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Scale-Up of Trisodium [(3β,5β,12α)-3-[[4(*S*)-4-[Bis[2-[bis[(carboxy-*kO*)methyl]amino-*kN*]ethyl]amino-*kN*]-4-(carboxy-*kO*)-1-oxobutyl]amino]-12-hydroxycholan-24-oato(6-)]gadolinate(3-)], a Gd(III) Complex under Development As a Contrast Agent for MRI Coronary Angiography

Pier Lucio Anelli,* Marino Brocchetta, Luciano Lattuada, Giuseppe Manfredi, Pierfrancesco Morosini, Marcella Murru, Daniela Palano, Marco Sipioni, and Massimo Visigalli

Centro Ricerche Bracco, Bracco Imaging Spa, via Ribes 5, 10010 Colleretto Giacosa (TO), d Italy

Abstract:

Process chemistry involved in the discovery and development routes to trisodium [(3β,5β,12α)-3-[[4(*S*)-4-[bis[2-[bis[(carboxy-*kO*)methyl]amino-*kN*]ethyl]amino-*kN*]-4-(carboxy-*kO*)-1-oxobutyl]-amino]-12-hydroxycholan-24-oato(6-)]gadolinate(3-)] (B22956/1) starting from L-glutamic acid and (3α,5β,12α)-3,12-dihydroxycholan-24-oic acid is described. The best process is based on seven chemical steps and overcomes difficult purification protocols. Such process has been successfully implemented to prepare multikilogram batches of the target compound in 20% overall yield from (3α,5β,12α)-3,12-dihydroxycholan-24-oic acid.

Introduction

Over the past decade, several complexes of paramagnetic metal ions have been tested as contrast agents for *in vivo* magnetic resonance imaging (MRI) and a few of them have been brought into clinical practice. Gd(III) complexes of ligands derived from diethylenetriaminepentaacetic acid (DTPA) have played a major role in this field.[1,2] Furthermore, the conjugation of DTPA-like ligands to bile acids has been pursued to achieve MRI contrast agents featuring improved hepatospecificity.[3] In addition, the quite strong binding to human serum albumin of the cholanoic residue proved able to guarantee a prolonged permanence in blood vessels, and accordingly, one of these conjugates, **B22956/1** (Scheme 1), has been selected for development as contrast agent for MRI coronary angiography.[4,5] Preliminary imaging results in animal models were very promising,[5,6] and presently, **B22956/1** has successfully completed phase 1 clinical studies.[7]

When **B22956/1** was first prepared in the lab, it was synthesized following the route previously reported for the analogous complex featuring a subunit of cholic acid (Scheme 1).[8]

Indeed, compound **1**, containing a DTPA skeleton and an additional carboxylic group for the amidation, is obtained from L-glutamic acid, a cheap starting material, and amino ester **2** can be obtained from the commercially available (3α,5β,12α)-3,12-dihydroxycholan-24-oic acid.[9] To introduce the amino group in position 3 the different reactivity of the two hydroxy groups, (i.e., the axial hydroxy group in position 12 is much less reactive than the equatorial one in position 3) was exploited.[10]

The amidation of **2** with **1** (DCC/HOBT/CH_2Cl_2, silica gel flash-chromatography, 94% yield) afforded the hexaester **3** which was deprotected to the hexaacid **4** [(i) TFA, (ii) aq NaOH, (iii) aq HCl; 98% yield]. The latter was complexed to **B22956/1** ($GdCl_3$/aq NaOH, 88% yield) in 81% overall yield (Scheme 1). As an alternative, the amidation was performed with diethyl cyanophosphonate and triethylamine in DMF (83%).[11]

The main critical issues for the scale-up of this synthetic approach were: (i) aminoester **2** was prepared following a too expensive route, and (ii) acid **1** was obtained in too-low yields. Furthermore, it slowly racemises on storage for long times. Accordingly, alternative synthetic routes leading to **4** had to be investigated.

Here, we report how the initial discovery route to **B22956/1** was modified into a process suitable for kilogram-scale preparations.

Results and Discussion

Synthesis of Aminoester 2. Aminoester **2** was a key intermediate of the above-described process, and its synthesis

* pier.lucio.anelli@bracco.com.

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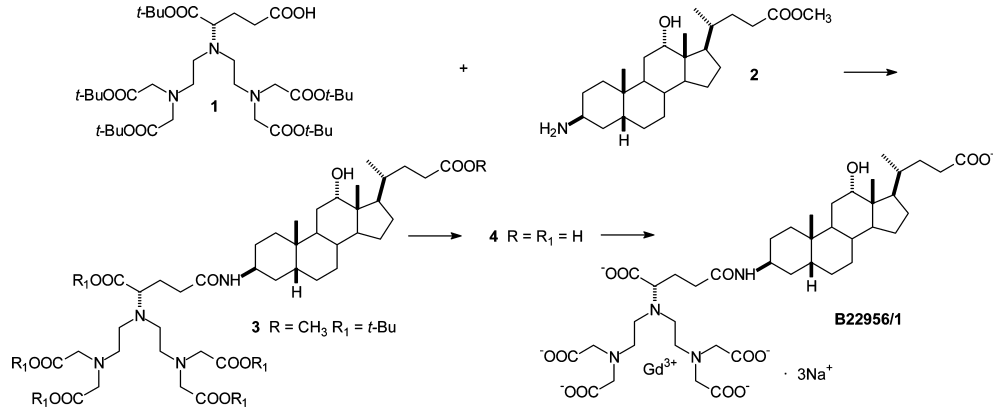
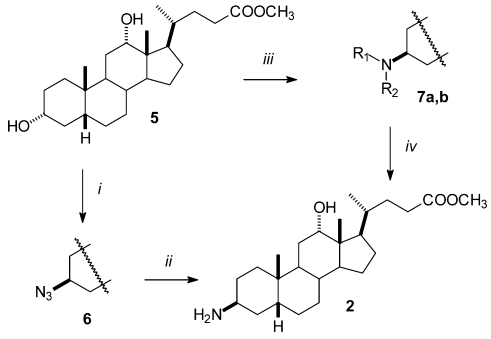
Scheme 1

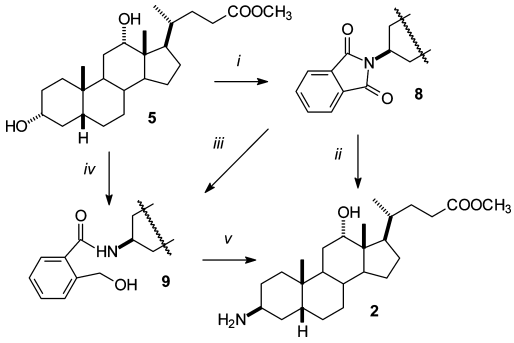


Scheme 2[a]



[a] Reagents and yields: (i) (1) MsCl/toluene/Et_3N followed by NaN_3 in H_2O/toluene/*n*-Bu_4NBr, 64%; or (2) MsCl/pyridine followed by NaN_3 in DMF, 71%; or (3) Ph_3P/DEAD/DPPA/THF, 70%; (ii) (1) Ph_3P/CH_3CN then H_2O, 86%;[9,13] or (2) H_2/Pd−C/THF, 30 °C, 50 bar, 80%; or (3) H_2/Ni Raney/THF, 30 °C, 1 bar, 80%; (iii) Ph_3P/DEAD/(diethoxyphosphinyl)carbamic acid 1,1-dimethylethyl ester/THF, 44% **7a** [R_1 = *t*-BuO_2C, R_2 = $(EtO)_2OP$];[14] Ph_3P/DEAD/imidocarbonic acid bis(phenylmethyl) ester/THF, 45% **7b** ($R_1 = R_2$ = Cbz);[15] (iv) HCl/MeOH for **7a**, 87%; H_2/Pd−C/MeOH for **7b**, 83%.

Scheme 3[a]



[a] Reagents and yields: (i) Ph_3P/DIAD/phthalimide/THF, 80%; (ii) (1) N_2H_4, 84%;[20] or (2) ethylenediamine/MeOH (76%);[21] or (3) other amines *i.e.* 2-aminoethanol, aq CH_3NH_2,[22] *n*-$BuNH_2$,[23] NH_4OH or CH_3NH_2/EtOH,[24] 5−50%; (iii) $NaBH_4$/DMA/phosphate buffer pH 8 then H_2O, 99%; (iv) Ph_3P/DIAD/phthalimide/DMA followed by $NaBH_4$/DMA/phosphate buffer pH 8 then H_2O, 77%; (v)HCl/MeOH then H_2O/NaOH, 85%.

was explored in depth. (3α,5*β*,12α)-3,12-Dihydroxycholan-24-oic acid was reacted in MeOH/PTSA·H_2O to afford **5** (93%), then several possibilities to transform the latter into **2**, e.g. through intermediates **6**, **7**, or **8** (Schemes 2 and 3), were investigated.

A reaction was needed which would transform the 3α-OH group of **5** into the 3*β*-NH_2 group of **2** with complete inversion of configuration. Accordingly, we investigated both classical S_N2 and Mitsunobu reactions.[12a,b]

An alternative transformation of **5** into **2** was explored reacting the methanesulfonate of **5** (MsCl in pyridine) with ammonia as well as classical and modified Gabriel reagents [potassium phthalimide, $Boc_2N^-K^+$, CF_3CONH_2, $NaN(CHO)_2$].[16] With potassium phthalimide the yield was very low (13%), while the other reagents gave complicated mixtures and no desired product.

An additional route, not depicted in Scheme 2, to **2** was also investigated starting with the oxidation of **5** under Oppenauer conditions [Al(*t*-BuO)$_3$/acetone or toluene][17] to afford a mixture of the desired 3-keto ester and the corresponding 3-keto acid in moderate yield. Pure 3-keto ester was obtained by oxidation with massive amounts of Ag_2CO_3 over Celite.[18] The reductive amination of the 3-keto derivatives (both methyl ester and acid) was performed as reported in the literature[19] using $AcONH_4$ and $NaBH_3CN$ in MeOH. However, this approach was abandoned as mixtures of the 3α and 3*β*-NH_2 derivatives were obtained.

Despite the quite good overall yield for the conversion of **5** into **2** with a two-step synthesis through **6**, the use of hazardous sodium azide raised some concern. As an alternative, we explored the routes depicted in Scheme 3.

The reduction with $NaBH_4$ of one of the carbonyl groups of the phthalimido residue to afford an intermediate like **9** which, in acidic medium, gives the desired amine and 1-isoben-

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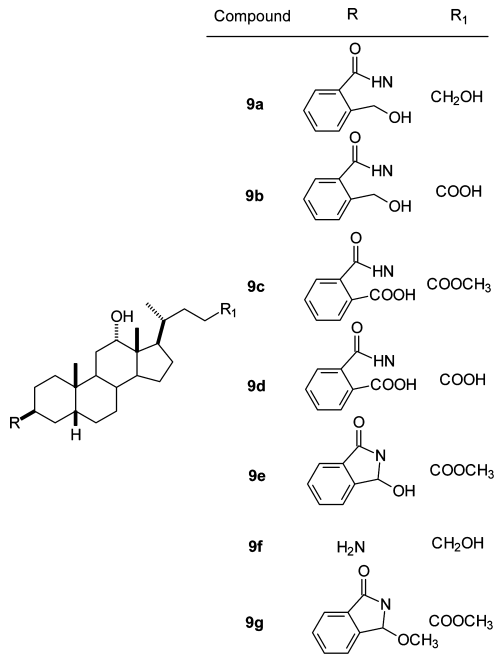

Figure 1

zofuranone is reported in the literature[25] as the mildest method for the deprotection of the phthalimido group. It has been especially applied to protected aminoacids, but has never been used for cholanoic derivatives. The reaction was studied in depth before reaching the final conditions (Scheme 3, reaction (iii)) as those reported in the literature (2-PrOH/H_2O, 5 mol equiv of $NaBH_4$, 20 °C) were not suitable due to the scarce solubility of **8**. Indeed, the reaction had to be performed under very dilute conditions (i.e., 2.5% concentration) which are not appealing from an industrial perspective. Furthermore, after 24 h at 40 °C, **9** was recovered in only 45% yield. Interestingly, several byproducts (**9a**−**g**, Figure 1) were isolated by silica gel chromathography and characterized.

Most of the byproducts stem from the presence of the methyl ester moiety, which is sensitive to both reduction and alkaline hydrolysis. In particular, working with 5 mol equiv of $NaBH_4$, compound **9a** was formed in about 25% yield (TLC) and could not be eliminated from **9** by crystallization from several solvents. The subsequent acidic hydrolysis converted **9a** into **9f**, whose very low solubility prevented its elimination from crude **2**. Compound **9e**, deriving from incomplete reduction of **8**, afforded during the following acidic hydrolysis compound **9g** that could be only partially eliminated from crude **2**. Compounds **9b**−**d** resulted from hydrolysis at alkaline pH values and lowered the reaction yield. However, in the following step, they afforded byproducts that could be eliminated to a great extent. As a consequence of the above-mentioned results, the reaction conditions were investigated to understand the effect of (1) solvent, (2) stoichiometry, (3) pH, (4) concentration, (5) temperature, and (6) reaction time, in reactions on the 200-g scale. The findings can be summarized as follows: (1) DMF proved a much better solvent, in comparison with 2-PrOH, to lower the formation of **9a** (about 3%) but was hydrolysed to some extent, affording the undesired dimethylamine. *N,N*-Dimethylacetamide (DMA) and *N*-methylpyrrolidone were quite stable, and the content of **9a** was around 2%. All the aforementioned solvents worked in concentrated solutions, and DMA was selected as the solvent of choice; (2) the excess of $NaBH_4$ was lowered from the initial 5.0 to 0.97 mol equiv, still observing a good reactivity. However, when the amount of $NaBH_4$ was further reduced to 0.85 or 0.7 mol equiv, the reaction was incomplete and afforded significant amounts (8−12%) of **9e**; (3) the first trials were performed in organic solvents/water mixtures. Under these conditions the final pH value largely exceeded 13, and as a consequence, the formation of **9b**−**d** was not negligible at all. The use of a 2 M phosphate buffer (pH 8) allowed a certain control of the pH in the earlier stage of the reaction. Indeed, although the mixture is strongly alkaline (pH 12.2) at the end of the reaction, hydrolytic cleavage of the labile functions is very limited: about 3−5% of **9c** is formed, along with traces of **9b** and **9d**; (4) the concentration of **8** was progressively increased up to 10%, which resulted in the upper limit. In fact, when it was further increased to 13%, **8** started to precipitate, and the amount of **9a** significantly increased (3.6%) in the reaction crude; (5) the reaction was performed at 45−48 °C in order to avoid the precipitation of both **8** and **9**; (6) the choice of the reaction time was quite crucial and was tentatively found after the other parameters were adjusted. After 55−60 min, the reaction was stopped by addition of AcOH because the undesired formation of **9a** started to become significant. When we changed the conditions and worked in DMA/phosphate buffer, **9** was conveniently precipitated by addition of water and recovered by filtration, thus leaving the inorganic salts in the mother liquor. The deprotection of isolated **9** was performed with conc HCl/MeOH at 50 °C. These conditions are advantageous since the byproduct **9b** is newly esterified, affording **9** which is the precursor of **2**.

The process was further improved when the isolation of **8** was avoided. In this respect, $NaBH_4$ was suspended in the solution of crude **8** in DMA, and after obtaining a solution, the reduction was performed at a temperature <50 °C. It must be underlined that **5** started to precipitate as a crystal containing 0.5 mol of MeOH when the scale of the esterification reaction was increased, working in more concentrated solutions. This was beneficial for the purification of **5** and simplified the workup but imposed the distillation of MeOH from the DMA solution in the next step, as MeOH would have been a reagent in the Mitsunobu reaction leading to **8**.

New Synthetic Approach to Ligand 4. Owing to the problems linked to the use of acid **1** in the discovery route (*vide infra*), a different strategy to afford the target ligand **4** was investigated. While in the discovery route the disconnection approach involved at first the formation of bonds **a** and then of bond **b** (Figure 2), in the development route the disconnection was turned the other way around, i.e. first bond **b** and then bonds **a** are formed.

This approach required the choice of a suitably protected L-glutamic acid derivative. The results of the amidation of **2** with *N*-Cbz-L-glutamic acid 1-*t*-Bu ester and coupling agents were discouraging because of the troublesome purification of the crude product and the consequent very low yield. The reaction with *N*-Cbz-L-glutamic acid anhydride, although oc-

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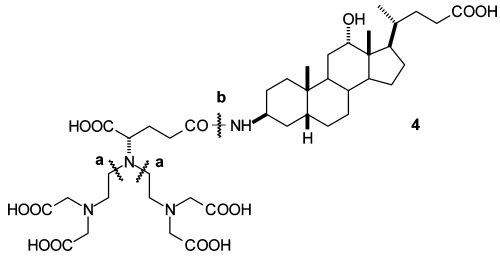
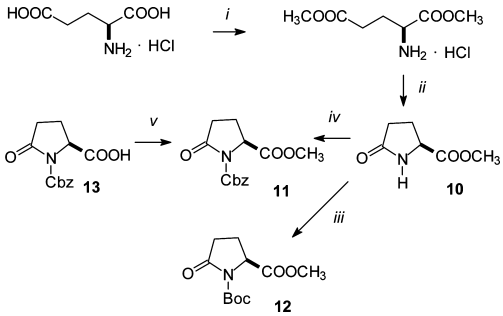


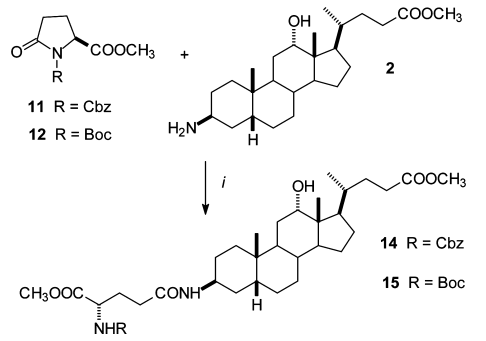
Figure 2

Scheme 4[a]



[a] Reagents and yields: (i) $SOCl_2$/MeOH; (ii) KOH/MeOH, 72% (steps i and ii);[28a,b] (iii) Boc_2O/EtOAc/DMAP, 91%;[29] 50%; (iv) CbzCl/CH_2Cl_2, 32%; (v) MeI/DIPEA/CH_2Cl_2, 98%.

Scheme 5[a]



[a] Reagents and yields: (i) **14**: (1) dioxane, 50 °C 24 h, 95 °C 29 h, chromatography, 75%; or (2) toluene, 100 °C, 24 h, chromathography, 70%; **15**: toluene, 90 °C, 24 h, 74%.

curring in good yields, was ruled out because of the formation of the unwanted regioisomer, i.e. the one deriving from amidation of the carboxy group in position 1. Eventually, the ring-opening of a protected pyroglutamate with amine **2** was taken into account. The only reference[26] found in the literature reports a reaction performed with a large excess of amine (6 mol equiv) and catalyzed by KCN under ultrasonic irradiation in order to shorten the reaction time. Obviously, such conditions are not suited for an industrial application. Conversely, several cases of ring-opening of protected pyroglutamates and lactams with other nucleophiles have been reported.[27a–d]

The preparation of two potential candidates, **11** and **12**, was performed as reported in Scheme 4.

We subsequently investigated the reaction of **11** with **2** to afford intermediate **14** (Scheme 5). This after deprotection and bisalkylation with **17** (Scheme 6) under Rapoport-like conditions,[30] affords hexaester **19** which is a useful precursor for the preparation of **B22956/1**.

We discovered that **14** could be obtained, although a silica gel chromatography was needed for its purification. In parallel, the analogous reaction of **2** and **12** (Scheme 5) performed in toluene afforded **15**, which spontaneously crystallized, as a solvate with toluene, on cooling the reaction mixture. A noticeable improvement was thus achieved as the chromatographic purification could be avoided. Furthermore, the crystalline solid **15**, unlike the waxy **14**, could be easily stored. An additional important advantage was related to the following deprotection (Scheme 6).

For the new route we still needed as alkylating agent mesylate **17**, which in our hands proved a convenient replacement to the corresponding bromide classically used in the Rapoport approach to DTPA frameworks.[30] The preparation of the precursor **16** underwent several optimisations and was finally carried out with a very simplified workup. Compound **18**, which was at first synthesised from **14** by hydrogenolysis of the Cbz protecting group (H_2/Pd−C/MeOH, 1 bar, rt, 95%), could be obtained, in a simpler and safer way, through Boc deprotection of **15**. The acidic deprotection did not induce racemisation at the stereogenic centre in the amino ester moiety. Several trials, performed using different solvents and acids, showed that **18** could be isolated as hydrochloride or methanesulfonate and obtained as free amine from MeOH/H_2O mixtures, after neutralisation with DIPEA.

The bisalkylation of **18** with **17** needed acetonitrile/pH 8 phosphate buffer (or H_2O maintained at pH 8.5 with NaOH) and gave **19** in 68% yield after silica gel flash chromatography. The use of H_2O/EtOH or H_2O/*i*-PrOH mixtures, as well as of pure solvents (EtOAc, DMA), gave worse results. Subsequent studies showed that the bisalkylation of **18** could also be performed in EtOAc in the presence of DIPEA (50 °C for 18 h, silica gel flash-chromatography, 74%) using crude **17** in its turn prepared in EtOAc. Compound **19** was prepared in kilogram-scale in EtOAc and needed a silica gel chromatography purification using EtOAc/*n*-hexane. This procedure, not suitable for scale-up, allowed the elimination of most of the byproducts, mainly deriving from incomplete alkylation or quaternisation of the nitrogen atoms and/or lactamisation. Alternative purifications of **19** such as liquid extraction with water/organic solvents mixtures, did not prove suitable. Subsequent studies showed that, using *n*-BuOAc instead of EtOAc as the reaction solvent, the amount of some byproduct was reduced, and accordingly, both preparations of **17** and of **19** were performed in *n*-BuOAc. Moreover, we realised that a thermal treatment of the crude, while not affecting the content of **19**, transformed most of the byproduct (e.g., quaternary onium salts) into others that, after the following alkaline hydrolysis, could be to a great extent eliminated by elution through a nonfunctionalized polystyrene

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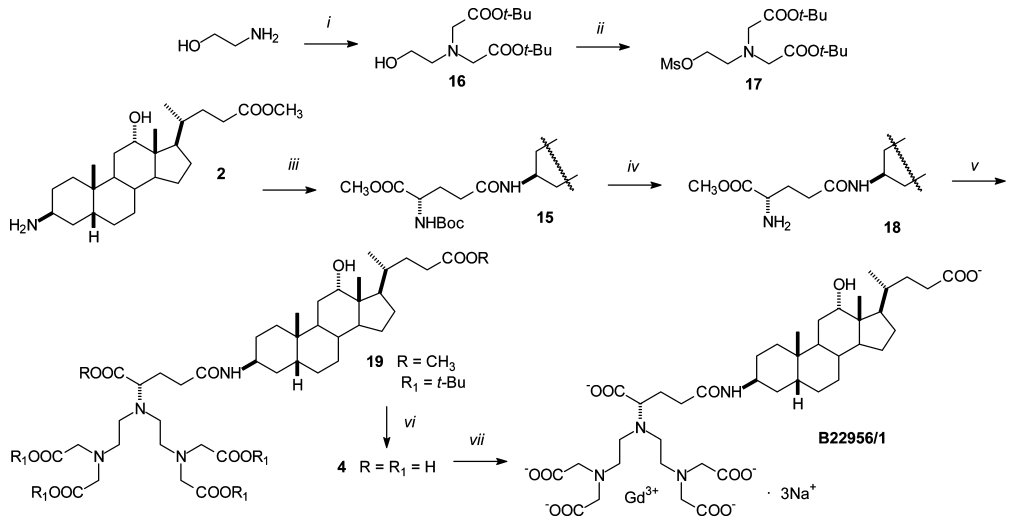
Scheme 6[a]



[a] Reagents and yields: (i) *tert*-butyl bromoacetate/DMA/Na_2CO_3 then H_2O, 76.5%; (ii) MsCl/EtOAc/DIPEA, chromatography, 71%; (iii) **12**/toluene, 77%; (iv) (1) $MeSO_3H$/MeOH, 85%; or (2) HCl/MeOH, 90%; (3) 37% aq HCl/MeOH, 80%; (v) **17**/EtOAc/DIPEA, 75%; (vi) LiOH/dioxane, 90%; (vii) H_2O/Gd_2O_3/NaOH, 83%.

resin. Consequently, the chromatographic purification was postponed to the following step.

For the deprotection of the *t*-butyl esters of **19**, the use of trifluoroacetic acid, although very efficient, was expensive and needed a further alkaline deprotection step for the methyl esters. Accordingly, we studied the possibility to deprotect both methyl and *tert*-butyl esters with a single reagent, and among others, LiOH proved the most effective. Compound **4** was at first obtained by treatment of pure, isolated **19** with LiOH in dioxane (rt, 72 h) followed by precipitation at pH 1.5 (90% yield). Later on, dioxane was replaced with the safer *i*-PrOH, crude **19** was directly used and NaOH employed as the base of choice. Besides being cheaper than LiOH, NaOH was preferable because sodium was the cation already present in the final product. The racemisation at the stereogenic centre in the DTPA moiety slightly increased, passing from LiOH to NaOH, but the epimer content could be maintained below 2%. In particular, we used at first *i*-PrOH/H_2O/LiOH, 25 °C, 20 h then *i*-PrOH/H_2O/NaOH, 15−20 °C, 3 h then 25 °C, 20 h. The solution containing crude **4** was then purified by elution through Amberlite XAD 16.00 loading the crude at neutral pH. This allowed to absorb on the resin the lipophilic impurities only and to remove the hydrophilic ones in the early eluting fractions. Compound **4** was recovered, in 60% yield (from **18**), with a total impurity content lower than 2%.

B22956/1 was prepared by complexation of **4** with Gd_2O_3 in the presence of NaOH, and then it was crystallized from acetone/water (90% overall), a procedure which increased the purity of the isolated compound, especially lowering the 3β(*R*) epimer content.

Stereochemical Issues. Besides controlling the chemical and stereochemical purity of the final product, we also needed to check the stereochemical purity of most intermediates, focusing on the two stereogenic carbon atoms which are likely to undergo stereochemical inversion under the experimental conditions employed. For the sake of clarity, we refer to the stereogenic carbon atom of the DTPA residue and the carbon atom in position 3 of the cholanoic moiety. Possible inversion at all the other stereogenic carbon atoms contained in the stereoidic residue was deemed unlikely. Besides the compounds of interest, i.e. the 3β(*S*) isomers reported in Scheme 5 (*note*: the stereochemical configuration of the stereogenic carbon atom present in the residue linked to the position 3 of the cholanoic skeleton is indicated in parentheses), the 3β(*R*) and/or the 3β(*RS*) epimeric mixtures were also prepared while for the 3α isomers only the combination with the (*S*) products was taken into account. Indeed, the formation of 3α(*R*) isomers during the synthesis was considered very unlikely because they would derive from reactions between impurities. Furthermore, it is noteworthy that racemisation at the stereogenic centre in the DTPA moiety can in principle occur under strongly acidic or basic conditions. Conversely, racemisation at the stereogenic carbon in position 3 of the cholanoic moiety, and therefore the conversion of 3β into 3α isomers, is extremely unlikely. The entry to 3α derivatives stemmed from the transformation of **5**, under Mitsunobu conditions, into the inverted formyloxy derivative[31] (Ph_3P/DEAD/HCOOH/THF, 95%) which was deprotected with HCl/MeOH (70%) to the 3β-OH epimer. From there on, the path through the azide depicted in Scheme 3 was followed so that the subsequent Mitsunobu reaction (Ph_3P/DEAD/DPPA/THF, 73%) again inverted the configuration, affording the 3α-azide, in its turn reduced (Ph_3P/CH_3CN then H_2O, 66%) to (3α,5β,12α)-3-amino-12-hydroxycholan-24-oic acid methyl ester. The latter was the starting material for the 3α(*S*) isomers of **15**, **18**, **19**, **4**, and **B22956/1**. The 3β(*R*) isomers were prepared, according to Scheme 5, using compound **12** with *R* configuration.

Conclusions

The discovery route to **B22956/1** through the versatile intermediate **1** was totally revised during development. Indeed, a different disconnection approach was pursued. L-Glutamic acid was conveniently incorporated into the scaffold after conversion

(31) Bose, A. K.; Lal, B.; Hofman, W. A.; Manhas, M. S. *Tetrahedron Lett.* **1973**, *18*, 1619–1622.

into an ester of *N*-Boc protected pyroglutamic acid. This species proved very effective for a smooth coupling with aminodeoxycholate **2**. The construction of the DTPA moiety was then achieved following a Rapoport-like approach. The new route allowed to produce multikilogram batches of **B22956/1** for the clinical trials in 20% overall yield from (3α,5*β*,12α)-3,12-dihydroxycholan-24-oic acid.

Experimental Section

(3α,5*β*,12α)-3,12-Dihydroxycholan-24-oic acid methyl ester (5). Monohydrate *p*-toluenesulfonic acid (152.7 g; 0.8 mol) was added to a suspension of (3α,5*β*,12α)-3,12-dihydroxycholan-24-oic acid (1570.3 g; 4.0 mol) in MeOH (6.15 L) stirred at rt. After 1 h a solution was obtained, and after additional 1 h, the desired product started to precipitate. The suspension was stirred for 23 h and then filtered, and the crystalline solid was suspended in H_2O (3 L) and stirred for 1 h. After filtration, washings with H_2O (3 × 0.8 L), and drying, **5** (1205.9 g; 2.85 mol) was obtained as a white solid containing 0.5 mol MeOH. The mother liquor was concentrated to 2.5 kg and stirred at rt for 20 h. Using the above-described methodology a second crop of **5**.0.5 MeOH (383.3 g; 0.907 mol) was obtained. Overall yield 94%. Mp 100−108 °C (Mp 67−70 °C for the compound devoid of MeOH) (in the literature,[32] mp 70−108 °C is reported); TLC: R_f 0.39 (eluent A).

(3*β*,5*β*,12α)-3-(1,3-Dihydro-1,3-dioxo-2H-isoindol-2-yl)-12-hydroxycholan-24-oic Acid Methyl Ester (8). Into a solution of **5** (512 g; 1.259 mol, as a product without MeOH was used) and triphenylphosphine (372.7 g; 1.421 mol) in THF (1.5 L), phthalimide (202.8 g; 1.378 mol) was suspended, and then a solution of diisopropyl azodicarboxylate (284.7 g; 1.408 mol) in THF (0.49 L) was dropped over 1.5 h, maintaining the temperature of the reaction mixture at 15 °C. The solution thus obtained was left at rt for 18 h, and then the solvent was distilled, and the oily crude (1518 g) was dissolved with MeOH (3.2 L) and stirred for 20 h. The crystalline solid which precipitated was filtered, washed with MeOH (700 + 2 × 350 mL), and dried to afford **8** (481.3 g; 0.898 mol) as a white solid. The mother liquor and the washings were concentrated to 2.35 kg, and after stirring for 20 h at 3 °C, a second crop of **8** (61.6 g; 0.114 mol) was recovered. Overall yield 80%. Mp 161.1−161.8 °C; TLC R_f 0.90 (eluent D); HPLC 98.7% (method A).

(3*β*,5*β*,12α)-12-Hydroxy-3-[[2-(hydroxymethyl)benzoyl]-amino]cholan-24-oic Acid Methyl Ester (9). A solution of **8** (225 g; 0.42 mol) in DMA (1.75 L), vigorously stirred at 34 °C, was diluted with 2 M phosphate buffer (pH 8, 0.5 L) and a cloudy mixture at 43 °C was obtained. Solid $NaBH_4$ (15.45 g; 0.408 mol) was added in 5 min, and the suspension was stirred at 47−48 °C. After 55 min an almost clear solution was obtained in which AcOH (50 mL) was added to neutralise the hydride excess and to correct the pH, from 12.8 to about 8. The mixture was poured into H_2O (4.6 L) and stirred for 3 h, the precipitate was filtered and suspended in H_2O (4 L) then stirred for 30 min. Finally, the precipitate was filtered, washed with H_2O (1.5 L), and dried to obtain **9** (224.6 g; 0.416 mol) as a white solid. Yield 99%. Mp 190.2−192.7 °C; TLC R_f 0.80 (eluent D); HPLC 93% (method A).

(32) Chang, F. C.; Wood, N. F.; Holton, W. G. *J. Org. Chem.* **1965**, *30*, 1718–1723.

Transformation of 5 into 9 without Isolation of 8. A solution of **5**.0.5 MeOH (75 g; 0.177 mol) in DMA (240 mL) was concentrated under reduced pressure to 230 g and then diluted with DMA (60 mL) and cooled to rt. Triphenylphosphine (52.5 g; 0.2 mol) and phthalimide (28.5 g; 0.194 mol) were added, the resulting solution was cooled to 15 °C, and then a solution of diisopropyl azodicarboxylate (39.9 g; 0.197 mol) in DMA (70 mL) was dropped over 75 min maintaining the temperature at 15−20 °C. After 24 h at rt the reaction mixture was diluted with DMA (0.33 L), vigorously stirred at 35 °C, and diluted with 2 M phosphate buffer (pH 8, 0.25 L). Solid $NaBH_4$ (7.5 g; 0.198 mol) was added over 5 min, and the suspension was stirred at 48 °C for 2 h. AcOH (22 mL) was added to destroy the hydride excess, and in the meanwhile a clear solid started to crystallize. The suspension was stirred for 15 h at rt, and then the solid was filtered, washed at first with 65/25 DMA/H_2O (2 × 90 mL), and then with H_2O (2 × 100 mL). The wet solid was suspended in H_2O (0.4 L), vigorously stirred for 2 h, filtered, washed with H_2O (3 × 50 mL), and dried to afford **9** (73.9 g; 0.137 mol) as a white solid. Yield 77%. HPLC 95.7% (method A).

(3*β*,5*β*,12α)-3-Amino-12-hydroxycholan-24-oic Acid Methyl Ester (2). A suspension of **9** (4.38 kg; 8.11 mol) in MeOH (22 L) and 34% HCl (1.13 kg; 10.5 mol) was heated to 65 °C under stirring. In about 1.5 h a solution was obtained, and after an additional 1.5 h, the reaction mixture was cooled to 20 °C and the pH adjusted to about 3.5 with 30% NaOH. The solvent was evaporated at reduced pressure to about half volume; *n*-BuOAc (31 L) was added, and distillation was continued to eliminate MeOH. After 30 min at 50 °C, the solution was stirred at 17−20 °C for 3 h, and then the precipitated hydrochloride was filtered and washed with *n*-BuOAc. The wet product was dissolved in MeOH (15.8 L), and then 30% NaOH was added to completely neutralise the hydrochloric acid present in the salt, which had been previously titrated, and to reach pH 9.8−10.1. Water (37.5 kg) was then added at 20 °C to obtain crystallization, and after 2 h, the suspension was filtered. The solid was washed first with 7/3 H_2O/MeOH (11 L) and then with H_2O (2 × 31 L) and dried to afford **2** (2.8 kg; 6.9 mol) as a white solid. Yield 85%. Mp 155−155.5 °C; TLC R_f 0.69 (eluent D); HPLC 96.5% (method B); GC 96.3% (method A).

(*S*)-5-Oxo-1,2-pyrrolidinedicarboxylic Acid 1-(1,1-Dimethylethyl) 2-Methyl Ester (12). $SOCl_2$ (732 g; 6.15 mol) was added over 2 h to a suspension of L-glutamic acid hydrochloride (551 g; 3 mol) in MeOH (3 L) stirred at 0−5 °C. After about 3.5 h at rt the reaction mixture turned into a clear solution that was stirred for 20 h. The solvent was evaporated to give crude L-glutamic acid dimethyl ester hydrochloride (650.8 g, TLC: R_f 0.79 (eluent E); argentometric titer: 105.3%) as a thick oil. Into a solution of the latter in MeOH (1 L), was added 3 M KOH in MeOH (1.08 L; 3.24 mol) over 1.5 h, causing the precipitation of KCl that was filtered off. The clear solution was concentrated and again filtered and evaporated to a residue (600 g) which was heated at 115 °C at atmospheric pressure for about 1 h while distilling off MeOH produced by the cyclization reaction. The residue containing **10** (bp 127−130 °C/0.2 kPa; TLC: R_f 0.71 (eluent E); HPLC: *S*/*R* ratio 99.0/1.0 with method C) was diluted with EtOAc (3 L) and DMAP (7.3

g; 0.06 mol) was added, then di-*tert*-butyl dicarbonate (673 g; 3.1 mol) was added, over 1 h, to the mixture stirred at 15−17 °C. After 3 h the solution was washed with 0.066 M phosphate buffer (pH 5.8, 3 × 0.7 L) and water (2 × 0.5 L) and was dried (Na_2SO_4). The filtered solution was evaporated to a thick oil (670 g) which was taken with EtOAc (0.35 L), obtaining a solution that was diluted with *n*-hexane (0.95 L). After 24 h at rt the suspension was filtered, and the solid was washed with *n*-hexane (3 × 0.25 L) and dried (320.8 g; 1.318 mol). The mother liquor and the washings were combined and evaporated, and then the residue was analogously crystallized to afford a second crop of **12** having similar purity (160.8 g; 0.66 mol). Overall yield 66%. Mp 70−71.5 °C (lit.[33] 72−72.5 °C); TLC R_f 0.74 (eluent G); HPLC 99.6% with method A; HPLC S/R ratio 100/0 with method D.

(3*β*,5*β*,12α)-12-Hydroxy-3-[[5-methoxy-1,5-dioxo-(4*S*)-4-[[(1,1-dimethylethoxy)carbonyl]amino]pentyl]amino]cholan-24-oic Acid Methyl Ester (15). A suspension of **2** (5.36 kg; 13.2 mol) and **12** (3.31 kg; 13.6 mol) in toluene (12.3 L) was slowly heated to 90 °C to obtain a solution which was kept at the same temperature for 24 h. The temperature was slowly decreased over 4 h to 17 °C, and the suspension thus obtained was stirred at the same temperature for 4 h. After filtration and washing with toluene (6 L), the wet product was analogously crystallized with toluene (12.5 L) to afford, after drying, **15** (7.35 kg) as a white solid. The compound contained toluene (about 10%, roughly corresponding to 0.75 mol), so the yield based on the dry product (6.6 kg) resulted 77%. Mp 172−173 °C; TLC R_f 0.76 (eluent D); HPLC 99.0% with method A.

(3*β*,5*β*,12α)-3-[[(4*S*)-4-Amino-5-methoxy-1,5-dioxopentyl]-amino]-12-hydroxycholan-24-oic Acid Methyl Ester (18). $MeSO_3H$ (1.12 kg; 11.7 mol) was added to a solution of **15** (6.5 kg corresponding to 5.85 kg without toluene; 9 mol) in MeOH (17 L), and then the reaction mixture was stirred at 50 °C for 3 h. After cooling to 10 °C, DIPEA (1.51 kg; 11.7 mol) was added, the temperature was further lowered to 0 °C, and H_2O (14.5 L) was dropped in, causing the precipitation of a white solid. The suspension was stirred overnight at 0 °C and filtered, and the solid, washed with 1/1 MeOH/H_2O (3 L) and H_2O (3 L), was dried to afford **18** (4.2 kg; 7.65 mol). Yield 85%. Mp 121 °C; TLC R_f 0.64 (eluent D); HPLC 96.5% (method A).

***N*-[2-(1,1-Dimethylethoxy)-2-oxoethyl]-*N*-(2-hydroxyethyl)glycine 1,1-Dimethylethyl Ester (16).** Na_2CO_3 (0.667 kg; 6.29 mol) was added to a solution of *tert*-butyl bromoacetate (1.17 kg; 5.99 mol) in DMA (0.77 kg), and then ethanolamine (0.19 kg; 3.11 mol) was dropped in about 2 h, keeping the temperature below 40 °C. After 22 h at 40 °C and cooling to 20 °C, H_2O (7 kg) was added in order to dissolve the salts and induce the precipitation of **16**. The suspension was kept at 17 °C for 2 h and then filtered, and the solid was washed with H_2O (1.6 kg) and dried to afford **16** (6.9 kg; 2.38 mol). Yield 76.5%. Mp 55−60 °C; GC 99.8% (method B).

***N*-[2-(1,1-Dimethylethoxy)-2-oxoethyl]-*N*-[2-[(methylsulfonyl)oxy]ethyl]glycine 1,1-Dimethylethyl Ester (17).** MsCl (1.4 mL; 18.1 mmol) was dropped at −10 °C into a solution of **16** (5 g; 17.3 mmol) and DIPEA (2.89 mL; 20.7 mmol) in EtOAc (12 mL). The resulting suspension was stirred at 0 °C for 1.5 h, then the precipitate was filtered and washed with cold EtOAc (2 × 20 mL). The clear solution was evaporated under reduced pressure, and the crude was purified by silica gel flash-chromatography (7/3 *n*-hexane/EtOAc) to afford **17** (4.5 g; 12.25 mmol) as a colourless oil. Yield 71%. TLC: R_f 0.27 (eluent C).

(3*β*,5*β*,12α)-3-[[(4S)-4-[Bis[2-[bis[2-(1,1-dimethylethoxy)-2-oxoethyl]amino]ethyl]amino]-5-methoxy-1,5-dioxopentyl]-amino]-12-hydroxycholan-24-oic Acid Methyl Ester (19). MsCl (9 mL; 138 mmol) was dropped at −10 °C into a solution of **16** (33.3 g; 115 mmol) and DIPEA (23.5 mL; 138 mmol) in EtOAc (80 mL). The resulting suspension was stirred at 0 °C for 1.5 h, and then the precipitate was filtered and washed with cold EtOAc (2 × 50 mL). The clear solution was diluted with EtOAc (40 mL), and then **18** (27.4 g; 50 mmol) and DIPEA (23.5 mL; 138 mmol) were added. The mixture was stirred for 18 h at 50 °C, cooled to rt, and filtered, and the solid was washed with EtOAc (2 × 50 mL). The mother liquor and the washings were evaporated to dryness, and the crude was purified by silica gel flash chromatography (gradient elution 7/3 → 2/8 *n*-hexane/EtOAc) to afford **19** (41.2 g; 38 mmol) as a white solid. Yield 75%. Mp 56−58 °C; TLC R_f 0.45 (eluent A); HPLC 99.3% (method A).

(3*β*,5*β*,12α)-3-[[(4*S*)-4-[Bis[2-[bis(carboxymethyl)amino]-ethyl]amino]-4-carboxy-1-oxobutyl]amino]-12-hydroxycholan-24-oic Acid (4) by Reaction of 17, Directly Obtained from 16, with 18 and Hydrolysis of Not Isolated 19. MsCl (1.19 kg; 10.4 mol) was dropped in 1 h into a solution of **16** (3.02 kg; 10.4 mol) and DIPEA (1.55 kg; 12 mol) in *n*-BuOAc (6.8 L), initially stirred at −5 °C, maintaining the temperature around 0 °C. The resulting suspension was stirred at 0−2 °C for 1.5 h, and then the precipitate was filtered and washed with cold *n*-BuOAc (2 × 2.3 L). The solution containing **17** was loaded at 17 °C, washing the reactor with *n*-BuOAc (3.4 L), into a solution of **18** (2.6 kg; 4.74 mol) and DIPEA (1.62 kg; 12.5 mol) in *n*-BuOAc (6 L). After stirring for 20 h at 50 °C and 7 h at 80 °C, the mixture was cooled to rt and filtered, and the solid was washed with cold *n*-BuOAc (2 × 1.8 L). The mother liquor and the washings were collected, and the solvent was evaporated under reduced pressure to the minimum stirred volume, and then 2-PrOH (8.5 L) was added. The mixture was concentrated at reduced pressure, 2-PrOH (4.2 L) was added and the procedure repeated. Finally, the residue was dissolved with 2-PrOH (8.6 L) and the solution diluted with H_2O (32 L). Into the mixture containing crude **19**, 30% NaOH (9.5 kg; 71 mol) was loaded in 0.5 h at 17 °C obtaining a complete dissolution of the initially formed two phases after 3 h at the same temperature. The solution was stirred at 25 °C for 22 h, and then the pH was adjusted to 6.5−7.0 with 34% HCl, and 2-PrOH was evaporated until there was a concentration around 10% in the final product. The mixture was then loaded onto an Amberlite XAD 16.00 column (118 L) which was eluted with water at a flow rate of 1 BV/h. The eluate was partially desalted and concentrated by nanofiltration to about 50 kg, then slowly added at 20 °C into a 0.5 M aqueous solution of HCl (42.6 L; 21.3 mol). The suspension was centrifuged, and the solid was

(33) Yoshifuji, S.; Tanaka, K.; Kawai, T.; Nitta, Y. *Chem. Pharm. Bull.* **1986**, *34*, 3873–3878.

washed with H_2O (2 × 9 L), suspended in H_2O (40 L), and again centrifuged and washed with H_2O (9 + 4 L). After drying at 35 °C under reduced pressure, **4** (2.39 kg; 2.84 mol) was obtained as a white solid. Yield 60%. Mp 185−190 °C dec; TLC R_f 0.16 (eluent F); HPLC 98.9% (method E).

Trisodium [(3*β*,5*β*,12α)-3-[[4(*S*)-4-[Bis[2-[bis[(carboxy-*kO*)-methyl]amino-*kN*]ethyl]amino-*kN*]-4-(carboxy-*kO*)-1-oxobutyl]-amino]-12-hydroxycholan-24-oato(6-)]gadolinate(3-)] (B22956/1). A suspension of **4** (2.4 kg; 2.86 mol) in H_2O (15 L) was dissolved with 30% NaOH (1.03 kg; 7.72 mol), adjusting the pH to 4.8, and then Gd_2O_3 (0.526 kg; 1.45 mol) was added. The suspension was stirred for 15 h at 50 °C until complete dissolution, and then the pH was corrected to 6.7−7.2 with 30% NaOH (0.115 kg; 0.86 mol) and the solution heated to 100 °C for 1 h. After cooling to 50 °C, decolourisation with Carbopuron 4N and filtration, the solution was concentrated up to 28 ± 3% of the initial weight by evaporation at reduced pressure. Acetone (9.1 L) was added in about 0.5 h to the solution stirred at 48 °C which was subsequently cooled in about 6 h to 17 °C. After 12 h stirring at the same temperature, a second portion of acetone (9.1 L) was added over 2 h, and the suspension stirred for 1 h. The subsequent filtration followed by washings with acetone (2 × 4 L) and drying afforded **B22956/1** (2.7 kg; corr. to 2.51 kg anhydrous; 2.37 mol) as a white solid. Yield 82.9%. Mp > 300 °C; HPLC 99.8% (method F).

Supporting Information Available

Experimental general information as well as NMR spectra, MS spectra and elemental analyses of the compounds described in the Experimental Section. This material is available free of charge via the Internet at http://pubs.acs.org.

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