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February 8, 2008

Licensing Assistant Division
Nuclear Materials Safety Branch
U.S. Nuclear Regulatory Commission
475 Allendale Road
King of Prussia, PA 19406-1415

03034011

Subject: **Amendment Request for NRC License #29-30262-01**

Dear Dr. /Sir/Madam,

By way of this letter, Bracco Research USA requests the NRC to add 50 mCi of Germanium-68 and 50 mCi of Gallium-68 to our license, to be used for research and development studies in cells and in animals.

These isotopes will be provided as a commercially available $^{68}Ge/^{68}Ga$ generator (IGG100 Model No. 3131-0901, -0902 or -0903) produced by Eckert & Ziegler Isotope Products (Burbank, CA). The ^{68}Ge ($t_{1/2}$ =270 days) is immobilized on a titanium dioxide column, enclosed in a tungsten/lead shield. Over time, ^{68}Ga ($t_{1/2}$ = 68 min) grows in, and is eluted from the generator with dilute HCl.

Technical data for the device is enclosed, along with instructions for handling and milking the generator. Handling, milking and assay procedures for ^{68}Ge breakthrough and ^{68}Ga have been provided by the manufacturer and will be followed. The generator will not be disassembled.

The generator will be stored in controlled areas at Bracco Research USA that are approved for the use of radioactivity. Labeling studies with the generator eluant will be performed in these same labs. Labeling will either take place in sealed vials with suitable shielding, or using a synthesis box designed for the preparation of positron-emitting isotopes. A hot cell is available for shielding.

The generator has a useful shelf-life of about 9 months. When no longer useful, it will be returned to the manufacturer for proper disposal.

If you have any questions about this request, please feel free to contact us. Thank you in advance for your assistance.

Best Regards,

Dr. Michael F. Tweedle

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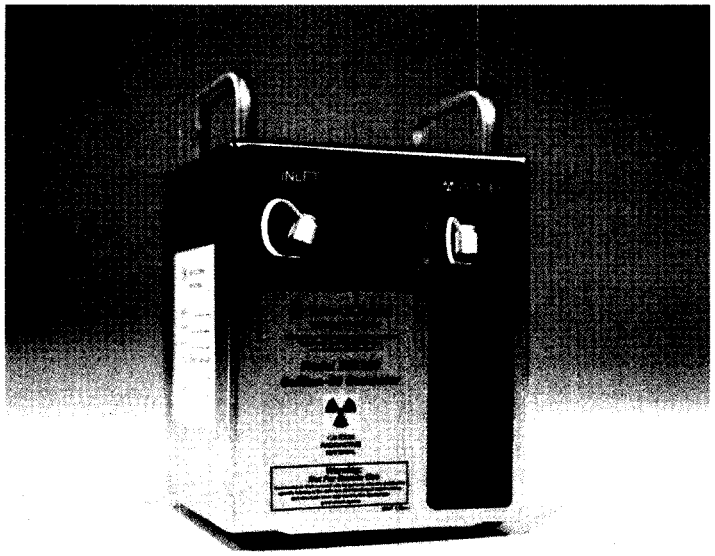
NMSS/RGN1 MATERIALS-002

IGG100 Gallium-68 Generator

Product Information



The Eckert & Ziegler Isotope Products IGG100 Gallium-68 Generator provides a positron emitter for the synthesis of radiopharmaceuticals for PET independent of a cyclotron.

■ Description

The Eckert & Ziegler Isotope Products (EZIP) Ionic Gallium Generator is a closed system consisting of a borosilicate glass column containing a titanium dioxide bed on which Germanium-68 is absorbed. Gallium-68 is continuously produced by decay of its radioactive parent and is eluted with 0.1M HCl.

This generator is designed to minimize both Ge-68 content and metal impurities in the eluate. The elution profile of the IGG100 generator has been optimized for maximum activity concentration per volume. The generator can be fully eluted using 5 mL with over 75% of the activity concentrated in just 1.5 mL.

To improve safety, reliability and simplicity, the IGG100 requires no disassembly. It is shipped ready-to use. Both the inlet and outlet lines use Luer female connections with attached caps for easy installation. EZIP maintains a comprehensive quality and regulatory compliant program based on a number of industry standards and regulations ensuring the production of consistently high value quality products. The IGG100 Generator device is made according to these regulated standards and good practices.

■ Advantages

EZIP's IGG100 Generator is ready to use as a stand alone device and is fully shielded. The IGG100 can be easily handled and installed due to its small size and light weight. There is no metal in contact with the eluate. It can be implemented in the Modular-Lab synthesis system for Ga68-peptides (see fact sheets on Ga-DOTA-conjugated peptides) and is eluted fully automatic therein.

■ Warning

- This product is not intended for use in humans.
- It has not been validated as a pharmaceutical product.
- Verification of its suitability for use in humans or as a clinical diagnostic reagent, as well as compliance with all required regulatory agencies are the sole responsibility of the purchaser.

*patent pending

Eckert & Ziegler
Eurotope

■ Technical Specifications

General Data	
Dimensions	130 x 130 x 155 mm
Weight	10 kg
Output	The actual Ge-68 activity at the assay date is indicated on the label at the side of the generator. A new generator in full equilibrium typically yields approximately 70% Ga-68 for each elution. In practice, a generator requires at least 7 hours to achieve full yield after being eluted. Output, of course, will decrease with decay of the Ge-68 parent. For example, after 9 months' decay (271 days) the activity of the eluted Ga-68 will be reduced by one-half. Elution efficiency of Ga-68 can drop a few percent if the generator is not eluted for a few days.
Breakthrough	A small amount of Ge-68 is washed from the column with each elution. The breakthrough for this generator typically ranges from 3 x 10e-5 % when new to 5.0 x 10e-3 % after 200 elutions. This breakthrough is measured as percent of Ge-68 in eluate per Ge-68 on the column. The user can significantly lower the amount and percentage of breakthrough by using only the portion of the 5mL eluate that contains the most Ga-68 activity. Peak activity can be determined by generating an elution profile from small fractions of the 5mL eluate (1mL or less). Breakthrough of Ge-68 can increase up to 1.0 x 10e-3% if the generator is not eluted for a few days. After a break in use, the generator should be pre-eluted with 10 mL of 0.1 M HCl one day prior to the intended use. The initial profile for the generator is contained in the technical data package.
Radiation	Average surface or contact radiation is less than 0.5 mR per hour per mCi of Ge-68. For example, a 20 mCi will read 10 mR per hour maximum. For high activity generators, it is recommended that the generator be stored within auxiliary shielding to minimize dose to operating personnel.
Decay characteristics	
Half-lives	Ge-68: 271 days Ga-68: 68 minutes
Radiation Type	Positrons: 1.90 Mev from Ga-68 daughter 89% abundance Photons: 0.51 Mev positron annihilation radiation 178% abundance 1.08 Mev gamma radiation 3.2% abundance
Order Information	
Art. no.: 3131-0902	10mCi (370 MBq)
Art. no.: 3131-0903	20mCi (740 MBq)
Art. no.: 3131-0901	30mCi (1.11 GBq)

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7131-0032a / Rev. 03 / 01.2008

DRAFT: IGG 100 INSTRUCTIONS FOR USE

Eckert & Ziegler

Isotope Products Laboratories

IGG100

Ionic Positron Generator

Description

The Isotope Products Laboratories Ionic Gallium Generator (IGG100) is a closed system consisting of a borosilicate glass column containing a titanium dioxide bed on which Ge-68 is adsorbed. Ga-68 is continuously produced by decay of its radioactive parent, Ge-68, and is eluted with 0.1M HCl.

This generator is designed to minimize both Ge-68 breakthrough and metal impurities. No metals are used within the closed system and all components are either medical grade or made from materials approved for medical use in other applications. The generator itself is produced at Eckert & Ziegler, Isotope Products in Burbank, California which is an ISO 13485 and ISO 14971 approved site.

Intended Use

The intended use for the generator is for research only. **WARNING: This product is Not For Human Use.**

Construction

The ion exchange column consists of a borosilicate glass tube and PEEK (Polyetheretherkeytone) end plugs which are attached to PVC inlet and outlet lines via barbed fittings. These lines extend through the shield and are attached to secondary inlet and outlet lines that then pass through the outer case of the generator. This intermediate connection provides a positive 'stop' as the lines are extended from the generator.

The column is contained within the tungsten/lead shield assembly. The average shield thickness is 3.8 cm (lead equivalent). The shield assembly is secured in a stainless steel outer box with two handles and recessed inlet and outlet ports.

Authorized Representative

EU Authorized Representative:

Eckert & Ziegler BEBIG, GmbH
Robert-Rössle Str. 10
D-13125
Berlin, GERMANY

DRAFT: IGG 100 INSTRUCTIONS FOR USE

Technical Specifications

Output

The generator is sold on the basis of the Ge-68 adsorbed on the column; therefore a nominal 20mCi generator will have 20 +/-10% mCi of Ge-68. The actual Ge-68 activity at the assay date is indicated on the label at the side of the generator. A new generator in full equilibrium typically yields approximately 70% Ga-68 for each elution. In practice, a generator requires at least 7 hours to achieve full yield after being eluted. Output, of course, will decrease with decay of the Ge-68 parent. For example, after 9 months' decay (271 days), the Ga-68 yield will be reduced by one-half.

Breakthrough

A small amount of Ge-68 is washed from the column with each elution. Ge-68 breakthrough is expressed as a percentage of total Ge-68 on the column, corrected for decay. The breakthrough for this generator typically begins at about **3×10^{-5} %** when new and gradually rises with the number of elutions. When used according to these instructions, the breakthrough should stay below **5.0×10^{-3} %** for 300 elutions.

The user can significantly lower the amount and percentage of breakthrough by using only the portion of the 5ml eluate that contains the most Ga-68 activity. Peak activity can be determined by generating an elution profile from small fractions of the 5ml eluate (1ml or less). The initial profile for this generator is contained in the technical data package.

<u>Warning: Breakthrough of Ge-68 can increase up to 1.0×10^{-3}% if the generator is not eluted for more than 3 days. If the generator has not been used for several days, it should be pre-eluted with 10 mL of 0.1 M HCl 7-24 hours prior to the intended use.</u>

Decay Characteristics

Half-life:

Ge-68: 271 Days

Ga-68: 68 Minutes

Radiation Type:

Positrons: 1.90 Mev from Ga-68 Daughter
89% abundance

Photons: 0.51 Mev positron annihilation radiation
1.08 Mev gamma radiation
3.2% abundance

At full growth Ga-68 is in secular equilibrium with its Ge-68 parent.

Radiation

Average surface or contact radiation is less than 0.5 mR per hour per mCi of Ge-68. For example, a 20 mCi will read 10 mR per hour maximum. For high activity generators, it is recommended that the generator be stored within auxiliary shielding to minimize dose to operating personnel.

Generator Life

Expected life of the generator is dependent upon several factors including, frequency of use, rate of elution, time from manufacture, purity of HCl solution and others. Isotope Products recommends replacement when Ge-68 breakthrough increases to a point above 5.0×10^{-3} %. When used according to these instructions, the generator should yield approximately 300 elutions before reaching this point. Radiation also affects the column over time; it is therefore recommended that the generator be replaced 1 year after receipt from the manufacturer regardless of the number of elutions.

Physical Characteristics

Size: Height 19cm; Width 13cm; Depth 13cm

Weight: Approximately 10kg (22lbs)

Elution location: Bottom. There is sufficient excess tubing to bring eluent line near the top of the generator if the generator is used in a recessed location.

Handling features: Two handles placed on the top of the generator can be used to move/handle the generator. They are also compatible with Eckert & Ziegler Modular Lab ™ stackable components.

DRAFT: IGG 100 INSTRUCTIONS FOR USE

Unpacking the Generator

1. **Check outer box for shipping damage**. If damaged, perform radiation wipe survey of the damaged area. If counts exceed 2200 dpm per 100 cm^2 notify you Radiation safety officer.

2. **Cut security tape** on top of shipping carton. Remove the inner Styrofoam support from the box. Separate the foam halves carefully so that the internal box does not fall.

3. **Carefully open the inner box** and remove generator. Perform radiation survey. CAUTION: Drop hazard: The radiator weighs approximately 10Kgs and has sharp edges. Handle with care to avoid potential injuries. If generator is dropped or if shipping damage extends into the inner box, check for leaks and perform a wipe survey of the generator. Also check for internal damage by slowly tilting the generator 90°. Listen for broken/loose metal parts. If the generator has sustained internal damage, call technical service at (661) 309-1010.

4. **Perform wipe survey** of box inserts and generator outer surface. If wipes exceed 2200 dpm per 100 cm^2, notify your Radiation Safety Officer. Call Technical Service at (661) 309-1010 for further information if necessary.

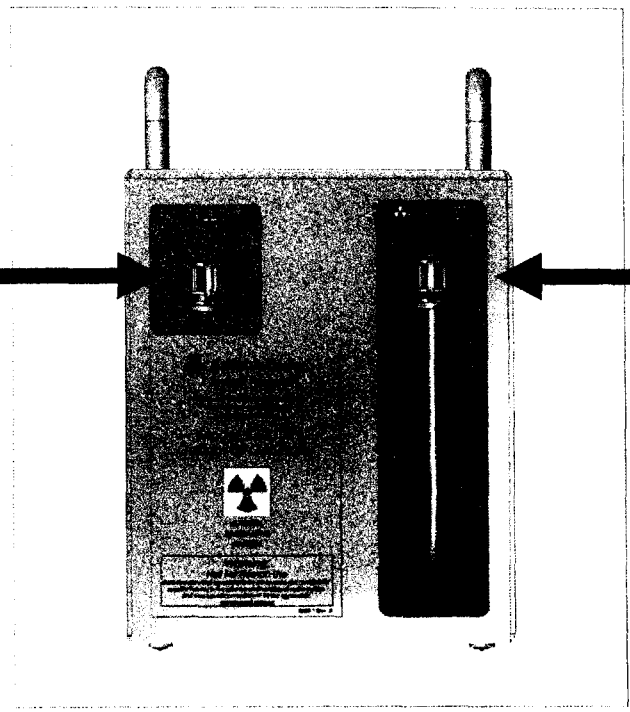
5. **Pull the inlet and outlet tubes** from their respective recesses until all of the excess tubing is exposed. Check the length to ensure the desired input and output connections can be reached.

6. **The Generator is now ready to use,** please completely read the following "Elution instruction" to properly use the Generator.

DRAFT: IGG 100 INSTRUCTIONS FOR USE

Elution Instruction

Eluent INLET:
0.1M Ultra-pure HCl



Eluate OUTLET:
Ga-68 solution

Please read the entire Instructions For Use before you start.

Prepare/assemble necessary materials:

a) Personal Protective equipment: elutions should be performed while wearing eye and hand protection.

b) Syringe capable of accurately eluting 10ml

c) Shielded receiving vessel

d) Supply of ultra-pure 0.1M HCl. (Note: Ultra Pure 0.1M HCl solution can be prepared from commercially available concentrated ultra-pure HCL diluted by quartz distilled water) Caution: Use only 0.1M HCl acid for elution. Acids of higher concentration or lower purity will lead to higher Ge-68 breakthrough and higher impurities. HCl is hazardous to human tissue. Handle with care. See MSDS for further information.

e) Shielding for eluate line during elution

1. Fill the syringe with at least 5ml of 0.1 M HCl.

2. Carefully discharge all air from syringe and luer hub; then connect to the female luer fitting on inlet line. The inlet line is located in the GREEN port. Caution: 1) Be sure to introduce eluent through the inlet (GREEN) port. Eluting the generator in reverse will damage the generator. 2) Elution efficiency may be reduced if air is introduced to the generator bed.

3. Connect a syringe or other receiving vessel to the outlet line using the female luer connector. The vessel must have sufficient capacity to accept up to 10 ml of eluate.

4. Introduce the 0.1 M HCl eluent at a rate no greater than 2ml/minute. Eluting at a faster rate may reduce the life of the generator. 5ml of eluent will fully elute the generator. If high resistance is encountered, do not force solution into generator. If a peristaltic pump is used to elute the generator it should be set to a volume rate of 2 ml/minute or less. The user should also verify that eluent is flowing without unusual

resistance. If high resistance is noted, discontinue elution and Call U.S. Technical Service at (661) 309-1010.

5. Collect eluate in receiving vessel.

6. Assay solution in receiving vessel with a calibrated ion chamber to determine yield of generator.

7. If less than 5 ml of eluate has been collected, assay may not represent the total potential yield of generator.

8. The by-products of radiolysis and free Ge-68 ions accumulate within the column over time. These by-products can affect the efficiency of some Gallium labeling processes . Therefore, if the generator has not been used for a period of 3 days or greater, it is recommended that the column be eluted once at least 7-24 hours prior to eluting for labeling studies. This elution should be done using twice the volume (10ml of 0.1MHCl) of an ordinary elution to fully wash the impurities from the column.

9. If fluid leaks are observed at any time, immediately stop eluting, install end caps on both the inlet and outlet lines, attempt to contain the leaking fluid and call Technical Service at (661) 309-1010.

Disassembly: Do not disassemble the Generator for any reason. Doing so may damage the internal components and possibly lead to a leak of radioactive material. Also, disassembly of stainless steel casing will expose lead shielding to operator.

Disposal: After its useful life, the generator must be disposed of as radioactive waste. Contact Eckert & Ziegler Isotope Laboratories for disposal guidance.

Note: Since Ga-68 has a 68-minute half-life, you must correct for the elapsed time between elution and measurement to determine actual yield at the elution time. Refer to the decay chart on the next page. Select the appropriate decay factor, based on the elapsed time, as defined above. Divide the measured mCi value by this factor to obtain the mCi yield at time of elution.

GALLIUM-68 DECAY CHART

Half-life 68.0 minutes

ElapsedTime in Minutes	Decay Factor	ElapsedTime in Minutes	Decay Factor
1	0.990	35	0.700
2	0.980	36	0.693
3	0.970	37	0.686
4	0.960	38	0.679
5	0.950	39	0.672
6	0.941	40	0.665
7	0.931	41	0.658
8	0.922	42	0.652
9	0.912	43	0.645
10	0.903	44	0.639
11	0.894	45	0.632
12	0.885	46	0.626
13	0.876	47	0.619
14	0.867	48	0.613
15	0.868	49	0.607
16	0.850	50	0.601
17	0.841	51	0.595
18	0.832	52	0.589
19	0.824	53	0.583
20	0.816	54	0.577
21	0.807	55	0.571
22	0.799	56	0.565
23	0.791	57	0.559
24	0.783	58	0.554
25	0.775	59	0.548
26	0.767	60	0.543
27	0.759	61	0.537
28	0.752	62	0.532
29	0.744	63	0.526
30	0.737	64	0.521
31	0.729	65	0.516
32	0.722	66	0.510
33	0.714	67	0.505
34	0.707	68	0.500

Pergamon

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Bioorganic & Medicinal Chemistry Letters 13 (2003) 3401–3404

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis of *Para*-Alkyl Aryl Amide Analogues of Sphingosine-1-phosphate: Discovery of Potent S1P Receptor Agonists

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Abstract—Sphingosine-1-phosphate (S1P) is a biologically active lysophospholipid with the capacity to induce a broad range of cellular responses via its interaction with the S1P family of G-protein coupled receptors. This report describes the synthesis of several potent S1P receptor agonists. For instance, compound **9c** displayed an $EC_{50}=8.6$ nM at the $S1P_1$ receptor using a [γ-^{35}S]GTP binding assay as compared to an $EC_{50}=4.5$ nM for the endogenous ligand. We also report the effects associated with introduction of a phenyl ring between the 'linker' and 'lipophilic tail' regions of the analogues, for example total loss of activity at $S1P_2$ and increased agonism at $S1P_5$.
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Sphingosine-1-phosphate (S1P) has been demonstrated to act as both an intercellular signaling molecule and an intracellular second messenger (Fig. 1).[1] Extracellular S1P binds a specific subset of seven trans-membrane G-protein coupled receptors, namely the $S1P_1$ (formerly Edg-1), $S1P_2$ (Edg-5), $S1P_3$ (Edg-3), $S1P_4$ (Edg-6), and $S1P_5$ (Edg-8) receptors.[2] Through its interaction with these receptors, S1P has been shown to induce a wide variety of cellular effects including differentiation, motility, and escape from apoptosis.[3]

The ability of S1P to mediate biological processes such as immune modulation make S1P receptor agonists and antagonists attractive candidates for use as therapeutic agents.[4] For example, the sphingosine analogue, FTY720, is a novel immunosuppressive agent that acts by reversibly sequestering lymphocytes to secondary lymphoid tissues.[5] FTY720 is a pro-drug that, after *O*-phosphorylation by a yet to be defined kinase, elicits lymphopenia via its interaction with one or more of the S1P receptors.[5,6] Phosphorylated FTY720 (phospho-FTY720) shares several structural similarities with S1P including a lipophilic tail region, a 2-amino group, and a phosphate head group.

To understand better the processes associated with S1P binding to its receptors, it is vital to establish a structure–activity relationship (SAR) for S1P with respect to each individual receptor. The generation of S1P analogues with different binding profiles across the S1P receptors proves useful in elucidating this SAR. More specifically, the synthesis of S1P receptor specific agonists and antagonists could prove useful in the discovery of novel drugs. To supplement our previously reported $S1P_1/S1P_3$ receptor agonist,[7] we now wish to report the synthesis of potent $S1P_1$, $S1P_3$, $S1P_4$ and $S1P_5$ receptor agonists along with their activity profiles across all five recombinant human S1P receptors. We also report several interesting effects in activity with the incorporation

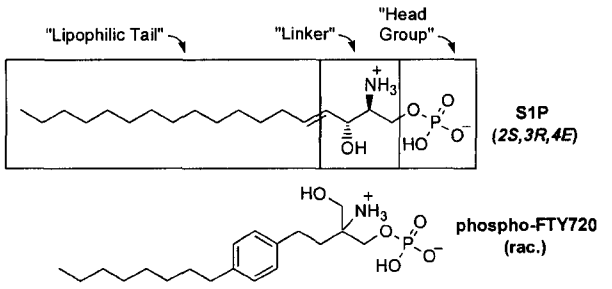

Figure 1. Structures and regions of S1P and phospho-FTY720.

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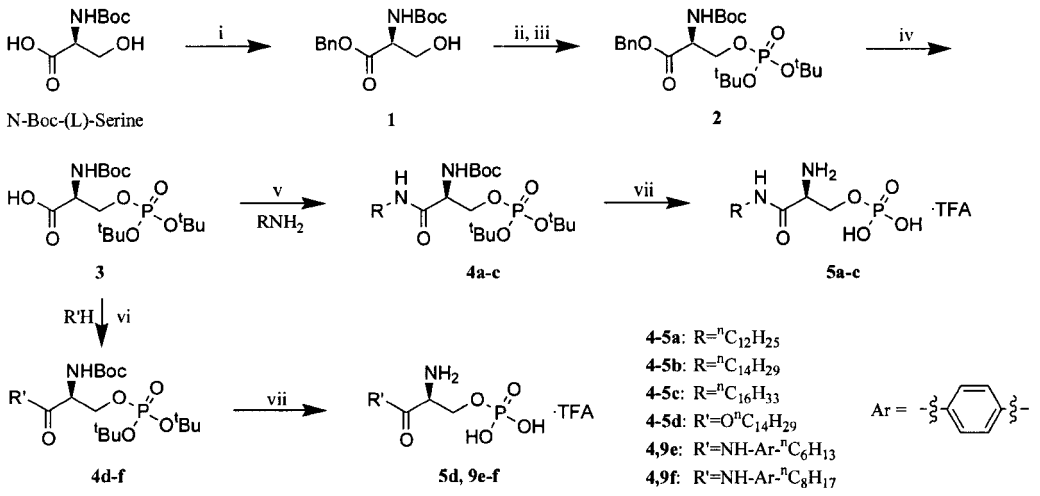
of a phenyl ring between the 'linker' and 'lipophilic tail' regions of the S1P analogues, as is the case in phospho-FTY720. These effects include a total loss of activity at $S1P_2$, increase in agonism at $S1P_5$, and a loss of C2-stereospecificity at the $S1P_1$ and $S1P_3$ receptors.

Our preparation of S1P analogues began with the synthesis of the (2*S*)-*N*-alkyl amide, (2*S*)-*O*-alkyl ester, and (2*S*)-*N*-aryl amide compounds, **5a–c**, **5d**, and **9e–f**, respectively (Scheme 1).[7] N-Boc-(L)-Serine was protected as the benzyl ester using benzyl bromide under standard conditions. The protected amino acid **1** was then phosphorylated using di-*tert*-butyl diisopropylphosphoramidite with subsequent hydrogen peroxide oxidation of the phosphite to afford **2** which underwent hydrogenolysis to give acid **3**. Coupling of **3** to the appropriate alkyl amine using DCC afforded the protected *N*-alkyl amides **4a–c**. Coupling of **3** to 1-tetradecanol using the PyBOP reagent gave the protected *O*-alkyl ester **4d**. Coupling of **3** to the appropriate *p*-alkyl aniline using the PyBOP reagent afforded the protected *N*-aryl amides **4e–f**. Deprotection of the amides **4a–c** and **4e–f** and ester **4d** using 1:1 TFA/CH_2Cl_2 gave the final products **5a–d** and **9e–f** as the TFA salts.

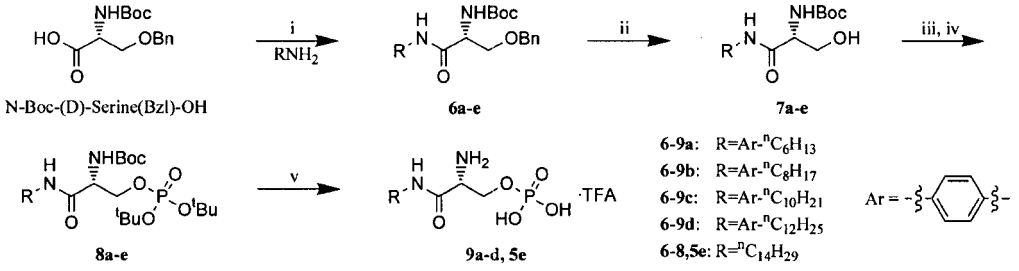
The synthetic route to the (2*R*)-*N*-alkyl amide and (2*R*)-*N*-aryl amide compounds, **5e** and **9a–d**, respectively, commenced with PyBOP mediated coupling of *N*-Boc-(D)-Serine(Bzl)–OH with the appropriate alkyl amine or *p*-alkyl aniline (Scheme 2).[7] The resulting amides **6a–e** were then subjected to hydrogenolysis to afford the deprotected alcohols **7a–e**. These alcohols were then phosphorylated using di-*tert*-butyl diisopropylphosphoramidite with subsequent oxidation of the phosphite by hydrogen peroxide to give the protected phosphates **8a–e**. Deprotection of **8a–e** using 1:1 TFA/CH_2Cl_2 gave the final products **5e** and **9a–d** as the TFA salts.

Receptor activation by S1P and the synthetic analogues was determined in vitro by measuring the ligand dependant binding of [γ-^{35}S]GTP to membranes containing each of the five human S1P receptors expressed in HEK293T cells.[8] None of the compounds in these series showed any activity at the related lysophosphatidic acid receptors (LPA_{1-3}) at concentrations up to 10 μM in this assay (data not shown). As was previously reported, *R*-configuration at the C2 position of the *N*-alkyl amide analogues appears to result in increased binding at $S1P_1$ and $S1P_3$, but not at the other S1P receptors (Table 1).[7]

Although the *N*-alkyl amide and *O*-alkyl ester compounds were poor agonists at $S1P_2$, introduction of a phenyl ring between the 'linker' and 'lipophilic tail'



Scheme 1. Reagents and conditions: (i) Cs_2CO_3, BnBr, DMF, rt, 12 h, quant.; (ii) tetrazole, di-*tert*-butyl diisopropylphosphoramidite, 1:1 CH_2Cl_2/THF, rt, 12 h; (iii) H_2O_2, rt, 4 h, quant. (2 steps); (iv) H_2, 10% Pd/C, EtOH, rt, 12 h, 91%; (v) DCC, DMAP, HOBT, CH_2Cl_2, rt, 12 h, 26–41%; (vi) PyBOP, DIEA, CH_2Cl_2, rt, 6 h, 43–60% (15% for **4d**); (vii) 1:1 TFA/CH_2Cl_2, rt, 4 h, 96–100%.



Scheme 2. Reagents and conditions: (i) PyBOP, DIEA, CH_2Cl_2, rt, 6 h, 65–77%; (ii) H_2, 10% Pd/C, EtOH, rt, 12 h, 84–96%; (iii) tetrazole, di-*tert*-butyl diisopropylphosphoramidite, 1:1 CH_2Cl_2/THF, rt, 12 h; (iv) H_2O_2, rt, 4 h, 60–100% (2 steps, 9% for **9d**); (v) 1:1 TFA/CH_2Cl_2, rt, 4 h, 56–100%.

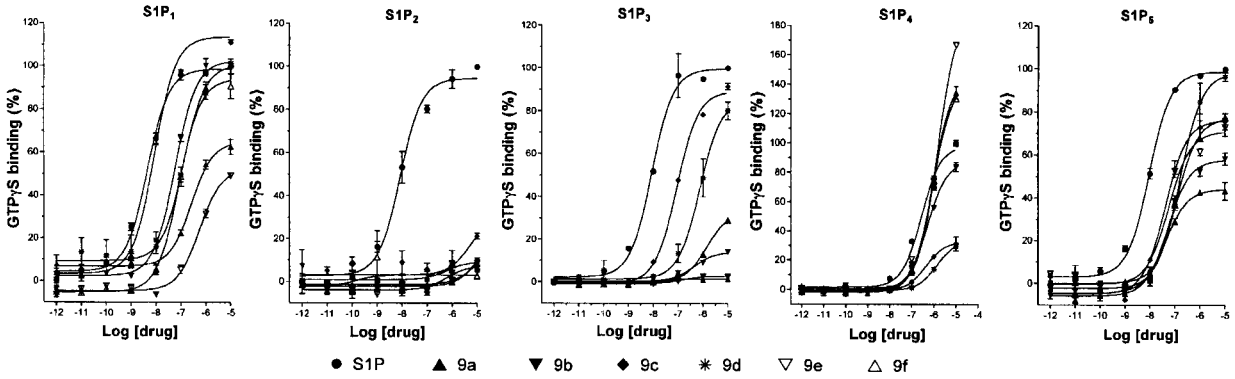


Figure 2. [γ-^{35}S]GTP binding to HEK293T cell membranes in response to S1P and *N*-aryl amide S1P analogues. Each data point represents the mean of three determinations.

regions of the analogues, as in compounds **9a–f**, resulted in a complete loss of activity at that receptor type (Table 1, Fig. 2). This structural modification also resulted in increased binding affinity towards $S1P_5$. In contrast to the *N*-alkyl amide compounds, stereochemical configuration at the C2 position of the *N*-aryl amide analogues does not appear to have an effect on activity at any of the S1P receptors. This finding also differs from data reported by Lynch, et al. for similar compounds containing 2-methyl groups, (*S*)-AFD and (*R*)-AFD, in which only the 2*R* enantiomer acted as a potent agonist at the S1P receptors (Fig. 3).[6]

Table 1. EC_{50} Values (nM) for S1P and *N*/*O*-alkyl amide/ester and *N*-aryl amide analogues at S1P receptors determined by a [γ-^{35}S]GTP binding assay[a,b]

N/*O*-Alkyl Compd	$S1P_1$	$S1P_2$	$S1P_3$	$S1P_4$	$S1P_5$
S1P	0.9	2.9	1.1	nd	43.9
5a	598.4	973.2	845.4	nd	645.5
5b	397.0	2685.0	862.4	nd	1606.0
5c	1805.0	>5000	878.6	nd	1220.0
5d	322.1	2760.0	601.9	nd	>5000
5e	12.7	2107.0	50.8	nd	>5000
N-Aryl Compd	$S1P_1$	$S1P_2$	$S1P_3$	$S1P_4$	$S1P_5$
S1P	4.5	8.3	8.7	270	9.2
9a	260	na	na	880	43
9b	58	na	450	500	52
9c	8.6	na	89	450	41
9d	130	na	740	1400	160
9e	550	na	na	1500	55
9f	82	na	1500	910	110

[a]Values are means of three experiments (na = not active, nd = no data).
[b]Data for *N*/*O*-alkyl compounds are taken from ref 7.

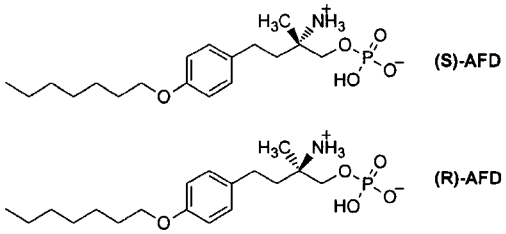


Figure 3. Structures of (*S*)-AFD and (*R*)-AFD (see ref 6).

Regarding the $S1P_1$ and $S1P_3$ receptors, the length of the alkyl chain of the *N*-aryl amide compounds was optimized at 10 carbons, compound **9c** displayed EC_{50}s = 8.6 nM and 89 nM, respectively, on those receptors. With respect to the $S1P_4$ receptor, shorter chain lengths of six and eight carbons on the *N*-aryl amide compounds exhibited higher potency and efficacy than the longer chain lengths of 10 and 12 carbons. At the $S1P_5$ receptor, the greatest efficacy of the *N*-aryl amide compounds was achieved with the longest chain length, 12 carbons. The potency, however, of the *N*-aryl amide compounds on $S1P_5$ was approximately equal, about one log order less than that of S1P itself.

To summarize, we have synthesized a second generation of S1P analogues that incorporate a phenyl ring between the 'linker' and 'lipophilic tail' regions of the pharmacophore, as is the case in phospho-FTY720. This structural modification has resulted in the generation of highly potent, fully efficacious agonists on four of the S1P receptors; $S1P_1$, $S1P_3$, $S1P_4$ and $S1P_5$. In particular, introduction of the phenyl ring between the 'linker' and 'lipophilic tail' regions of the S1P analogues described here results in complete loss of activity at $S1P_2$, increased activity at $S1P_5$, and a loss of C2-stereospecificity at the $S1P_1$ and $S1P_3$ receptors. These findings have helped to decipher further the SAR of S1P on the five S1P receptors and will serve as the basis for additional studies along this route.

Acknowledgements

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References and Notes

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8. Brief assay protocol: S1P was purchased from Biomol (Plymouth Meeting, PA). [γ-^{35}S]GTP was purchased from New England Nuclear (Boston, MA). HEK293T cells were a gift from Dr. Judy White (Department of Cell Biology, University of Virginia). First, 5 μg of membranes from the appropriate receptor and G-protein expressing HEK293T cells were incubated in 0.1 mL of GTP-binding buffer (in mM: HEPES 50, NaCl 100, $MgCl_2$ 5), pH 7.5 containing 5 μg saponin, 10 μM GDP, 0.1 nM [γ-^{35}S]GTP (1200 Ci/mmol) and test lipid. After incubating for 30 min at 30 °C, bound radionuclide was separated from free by filtration through Whatman GF/C paper using a Brandel Cell Harvester (Gaithersburg, MD). Bound radionuclide was detected with a Packard Top Count liquid scintillation counter.

Note: This summary of the IGG 100 is based on data generated from test units. It is not a specification, marketing claim or guarantee of performance characteristics.

Eckert & Ziegler Isotope Products
IGG 100 Gallium Generator

Description

The Eckert & Ziegler Isotope Products Ionic Gallium Generator (IGG100) is a closed system consisting of a borosilicate glass column containing a titanium dioxide bed on which Ge-68 is adsorbed. Ga-68 is continuously produced by decay of its radioactive parent and is eluted with 0.1M HCl.

This generator is designed to minimize both Ge-68 breakthrough and metal impurities. No metals are used within the closed system and all components are either medical grade or made from materials approved for medical use in other applications.
NOTE: THIS GENERATOR IS NOT INTENDED FOR HUMAN USE.

Break-through

IGG100 Break-through	Initial	End of life
Typical	**0.00001%**	**0.001%**
Specification	0.001%	0.005%

Efficiency

(Ga-68 produced)/(Ge-68 loaded) X 100%= **70+/-5%***

* corrected for decay

Impurities

Impurities can compete with synthesis and radio-labeling processes. The IGG 100 was designed to reduce or eliminate all metal impurities. ICPMS testing of the IGG 100 elutions confirm that most metals are not detectable (typical limit 0.001 ppm). The sum total of all metals (less Na, B, K, Si) is typically **less than 1 ppm.** See attached test report

External dose rate

The IGG 100 is well shielded, providing much lower external dose than other Gallium generators. This was done to ease shielding requirements in the space confined areas in which they are used. Typical surface dose rates for a 30mCi (1,110 MBq) generators are shown below:

	µSv/hr	Mr/hr
IGG100	**100**	**10**
Other Gen	480	48

Activity Concentration:

The IGG 100 can be fully eluted using just **5 ml** and over **75%** of the eluted activity is concentrated in just **1.5ml.**

Compact/Flexible Geometry

To reduce the amount of hood space required for the generator, The IGG 100 was designed to be smaller and lighter than all other Gallium generators in the market. It is **15cm high by 13cm wide** and has a mass of only **10Kg**. In addition, the eluate (outlet) line can be eluted both at the base (for hoods with one working surface) or near the top (for hoods with recessed areas).

Compatible with Modular Lab™

The IGG 100 is compatible with Eckert & Ziegler's Modular Lab™ components. Modular lab components can be stacked on the generator and all materials and connections are compatible with Modular Lab™ synthesis hardware and processes.

Ready-to-use

Some generators require the operator to disassemble the unit, pierce a septum with a needle and charge the column with the elution fluid. To improve safety, reliability and simplicity, the IGG 100 requires none of these operations. It is shipped in a ready to use condition. Both the inlet and outlet lines use Luer female connections with attached caps facilitating easy installation into any Radiation lab area.

This is to acknowledge the receipt of your letter/application dated

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AMEND. 29-30262-01

☑ There were no administrative omissions. Your application was assigned to a technical reviewer. Please note that the technical review may identify additional omissions or require additional information.

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