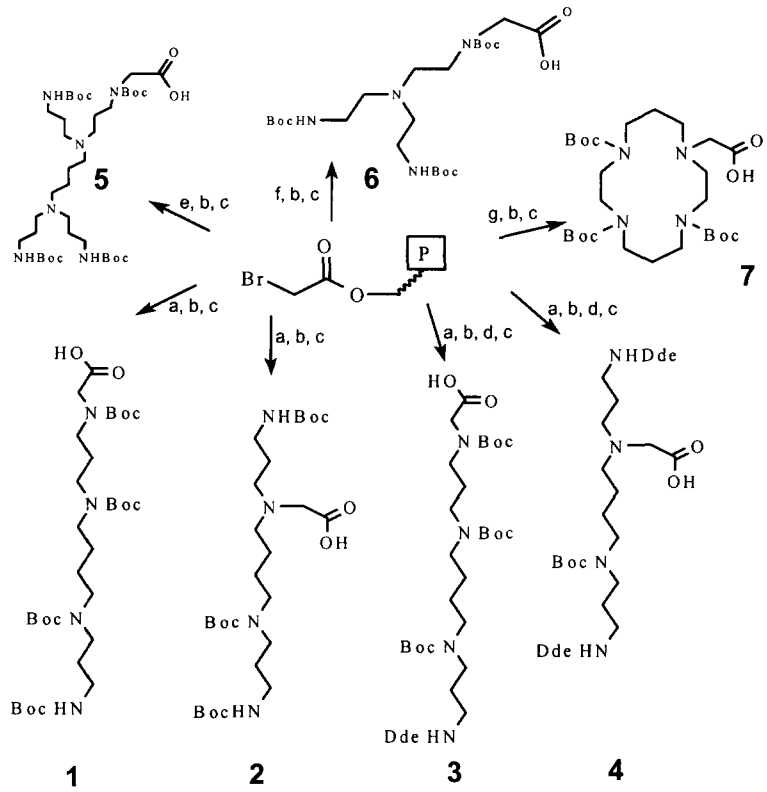

Figure 1. Synthesis of functionalized polyamines by solid phase strategy, a: Spermine in CH_2Cl_2, b: $(Boc)_2CO_2$ in CH_2Cl_2, c: Cleavage with trifluoroethanol/CH_2Cl_2, d: DdeOH, e: Tetra-(3-aminopropyl)-diaminobutane, f: Tris(2-aminoethyl)amine, g: 1,4,8,11-tetraazacyclotetradecane

In conclusion, we describe a new, rapid and easy route for the synthesis of geometrically differing unsymmetrically functionalized polyamines by application of a solid phase synthesis methodology. This solid

phase strategy in combination with orthogonal protection of the resulting functionalized polyamines with DdeOH[3] and Boc, implies a significant extension of the solid phase chemistry of polyamines, opening new possibilities for novel applications in the field of chemical combinatorial libraries[7]. These are, for example, the use of polyamines as scaffolds for drug discovery, synthesis of novel analogs of polyamine-containing toxins[8], introduction of substituted polyamines into peptides[9], synthesis of functionalized metal-complexing cryptands[10,11] or synthesis of novel cationic lipids for gene delivery presently being explored in our laboratory.

Experimental

1- Anchorage of the acidic function to the polymeric support: briefly, O-chloro trityl chloride resin (5 g, 1.2 mmol Cl/g resin from Nova) was placed in the solid phase synthesis flask, 50 ml CH_2Cl_2 were added followed by bromoacetic acid (1.05 g, 7 mmol) or acrylic acid (0.5 g, 7 mmol) and DIEA (0.95 ml, 7.5 mmol). The flask was placed into a motor flask shaker and was shaked for two hours at room temperature. The solution was then filtered and the resin beads were washed alternatively with CH_2Cl_2 and iPrOH (3X 50 ml) and MeOH (2X50 ml) and dried on N_2.

2- Reaction of the polyamine with the Bromoacetyl or acriloyl resins: ten fold molar excess of polyamines were dissolved in 50 ml DMF or CH_2Cl_2, added to the flask and agitated for two hours. The solvent was filtered and the resin was washed alternatively with CH_2Cl_2 and iPrOH (3X 50 ml) Kaiser tests (cold and 110 °C) are positive.

3- Protection of the amine groups of the functionalysed polyamine on the solid support:

Dde/Boc stepwise protection: DdeOH (1.99 g; 10.3 mmol) were dissolved in DMF (50 ml) and added to the flask shaker, the reaction was left 2 h at room temperature under shaking. The solvent was filtrated and the resin was washed alternatively with CH_2Cl_2 and iPrOH (4 X 50 ml), cold Kaiser test after 10 min is negative, and positive after 10 min. at 110 C° (violet). Di-tert butyl dicarbonate (27 mmol) and DIEA (13.8 mmol) were dissolved in dichloromethane (50 ml) and added to the flask shaker, the reaction was left overnight at room temperature under shaking. The solvent was filtrated and the resin was washed alternatively with CH_2Cl_2 and iPrOH (4 X 50 ml), MeOH (2X50 ml) and ether (2X 50 ml) and dried on N_2, Kaiser test was negative.

Direct Boc protection: Di-tert butyl dicarbonate (48 mmol) and DIEA (50 mmol) were dissolved in dichloromethane (50 ml) and added to the flask shaker, the reaction was left overnight at room temperature under shaking. The solvent was filtrated and the resin was washed alternatively with CH_2Cl_2 and iPrOH (10 X 50 ml), MeOH (2X50 ml) and ether (2X 50 ml) and dried on N_2. Kaiser test was negative.

4- Cleavage of the protected polyamino acids from the resin:

The resin was placed in a 250 ml round bottomed flask equipped with a magnetic stirrer. A solution composed of 50 ml CH_2Cl_2 and 25 ml CF_3CH_2OH was added and stirred for two hours at room temperature. The solution was filtered and the resin washed with 100 ml CH_2Cl_2. The organic fractions were collected and evaporated. The crude products were purified by flash chromatography on SiO_2 with $CHCl_3$/MeOH (9:1) as eluent and 8:2 for product **3**. The fractions containing the good products were identified by T.L.C. and characterized by mass spectroscopy and NMR.

$BocNH(CH_2)_3NBoc(CH_2)_4NBoc(CH_2)_3NBocCH_2CO_2H$ 1:

Yield 40 %, TLC: R_f= 0.9 ($CHCl_3$/MeOH, 8:2), HPLC, R_t= 4,22 min, (H_2O/MeCN: 3 min [40/60], 3-20 min [0/100], 35 min [0/100] , ^{1}H NMR(400 MHz, $(CD_3)_2SO$ d6 with some drops of CD_3COOD d4, δ in ppm) : 1,40 (4 s, 36H : $C(CH_3)_3$) ; 1,46 (mt, 4H : CH_2 CH_2 central butylene) ; 1,64 et 1,74 (2 mts, 2H each: CH_2 central propylene) ; 2,96 (t, J = 7 Hz, 2H : CH_2NCOO) ; 3,15 (mt, 8H : CH_2N CH_2) ; 3,23 (t, J = 7,5 Hz, 2H : CH_2NCOO) ; 3,83 (s, 2H : OCON CH_2COO). MH^+: 661

$BocNH(CH_2)_3NBoc(CH_2)_4N[(CH_2)_3NHBoc]CH_2CO_2H$ 2:

During the synthesis of product [1], product [2] is isolated after SiO_2 purification. Yield: 8 %. TLC Rf= 0.5 ($CHCl_3$/MeOH, 8:2); ^{1}H NMR (400 MHz, $(CD_3)_2SO$ d6, δ in ppm) : 1.30-1.60 (mt, 4H : $(CH_2)_2$ centrals butylene) ; 1,40 (s, 27H : $C(CH_3)_3$) ; 1,56 (mt, 4H : CH_2 propylenes) ; 2,68 and 3,11 (respectively t large and t, J = 7 Hz, 4H each : NCH_2 butylene and NCH_2 propylenes) ; 2,90 and 2,96 (2 q, J = 7 Hz, 2H each: $BocNHCH_2$ propylenes); 3,18 (s, 2H : N CH_2COO). MH^+: 561

$DdeNH(CH_2)_3NBoc(CH_2)_4NBoc(CH_2)_3NBocCH_2CO_2H$ 3:

Yield 17 % , TLC: R_f =0.55 ($CHCl_3$/MeOH, 8:2), HPLC, R_{t1}= 6.2 min (H_2O/MeCN: 3 min [40/60], 3-20 min [0/100], 35 min [0/100] , Rt2 = 16.22 (H_2O/MeCN: 3 min [80/20], 3-20 min [0/100], 35 min [0/100] ^{1}H NMR (400 MHz, $(CD_3)_2SO$ d6, at temperature of 413K, δ in ppm) : 1.02 (s, 6H : CH_3) ; 1.46 (s, 27H : $C(CH_3)_3$) ; 1.55 (m, 4H : $(CH_2)_2$ of butyl) ; 1.78 and 1.88 (2 m,

2H each : CH_2 of propyl) ; 2.32 (s, 3H : CH_3) ; 2.50 (m overlapped by the DMSO signal : $COCH_2$) ; from 3.15 to 3.35 and 3.46 (2 m, respectively 10H and 2H : NCH_2 of butyl and NCH_2 of propyl) ; 3.72 (s, 2H : NCH_2COO) ; 13.20 (mf, 1H : COOH). MH^+: 725

$DdeNH(CH_2)_3NBoc(CH_2)_4N[(CH_2)_3NHDde]CH_2CO_2H$ 4:

Yield 12 %, TLC: R_f =0.26 ($CHCl_3$/MeOH, 8:2), HPLC, R_t= 11,4 m (H_2O/MeCN: 3 min [80/20], 3-20 min [0/100], 35 min [0/100]. ^1H NMR (400 MHz, $(CD_3)_2SO$ d6, at temperature of 413K, δ in ppm) : 1.02 (s, 9H : $C(CH_3)_3$) ; 1.45 (s, 12H : CH_3) ; 1.55 (m, 4H : $(CH_2)_2$ of butyl) ; 1.78 and 1.87 (2 m, 2H each : CH_2 of propyl) ; 2.32 (s, 6H : CH_3) ; 2.52 (m overlapped by the DMSO signal : CH_2CO) ; 2.65 and 2.72 (2 bt, J = 7 Hz, 2H each : CH_2NCH_2) ; 3.20 (s, 2H : NCH_2COO) ; from 3.15 to 3.55 (4 m, 2H each : NCH_2). MH^+: 689

$\{BocNH(CH_2)3\}_2N(CH_2)_4N\{(CH_2)_3NHBoc\}(CH_2)_3NBocCH_2$ CO_2H 5:

Yield: 35 %, TLC: R_f= 0,2 ($CHCl_3$/MeOH, 8:2), ^1H NMR (400 MHz, $(CD_3)_2SO$ d6 with some drops of CD_3COOD d4, temperature of 433K, δ in ppm) : 1,42 (s, 36H : $C(CH_3)_3$) ; 1,56 (mt, 4H : CH_2 CH_2 central butylene) ; from 1,65 to 1,85 (mt, 8H : CH_2 central propylenes) ; 2,76 (mt, 12H : $CH_2N(CH_2)_2$) ; 3,06 (t, J = 6,5 Hz, 6H : OCON CH_2) ; 3,29 (mt, 2H : N CH_2) ; 3,86 (s, 2H : OCON CH_2COO). MH^+: 775

$\{BocNH(CH_2)2\}_2N(CH_2)_2NBocCH_2$ CO_2H 6:

Yield: 29 %, TLC, R_f= 0,55 ($CHCl_3$/MeOH, 8:2), ^1H NMR (400 MHz, $(CD_3)_2SO$ d6 temperature of 393 K, δ in ppm) : 1,44 (s, 27H : $C(CH_3)_3$) ; 2,58 (t, J = 6,5 Hz, 4H : CH_2N CH_2) ; 2,66 (t, J = 7 Hz, 2H : N CH_2) ; 3,04 (q, J = 6,5 Hz, 4H : OCON CH_2) ; 3,28 (t, J = 7 Hz, 2H : OCON CH_2) ; 3,76 (s, 2H : OCON CH_2COO); 6,06 (mf, 2H : CONH). MH^+: 505

(1,4,8-tri-Boc-1,4,8,11-tetraazacyclotetradecane-11-yl)-acetic acid 7:

Yield: 37 %, TLC, R_f= 0,57 ($CHCl_3$/MeOH, 1:1), HPLC, R_t= 12.1 m (H_2O/MeCN: 3 min [80/20], 3-20 min [0/100], 35 min [0/100]. ^1H NMR (400 MHz, $(CD_3)_2SO$ d6, at temperature of 383K, δ in ppm) : 1.44 (s, 27H : $C(CH_3)_3$) ; 1.66 and 1.88 (2 m, 2H each : CH_2 of propyl) ; 2.66 and 2.82 (2 bt, J = 6.5 Hz, 2H each : CH_2NCH_2) ; 3.12 (s, 2H : NCH_2COO) ; from 3.25 to 3.45 (m, 12H : $OCONCH_2$). MH^+ : 559

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